Research Article

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Bioactivity and Antioxidant Capacity of *Anabaenopsis sp.* (Cyanobacteria) Extracts

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ABSTRACT

In the present study, methanol, ethanol, dichloromethane and water extracts of *Anabaenopsis sp.* from Turkey were screened for their potential antimicrobial and antioxidant activities. The antimicrobial activities of the extracts were determined against five fish pathogen and twelve food-borne and clinical human pathogen microorganisms with disc diffusion and micro-dilution broth methods. The highest inhibitory activities were determined against *L. garviae* in the ethanol extract (18.51 mm) of all fish pathogens and *B.cereus* RSKK 863 in the methanol extract (21.28 mm) among human pathogen microorganisms. The MBC or MFC values of the extracts were in the range of 1.25-20.00 mg/ml for all tested microorganisms. Antioxidant capacity of the extracts was investigated by different assays, namely, total antioxidant activity, free radical scavenging activity (DPPH assay) and β-carotene-linoleic acid assay. The total phenolic and flavonoid content of extracts were also determined. The highest inhibition activity against linoleic acid oxidation was exhibited by dichloromethane extract of 83.63%, followed by methanol (81.19%), ethanol (77.82%) and water extract (17.34%). The findings indicated that the freshwater cyanobacteria *Anabaenopsis* sp. extracts are promising sources of new bioactive and antioxidative natural products.

Keywords: Antibacterial, antifungal, fish pathogen, human pathogen, free radical scavenging

INTRODUCTION

Cyanobacteria are a remarkable group of photosynthetic prokaryotes, comprising more than 150 genera and 2000 species, which play diverse yet significant roles in aquatic and terrestrial ecosystems (Prasanna *et al*., 2010). Excessive growth of cyanobacteria, broadly referred to as "blooms", represents a serious threat to aquatic ecosystems (Ghadouani *et al*., 2004). Bloom formation contributes to degradation of water quality and recreational value of lakes and reservoirs (Skulberg, 2000; Ghadouani *et al*., 2004, Fistarol *et al*., 2005). Freshwater cyanobacteria are acknowledged synthesizers of biologically active and structurally diverse secondary metabolites (LeFlaive and Ten-Hage, 2007).

Over the past decade, cyanobacteria have been recognized as a major source of active natural products with potential therapeutic applications in the treatment of cancer and HIV related diseases (Mahdi and Fariba, 2012; Harrigan *et al*., 1998). Cyanobacterial metabolites are also now being explored as important sources of pharmacologically active compounds useful in diagnostics or pigments as fluorescent probes and as nutraceuticals and food/feed supplements.

Free radicals have been implicated in the causation of several diseases such as liver chirrhosis, atherosclerosis, cancer, diabetes, etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Wilson, 1988; Geber *et al*., 2002; Kris-Etherton *et al*., 2002; Serafini *et al*., 2002; Di Matteo and Esposito, 2003; Behera *et al*., 2006). Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species (Lollinger, 1982; Tutour, 1990). The commonly used synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene have potential health risks and toxicity. Therefore, these need to be replaced with natural antioxidants (Jamuna and Rai, 2011).

Microbial resistance to antibiotics is a world-wide problem in human and veterinary medicine. It is generally accepted that the main risk factor for the increase in the antibiotic resistance is an extensive use of antibiotics. In fact, for the last 50 years, high levels of antibiotics are commonly used for treatment and prevention of infectious diseases in humans and animals. This led to emergence and dissemination of resistant bacteria and resistance genes in wild populations (Bogaard and Stobberingh, 2000). The antimicrobial agents used in animal care are also significant, both in increasing resistance in animal pathogens, and in transmission of resistant bacteria from animals to humans. Hence the need of the hour is a search for novel antibacterial compounds with therapeutic potential for which the pathogens may not have resistance (Patil *et al*., 2001).

Due to the significant role of cyanobacteria, it was considered worthwhile to examine antimicrobial and antioxidant activities for possible biotechnological and pharmaceutical applications. There is a growing interest in natural additives as potential antimicrobials and antioxidants. In this study, antimicrobial and antioxidant activities of *Anabaenopsis sp.* extracts of located at Mamasın Dam Lake, Aksaray, Turkey were studied.

MATERIAL AND METHODS

Samples collection

Algal bloom of *Anabaenopsis* sp. was observed in Mamasın Dam Lake (Central Anatolia, Turkey) on 20.09.2012. The samples were filtered by using a plankton net with 10 µm mesh size and collected to 25 liters plastic bucket. After

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collection, examination of the samples under a microscopy showed that *Anabeanopsis* sp. was dominant and still some zooplankton specimens were present. To remove the zooplankton specimens from the samples, samples were filtered by using 40 µm mesh size plankton net. Following the removal of zooplankton specimens and particles, *Anabeanopsis* sp. specimens were washed with distilled water many times. Then a detailed examination of *Anabeanopsis* sp. samples under a stereo and binocular microscope was carried out and pure *Anabeanopsis* sp. specimens were obtained.

Preparation of cyanobacterial extracts

The algal biomass was harvested by centrifugation at 5000 rpm for 15 minute. The pellet was washed twice with distilled water. Cyanobacterial cells were dried at 60°C, and then the cells were extracted with methanol (M), ethanol (E), dichloromethane (DCM) and water (W) at a concentration of 100 mg/ml (dried material/ml of solvent) in sterile tubes. Solutions were sonicated with an ultra-sonic bath (Bandelin Sonorex RK 100H) for 3x2 min on ice. The solutions were centrifuged at 5000 rpm for 15 min, the supernatants recovered and sterilized by filtration using 0.45 µm Millipore and used as stock solution for further assays.

Determination of antimicrobial activity

Microbial strains and growth conditions

The following pathogenic fish bacteria were used in the screening of antibacterial activity: *Lactococcus garvieae*, *Yersinia ruckeri*, *Vibrio anguillarum* (M1 and A4 strains, from two different companies) and *Vibrio alginolyticus*. *Y. ruckeri* and *L. garviae* were grown on TSA. *V. anguillarum* and *V. alginolyticus* were cultured in TSA supplemented with 2% NaCl. All bacterial cultures were incubated at 25 ºC for 24 h. Antimicrobial activity was also screened against clinical and food-borne pathogenic bacteria: *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375,*Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* Mu:57, *Yersinia enterocolitica* NCTC 11175). A yeast (*Candida albicans* ATCC 10231) was also used. Nutrient agar (NA) and Tryptic Soy Agar (TSA) were used for the cultivation of bacteria while YPD medium was used to culture the yeast. All bacterial cultures were incubated at 37 ºC for 24 h whereas the yeast culture was incubated at 30 ºC for 48 h.

Inhibitory effect with the disc diffusion method

The disc diffusion method was employed for the determination of the antimicrobial activity (Murray *et al*., 1995). The culture suspensions were adjusted with comparing with 0.5 McFarland. 100 µl of suspension of the test microorganisms was spread on solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 µl extract (1500 µg extract/disc) and then placed on the inoculated plates. Afterwards, they were kept for 2 hours in a refrigerator to enable prediffusion of the extracts into the agar. Then, the inoculated plates were incubated for 24 h and 48 h for bacterial and yeast strains respectively. Antibiotic discs of Ampicillin (Amp, 10 µg/disc), Gentamicin (CN, 10 µg/disc), and Amikacin (AK, 30 µg/disc) were also used as positive controls. Negative controls were performed with paper discs loaded with solvents (ethanol, methanol, water and dimethyl sulfoxide (DMSO). The diameters of inhibition zones were used as a measure of antimicrobial activity, and activity was expressed as mm. Each assay was repeated twice.

Determination of minimal bactericidal (MBC) or fungicidal (MFC) concentration

MBC/MFC values of extracts were determined with a 2-fold serial dilution method with some modifications (Chandrasekaran and Venkatesalu 2004), and studied for the microorganisms which are sensitive to the extracts in the disc diffusion assay. The test samples were added to growth broth medium to obtain a final concentration. The serial dilutions of the extracts in the tubes were made in a concentration range from 40.00 to 0.63 mg/ml with broth growth medium. The final volume in each tube was 100 µl. 2.5 µl of standardized suspension of each tested microorganism (at 0.5 McFarland standard turbidity) was transferred to each tube. A positive control (containing 2.5 µl inoculum and 100 µl growth medium) and a negative control (containing 2.5 µl of extract, 100 µl growth medium without inoculum) were included in each microtube. The contents of the tubes were mixed by pipetting and they were incubated for 24 h. 5 µl samples from all tubes were plated on solid growth medium. The MBC and MFC were recorded as the lowest concentration of the extract that did not permit any visible bacterial and fungal colony growth on the appropriate agar plate after the period of incubation (Chandrasekaran and Venkatesalu, 2004). The concentrations of the extracts that prevent the growth of a microorganism on the solid media were evaluated as MBC/MFC values in this study.

Antioxidant Assays

Assay for total phenolics

Total phenolic constituents of the extracts were determined by employing the methods given in the literature (Slinkard and Singleton, 1977) involving Folin-Ciocalteu reagent and gallic acid as standard. One milliliter of extract solution

ISSN: 2229- 6905

containing 2 mg extract was added to a volumetric flask. Forty-five milliliter distilled water and 1 ml Folin–Ciocalteu reagent was added and flask was shaken vigorously. After 3 min, 3 ml of Na_2CO_3 (2%) solution was added and the mixture was allowed to stand for 2 h by intermittent shaking. Absorbance was measured at 760 nm (Shimadzu-UV1800). The total phenolic content was determined as gallic acid equivalents (mg GAE/g extract).

Assay for total flavonoids

The total flavonoid content in extracts was determined spectrophotometrically according to Arvouet-Grand *et al*. (1994). Briefly, 1 ml of 2% aluminum trichloride ($AlCl_3$) methanolic solution was mixed with the same volume of extract solution (at 2 mg/ml concentration). The absorbance values of the reaction mixtures were determined at 415 nm after 10 min duration against a blank. Quercetin was used as the standard and the total flavonoids content of the extracts was expressed as µg quercetin equivalents per gram of extract (µg QE/g extract).

Evaluation of total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacities of extracts were evaluated by phosphomolybdenum method according to Prieto *et al*. (1999). 0.3 ml of extracts (2 mg/ml) was combined with 3 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95ºC for 90 min. Then the absorbance of the solution was measured at 695 nm against a blank. The antioxidant capacity of extracts was expressed as equivalents of ascorbic acid (mg AE/g extract).

β-Carotene-linoleic acid assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius *et al*., 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 µl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.35 ml of the extracts (2 mg/ml) were added and the emulsion system was incubated for up to 2 h at 50ºC. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared. The bleaching rate (*R*) of β-carotene was calculated according to the following equation:

$$R = \ln(a/b)/t$$

where ln = natural log, a = absorbance at time 0, b = absorbance at time *t* (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using Eq. (1).

$$AA = [(R_{Control} - R_{Sample})/R_{Control}] \times 100$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 2.0 mg/ml.

Scavenging activity on DPPH

The hydrogen atoms or electrons donation ability of the corresponding extracts and were measured from the bleaching of purple colored methanol solution of DPPH. The effect of *Anabaenopsis* extracts on DPPH radical was estimated according to Sarikurkcu *et al*. (2009). One milliliter of various concentrations of the extracts was added to a 4 ml of a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Inhibition activity was calculated in following way (Eq. (2)):

$$I(\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard.

RESULTS AND DISCUSSION

Antimicrobial activity

A number of important advances have occurred in cyanobacterial biotechnology in the recent years. Worldwide attention is drawn towards cyanobacteria for their possible use in mariculture, as food, feed, fuel, fertilizers, colorants, and for the production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals and pharmacological probes, and for pollution abatement (Tredici *et al*., 2009). Emergence of microbial diseases in

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pharmaceutical industries implies serious loss. Usage of commercial antibiotics for human and fish disease treatment produces undesirable side effects. Cell extracts and active constituents of various algae may be potential bioactive compounds of interest in the pharmaceutical industry (Rodrigues *et al*., 2004).

The antimicrobial activities of *Anabaenopsis sp.* extracts were employed against the fish and human pathogens and their activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and MBC or MFC values. The inhibition zones formed by standard antibiotic discs and those discs (Amp, CN and AK) are also listed in Table 1 and Table 2.

The results of the antimicrobial screening of the extracts against fish pathogens are shown in Table 1. These bacterial fish pathogens are the ones that commonly occur in the aquaculture sector and cause serious infectious diseases and mortality in fish (Buller, 2004). The disc diffusion assay results showed that the extracts of *Anabaenopsis sp.* showed various antimicrobial activities (7.66 - 18.51 mm) against the tested fish pathogen microorganisms used in this study. The highest inhibitory activities were determined against *L. garviae* in the ethanol extract. The weakest inhibitory activity was determined against *V. anguillarum* A4 from DCM extract. In general, the highest antibacterial activity was determined for ethanolic extract, followed by methanolic and DCM extracts. The water extract did not exhibit any activity against all the tested bacteria. Similarly, several researchers (Martin, 1995; Paz *et al*., 1995; Vlientinck *et al*., 1995) have generally reported that water extracts of plants do not have much activity against bacteria. The antimicrobial activities of the plants extracted in different solvents varied greatly because there are many factors that influence the active principle present in the plant (Parekh and Chanda, 2006). Here the polarity of the extracting solvents could influence the antimicrobial property (Table 1). The ethanolic extract has shown better antibacterial activities against 3 out of 5 bacteria when compared with standard Amp, CN and AK (Table 2). The MBC values of the extracts were in the range of 1.25-20.00 mg/ml. The lowest MBC value was found in the DCM extract, followed by the ethanolic and methanolic extracts.

The results of antimicrobial activity of the extracts of *Anabaenopsis sp.* against reference clinical and food-borne pathogens are shown in Table 2. All the extracts except for water showed antimicrobial activity against all the microorganisms. The water extract had no inhibitory activity against all the tested reference clinical and food borne pathogens extract similar to results of this study against fish pathogens. In general, the susceptibility of the tested microorganisms was determined against ethanolic extract, followed by methanolic and DCM extracts similar to fish pathogen results. The ethanolic extract has shown better antibacterial activity against 8 out of 11 bacteria when compared with standard Gentamicin (Table 2). MBC values for the microorganisms which were sensitive to the extracts were in the range of 10.00-20.00 mg/ml.

In a previous study, Rania and Taha (2008) tested antibacterial and antifungal activity of three cyanobacteria (*Anabaena oryzae*, *Tolypothrix ceytonica* and *Spirulina platensis*) and two green microalgae (*Chlorella pyrenoidosa* and *Scenedesmus quadricauda*) against human and plant pathogen microorganisms by using agar well diffusion method. In their study, it was found that *S. platensis* and *A. oryzae* had the highest antibacterial and antifungal activity towards the tested bacteria and fungi. However, *A. oryzae* methanol extract (5 mg/well) showed lower antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* when compared to our study by using disc diffusion method (1.5 mg/disc). The activity differences in these two studies may derive from the different cyanobacteria and microorganism strains that were used and the methods used in determination of antimicrobial activity.

Sethubathi and Prabu (2010) determined antimicrobial activity of three marine cyanobacterial species namely *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscule* isolated from Adirampattinam coast against the human pathogenic bacteria such as *Streptococcus mutants*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae.* In their study, *Oscillatoria* sp. showed the maximum inhibition against pathogen strain compared to other species and the minimum inhibition activity was observed in the extract of *L. majuscule.*

In another study, fifteen cyanobacteria belonging to *Anabaena*, *Nostoc*, *Scytonema* and *Microcystis* species isolated from various aquatic and terrestrial habitats were screened for antimicrobial activity by using disc diffusion method (Yadav *et al*., 2012). In the study, ethanolic extracts of *Anabaena* BT2, *Nostoc* Brf02, *Nostoc* Brf04 and *Scytonema* Br1 at a concentration of 500 µg/disc showed prominent zone of inhibition against two gram-negative bacteria (*Pseudomonas* sb1 and sb3).

Najdenski *et al.* (2013) evaluated in vitro activity of nine cyanobacterial and ten microalgal newly isolated or culture collection strains against eight significant food-borne pathogens. Water extracts and culture liquids of *Gloeocapsa* sp. and *Synechocystis* sp. demonstrated the widest spectrum of activity with minimal inhibitory concentration (MIC) ranging from 1.56 to 12.5 mg/ml. Culture liquid of *Anabaena* sp. had the highest activity (MIC = 0.39 mg/ml).

Cell extracts and active constituents of various algae may be potential bioactive compounds of interest in the pharmaceutical industry (Rodrigues et al., 2004). Al-Wathnani *et al*. (2012) analyzed bioactive natural compounds from algae such as cyanobacteria and determined their activity against human pathogenic bacteria and yeast. In their study, they found that all the algal species extracts which were extracted with acetone/methanol/di-ethyl-ether showed strong inhibition against *S. sonnei*, similarly to our results. Emergence concerns have been raised to establish structural and functional

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properties of the bioactive compounds described in algal crude extracts, up to date, over 2,400 bioactive metabolites have been isolated and identified from a diverse group of algal communities (Faulkner, 2001). In this preliminary study, antimicrobial activity of ethanol, methanol, DCM and water extracts of *Anabaenopsis sp.* was determined. The results showed that the extracts except for water showed various degree of antimicrobial activity. Analysis and purification of bioactive compounds from *Anabaenopsis sp.* may be studied in the further studies to explain their usefulness in the pharmaceutical and feed industry.

Table 1. Antibacterial activity of *Anabaenopsis* sp. extracts against different bacterial fish pathogens

Test microorganisms	MBC[a] (mg/ml)				Inhibition zone diameter[b] (mm)				Antibiotics Inhibition zone diameter[b] (mm)		
	M-E	E-E	W-E	DCM-E	M-E	E-E	W-E	DCM-E	Amp	CN	AK
L. garviae	20.00	20.00	-	-	16.93 ±0.06	18.51 ±0.28	-[c]	-	33.10 ±0.12	15.19 ±0.10	10.30 ±0.08
Y. ruckeri	20.00	10.00	-	5.00	14.65 ±1.57	17.32 ±0.18	-	7.66 ±0.01	32.30 ±0.15	18.85 ±0.05	18.69 ±0.12
V. anguillarum M1	20.00	10.00	-	2.50	12.30 ±0.02	17.33 ±2.72	-	8.33 ±1.00	9.02 ±0.04	12.38 ±0.09	9.46 ±0.12
V. anguillarum A4	20.00	10.00	-	10.00	13.56 ±0.52	17.54 ±0.46	-	8.96 ±0.37	9.40 ±0.11	15.13 ±0.15	12.07 ±0.13
V. alginolyticus	10.00	10.00	-	1.25	10.03 ±0.21	15.57 ±0.64	-	10.19 ±0.06	13.57 ±0.09	15.06 ±0.07	15.03 ±0.03

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of two separate experiments.
[c]: Indicates no antimicrobial activity.

Table 2. Antimicrobial activity of *Anabaenopsis* sp. root extracts against test microorganisms

Test microorganisms	MBC[a]/MFC[b] (mg/ml)				Inhibition zone diameter[c] (mm)				Antibiotics Inhibition zone diameter[c] (mm)		
	M-E	E-E	W-E	DCM-E	M-E	E-E	W-E	DCM-E	Amp	CN	AK
B. cereus RSKK 863	10.00	10.00	-	10.00	21.28 ±0.15	18.03 ±0.32	-[d]	18.27 ±0.16	37.68 ±0.03	18.02 ±0.11	18.72 ±0.07
E. coli O157:H7	20.00	20.00	-	10.00	16.45 ±0.07	15.32 ±0.18	-	8.57 ±0.17	25.92 ±0.15	18.37 ±0.17	22.58 ±0.09
S. sonnei Mu:57	20.00	20.00	-	10.00	16.11 ±0.26	17.59 ±0.12	-	8.21 ±0.21	38.43 ±0.16	19.49 ±0.05	27.07 ±0.04
M. luteus NRRL B-4375	10.00	10.00	-	10.00	13.89 ±0.08	16.22 ±0.18	-	10.29 ±0.34	34.65 ±0.12	13.48 ±0.22	19.55 ±0.14
Y. enterocolitica NCTC 11175	10.00	20.00	-	20.00	13.41 ±0.27	17.55 ±0.24	-	9.98 ±0.14	11.58 ±0.09	16.17 ±0.11	21.19 ±0.07
E. coli ATCC 11229	20.00	20.00	-	10.00	14.35 ±0.12	15.70 ±0.09	-	9.08 ±0.05	27.99 ±0.14	14.98 ±0.12	19.81 ±0.13
P. aeruginosa ATCC 27853	20.00	20.00	-	10.00	14.88 ±0.13	18.31 ±0.19	-	8.79 ±0.17	-	15.89 ±0.05	19.71 ±0.08
S. aureus ATCC 25923	20.00	20.00	-	10.00	16.49 ±0.07	18.59 ±0.05	-	8.80 ±0.15	34.82 ±0.06	15.52 ±0.14	19.46 ±0.16
E. coli ATCC 35218	20.00	20.00	-	20.00	14.11 ±0.21	16.88 ±0.12	-	8.28 ±0.23	25.78 ±0.19	12.17 ±0.21	20.03 ±0.09
S. enteritidis ATCC 13076	20.00	10.00	-	10.00	18.16 ±0.31	19.04 ±0.34	-	8.81 ±0.18	29.49 ±0.15	16.38 ±0.17	17.27 ±0.11
L. monocytogenes ATCC 7644	20.00	10.00	-	10.00	16.95 ±0.15	19.83 ±0.28	-	8.84 ±0.11	25.13 ±0.06	20.63 ±0.16	20.52 ±0.21
C. albicans ATCC 10231	20.00	20.00	-	10.00	13.31 ±0.23	17.22 ±0.32	-	10.83 ±0.14	-	-	-

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Minimal Fungicidal Concentration (MFC)
[c]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of two separate experiments.
[d]: Indicates no antimicrobial activity

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Antioxidant Activity

The occurrence of phenolic compounds in plants is well documented and these compounds are known to possess antioxidant activity in biological systems but the antioxidant characteristics of algae and cyanobacteria are less well documented (Colla *et al.*, 2007). Phenolic compounds contribute to multiple biological effects, including antioxidant activity. Total phenol contents in three different extracts (dichloromethane, water and methanol) were found as 8.73, 13.73 and 2.59 mg GAE/g, respectively. However, total phenol content was not detected in the ethanol extract (Table 3).

The total flavonoid content was expressed as quercetin equivalents. DCM extract had the highest of flavonoids, whereas the contents obtained from ethanol were much smaller. Phosphomolybdenum assay was performed in order to evaluate total antioxidant capacity of *Anabaenopsis* sp. Total antioxidant capacity of *Anabaenopsis* sp. showed a similar trend to total flavonoid (Table 3).

Table 3. Total phenolics, flavonoid contents and antioxidant capacities of various extracts from *Anabaenopsis* sp.[a]

	Anabaenopsis sp.		
	TPC	**TFC**	**TAC**
DCM	8.73±0.64[a]	43.86±0.61	34.15±7.61
Water	13.73±0.64	-	-
Methanol	2.59±0.96	16.92±0.25	13.77±3.81
Ethanol	-	7.65±0.18	-

[a] Values are reported as means ±SD.
TPC, total phenolic content (mg GAE/g extract); TFC, total flavonoid content (µg QE/g extract); TAC, total antioxidant capacity (mg AE/g extract).

The highest inhibition activity against linoleic acid oxidation was exhibited dichloromethane extract of 83.63%, followed by methanol (81.19%), ethanol (77.82%) and water extract (17.34%) (Figure 1).

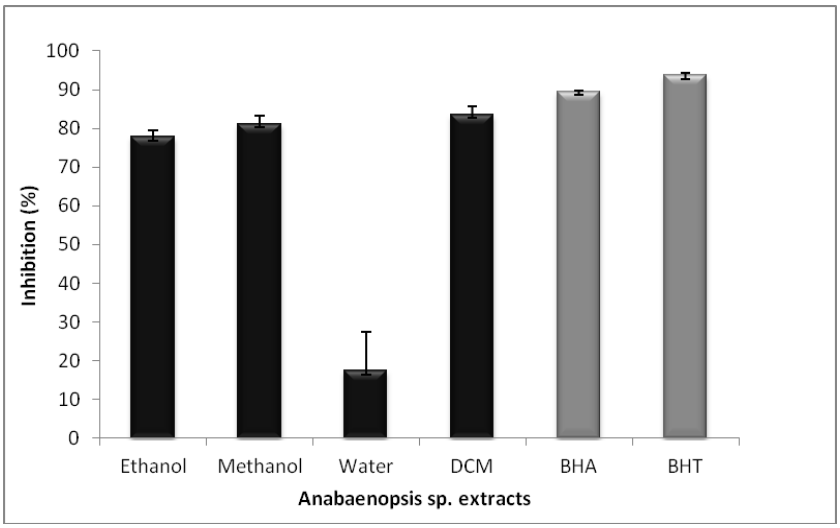

Figure 1. Inhibition activity of *Anabaenopsis* sp. extracts against on linoleic acid oxidation

In DPPH method, dichloromethane, water, methanolic and ethanolic extracts of *Anabaenopsis* sp. showed antioxidant activity, with inhibition values of 10.19, 10.78, 11.29 and 12.31%, respectively (Figure 2). Suhail *et al.* (2011) determined antioxidant potential of the methanol extracts of different cyanobacteria (*Plectonema boryanum*, *Scytonema* sp., *Osillatoria* sp., *Chroococcus* sp., *Anabaena variabilis*, and *Nostoc* sp.). They reported that the methanolic extracts of *Plectonema boryanum* and *Scytonema* sp. exhibited greater antioxidant activity as it was 30% and 27% inhibition of DPPH than the positive control (ascorbic acid, 25%) at 50 µg/ml. Other species have shown potent radical scavenging activity include *Osillatoria* sp., *Chroococcus* sp., followed by *Anabaena variabilis*, and *Nostoc* sp.

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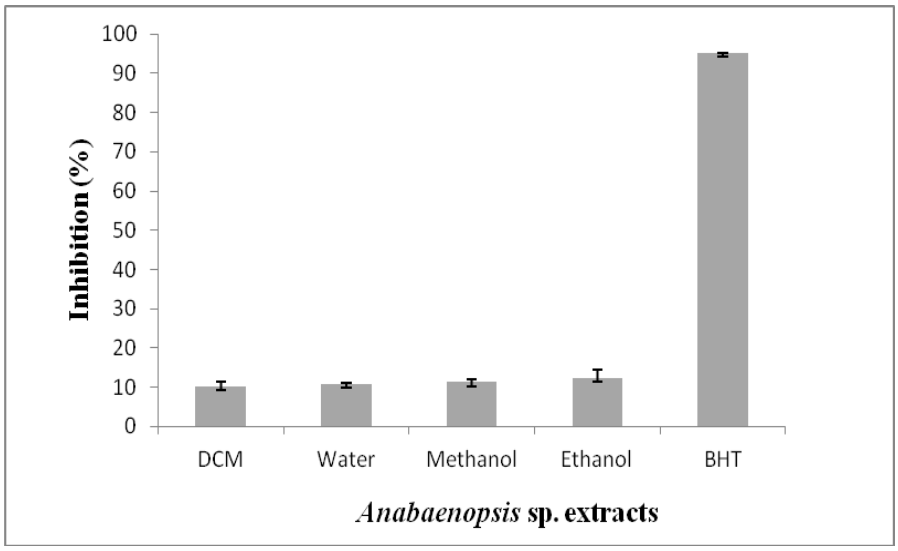

Figure 2. Free radical scavenging effect of different solvent extracts from *Anabaenopsis* sp.

Conclusions

In this study, the antimicrobial effect and antioxidant capacity of the extracts of *Anabaenaopsis* sp. from Turkey were determined. Our results prove that the freshwater cyanobacteria *Anabaenopsis* sp. from Turkey (Mamasın Dam Lake) is a promising source of new bioactive and antioxidative natural products. Further studies have to be made on separation of the extracts to determine the principle antibacterial and antioxidant compounds.

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