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ORIGINAL RESEARCH

Antimicrobial and antioxidant activities with acute toxicity, cytotoxicity and mutagenicity of *Cystoseira compressa* (Esper) Gerloff & Nizamuddin from the coast of Urla (Izmir, Turkey)

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Abstract The aim of the study was to evaluate the biological activities with toxic properties of the methanol, hexane, and chloroform extracts of *Cystoseira compressa* (Esper) Gerloff & Nizamuddin from the Coast of Urla in the Aegean Sea. The extracts of *C. compressa* were tested for their antimicrobial and antioxidant activities in this study. Cytotoxic and mutagenic potentials of the extracts were also evaluated using cell culture and mutagenicity assays. Hexane extract was found to have higher total flavonoid and phenolic contents than the other extracts and exerted higher antioxidant activity than other extracts. All extracts exhibited moderate antimicrobial activity against tested microorganisms (minimum inhibitory concentration ranges are 32–256 μg/mL). The results indicated that the extracts had no significant cytotoxic activity against human hepatocellular carcinoma Hep 3B cell line in all treated concentrations (5–50 μg/mL) and did not show mutagenicity in the Ames test. Lethality was not observed among mice treated with oral doses of the extracts. In conclusion, results of investigations indicate that brown alga *C. compressa* is a natural source of antioxidant. It has moderate antimicrobial activities with no toxicity.

Keywords *Cystoseira compressa* · Antioxidant activity · Antimicrobial activity · Acute toxicity · Cytotoxicity · Mutagenicity

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Introduction

Seaweeds are considered to be an important nutrient source for diet and food additives because of their high content of essential and free amino acids (Heiba et al. 1993; Mohamed et al. 2012). The ability of seaweed to produce secondary metabolites of potential interest has been extensively documented (Faulkner 1993). Numerous studies are being carried out globally in order to check the bioactivities, such as antibacterial, antiviral, antineoplastic, anti-inflammatory, cytotoxic and antimitotic activities of marine algae or to isolate

compounds for different purposes such as pharmaceutical, cosmetics and food preservatives, antifouling coatings, etc. (Fenical and Paul 1984; Hodgson 1984; Scheuer 1990; Ballesteros et al. 1992; Sastry and Rao 1994; Armstrong et al. 2000; Gonzalez del Val et al. 2001; Bhosale et al. 2002; Ozdemir et al. 2006; Salvador et al. 2007; Tierney et al. 2010; Wada et al. 2011; Liu et al. 2012).

Cystoseira C. Agardh (Sargassaceae) is a widely distributed genus of brown algae and shows distribution in Atlantic, Mediterranean, Aegean and Black Seas. The secondary metabolites such as diterpenes and sterols from Mediterranean species of this genus have been widely studied (Amico 1995; Valls et al. 1995; Culioli et al. 2004). The species from *Cystoseira* genus are known to contain enzyme inhibitors, cell division inhibitors, antibacterial, antifungal, antitumoral and cytotoxic constituents (Faulkner 1986; Amico et al. 1988; Abourriche et al. 1999; Bennamara et al. 1999; Ayyad et al. 2003). *Cystoseira compressa* (Esper.) Gerloff & Nizamuddin, which is synonymous with *Cystoseira fimbriata* Bory de Saint-Vincent is known as an important element of intralittoral benthic vegetation, very tolerant of hydrodynamic conditions (Huve 1972). *C. compressa* has a discoid base with several spined axes and the axes have denticulate margins. It is irregularly branched with compressed primary ramifications, and compact crawling receptacles. *C. compressa* has an olive-brown coloration. In the literature, there is no biological activity study on *C. compressa*.

Despite the diversity in quality and quantity of the Turkish coast marine flora, with its large contains of seaweeds, most of them have not yet been investigated for biological activities. Therefore, the objective of this research was to evaluate the antimicrobial and the antioxidant activities with toxic and mutagenic properties of chloroform, hexane and methanol extracts of *C. compressa*.

Materials and methods

Seaweed materials

The marine algae *C. compressa* samples were collected at a depth of 1–2 m from the coast of Urla, Izmir in April 2012 and were identified by Prof. Dr. Atakan Sukatar. Voucher specimens (number: 40796) were deposited in the Hydrobiology Laboratory at the Ege University, Faculty of Science, Department of Biology. The samples were washed three times with tap water to remove the salt, epiphytes, and sand attached to the surface, then carefully rinsed with fresh water, and maintained in a refrigerator at −20 °C.

Extract preparation

Algae samples were dried at 45 °C. Powdered material (71.07 g) was extracted with n-hexane (Merck, Darmstadt, Germany), chloroform (Merck, Darmstadt, Germany) and methanol (Merck, Darmstadt, Germany), chloroform (Merck) and methanol (Merck) at room temperature in an ultrasonic bath (SONOREX Super, Bandelin Electronic GmbH & Co. KG, Berlin-Lichterfelde, Germany) (3 x 1 L for 24 h). The combined extracts were evaporated separately under reduced pressure to dryness which afforded 166, 228 and 3,902 mg, respectively.

Determination of total phenolic and flavonoid contents

Total phenolic content was determined by Folin–Ciocalteu method (Meda et al. 2005). Briefly, 0.1 mL of each extract was mixed with 2.8 mL of deionized water. This solution was mixed with 2 mL of 2 % sodium carbonate (Sigma-Aldrich Co., St Louis, MO, USA) and 0.1 mL of 0.1 N Folin-Ciocalteu reagents (Sigma-Aldrich Co.). After incubation at room temperature for 30 min, the absorbance of the mixture was measured at 750 nm against a deionized water blank on a UV/Vis spectrophotometer (UNICAM 8625, Durban, RSA). The data were expressed as Gallic acid equivalence (GAE).

Total flavonoid content was determined by the aluminum chloride colorimetric method described by Chang et al. (2002). Half a milliliter of the extracts was mixed with 1.5 mL of ethanol (Merck), 0.1 mL of 10 % aluminum chloride (Sigma-Aldrich Co.) and 2.8 mL of distilled water. The mixture was kept at room temperature for 30 min and the absorbance was recorded at 415 nm with the help of a UV/Vis spectrophotometer (UNICAM 8625). The total flavonoid content was expressed as quercetin equivalence (QE).

Determination of antioxidant activity

The antioxidant activity of extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH)(Sigma-Aldrich Co.) free radical scavenging ability (Okada and Okada 1998), formation of phosphomolybdenum complex (Prieto et al. 1999), and $ABTS^{\cdot+}$ radical cation decolorisation assays (Re et al. 1999; Arumagam et al. 2006).

DPPH radical scavenging activity assay: The extracts were screened for radical scavenging potential using a DPPH in vitro model system. The stock solutions of extracts were prepared in methanol (1,000 μg/mL). Dilutions were made to obtain concentrations of 500, 250 and 125 μg/mL. One milliliter of each diluted solution was added to 4 mL of 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature in darkness, the absorbance of the mixture was measured at 517 nm against methanol as blank using a UV/Vis spectrophotometer (UNICAM 8625). Free radical scavenging activity of α-tocopherol as reference compound was also determined. The inhibition percent of the tested samples were evaluated by comparison with a control. The inhibition of free radical by DPPH in percent (I %) was calculated using the formula:

$$\text{Inhibition } \% = (A_c - A_s/A_c) \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance of the tested sample after 30 min. The 50 % inhibitory concentrations of each extract (IC_{50}) was also obtained by Finney probit analysis. The antioxidant activity of each sample was expressed as α-tocopherol equivalent antioxidant activity using the following linear equation established as

$$A = 1.8652C - 5.9279. \quad R^2 = 0.9985$$

where A is the absorbance at 517 nm and C is the concentration as α-tocopherol equivalent (μg/mL). The values were expressed as average of three analyses ± standard deviation (SD).

Formation of phosphomolybdenum complex: The assay is based on the reduction of hexamolybdate to pentamolybdate. The absorbance of the subsequent green phosphomolybdenum complex at acid pH is measured at 695 nm. One milliliter of extract solution was combined with 3 mL of reagent solution containing 4 mM ammonium molybdate and 28 mM sodium phosphate in 0.6 M sulphuric acid. The reaction mixture was incubated in a water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of the colored complex was measured at 695 nm against a blank. The antioxidant capacity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC) using the following linear equation established using trolox as standard:

$$A = 0.0126C - 0.0317. \; R^2 = 0.9969$$

where A is the absorbance at 695 nm and C is the concentration as trolox equivalent (μg/mL). The values are presented as the mean ± SD of triplicate analysis.

$ABTS^{+}$ radical cation decolorisation assay: The total antioxidant activities of extracts were estimated by using the $ABTS^{+}$ (Sigma-Aldrich Co.) decolorization method. ABTS with potassium persulfate (Sigma-Aldrich Co.) generates blue/green $ABTS^{+}$. The radical formed shows a maximum absorbance at 734 nm. The antioxidants cause discoloration by transferring a hydrogen atom to a radical cation. In this experiment, 5 mL, 7 mM ABTS and 88 μL, 140 mM $K_2S_2O_8$ were mixed and allowed for the completion of radical generation for 12–16 h in the dark at room temperature. The stock solution was diluted with phosphate buffer solution (PBS) (pH 7.4) to give absorbance of 0.75 at 734 nm. One milliliter of each extract was added to 2 mL of diluted stock solution and the absorbance was measured at 734 nm 5 min after the initial mixing, using PBS as the blank. The total antioxidant activity percentage (TAA%) was calculated by the equation given below. In addition, trolox was prepared (0.5 μg/mL) as positive control and the relative antioxidant activity (RAA) of the extracts was calculated.

$$TAA\% = A_c - A_s/A_c \times 100.$$

A_c is the absorbance of stock solution and A_s is absorbance of the extract. RAA = TAA% extract/TAA% standard antioxidant compound (0.5 μg/mL trolox). All determinations were performed in triplicate and results were presented as mean ± SD.

Determination of antimicrobial activity

In vitro antimicrobial studies were carried out against eight bacteria strains, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538/P, *Streptococcus epidermidis* ATCC 12228, *Enterococcus faecalis*

ATCC 29212, *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumonie* ATCC 13883, *Bacillus cereus* ATCC 7064, *Pseudomonas aeruginosa* ATCC 9027, which were obtained from the Microbiology Department of the Faculty of Science, Ege University.

Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by NCCLS (2003) with some modifications. Dilution series of the extracts were prepared in test tubes and then transferred to the broth in 96-well microtiter plates. Final concentrations in the medium were 512–0.5 μg/mL. Before inoculation of the test organisms, the bacteria strains were adjusted to 0.5 McFarland standards (Thermo Oxoid Remel, Basingstoke, Hampshire, UK) and diluted 1:100 (v/v) in Mueller–Hinton broth (Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 35 °C for 18–24 h. All tests were performed in broth and repeated twice. The MIC was defined as the lowest concentration that showed clear against a black background (no visible growth). Samples from clear wells were subcultured by plotting on to Mueller–Hinton agar. Gentamycin and Ampicillin were used as standard antibacterial agents. All antibiotics were purchased from Sigma-Aldrich Co. and dilutions were prepared at concentrations ranging from 128 to 0.25 μg/mL in microtiter plates.

Determination of cytotoxicity and MTT assay

Human hepatocellular carcinoma Hep 3B cell line was generously donated by the Institute of Nuclear Science, Ege University. The cells were maintained in RPMI 1640 (PAA Laboratories GmbH, Cölbe, Germany) medium supplemented with 10 % heat-inactivated fetal bovine serum (PAA Laboratories GmbH), 1 % L-glutamine (PAA Laboratories GmbH) and 1 % Gentamycin (PAA Laboratories GmbH) in a humidified atmosphere with 5 % CO_2 at 37 °C. The cells were subcultured twice a week.

Screening of the extracts for cytotoxicity, based on metabolic cell viability, was carried out by using a modified MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide)] assay which determines the mitochondrial activity of viable cells (Mossman 1983; Surakka et al. 2005). The cytototoxic compounds destroy cells by interfering the cell division and growth. The affected cells become damaged and eventually die. The survival of viable cells after treatment of the extracts in monolayer culture was determined. Hundred μL of Hep 3B cells at 3×10^4 cells/mL as initial concentrations were cultivated in 96 well microplates for 24 h. After that the culture was treated with different dilutions (0.5–50 μg) of the extract and incubated for 48 h. Growth inhibition was estimated as the 50 % effective concentration (IC_{50}).

The assay is based on the cleavage of the yellow tetrazolium salt, MTT (Sigma-Aldrich Co.), which forms water-insoluble, dark blue formazan crystals. This cleavage only takes place in living cells by the mitochondrial enzyme succinate-dehydrogenase. The water-insoluble, dark blue formazan crystals are solubilized by using dimethyl sulfoxide. Optical density of the dissolved material was measured at 570 nm (reference filter, 690 nm) with UV–Vis spectrophotometer (Molecular-Devices, Winnersh, UK).

Bacterial reverse mutation assay (Ames test)

The assay was performed in two histidine-requiring strains of *Salmonella typhimurium*, tester strains TA98 and TA100, according to Maron and Ames (1983). Two separate experiments were performed, using triplicate plates, in the presence and absence of metabolic activation by an Aroclor 1254 (Sigma-Aldrich Co.)-induced (500 mg/kg body weight) Swiss albino mice liver post-mitochondrial fraction (S9). The post mitochondrial fraction was used at a concentration of 10 % v/v in the S9 mixture (metabolic activation). The S9 mixture was freshly prepared for each experiment according to the method of Maron and Ames (1983). Negative controls and positive controls were tested in all strains in both experiments. Dimethyl sulfoxide (DMSO) was used as a reference negative control. Benzo[a]pyrene (Sigma-Aldrich Co.) (5 μg/plate), 2-nitrofluorene (Sigma-Aldrich Co.) (5 μg/plate) and sodium azide (Sigma-Aldrich Co.) (10 μg/plate) were used as positive controls. Fresh cultures of tester strains were grown to approximately 10^9 cell/mL in 5 mL Oxoid nutrient broth (Oxoid). The cultures were incubated for 10–12 h at 37 °C in a gyratory incubator in order to insure adequate aeration. The strains were periodically raised from a single colony to check the genetic markers.

The extracts with the amounts of 1, 2.5 and 5 mg per plate were tested on TA98 and TA100. All positive and negative controls as well as the extracts were prepared in the absence (0.5 mL/plate) and presence of S9

mixture (0.5 mL/plate). The mixtures containing positive and negative controls and extracts and 0.1 mL of overnight bacterial cultures with or without S9 was vortexed and pre-incubated at 37 °C for 30 min. It was then plated in 2 mL of top agar on glucose-supplemented minimal agar. After 48 h of incubation at 37 °C, revertant colonies (his^+) were counted. The results of the test were presented as the mean ± SEM. Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of $p \leq 0.05$ were regarded as statistically significant (Snedecor and Cochran 1968).

Acute toxicity test

In this study, the up and down procedure was used to determine the lethal dose (LD_{50}) of a substance that will kill 50 % of test animals (Derelanko and Hollinger 1995; Barile 2008). In the test, male and female albino mice weighing 15–20 g each were used (n = 2 for each group). The protocol was approved by the Ege University, Local Ethical Committee of Animal Experiment (25.04.2012, no. 2012-062). Animals were housed in a room maintained at 22 ± 1 °C with an alternating 12 h light–dark cycle. Food and water were available ad libitum. The animals were transported to a quiet laboratory at least 1 h before the experiment. All experiments conformed to ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). Each animal was used once only and was humanely sacrificed immediately after completion of testing. The limit dose (2,000 mg/kg body weight) for acute oral toxicity was used according to the Environmental Protection Agency (EPA) and The Organization for Economic Cooperation and Development (OECD).

Results and discussion

In this study, we have investigated the biological activities and acute toxic, cytotoxic and mutagenic potentials of *C. compressa* extracts. According to our searches, this is the first paper to show the biological activities and toxicity profiles for *C. compressa*. Recent researches showed that the search for natural antioxidant compounds has gained considerable attention (Huang et al. 2005; Zubia et al. 2007).

Table 1 Total flavonoid and phenolic contents in extracts of *C. compressa*

Extracts (2%)	Flavonoid content (QE mg/g)	Phenolic content (GAE mg/g)
Methanol	0.291 ± 0.02	0.161 ± 0.08
Hexane	0.800 ± 0.07	1.541 ± 0.09
Chloroform	0.804 ± 0.07	0.454 ± 0.04

Data expressed as mean ± SD

QE quercetin equivalent, *GAE* gallic acid equivalent

Table 2 DPPH radical scavenging activity of *C. compressa* extracts

Extracts (1 mg/mL)	Inhibition (%)	α-Tocopherol equivalent antioxidant activity values (μg/mL)	IC_{50} (mg/mL)
Methanol	11.69 ± 0.07	9.45 ± 0.06	15.94 ± 0.05
Hexane	21.19 ± 0.04	14.54 ± 0.01	5.00 ± 0.01
Chloroform	15.53 ± 0.01	11.50 ± 0.04	7.46 ± 0.02

Data expressed as mean ± SD

IC_{50}, concentrations of extracts for 50 % inhibition of DPPH free radicals

Antioxidant compounds play an important role against various diseases such as chronic inflammation, cancer and cardiovascular disorders and ageing processes, which explains their considerable commercial potential in medicine, food production and the cosmetic industry. Moreover, interest in employing antioxidants from natural sources is considerably enhanced by consumer preference for natural products and concern about the potential toxic effects of synthetic antioxidants (Safer and al-Nughamish 1999).

Our results showed that hexane extract of *C. compressa* had higher total flavonoid and phenolic contents than the other extracts (Table 1). Total flavonoid and total phenolic contents of *C. compressa* hexane extract were calculated as 0.800 and 1.541 mg/g, respectively. The antioxidant activities of *C. compressa* extracts are also reported in Tables 2 and 3. The results showed that hexane extract exerted higher antioxidant activity than other extracts with all tested assays. A lower IC_{50} value indicates higher antioxidant activity. The IC_{50} value of hexane extract of *C. compressa* for DPPH scavenging activity was calculated as 5.00 mg/mL. Marine organisms are rich

Table 3 Total antioxidant activity of *C. compressa* extracts by ABTS and formation of phosphomolybdenum complex assays

Extracts (1 mg/mL)	TAA (%)	RRA	TEAC (μg/mL)
Methanol	62.32 ± 0.07	0.46 ± 0.002	7.75 ± 0.04
Hexane	88.92 ± 0.04	0.66 ± 0.002	51.19 ± 0.02
Chloroform	73.15 ± 0.06	0.54 ± 0.003	23.31 ± 0.06

Data expressed as mean ± SD

TAA, total antioxidant activity with ABTS assay; RAA, relative antioxidant activity (TAA % extract/TAA % standard antioxidant compound); TEAC, trolox equivalent antioxidant capacity with formation of phosphomolybdenum complex

sources of structurally new and biologically active metabolites (Ely et al. 2004). Fresh seaweeds are known to contain reactive antioxidant molecules (such as ascorbate and glutathione) as well as secondary metabolites, including carotenoids (mycosporine-like amino acids, catechins and tocopherols (Takamatsu et al. 2003). Hexane extract of *C. compressa* were found to have the highest phenolic and flavonoid contents and exerted higher antioxidant activity than other extracts. Several studies have shown a highly significant correlation between the phenolic content and the antioxidant activity in seaweed extracts. In addition, phenolic compounds with antioxidant activity comparable to that of synthetic antioxidants, butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) were previously reported (Takamatsu et al. 2003; Zubia et al. 2007).

The antimicrobial potential of *C. compressa* extracts was examined by MIC method. All extracts exhibited moderate antimicrobial activity against assayed Gram (+) and Gram (−) microorganisms when compared with standard antibiotics (Table 4). It has been previously shown that marine macro algae can inhibit the growth of some bacteria (Reichelt and Borowitzka 1984; Sastry and Rao 1994; Gonzalez del Val et al. 2001) and the efficacy of algae extracts against microorganisms is influenced by factors such as location and seasonality (Febles et al. 1995). The type of extraction solvent had a big influence on the antimicrobial properties of obtained extracts, suggesting that antimicrobial activity depends on both algal species and the efficiency of the extraction method. Several authors have documented the antimicrobial potency of organic extracts and terpenes with antimicrobial activity were isolated from marine algae of the genus of *Cystoseira* (Caccamese et al. 1985; Amico et al. 1987). It is important to consider that these extracts were unpurified and may contain both polar and nonpolar compounds; therefore the antimicrobial activity may be due to presence of compounds with different polarities.

Toxicity screenings of seaweed species from various geographic regions have shown that seaweeds are an important source of bioactive products (Martí et al. 2004). Consequently, seaweeds have often been targeted in the search for compounds with pharmacological properties. In this study, the cytotoxic effect of methanol, hexane and chloroform extracts of marine algae samples were tested on Hep 3B cells by MTT assay. The results indicated that algal extracts had no significant cytotoxic activity against Hep 3B cells in all treated concentrations (5–50 μg/mL). Previous studies indicated that the antioxidant activities may have synergistic effects on anticancer activity, proliferation inhibition, and apoptosis of human tumor or cancer cell lines (Seeram et al. 2004; Shao et al. 2004; Hsu et al. 2005). In our study, the result of cytotoxicity has shown correlation between the results of antioxidant and cytotoxic activity since there was no cytotoxicity on Hep 3B cells with moderate antioxidant activity of the extracts.

The extracts (1, 2.5 and 5 mg) were also evaluated on the *Salmonella typhimurium* TA 98 and 100 strains, both in the presence and the absence of metabolic activation. When the chemical concentrations and resulting his^+ revertants produced by the extracts were compared with those produced by controls, even 5 mg/mL concentration of the extracts demonstrated no mutagenicity (Table 5). In vivo acute oral toxicity study showed that, no lethality was observed among mice treated with oral doses of *C. compressa* extracts. The acute LD_{50} value of the extracts after oral administration in mice was >2,000 mg/kg in 24 h. In this study, it was determined that the methanol, hexane and chloroform extracts of *C. compressa* has no in vivo and in vitro toxicity.

Conclusion

In this research, the antioxidant and antimicrobial activity of *C. compressa* from the Coast of Urla in the Aegean Sea was investigated. Marine organisms are currently undergoing detailed investigations with the objective of isolating biologically active molecules

Table 4 Minimum inhibitory concentration (MIC) results of *C. compressa* extracts

Microorganisms	MIC (μg/mL)			Standard antibiotics (μg/mL)	
	Methanol	Hexane	Chloroform	Gentamycin	Ampicilin
Escherichia coli ATCC 8739	256	256	128	1	8
Staphylococcus aureus ATCC 6538/P	64	256	64	1	1
Streptococcus epidermidis ATCC 12228	32	128	64	1	2
Enterococcus faecalis ATCC 29212	64	128	64	16	2
Enterobacter cloacae ATCC 13047	64	128	32	2	4
Klebsiella pneumonie ATCC 13883	256	128	128	4	8
Bacillus cereus ATCC 7064	128	128	256	4	8
Pseudomonas aeruginosa ATCC 9027	256	256	256	2	16

Table 5 his^+ revertants in the Bacterial reverse mutation assay

Extracts	Concentration (mg/plate)	his^+ revertants/plate (X ± SEM)			
		TA 98		TA 100	
		+S9 mix	−S9 mix	+S9 mix	−S9 mix
DMSO	10 μl	34 ± 5	41 ± 8	133 ± 10	114 ± 11
Methanol	1	31 ± 2	16 ± 3	134 ± 9	134 ± 12
	2.5	40 ± 2	36 ± 2	132 ± 11	118 ± 15
	5	54 ± 1	24 ± 1	129 ± 13	197 ± 9
Hexane	1	36 ± 4	61 ± 1	127 ± 7	124 ± 10
	2.5	23 ± 3	16 ± 1	123 ± 8	104 ± 10
	5	24 ± 2	17 ± 1	124 ± 10	135 ± 12
Chloroform	1	31 ± 2	17 ± 1	117 ± 9	113 ± 11
	2.5	20 ± 1	20 ± 2	191 ± 7	111 ± 10
	5	20 ± 1	19 ± 3	183 ± 8	166 ± 12

Chemicals	Concentration/plate (μg)	Strain	S9	his^+ revertants/plate (X ± SEM)
Benzo[a]pyrene	5	TA98	+	672 ± 15
		A100	+	764 ± 42
2-Nitrofluorene	5	TA98	–	935 ± 25
Sodium azide	10	TA100	–	1,157 ± 28

along with the search for new compounds. Moreover, it was indicated that the Aegean Sea is a potential source of a variety of biologically active marine organisms and present results will provide a starting point for investigations aimed at exploiting new natural antioxidant substances present in the extracts

of algae collected from the Coast of Urla (Izmir, Turkey). These results may also support the use of the alga as traditional remedies for nutrient source for diet and food additives.

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Conflict of interest The authors have declared that they have no conflict of interest.

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