TÜBİTAK

Turkish Journal of Biology

Volume 30 | Number 3 Article 10

1-1-2006

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Turk J Biol
30 (2006) 171-175

Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Urla (İzmir, Turkey)

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Received: 09.03.2006

Abstract: Methanol, acetone, diethyl ether, and ethanol extracts of 11 seaweed species from the coast of Urla were tested in vitro for their antimicrobial activities against *Candida* sp., *Enterococcus faecalis, Staphylococcus aureus, Streptococcus epidermidis, Pseudomonas aeruginosa, and Escherichia coli* with the disc diffusion method. Diethyl ether was the best solution for extracting the effective antimicrobial materials from the algae species used in this experiment, with the exception of *D. linearis*, for which ethanol was the most effective extraction solution. Diethyl ether extracts of fresh *Cystoseira mediterranea, Enteromorpha linza, Ulva rigida, Gracilaria gracilis,* and *Ectocarpus siliculosus* showed effective results against all test organisms. However, diethyl ether extracts of some species, such as *Padina pavonica, Colpomenia sniosa, Dictyota linearis, Dictyopteris membranacea, Ceramium rubrum,* and *Acanthophora nojadiformis*, gave different results. A significant difference in antimicrobial activity was not observed between the acetone and methanol extracts of each alga. In addition, as a result of the comparison of dried and fresh extract antimicrobial activity, it was found that all test organisms were more sensitive to fresh extracts of the algae. Although fresh extracts of *G. gracilis, D. linearis,* and *E. siliculosus* inhibited the tested bacteria and yeast, their dried extracts had no inhibition activity on either Gram-negative or Gram-positive bacteria.

Key Words: Antimicrobial activity, macro-algae, algal extract

Urla Kıyılarında Bulunan Deniz Alg Ekstraktlarının Antimikrobiyal Aktiviteleri

Özet: Urla kıyılarından toplanan 11 deniz algi türünden elde edilen methanol, aseton, dietil eter ve etanol ekstraktlarının antimikrobiyal aktiviteleri in vitro koşullarda, *Candida* sp., *Enterococcus faecalis, Staphylococcus aureus, Streptococcus epidermidis, Pseudomonas aeruginosa* ve *Escherichia coli* türleri üzerinde disk difuzyon yöntemi ile araştırılmıştır. Dietil eter, Dictyota linearis'in etanol ekstraktı dışında, tüm türlerde en iyi sonucu vermiştir. *Cystoseira mediterranea, Enteromorpha linza, Ulva rigida, Gracilaria gracilis* ve *Ectocarpus siliculosus* türlerinin yaş ekstraktları tüm test organizmalarına karşı etkinlik göstermiştir. Ancak *Padina pavonica, Colpomenia sniosa, Dictyota linearis, Dictyopteris membranacea, Ceramium rubrum,* ve *Acanthophora nojadiformis* gibi bazı türlerin dietil eter ekstrakları farklı sonuçlar vermiştir. Kullanılan alg türlerinin aseton ve methanol ekstraktlarının etkileri arasında belirgin bir farklılık bulunamamıştır. Bunlara ek olarak, örneklerin kuru ve yaş ekstraklarının antimikrobiyal aktiviteleri karşılaştırıldığında, tüm test mikroorganizmalarının yaş ekstraklara karşı daha duyarlı olduğu gözlenmiştir. *Gracilaria gracilis, Dictyota linearis* ve *Ectocarpus siliculosus* türlerinin yaş ekstraktları test organizmaların inhibe ederken, kuru ekstraktlarında gram negatif ve gram pozitif bakterilere karşı böyle bir etki gözlenmemiştir.

Anahtar Sözcükler: Antimikrobiyal aktivite, makro alg, alg ekstraktı

Introduction

Marine organisms are a rich source of structurally novel and biologically active metabolites (1,2). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (3). To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals (3,4).

The cell extracts and active constituents of various algae have been shown to have antibacterial activity in vitro against Gram-positive and Gram-negative bacteria. (1). A wide range of results of in vitro anti-fungal activities of extracts of green algae, diatoms, and dinoflagellates have also been reported (1,5). Similarly, to date, some micro-algae screened contain and/or excrete pharmacologically active compounds. For example, the dinoflagellates *Gymnodinium* sp. and *Gonyaulax* sp. produce an alkylguanidine compound that

effects the central nervous system. In *Rivularia firma*, pharmacological activity is due to brominated bi-indoles. A red alga, *Gracilaria lichenoides*, excretes prostaglandins and the compound C_{20} (6).

In the present study, we describe the antimicrobial characteristics of methanol, acetone, diethyl ether, and ethanol extracts of some marine algae obtained from the coast of Urla (İzmir, Turkey).

Materials and Methods

Extract Preparation

Seaweeds were collected at a depth of 1-2 m from the coast of Urla, Izmir (lat 38°21'32N, long 26°46'59E) in May 2005 and were identified by Dr. Atakan Sukatar.

Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris. Half of these cleaned fresh materials were air-dried. As described by González del Val et al. (2001), 25 g of each fresh and air-dried algal sample were extracted in 50 ml of methanol, acetone, diethyl ether, and ethanol.

Test microorganisms

The strains of *Candida* sp., *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli* were obtained from the culture collection of the Basic and Industrial Microbiology Section of Ege University, İzmir, Turkey, and were maintained on Brain Heart Infusion (BHI) agar medium at 4 °C until testing.

Antimicrobial testing

Antimicrobial activity was evaluated using the agar diffusion technique in petri dishes (3). Briefly, 25 µl of each extract was loaded on sterile filter paper discs 6 mm in diameter (E-760), and air-dried. Indicator microorganisms were spread on Mueller-Hinton agar plates with sterile effusion and the discs were placed on plates. After incubation for 24 h at 30 °C, a clear zone around a disc was evidence of antimicrobial activity. Diameters of the zones of inhibition were measured in millimeters. Each test was prepared in duplicate. Discs loaded with the extracting agents were tested as controls.

Results and Discussion

Extracts of 11 species of seaweed were tested against bacteria (*E. faecalis*, *P. aeruginosa*, *E. coli*) and yeast (*Candida* sp.). The results of primary screening tests are summarized in Table 1, which show that the extracts of 6 algal species possessed antibacterial activity. For some species, the antimicrobial activity we observed was similar to previous screening studies (1,2).

In this study, diethyl ether was the best solution for extracting the effective antimicrobial materials from the algae species used in this experiment, with the exception of *D. linearis,* for which ethanol was the most effective extraction solution. (Table 1). A significant difference in antimicrobial activity was not found between the acetone and methanol extracts of each alga. Antibacterial activity depends on both algal species and the efficiency of the extraction method. For instance, diethyl ether extracts of fresh *C. mediterranea, E. linza, U. rigida*, *G. gracilis*, and *E. siliculosus* showed effective results against all test organisms; however, the diethyl ether extracts of some species, such as *P. pavonica, C. sniosa, D. linearis, D. membranacea, C. rubrum,* and *A. nojadiformis,* gave different results. Although it is known that the diethyl ether extract of *D. linearis* was ineffective against microorganisms, the ethanol extract of the *D. linearis* indicated an antimicrobial activity against Gram-negative bacteria and *Candida* sp. This result could be related to the presence of bioactive metabolites in *D. linearis*, which are soluble in ethanol, but not in diethyl ether. The structures of the bioactive metabolites of the species will be examined in our next investigation.

As a consequence, the diethyl ether extracts and ethanol extracts with the most effective antimicrobial activity were selected from 11 algal species for detailed antimicrobial producer tests. Regarding the obtained results, fresh-extracted and dry-extracted samples of the best halo-zone producers were assayed against 3 Gram (+) and 2 Gram (-) bacteria, and 1 yeast. Table 2 shows the effects of the different algae extracts collected from pathogenic microorganisms.

As seen in Table 2, dried extracts have less or no effects on bacteria in comparison to the fresh extracts. This result can be related to volatile antimicrobial compounds in the samples, such as hydrogen peroxide, terpenoid, and bromo-ether compounds (6,7). Another reason might be the loss of active materials that may be

Table 1. Antibacterial activity of different fresh extracts of marine algae.

Species		*Candida* sp.	*E. faecalis*	*P. aeruginosa*	*E. coli*
Ulva rigida	Acetone	-	-	-	-
	Methanol	-	-	+	-
	Diethyl ether	++	++	++	++
	Ethanol	+	+	-	+
Enteromorpha linza	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	++	++	++	++
	Ethanol	+	+	-	+
Padina pavonica	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	-	-	-	-
	Ethanol	+	+	+	+
Colpomenia sniosa	Acetone	-	-	-	+
	Methanol	-	-	-	-
	Diethyl ether	-	-	-	-
	Ethanol	-	-	-	-
Dictyota linearis	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	-	-	-	-
	Ethanol	++	-	+	++
Dictyopteris membranacea	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	+	+	-	-
	Ethanol	+	+	-	-
Cystoseira mediterranea	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	++	++	++	++
	Ethanol	+	+	+	+
Ectocarpus siliculosus	Acetone	-	+	+	-
	Methanol	-	-	-	-
	Diethyl ether	++	++	++	++
	Ethanol	+	+	+	+
Ceramium rubrum	Acetone	-	-	-	-
	Methanol	-	-	-	-
	Diethyl ether	-	-	-	+
	Ethanol	-	-	-	+
Gracilaria gracilis	Acetone	+	-	-	+
	Methanol	+	+	-	-
	Diethyl ether	++	++	++	++
	Ethanol	+	+	+	-
Acanthophora nojadiformis	Acetone	-	-	-	+
	Methanol	-	-	-	-
	Diethyl ether	+	+	+	+
	Ethanol	+	+	+	-

(-) No activity, (+) low activity (7-10-mm halo), (++) high activity (10-15-mm halo).

Table 2. Antimicrobial activity of the most inhibiting ethanol and diethyl ether extracts of the marine algae.

Organic Solvent	Alga	Yeast	Bacteria (Gram-positive)		Bacteria (Gram-negative)		
		Candida spec.	*E. faecalis*	*S. epidermidis*	*S. aureus*	*E. coli*	*P. aeruginosa*
Diethyl ether	*G. gracilis* (fw)	18.5*	28.5	15	18.5	16.5	18.5
	G. gracilis (dw)	9.5	-	-	-	-	-
	E. linza (fw)	24.5	45	43	51	22	23.25
	E. linza (dw)	9	13	11	9	7.5	10
	C. mediterranea (fw)	16	35	42	26.5	20	23
	C. mediterranea (dw)	10	9	-	-	-	-
	U. rigida (fw)	14.5	33	47	38	22.5	25
	U. rigida (dw)	8.5	10	-	-	8.5	9
	E. siliculous (fw)	16	32.5	43.5	30	22	22
	E. siliculous (dw)	-	-	-	-	-	-
Ethanol	*D. linearis*(fw)	10.5	-	-	-	12	9
	D. linearis(dw)	-	-	-	-	-	-

fw: fresh weight, dw: dry weight
*measured in millimeters

present in algae, like volatile fatty acids, during the drying process.

Ethanolic and diethyl ether fractions appear to be specific, particularly against the tested Gram-positive bacteria. In addition to their antibacterial activity, diethyl ether extracts of *C. mediterranea, E. linza, U. rigida*, *G. gracilis*, and *E. siliculosus* also inhibited the growth of yeasts.

Another significant result of the present study was that the ethanol extracts of *D. linearis* (fw) showed antifungal and antibacterial activity against the *Candida* sp. and Gram-negative bacteria, respectively. Similarly, Moreau et al. (1988) reported the inhibitive effect of hexane extracts of *D. dichotoma* on fungal growth.

Lima-Filho et al. (2002) found that the hexane extract of *Gracilaria* sp. inhibits only *Bacillus subtilis*. In contrast, our results showed that the diethyl ether extract of *G. gracilis* (fw) inhibited *Candida* sp., *E. faecalis, S. epidermidis, S. aureus, E. coli,* and *P. aeruginosa*. The activity against Gram-negative bacteria was less effective compared to Gram-positive bacteria.

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate (5,7,8), whereas others report that chloroform is better than methanol and benzene (3). It is clear that using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water-based methods (4,6). According to our experimental results, diethyl ether caused better halo-zones than methanol, acetone, and ethanol.

Perez et al. (1990) found that the extract of *Ulva lactuca* had no antimicrobial activity. In contrast, our results showed that the diethyl ether extract of *U. rigida* inhibited all the test organisms. This difference may have been due to species variation.

In 2001, Gonzalez del Val wrote that methanol extracts of *Padina pavonica* show antibacterial activity only against *B. subtilis*; however, in our experiments, 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*, *E. faecalis*, *P. aeruginosa*, and *E. coli*.

Gonzalez del Val (2001) also demonstrated the antibacterial or antifungal activities of the methanol extract of *Enteromorpha compressa*. According to our results, diethyl ether and ethanol extracts of *E. linza*

showed high and low antimicrobial activities, respectively.

The remarkable differences between our results and the results obtained in previous studies may be due to several factors. First of all, this can be because of the intraspecific variability in the production of secondary metabolites, occasionally related to seasonal variations, and these variations are seen in other published reports (4,5). Secondly, there may also be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods that would result in different susceptibilities of the target strains (9,10). This is an inevitable fact for all biochemical research because test materials have trace impurities.

Finally, we conclude that macro-algae from the Urla coast of Turkey are potential sources of bioactive compounds and should be investigated for natural antibiotics; however, further work is required to identify these active compounds.

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