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Investigation of Biological Activities of Some Microalgae Extract Isolated from Kabakli Pond (Diyarbakır) Turkey

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Abstract

The antimicrobial, antioxidant and mutagenic activities of methanolic extracts of microalgae species isolated from Kabakli Pond (37° 55' 23N, 40°17' 40E, Diyarbakır) and identified as *Chlorella vulgaris* Beyerinck and *Chroococcus limneticus* Lemmermann were

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investigated. The highest antioxidant activity was observed in the ABTS•+ assay of *C. vulgaris* (36.63%) and *C. limneticus* (28.48%) at a concentration of 100 µg/ml concentration. The DPPH and CUPRAC assays showed weak activity. The antioxidant activity did not appear to be significant for either species when compared with the positive controls. *C. vulgaris* showed high antimicrobial activity with inhibition zone and MIC value against S. aureus. There seemed to be no mutagenic activities; however, *C. limneticus* showed an effect on the colony structure of S. *typhimurium strain TA98. C. vulgaris* has a good antimicrobial potential, whereas *C. limneticus* has relatively weak potential. *C. limneticus* a relatively has a higher antioxidant activity compared to *C. vulgaris*, but the activities of both extracts are weak compared to the controls. None of the tested extracts showed mutagenic activity against *S. typhimurium* TA98 at any of the concentrations. These organisms via different solvents or extraction systems since they may affect various biological systems.

Keywords: Microalgae; Mutagenicity; Biological activity; Antioxidant; Antimicrobial**.**

Kabaklı Göleti'nden (Diyarbakır) İzole Edilen Bazı Mikroalg Ekstraktlarının Biyolojik Aktivitelerinin Araştırılması

Öz

Kabaklı Göleti'nden (37° 55' 23K, 40°17' 40D, Diyarbakır) izole edilen ve *Chlorella vulgaris* Beyerinck ve *Chroococcus limneticus* Lemmermann olarak tanımlanan mikroalg türlerinin metanol ekstraktlarının antimikrobiyal, antioksidan ve mutajenik aktiviteleri araştırıldı. En yüksek antioksidan aktivite, 100 µg/ml konsantrasyonunda *C. vulgaris* (%36,63) ve *C. limneticus* (%28,48) için ABTS•+ analizinde gözlendi. DPPH ve CUPRAC analizleri zayıf aktivite gösterdi. Antioksidan aktivite, pozitif kontrollerle karşılaştırıldığında her iki tür için de önemli görülmedi. *C. vulgaris* metanol ekstraktlarının *S. aureus*'a karşı inhibisyon zonu ve MİK değeri ile yüksek antimikrobiyal aktivite gösterdi. *C. limneticus* metanol ekstraktlarının *S. typhimurium* TA98 üzerinde herhangi bir mutajenik aktivitesi gözlenmedi. *C. vulgaris* metanol ekstraktı iyi bir antimikrobiyal potansiyele sahipken, *C. limneticus* ektraktı nispeten zayıf bir potansiyele sahiptir. *C. limneticus* metanol ekstraktı, *C. vulgaris* metanol ekstraktına kıyasla nispeten daha yüksek bir antioksidan aktiviteye sahip ancak her iki özütün aktiviteleri kontrollerle karşılaştırıldığında zayıf olarak gözlendi. Test edilen özütlerin hiçbiri, herhangi bir konsantrasyonda *S. typhimurium* TA98'e karşı mutajenik aktivite göstermedi. Bu organizmalar

farklı çözücüler veya ekstraksiyon sistemleri aracılığıyla çeşitli biyolojik sistemleri etkileyebilirler.

Anahtar Kelimeler: Mikroalg; Mutajenite; Biyolojik aktivite; Antioksidan, Antimikrobiyal.

1. Introduction

Algae, which are multicellular or single-celled organisms, are classified as macro- or microalgae depending on their size [1]. Microalgae are the most important members of aquatic ecosystems because they accumulate important metabolites in their cells. They also play a key role at the top of the food pyramid as photosynthetic eukaryotes, which are among the oldest life forms on the planet. Microalgae have long attracted attention, both as a food source and for their use in many fields such as cosmetics, and this interest has increased in recent years [2, 3].

Although microalgae range from prokaryotic cyanobacteria to eukaryotic microalgae, this diversity is not yet fully understood. Recent research shows that although more than 50,000 different species of microalgae are found in oceans and fresh water (lakes, ponds and rivers), only 30,000 of these species have been studied [4–7].

Microalgae are unicellular and fast-growing organisms that produce various bioactive compounds through photosynthesis and are more productive than terrestrial plants [8, 9]. In addition, the high adaptability of microalgae to changing environmental conditions, such as ambient temperature, pH, humidity and salinity, makes them good candidates for drug discovery [3, 10–13]. In fact, microalgae have been shown to produce different and higher levels of metabolites under stress and laboratory conditions than they can produce in their natural environment [14].

Plants have been used as medicines for many years because these natural products have therapeutic potential and have led to the development of new medicines [15, 16]. Microalgae have also been used for the same purpose for a long time. The biologically active metabolites present in microalgae can be derived from a multitude of molecular structures, including various fatty acids; peptides, carbohydrates, lipopeptides, polyketides, lactones, amides, alkaloids, amino acid derivatives, aromatic substances, terpenoids, and terpenes. On the other hand, although most metabolites accumulate in the cells, some can be released into the environment, examples of the biological activity of extracellular metabolites can be attributed to five antibacterial diterpenoids derived from *Nostoc commune* [17], a brominated indole alkaloid of *Anabaena constricta* that possesses antimicrobial activity [18], the antifungal peptides produced by *Tolypotrix byssoidea*

[17], *Fischerella ambigua* excretes a broad-spectrum antibacterial and antifungal substance called "parsiguine," which was also gathered from paddy fields [19]. Moreover, it has been demonstrated that isolates of cyanobacteria from Brazil are capable of producing antimicrobial non-ribosomal peptides [20], and lipophilic extracts of *Synechocystis* sp. have antimicrobial fatty acids and volatiles [21].

Kabaklı pond is a natural pond located at the provincial borders of Diyarbakır in Türkiye (37° 55' 23N, 40°17' 40E), and 94 algae taxa have been recorded in Kabaklı pond in previous studies. The pond is exposed to a significant amount of organic pollution because of sewage waste and animal husbandry in the surrounding area; therefore, the number of algae in the water reaches millions per liter. The pond is exposed to a significant amount of organic pollution because of sewage waste and animal husbandry in the surrounding area; therefore, the number of algae in the water reaches millions per litter [22].

In this study, the antioxidant, antimicrobial, and mutagenic activities of methanol extracts of *Chlorella vulgaris* Beyerinck [Beijerinck] (*C. vulgaris***)** and *Chroococcus limneticus* Lemmermann (*C. limneticus*) were investigated.

2. Materials and Methods

2.1. Sample preparation

Two freshwater species of microalgae, *C. vulgaris* and *C. limneticus*, were collected from Kabaklı Pond (37° 55' 23N, 40° 17' 40E, Diyarbakır, southeastern Türkiye) and isolated using subculture BG-11 agar medium. Colonies were photographed using a Nikon 80i microscope with a 100x objective and an attached digital camera. The samples were then identified according to Prescott [23].

2.2. Extraction procedure

The microalgae species were harvested by centrifugation (4500 rpm for 5 min) in the stationary phase, and the supernatants were discarded. The wet pellets (about 1 g) were re-suspended in 18 ml of methanol: water (5:1) and the cells were disrupted by sonication using a Soniprep 150 sonicator. Sonication was performed on ice for a total of 5 min (5 pulses of 30 s each at 30-second intervals to keep it out from any temperature rise). The cells were then incubated on ice for 2 h by shaking at 120 rpm and centrifuged at 4800 rpm for 15 min to remove cell debris. Subsequently, the supernatants were collected and evaporated using a rotary vacuum evaporator at 40°C [24].

2.3. GC-MS analysis of essential oils

The sample was analyzed using an Agilent Technologies 6890N Network GC System, Agilent Technologies 5973 Inert Mass Selective Detector, and Columns: 19091N-136 HP-INNOWAX Length (meters) =60; I.D. (mm)=0.250; Film (micrometer) = 0.25. Temperature Limits: From 40°C to 260°C. 1- The detector was set to 250 °C. The stepped temperature program was held at 60 °C for 10 minutes initial, followed between 60-250 °C by 50 minutes at a rate of 5 °C/min. The GC-MS interface temperature was 250 °C. The injection volume was 2 µL and the solvent delay was 2 minutes. The total run time was 98 minutes. Compounds identification were obtained by mass spectra of references in Mass Spectral Library.

2.4. Antioxidant activity of ethanol extract

2.4.1. DPPH radical scavenging assay

DPPH (2,2´-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the Blois [25].

The percentage of DPPH scavenging activity is calculated using the following formula: [Ac–As/Ac] × 100.

In this context, "AC" represents the absorbance value of the control sample, while "AS" denotes the absorbance value of a solution added as a test sample. Microsoft Excel was used to calculate these values. The tests were performed in triplicates. The values indicated are as the mean ± SD of three independent measurements.

2.4.2. ABTS assay

2,2'-Azinobis-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) was dissolved in water (7mM) then ABTS solution was combined with the $K_2S_2O_8$ solution and allowed to stand for 12-16 hours at room temperature in the absence of light. Subsequently, the ABTS solution was diluted to an absorbance of 0.70 at 734 nm. BHA and BHT were used as positive controls. 160 µL of ABTS•+ solution and 40 µL of different concentrations of samples (10, 25, 50, and 100 µg/ml) were added to 96 well plates. Following a 30-minute incubation period at room temperature, the absorbance was determined at 734 nm using a spectrophotometer [26].

$$I\% = (Acontrol – Asample / Acontrol) \times 100 \quad (1)$$

2.4.3. Copper (II) reductive antioxidant activity (CUPRAC) assay

The Cupric ion-reducing antioxidant capacity (CUPRAC) was determined using the method described by Apak et al. [27]. In summary, 61 µL $CuCl_2.2H_2O$, 61 µL neocuproine, and 61 µL ammonium acetate buffer were added to 67 µL of extract samples prepared at different concentrations (10, 25, 50,100 µg/mL). Following incubation for 1 h measurements were performed at 450 nm using a plate reader, and the half-maximal inhibitory concentration (IC50) values were calculated.

2.4.4. Antimicrobial activity

Antimicrobial activity of the extract samples prepared at different concentrations (200, 300, and 400 µg/mL) against Gram positive (*Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615), Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria and yeast (*Candida albicans* ATCC 10231) were determined using the disc diffusion method [28].

100 µL of each microorganism equal to 0.5 McFarland turbidity was seeded onto Mueller-Hinton agar plates. The 15 µL volume samples, which were prepared at different concentrations of all extracts, were impregnated on sterile paper discs placed in plates. Following incubation at 37°C for 24 h (bacteria) and 30°C for 48 h (yeast), the inhibition zone diameters were measured in millimetres, and the extracts were processed to determine the MIC value. Serial dilutions (100 µL) of extracts, 90 µL of nutrient broth, and 10 µL of microorganism overnight cultures (turbidity equal to 0.5, McFarland) were pipetted into 96 well sterile plates. Following incubation at 37°C for 24 h, the evaluations were performed. The concentration without visible growth was determined as the MIC value. All tests were performed in triplicate. As positive controls for bacteria and yeast, respectively, ampicillin and fluconazole were used.

2.4.5. Mutagenic activity

The *Salmonella typhimurium* TA98 strains were purchased from the Salmonella Genetic Stock Centre (University of Calgary, Canada), and their genetic backgrounds were controlled as previously described [29, 30].

Our experiment was performed using the TA98 strain of *S. typhimurium*. Positive and negative controls were used for each test. Compounds were considered mutagenic (reduction in the number of returning colonies). The data were ranked as follows.

Test compounds were considered mutagens if there was a two-fold increase in the number of returned colonies or a dose-dependent increase in the number of spontaneously returned colonies. Microalgae extracts were dissolved in dimethyl sulfoxide (DMSO). The sample concentrations were prepared as 12.5, 25, 50, 100, 200, 400 and 800 µg/plate. Daunomycin (in distilled water- 6 µg/ml) was used as the positive control and DMSO as the negative control.

2.5. Statistical analysis

The antimicrobial and antioxidant activity results are expressed as the mean ± standard deviation (SD) of three experiments. Analysis of variance (ANOVA) followed by Dunnett's test was used to compare the treated groups with the control group. P-values less than or equal to 0.05 were considered to indicate statistical significance.

3. Results

Both qualitative and quantitative GC-MS analysis results of *C. vulgaris* and *C. limneticus* ethanol extracts are shown in Tables 1 and 2. Methanol extracts of algae have been found to contain bioactive components. The chemical components of the algae used in this study for *C. limneticus* were oleic acid and hexadecanoic acid (Table 2, Fig. 2), and 9,12-Octadecadienoic acid was the major fatty acid for *C. vulgaris*.

Table 1: GC/MS Analysis of the Methanol Extract of *C. vulgaris*.

PK	RT	Area%	ID	Qual
1	29.702	0.10	Decanoic Acid	49
3	32.752	0.03	Heptanoic Acid	78
4	36.969	0.15	Octanoic Acid	90
5	43.309	0.07	Nonanoic Acid	95
6	50.570	0.03	Alpha-(Aminomethylene)glutaconic anhydride	49
7	51.360	0.04	(9E)-9-Octadecenoic Acid	58
8	55.938	-0.03	Undecanoic Acid	38
10	67.393	14.47	n-Hexadecanoic Acid	96
15	70.958	3.28	Heptaethylene glycol monododecyl ether	62
16	71.318	18.22	9-Octadecenoic acid, (E)	99
19	74.288	2.69	15-Crown-5	78
21	75.324	1.74	Octaethylene glycol	86
22	75.936	5.97	1,4,7,10,13,16-Hexaoxacyclooctadecane	90
32	83.975	1.94	2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol	86
37	91.545	2.68	18,18'-Bi-1,4,7,10,13,16-hexaoxacy clononadecane	86

PK: Peak; RT: Reiteration Time. Area% gives the percentage of that item from the peak area.

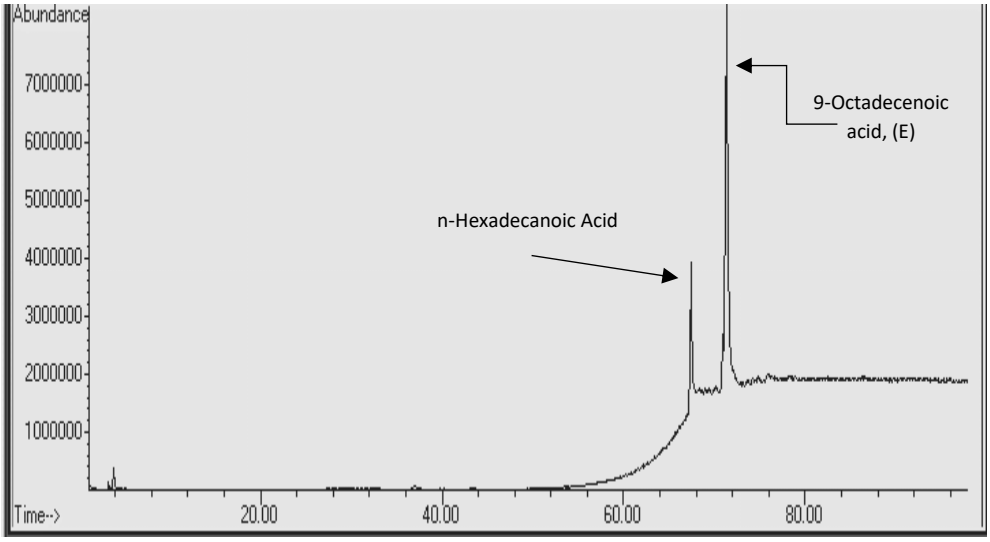

Figure 1: GC-MS analysis of methanol extract of *C. vulgaris*.

Table 2:GC/MS Analysis of the Methanol Extract of *C. limneticus*.

PK	RT	Area %	ID	Qual
1	28.804	0.25	Methyl d-glycero-beta-d-gulo-heptoside	59
2	31.499	0.09	Heptanoic acid	59
4	35.281	0.20	Octanoic acid	93
6	42.273	0.10	Nonanoic acid	91
8	51.165	0.06	Decanoic Acid	81
9	55.840	-0.01	Undecanoic Acid	83
11	58.438	0.06	(9Z)-1-(2,3-Dimethoxypropoxy)-9-OC Tadecene	72
12	67.393	15.25	n-Hexadecenoic acid	97
13	69.407	3.11	Octaethylene glycol monododecyl ether	83
15	71.324	29.60	Oleic Acid	94
16	73.115	1.70	18,18'-Bi-1,4,7,10,13,16-hexaoxacy clononadecane	86
20	75.913	5.72	Octaethylene glycol monododecyl ether	81
21	77.366	1.50	Octaethylene glycol	86
25	81.692	1.79	1,4,7,10,13,16-Hexaoxacyclooctadecane	90
31	89.279	0.79	2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol	86
33	91.488	2.25	Heptaethylene glycol monododecyl ether	80

PK: Peak; RT: Reiteration Time. Area% gives the percentage of that item from the peak area.

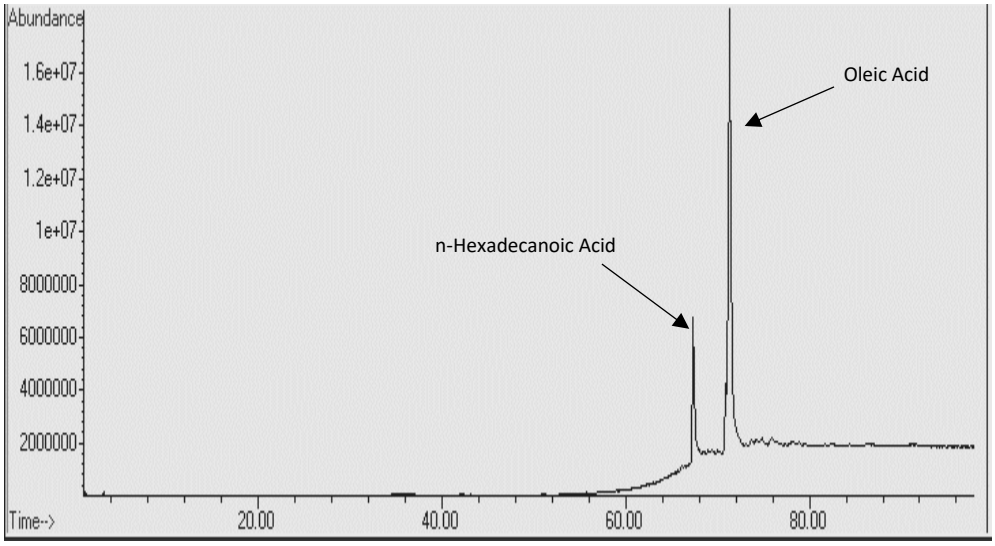

Figure 2: GC-MS analysis of methanol extract of *C. limneticus.*

3.1. Antioxidant activity

3.1.1. DPPH radical scavenging assay

The antioxidant activities of microalgae extracts were determined as percent inhibition according to the DPPH method at 10, 25, 50, and 100 µg/ml concentrations, and IC50 values are shown in Table 3 and Fig. 3, respectively. DPPH free radical scanning activities increased as concentrations increased, regardless of the non-significant antioxidant activity of the two microalgae extracts compared to that of the positive controls. An IC50 value of 325.89 µg/ml for *C. vulgaris* and an IC50 value of 471.26 µg/ml for *C. limneticus* was calculated (Fig. 3). *C. limneticus* showed stable antioxidant activity at concentrations below 50 µg/ml, whereas there was a significant increase from 50 µg/ml to 100 µg/ml. Taghavi et al. [31] demonstrated a 42.96% inhibition of ethanol extract at a concentration of 200 µg/ml in the DPPH assay.

Table 3: DPPH free radical-scavenging activities of *C. vulgaris* and *C. limneticus* methanol extracts. The results show the I% values. BHA and BHT were used as positive controls.

Concentrations (µg/ml)	***C. vulgaris***	***C. limneticus***	**BHA**	**BHT**
10	3.84±0.02	1.86±0.00	70.28±0.00	21.49±0.04
25	4.06±0.01	2.59±0.00	87.03±0.00	25.37±0.03
50	7.57±0.01	4.67±0.08	94.69±0.05	44.7±0.02
100	15.48±0.02	41.02±0.02	95.77±0.00	58.25±0.00

The results are presented as the mean ± standard deviation.

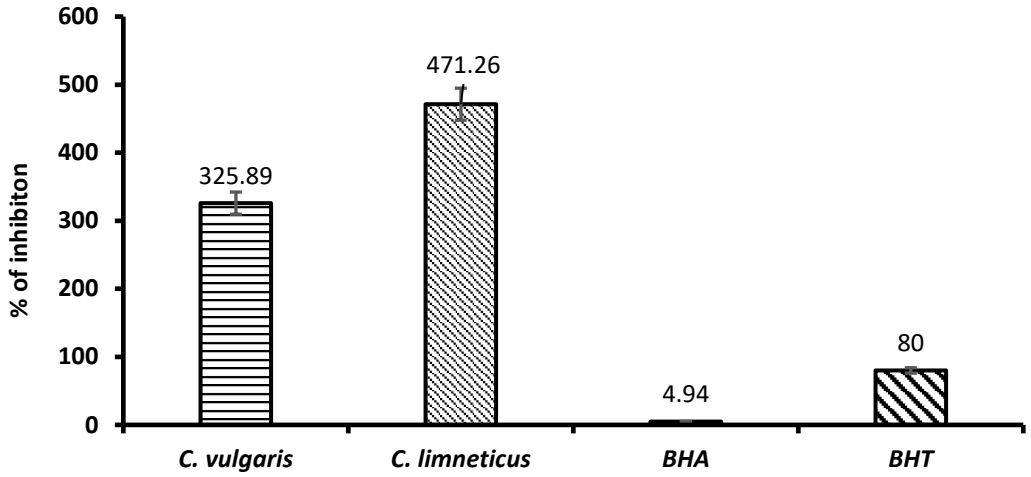

Figure 3: DPPH free radical-scavenging activities of *C. vulgaris* and *C. limneticus* methanol extracts. The results showed IC50 values (µg/ml) of I%. BHA and BHT were used as positive controls.

3.1.2. ABTS assay

The ABTS assay results of the extracts at concentrations of 10, 25, 50, and 100 µg/ml are shown in Table 4, and IC50 values are shown in Fig. 4. In this method, higher activity was obtained than in the DPPH method. However, no significant antioxidant activity was observed when the extracts were compared with the positive controls. The IC50 value of 137.32 µg/ml for *C. vulgaris* and an IC50 value of 172.83 µg/ml and *C. limneticus* was calculated (Fig. 4). In contrast, BHA and BHT possess values of 2.57 and 52.14 µg/ml IC50 respectively. The *C. vulgaris* extract showed better antioxidant activity than *C. limneticus* in both DPPH and ABTS assays, with no significant difference.

Table 4: ABTS cation radical-scavenging activity of *C. vulgaris* and *C. limneticus* methanol extracts. The results show the I% values. BHA and BHT were used as positive controls.

Concentrations (µg/ml)	***C. vulgaris***	***C. limneticus***	**BHA**	**BHT**
10	5.21±0.00	2.1±0.00	88.23±0.00	56.89±0.03
25	9.66±0.00	5.71±0.01	89.41±0.00	86.89±0.00
50	19.83±0.00	16.55±0.02	89.57±0.00	80.33±0.00
100	36.63±0.00	28.48±0.06	89.74±0.00	69.74±0.00

The results are presented as the mean ± standard deviation.

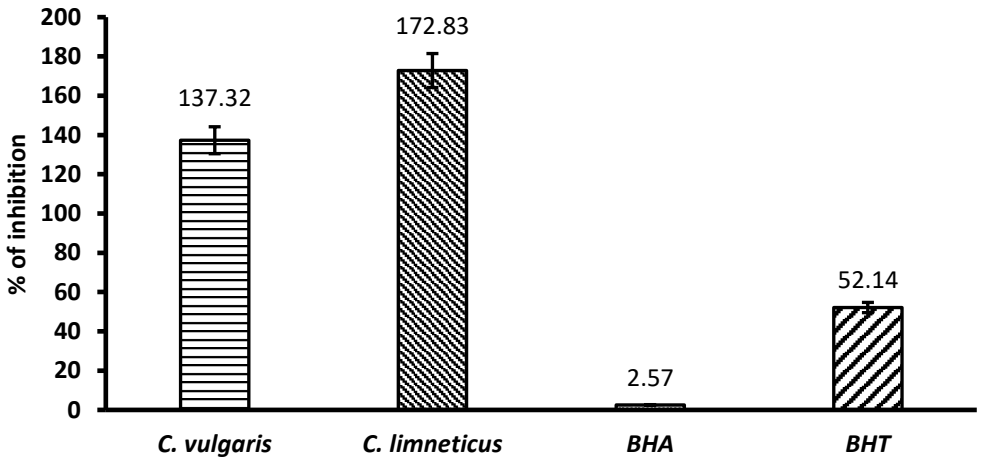

Figure 4: ABTS radical-scavenging activity of *C. vulgaris* and *C. limneticus* methanol extracts. The results showed IC50 values (µg/ml) of I%. BHA and BHT were used as positive controls.

3.1.3. Copper (II) reductive antioxidant activity (CUPRAC) assay

The copper (II) ion reduction capacities of the extracts and the positive control samples are presented in Table 5 and Fig. 5. In this method, the results are given as the absorbance value that increased absorbance value expressed increased activity. The extracts showed increased concentration-dependent activity, whatever there were no significant activities compared with the positive controls. As shown at Fig. 5, an A0.5 value of 198.22 µg/ml for *C. vulgaris* and an A0.5 value of 360 µg/ml for *C. limneticus* was calculated.

Table 5: CUPRAC-copper (II) ion-reduction capacities of *C. vulgaris* and *C. limneticus* methanol extracts. The results show the I% values. BHA and BHT were used as positive controls.

Concentrations (µg/ml)	***C. vulgaris***	***C. limneticus***	**BHA**	**BHT**
10	0.16±0,03	0.15±0.01	1.59±0.71	0.93±0.21
25	0.17±0,00	0.19±0.04	2.4±0.3	1.63±0.24
50	0.23±0,01	0.19±0.03	>4	3.67±0.1
100	0.32±0,00	0.24±0.05	>4	>4

The results are presented as the mean ± standard deviation.

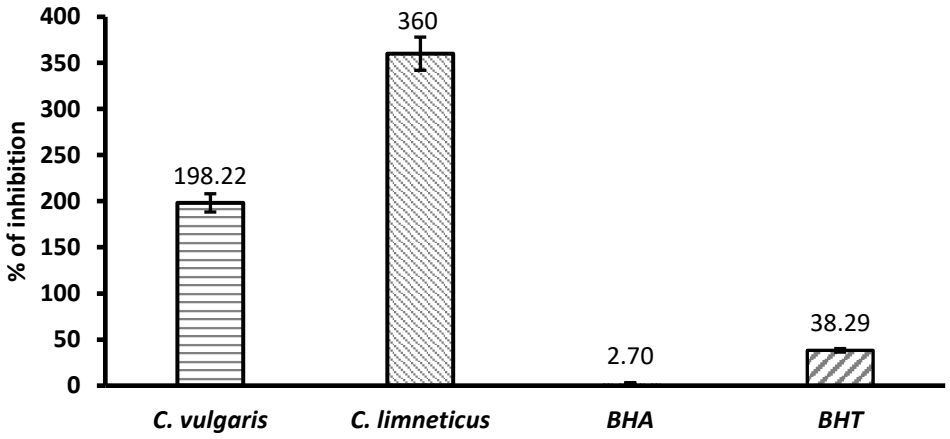

Figure 5. Copper (II) ion-reduction capacities of *C. vulgaris* and *C. limneticus* methanol extracts. The results showed IC50 values (µg/ml) of I%. BHA and BHT were used as positive controls.

3.1.4. Antimicrobial assay

Table 6 indicates the antimicrobial activity of the microalgae. The tested samples exhibited varying levels of antimicrobial activity. *C. vulgaris* was exhibited high activity with inhibition zone diameter greater than 22 mm and the MIC values range from 75 to 85 µg/ml. *C. limneticus* was exhibited weak activity with an inhibition zone diameter less than 12 mm and the MIC values range from 200 to 240 µg/ml. The major effectiveness (inhibition zone diameter 28±0.8 and MIC value 75 µg/ml) was recorded for *C. vulgaris* against *S. aureus*.

Table 6: Antimicrobial activities of *C. vulgaris* and *C. limneticus* methanol extracts.

Doses (µg/ml)		*E. coli*		*P. aeruginosa*		*S. aureus*		*S. pyogenes*		*C. albicans*	
		DD	**MIC**	**DD**	**MIC**	**DD**	**MIC**	**DD**	**MIC**	**DD**	**MIC**
C. vulgaris	**400**	24±0.3		22±0.8		28±0.8		23±0.8		22±1.0	
	300	20±0.9	85±1.0	16±0.5	75±0.3	24±1	75±0.9	19±1.0	85±0.8	18±0.8	85±0.8
	200	10±0.7		16±1.0		13±0.3		11±0.9		9±0.7	
C. limneticus	**400**	10±0.6		9±0.7		11±0.6		10±0.8		9±0.3	
	300	8±0.8	200±1.0	7±0.8	240±0.7	9±0.8	240±0.2	8±0.8	240±0.6	8±1.0	240±0.3
	200	7±0.8		NA		NA		7±0.8		NA	
PC		20±0.6	7.8±0.4	NA	NA	35±0.0	95±0.3	19±0.1	7.8±0.1	30±0.0	3.1±0.2

PC: positive controls that are ampicillin for bacteria and fluconazole for yeast, DD: disc diameter in mm, MIC as µg/ml, NA: not active. The results are presented as the mean ± standard deviation.

3.1.5. Mutagenic assay

Table 7: Plaque incorporation test results of *C. vulgaris* and *C. limneticus* methanol extracts on the *S. typhimurium* TA98 strain.

Concentrations (µg/Plate)	*Revertant colony number*	
	C. vulgaris	***C. limneticus***
Control	30.3±4.5	17±0.3
DMSO	32.1±2.2	15±0.1
Daunomycin	608±37.5	767±44.0
12.5	32.5±6.5	28.8±3.4
25	34.6±6.6	33.1±4.8
50	33.1±5.5	32.7±6.6
100	30.3±4.0	33.2±4.7
500	31.6±3.7	-30.9±6.4
1000	34.8±4.6	-32.3±2.8

Negative control: DMSO, Positive control: Daunomycin, Mean statistically significant at $p<0.05$ (Dunnett's t-test). The results are presented as the mean ± standard deviation.

The mutagenic activities of microalgae extracts are presented in Table 7. There were no mutagenic activities at all concentrations when comparing colony numbers of Control, DMSO, and Positive Control plates. Regardless of *C. limneticus* at any concentration, colony numbers could not be counted and the effect on the colony structure of *S. typhimurium* TA98.

4. Discussion and Conclusion

Microalgae are unicellular organisms that can survive extreme conditions. On the other hand, these cells are well-known sources of various secondary metabolites that are highly valuable in the pharmaceutical and cosmetic industries. These activities might have been due to the presence of biologically important phytoconstituents in the different algal extracts. In this study, we investigated the antimicrobial, antioxidant, and mutagenic activities of methanol extracts of two microalgae, *C. vulgaris* and *C. limneticus*, which were collected from Kabaklı pond in Diyarbakir-Türkiye. In the relevant literature, there are not enough studies reporting the activities of *C. limneticus* extracts. However, the *Chroococcus* genus has been relatively well studied, and its antimicrobial and antioxidant activities have been studied [32, 33]. *Chroococcus disperses* [19], and *Chroococcus minutus* [34].

GC-MS analysis of the ethanol extracts of these algae revealed the presence of several phytochemicals widely known for their biological activities (Table 1,2). These extracts are rich

in various bioactive compounds such as oleic acid, 9-Octadecenoic acid, (E), n-hexadecanoic acid, 1,4,7,10,13,16-Hexaoxacyclooctadecane, Heptaethylene glycol monododecyl ether, 15-Crown-5, 18,18'-Bi-1,4,7,10,13,16-hexaoxacy clononadecane, and octaethylene glycol monododecyl ether.

As shown in Table 3 and Fig. 3, DPPH free radical scanning activities increased in a dose-dependent manner, regardless of the non-significant antioxidant activity of the two microalgae extracts compared to the positive controls. Although *C. vulgaris* showed a dose-dependent increase in activity, *C. limneticus* showed stable antioxidant activity at concentrations of 50 µg/ml, whereas it significantly increased from 50 µg/ml to 100 µg/ml.

The ABTS radical scavenging assay results showed higher activity than the DPPH assay results for both *C. vulgaris* and *C. limneticus* extracts (Table 4). However, no significant antioxidant activity was observed when comparing the extracts with the positive controls. *C. vulgaris* extract showed better antioxidant activity than *C. limneticus* in both DPPH and ABTS assays, but the difference was not significant (Fig. 4). Copper (II) ion reduction capacity of algae extracts has increased concentration-dependent activity, although there were no significant activities compared with positive controls (Table 5 and Fig. 5). *C. vulgaris* showed antioxidant activity in similar studies, but it showed weak antioxidant activity compared to positive controls in our study [35]. The antioxidant and antimicrobial activities of ethanol extracts of microalgae are potentially attributable to the presence of specific chemical structures comprising hydroxyl groups and unsaturated carbon-carbon bonds. In both food systems and the human body, antioxidants play a critical role in reducing oxidative processes and the harmful effects of reactive oxygen species (ROS).

The antimicrobial activity of *C. vulgaris* and *C. limneticus* showed different levels of antimicrobial activity (Table 6) While *C. vulgaris* exhibited high microbial activity, *C. limneticus* exhibited weak activity. The major effectiveness (inhibition zone diameter 28±0.8 and MIC value 75 µg/ml) was recorded for *C. vulgaris* against *S. aureus*. As shown in Table 6 and Fig. 6, *C. vulgaris* showed high antimicrobial activity against *S. aureus* with 28 mm diameter and 75 MIC value and high activity against *E. coli* compared to the positive control. Similar studies have confirmed that *C. vulgaris* has the potential to become an antimicrobial agent [36, 37]. This is extremely important, as bacteria become resistant to a wide range of antibiotics used to treat bacterial diseases in humans.

The Salmonella microsome mutagenicity test (Ames test) is a test system used to understand both the mutagenic and antimutagenic effects of various chemicals and provides over

90% correlation in predicting genotoxicity [38]. In the Ames test, S. typhimurium strains with a mutation in the his-operon are used to detect the mutagenicity of substances [30]. Prokaryotic bioassays provide information about primary DNA damage and gene mutations induced by any agent [39]. In this study, the mutagenic activity of algae was determined using the Ames test, and the results showed that algae did not induce mutations in TA98 at all doses. Mutagenic activities of microalgae extracts (Table 7) were not shown any mutagenic activities at all concentrations. *C. vulgaris* and *C. limneticus* extracts affected on colony structure of *S. typhimurium* strain TA98. Because of water pollution, algal blooms occur when cyanobacteria produce toxins that are harmful to biological organisms. The mutagenic activity of *C. vulgaris* was not observed in the *S. typhimurium strain* TA98. A similar study with *C. vulgaris* has revealed that there is no mutagenic activity in *S. typhimurium* and working with rats [40, 41]

The chemical contents of microalgae used in our study oleic acid, hexadecanoic acid and 9,12-Octadecadienoic acid (Fig. 1,2) were the major fatty acid compounds in both *C. vulgaris* and *C. limneticus*. Wei et al. [42] reported that oleic acid exerted excellent antioxidative stress activity in vivo. Hexadecanoic acid is a saturated fatty acid (14:47%). Meanwhile, 9,12-Octadecadienoic acid is a polyunsaturated fatty acid (18:22%). Sawant and Mane [43] and Elshobary et al. [44] reported the antimicrobial activities of these fatty acids in *Chlorella emersonii*. In addition, several researchers have correlated the effects of fatty acids with different pathological conditions, such as positive effects against cardiovascular diseases and anticarcinogenic, anti-inflammatory, and antimicrobial activities [45].

The non-toxic and non-harmful natural antioxidants present an appealing alternative to synthetic chemical oxidants. Due to their non-toxic, non-carcinogenic and biodegradable nature, microalgae are regarded as a promising substitute for chemical antioxidants [46, 47].

A class of bioactive substances derived from microalgae, including polysaccharides, carotenoids, and phenolic compounds, is being increasingly recognised as a potent and durable source of natural antioxidants. The use of natural antimicrobials and antioxidants is a more attractive proposition than the use of synthetic chemical oxidants due to the non-toxic, non-harmful and biodegradable properties of the former. Hence, microalgae-derived bioactive compounds are an extremely valuable source of biologically active fractions that play a major role in the search for new drug candidates.

The results observed in this study demonstrated that although *C. vulgaris* has good antimicrobial potential, its activity is relatively weak. However, despite the relatively higher antioxidant activity of *C. limneticus* than that of *C. vulgaris*, the activities of both extracts were

weaker than those of the controls. Furthermore, the results showed that the extracts did not have mutagenic activity at any concentration tested.

Consequently, the results presented here show that *C. vulgaris* and *C. limneticus* may contain metabolites that affect various biological mechanisms. Therefore, this study may be considered as a starting point for investigating the biological activities of these microorganisms. Finally, we suggest further investigation of the biological activities of these organisms via different solvents or extraction systems since they may affect various biological systems.

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