

Phytochemical profiling and assessment of antioxidant and antibacterial activities of the green macroalga *Caulerpa prolifera* from the Antalya coast

Antalya kıyılarından toplanan yeşil makroalg *Caulerpa prolifera*'nın fitokimyasal profili, antioksidan ve antibakteriyel aktivite değerlendirmesi

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Received date: 14.11.2025

Accepted date: 09.02.2026

How to cite this paper:

Keskinçaya, H.B., Okudan, E.Ş., Altınok, B.Y., Koçak, M., & Gümüş, N.E. (2026). Phytochemical profiling and assessment of antioxidant and antibacterial activities of the green macroalga *Caulerpa prolifera* from the Antalya coast. *Ege Journal of Fisheries and Aquatic Sciences*, 43(1), 68-75. <https://doi.org/10.12714/egejfas.43.1.08>

Abstract: Macroalgae are increasingly supported for utilization and development in pharmacology, food, and biotechnology due to their high bioactive potential. This study aimed to characterize the phytochemical profile of *C. prolifera* extracts and to evaluate their in vitro antioxidant and antibacterial activities. The extracts exhibited total phenolic (TPC) and total flavonoid contents (TFC) values of 2.68±0.22 and 18.94±0.68 µg GAEs/mg, while flavonoid contents were determined as 51.05 and 7.26±0.09 µg QEs/mg. HPLC-DAD analysis identified major metabolites including chlorogenic acid, 4-hydroxybenzoic acid, t-ferulic acid, hydroxycinnamic acid, naringin, o-coumaric acid, rosmarinic acid, salicylic acid, quercetin, t-cinnamic acid, rutin, and naringenin. Antibacterial evaluation demonstrated variable susceptibility among Gram-negative and Gram-positive strains, with MIC values ranging from 7.5 to 5.25 mg/mL. While the ethanol extract exhibited the strongest antioxidant activity in the ABTS*[•] (IC₅₀: 333.16±0.74 µg/mL) and CUPRAC (A_{0.50}: 538.5±0.02) assays, all extracts showed limited DPPH scavenging capacity (IC₅₀: >800 µg/mL). The ethanolic extract also demonstrated the highest metal chelating capacity (IC₅₀: 327.15±0.63 µg/mL). In conclusion, this study demonstrates that ethanol is the most effective solvent for recovering antioxidant bioactive components from *C. prolifera* and recommends its use in extraction-based studies.

Keywords: Bioactive metabolites, HPLC-DAD, phenolic compounds

Öz: Makroalglerin yüksek biyoaktif potansiyelleri, farmakoloji, gıda ve biyoteknoloji alanlarında kullanım ve geliştirilmesini giderek daha fazla desteklemektedir. Bu çalışma, *C. prolifera* ekstrelerinin fitokimyasal profilini karakterize etmeyi ve in vitro antioksidan ve antibakteriyel aktivitelerini değerlendirmeyi amaçlamıştır. Ekstrelerin toplam fenolik içerik (TPC) ve toplam flavonoid içeriği (TFC) değerleri sırasıyla 2.68±0.22 ve 18.94±0.68 µg GAEs/mg olarak, flavonoid içerikleri ise 51.05 ve 7.26±0.09 µg QEs/mg olarak belirlenmiştir. HPLC-DAD analizi, klorojenik asit, 4-hidroksibenzoik asit, t-ferulik asit, hidroksisünamik asit, naringin, o-kumarik asit, rosmarinik asit, salisilik asit, kuersetin, t-sünamik asit, rutin ve naringenin gibi başlıca metabolitleri ortaya koymuştur. Antibakteriyel değerlendirme, Gram-negatif ve Gram-pozitif suşların farklı duyarlılık düzeyleri sergilediğini göstermiştir (MIC: 7.5–5.25 mg/mL). Etanol ekstresi, ABTS*[•] (IC₅₀: 333.16±0.74 µg/mL) ve CUPRAC (A_{0.50}: 538.5±0.02) testlerinde en yüksek antioksidan aktiviteyi gösterirken, tüm ekstreler DPPH radikal süpürme kapasitesi açısından düşük aktivite sergilemiştir (IC₅₀: >800 µg/mL). En yüksek metal şelatlama aktivitesi de etanol ekstresinde gözlenmiştir (IC₅₀: 327.15±0.63 µg/mL). Sonuç olarak bu çalışmada, etanolün *C. prolifera*'dan antioksidan biyoaktif bileşenleri geri kazanmak için en etkili çözücü olduğunu göstermektedir.

Anahtar kelimeler: Biyoaktif metabolitler, HPLC-DAD, fenolik bileşikler

INTRODUCTION

Macroalgae, as primary producers in aquatic ecosystems, play a pivotal role and hold considerable potential for use as dietary supplements and in pharmaceutical applications, largely owing to their diverse pigment profiles and pronounced antioxidant activities. In Türkiye, research on the systematics of algal species inhabiting aquatic ecosystems has a long-standing tradition (Aşıkutlu and Akköz, 2022). Complementing these systematic investigations, studies exploring the industrial and biotechnological potential of algae have been steadily increasing in recent years (Armagan et al., 2024; Gümüş, 2025).

Unsustainable industry and the increasing demand for wealth are further damaging natural ecosystems (Gümüş and

Buluş, 2020; Gümüş et al., 2024). Therefore, marine life has become even more important. Macroalgae constitute a highly diverse group of species that are indispensable components of marine ecosystems. Recognized as one of the most abundant natural reservoirs of biologically active secondary metabolites, they contain substantial quantities of bioactive compounds with demonstrated nutritional, pharmaceutical, and biotechnological relevance. Consequently, there has been a growing research interest in exploring these organisms as valuable sources for the development of functional ingredients, nutraceuticals, and potential pharmaceutical leads (Leandro et al., 2020; Taşkın, 2022). Macroalgae are organisms that exhibit particularly strong

antioxidant activity. (Park et al., 2004). They are increasingly acknowledged as valuable reservoirs of natural bioactive metabolites, with particular emphasis on compounds exhibiting potent antioxidant activity (Borowitzka, 2013). Remarkably, the structural integrity of macroalgae remains largely unaffected, and their stability during storage highlights the presence of well-developed cellular antioxidative defense mechanisms (Matsukawa et al., 1997). This multifaceted biotechnological potential highlights macroalgae as key contributors not only to sustainable resource utilization but also to the advancement of human health and well-being through the discovery of novel therapeutic and functional compounds (Keskinkaya et al., 2022).

Extensive research has established a strong link between oxidative stress in humans, characterized by excessive free radical production and cancer. Consequently, dietary intake of antioxidants or supplementation has been suggested as a preventive strategy against cancer (Stagos et al., 2012). Bioactive compounds, especially those obtained from marine macroalgae have shown significant antioxidant and anticancer effects in both in vitro and in vivo studies

(Goutzourelas et al., 2023; Keskinkaya et al., 2023). Despite these bioactive properties and their potential health benefits, marine macroalgae remain an underexploited natural resource. The present study aimed to evaluate the cytotoxic and antibacterial activities, along with the total phenolic and flavonoid contents, of *C. prolifera* extracts. Furthermore, the phenolic compounds contributing to the observed bioactivity were characterized using HPLC-DAD analysis.

MATERIALS AND METHODS

Collection and extraction of algal sample

Specimens of *Caulerpa prolifera* (Forsskål) J.V. Lamouroux 1809, a member of the division Chlorophyta inhabiting the infralittoral zone of the Mediterranean Sea (Antalya, Türkiye), were collected by scuba diving at a depth of 35 m from predetermined coordinates (36°27'40.47"N 30°32'38.18"E) (Figure 1). Macroalgal samples removed from epiphytes and other foreign substances were dried at 40°C for 24 hours, then ground into powder with a homogenizer and stored at room temperature until extraction.



Figure 1. Sampling site of *C. prolifera* indicated on the Antalya coastline, eastern Mediterranean region of Türkiye

Extraction of *C. prolifera* was performed using three different polar solvents ethanol, methanol, and aqueous via solid-phase extraction. All solvents, including methanol and ethanol, were of analytical (HPLC) grade and obtained from certified suppliers. For each extraction, 20 g of powdered algal material was subjected to Soxhlet extraction with 180 mL of the respective solvent at its boiling point for 6 hours. Sequential extraction was conducted on the same sample by applying the solvents in order of increasing polarity, from the least polar to the most polar.

The resulting extracts containing excess solvent were evaporated until approximately 1-2 mL of concentrated extract was obtained. The concentrated extracts were then completely dissolved in their respective solvents, transferred to Eppendorf tubes, and sealed in a nitrogen atmosphere (Keskinkaya et al., 2023).

Antioxidant activity

The antioxidant activities of *C. prolifera* extracts were evaluated using DPPH• radical scavenging, ABTS•⁺ radical scavenging, CUPRAC, and metal chelating assays (Decker and Welch 1990; Deveci et al., 2019). Ascorbic acid served as the standard reference. For radical scavenging assays, the concentration required to achieve 50% inhibition was determined and reported as IC₅₀, whereas for CUPRAC activity, the concentration corresponding to an absorbance of 0.50 was calculated and expressed as A_{0.50}. Results were calculated using the equation Inhibition (%) = [(A₀ - A_s) / A₀] × 100, (Blois, 1958) and IC₅₀ values were determined as the extract concentration required to achieve 50% inhibition from the dose-response curve.

Total phenolic (TPC) and total flavonoid contents (TFC)

TPC analyses of *C. prolifera* extracts were performed using the Folin Ciocalteu method (Slinkard and Singleton, 1977) and TFC analyses were performed using the aluminum nitrate method (Park et al., 2004).

HPLC analysis of phenolic compounds

Analyses were performed with an HPLC system (Agilent 1260 Infinity Series, DAD detector) using an Acegenerix 5C18 column operated at 30°C. Detection was performed at 300/200 nm wavelengths and in 500/100 nm reference mode (Türkan et al., 2020).

The mobile phase consisted of solvent A consisted of water containing 0.1% formic acid, while solvent C was acetonitrile (HPLC grade) and the following gradient program was applied: 83:17 (A:C, v/v) at 0 min, 85:15 at 7 min, 80:20 at 20 min, 75:25 at 24 min, 70:30 at 28 min, 60:40 at 30min, 50:50 at 32 min and 30:70 at 36 min. At the end of the program, the initial condition of 83:17 was returned at 40min. Compounds were identified and quantified by comparison with authentic standards. The flow rate was 1mL/min, the injection volume for sample solution was 20µL, and the column temperature was kept at 30°C.

Antimicrobial activity test (broth microdilution method, MIC)

The antimicrobial activity of *C. prolifera* extracts was assessed using the minimum inhibitory concentration (MIC) method as described by David et al. (2021). The test panel included Gram-positive and Gram-negative bacteria, as well as the yeast *Candida albicans*. Bacterial suspensions were prepared according to the 0.5 McFarland standard and diluted to 10⁵CFU/mL for inoculation purposes. Each well of a 96-well microplate contained 100µL of Mueller–Hinton broth. Extracts, initially prepared at 25mg/mL, were serially two-fold diluted to achieve final concentrations ranging from 6.25 to 0.0030 mg/mL. Subsequently, 100µL of the inoculum was added to each well. DMSO served as negative control, while gentamicin was used as the positive control. MIC values were defined as the lowest concentration of extract at which no microbial growth or pink/red coloration was observed (Akdag et al., 2025; Basri and Fan, 2005; Keskinaya et al., 2020; Lourens et al., 2004; Salie et al., 1996).

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using appropriate statistical software. A value of $p < 0.05$ was considered statistically significant. Dose–response curves and IC₅₀ values were calculated by nonlinear regression analysis.

RESULTS

HPLC-based characterization of phenolic compounds in *C. prolifera* extracts

The chromatographic profile of *C. prolifera* extract revealed the presence of several phenolic compounds with distinct retention times. The first major peak was identified as chlorogenic acid at 6.218min, representing one of the dominant early-eluting phenolics. Subsequent minor peaks included rutin at 19.441min and trans-ferulic acid at 20.111min. A more distinct signal was observed for hydroxycinnamic acid at 23.033min, followed by a clear peak for naringin at 27.603 min. The later part of the chromatogram (30–40 min) showed a cluster of broad and intense peaks, suggesting the presence of higher molecular weight or less polar compounds. Overall, chromatographic distribution indicates a diverse phenolic profile, with both early-eluting hydrophilic acids and later-eluting flavonoid-type compounds (Figures 2, 3, 4).

Only a limited number of studies have reported the chemical composition of *Caulerpa* species. In the present study, chromatographic analysis revealed that the ethanolic extracts of *C. prolifera* contained chlorogenic acid, 4-hydroxybenzoic acid, trans-ferulic acid, hydroxycinnamic acid, naringin, o-coumaric acid, rosmarinic acid, salicylic acid, quercetin, and trans-cinnamic acid. The aqueous extracts were found to include chlorogenic acid, rutin, trans-ferulic acid, hydroxycinnamic acid, and naringin. In methanolic extracts, HPLC analysis identified chlorogenic acid, naringin, and trans-cinnamic acid (Table 1).

Table 1. Phytochemical constituents identified in the extracts of *C. prolifera*

Compounds	CPA(µg/g)	CPM(µg/g)	CPE (µg/g)
Chlorogenic acid	2.57	3.14	1.18
t-Cinamic acid	ND	2.73	3.50
p-coumaric acid	0.15	ND	ND
Quercetin	ND	ND	1.20
t-Ferulic acid	2.82	ND	4.50
Hydroxy sinamic acid	3.16	ND	1.33
Naringin	1.07	3.57	7.43
o-Coumaric acid	ND	ND	1.52
Rosmarinic acid	ND	ND	3.92
4-Hydroxy benzoic acid	ND	ND	1.02

CPM: *C. prolifera* methanol extract, CPE: *C. prolifera* ethanol extract, CPA: *C. prolifera* aqueous extract.

Antioxidant activity

Because antioxidants exert their effects through multiple mechanisms, it is generally recommended to employ more than one analytical method rather than relying on a single assay. Accordingly, the antioxidant potential of *C. prolifera* extracts was evaluated using ABTS (% inhibition), metal chelating (% inhibition), DPPH radical scavenging, and CUPRAC assays.

The ABTS (% inhibition) results for methanolic, ethanolic, and aqueous extracts are summarized in Table 2. Among the tested extracts, the ethanolic extract displayed the highest ABTS•⁺ scavenging activity (IC₅₀: 333.16±0.74µg/mL), whereas none of the extracts exhibited significant DPPH radical scavenging activity (IC₅₀>800µg/mL). The highest metal chelating activity was observed in the hexane extract (IC₅₀:254.45±0.89µg/mL). Furthermore, the ethanolic extract

demonstrated the strongest CUPRAC antioxidant capacity (A_{0.50}:538.5±0.02). These results confirm that ethanol is the most effective solvent for extracting bioactive antioxidant constituents from *C. prolifera*.

As shown in Table 3, the aqueous extract of *C. prolifera* exhibited the highest total phenolic content (TPC) (18.94±0.68 µg GAEs/mg), while the ethanolic extract contained the highest total flavonoid content (51.05±0.54 µg QEs/mg).

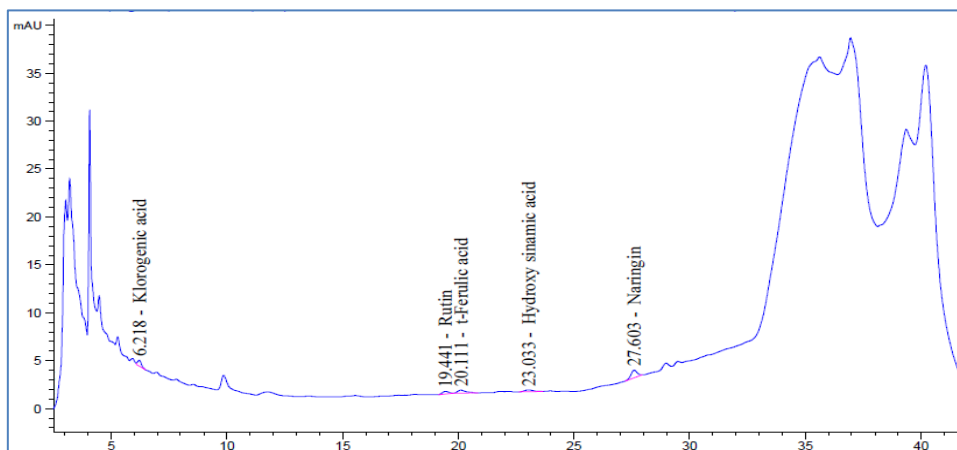


Figure 2. HPLC-DAD Chromatographic profile of *C. prolifera* aqueous extract

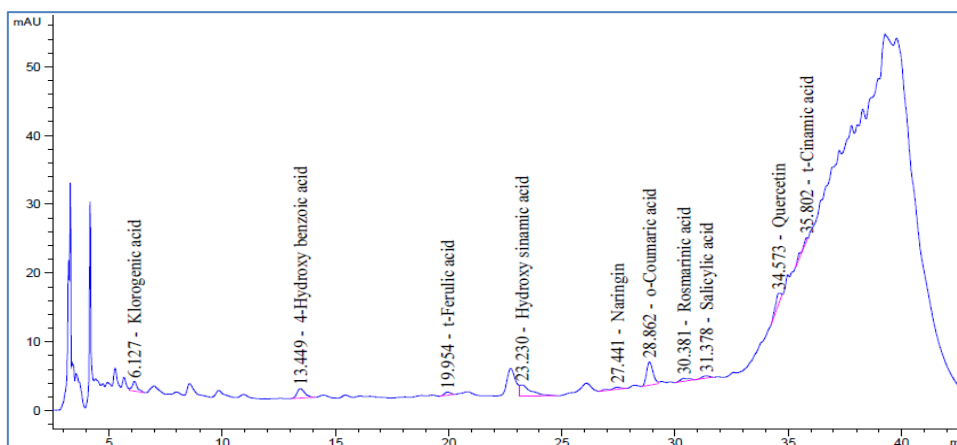


Figure 3. HPLC-DAD chromatographic profile of *C. prolifera* ethanolic extract

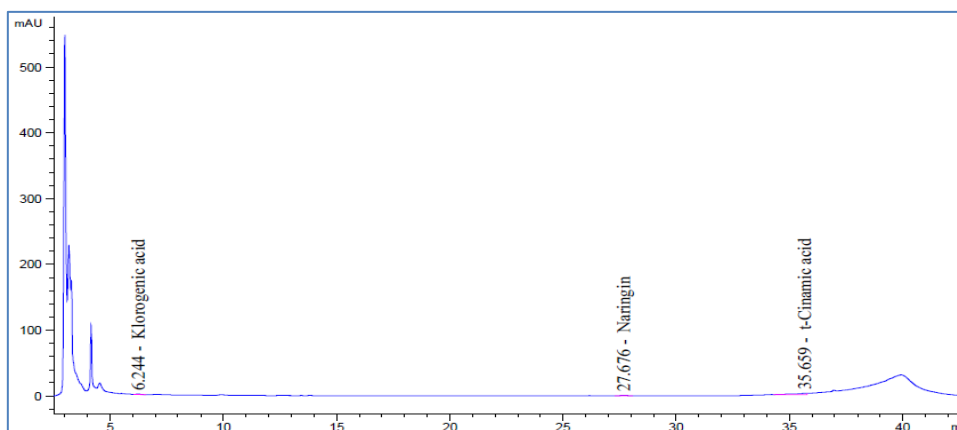


Figure 4. HPLC-DAD chromatographic profile of *C. prolifera* methanolic extract

Table 2. Antioxidant capacity of *C. prolifera* extracts

		Antioxidant Activity							
		DPPH• assay		ABTS•+ assay		CUPRAC assay		Metal Chelating assay	
		Inhibition (%) ^a	IC ₅₀ (µg/mL) ^b	Inhibition (%) ^a	IC ₅₀ (µg/mL) ^b	Absorbance ^c	A _{0.50} (µg/mL) ^d	Inhibition (%) ^a	IC ₅₀ (µg/mL) ^b
Extracts	CPM	-	>400	31.33±0.77	>400	0.16±0.00	>400	15.18±0.00	>400
	CPE	-	>400	56.70±0.37	333.16±0.74	0.33±0.01	538.5±0.02	55.41±0.12	327.15±0.63
	CPA	-	>400	33.04±0.92	748.21±0.51	0.14±0.02	>400	42.14±0.43	713.54±0.45
Standards	BHT	86.63±0.19	23.70±0.14	85.62±0.32	12.76±0.63	2.99±0.03	28.22±0.01	-	-
	BHA	88.19±0.10	22.90±0.59	87.52±0.74	12.07±0.97	3.23±0.01	26.55±0.02	-	-
	EDTA	-	-	-	-	-	-	90.21±0.24	4.29±0.06

CPM: *C. prolifera* methanol extract, CPE: *C. prolifera* ethanol extract, CPA: *C. prolifera* aqueous extract BHT: Butylated hydroxytoluene, BHA: Butylated hydroxyanisole

^aInhibition values % of 400 µg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements

^bIC₅₀ values are given as a mean ± D of three parallel measurements

^cAbsorbance values of 400 µg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements

^dA_{0.50} values are given as a mean ± SD of three parallel measurements.

Table 3. TPC and TFC contents of *C. prolifera* extracts^a

Extracts	TPC	TFC
	(µg GAEs/mg extract ^b)	(µg QEs/mg extract ^c)
CPE	16.42±1.26	51.05±0.54
CPM	6.50±1.03	7.26±0.09
CPA	18.94±0.68	11.69±0.18

CPM: *C. prolifera* methanol extract, CPE: *C. prolifera* ethanol extract, CPA: *C. prolifera* aqueous extract

^aThe results are given as a mean ±SD of three parallel measurements.

^bGAEs, gallic acid equivalent, $y=0.0123x-0.0155$ $r^2=0.9931$

^cQEs, quercetin equivalent, $y=0.0156x-0.0112$ $r^2=0.9985$

Antimicrobial activity

The antimicrobial activity was evaluated against three Gram-positive strains (*Staphylococcus aureus* ATCC 43300, *Bacillus cereus* ATCC 11778, *Sarcina lutea* ATCC 9341), four Gram-negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 70603, *Salmonella enteritidis* ATCC 13076), and one fungal strain (*Candida albicans*). Full details of the strains are provided in Table 4.

The aqueous extract of *C. prolifera* did not exhibit detectable antimicrobial activity against any of the tested microorganisms. In contrast, the methanolic and ethanolic extracts displayed variable levels of antimicrobial activity. Both extracts were active against *P. aeruginosa* (MIC:1.562 mg/mL), as well as *K. pneumoniae*, *S. aureus*, and *S. lutea* (MIC:3.125 mg/mL).

Table 4. MIC results of the aqueous, methanolic, and ethanolic extracts of *C. prolifera*

Test of Microorganism	CPA mg/ml	CPM mg/ml	CPE mg/ml	Gentamycin (0.1 mg/ml)	DMSO (%)
<i>Escherichia coli</i>	NA	NA	NA	<0.02	12.5
<i>Pseudomonas aeruginosa</i>	NA	1.562	1.562	<0.02	12.5
<i>Klebsiella pneumoniae</i>	NA	3.125	3.125	0.78	12.5
<i>Staphylococcus aureus</i>	NA	3.125	3.125	<0.02	25
<i>Salmonella enteritidis</i>	NA	NA	NA	0.04	12.5
<i>Sarcina lutea</i>	NA	3.125	3.125	<0.02	12.5
<i>Bacillus cereus</i>	NA	NA	NA	<0.02	12.5
<i>Candida albicans</i>	NA	NA	NA	<0.02	12.5

NA: Not active CPM: *C. prolifera* methanol extract, CPE: *C. prolifera* ethanol extract, CPA: *C. prolifera* aqueous extract

DISCUSSION

Chromatographic analysis of *C. prolifera* extracts revealed a diverse phytochemical profile, particularly rich in phenolic and flavonoid constituents. In the ethanolic extract, nine major compounds were identified, including chlorogenic acid (1.18µg/g), 4-hydroxybenzoic acid (1.02µg/g), trans-ferulic acid (4.50µg/g), hydroxycinnamic acid (1.33µg/g), naringin (7.43µg/g), *o*-coumaric acid (1.52µg/g), rosmarinic acid (3.92µg/g), quercetin (1.20µg/g), and trans-cinnamic acid (3.50µg/g). The aqueous extract contained chlorogenic acid (2.57µg/g), trans-ferulic acid (2.82µg/g), hydroxycinnamic acid (3.16µg/g), and naringin (1.07µg/g), whereas the methanolic extract was characterized by chlorogenic acid (3.14µg/g), naringin (3.57µg/g), and trans-cinnamic acid (2.73µg/g).

The findings of the present study demonstrate that the extracts of *C. prolifera* possess a remarkable antioxidant potential, which can be attributed to their high content of phenolic and flavonoid compounds. This observation is consistent with previous investigations reporting a strong correlation between phenolic richness and antioxidant capacity in macroalgae (Alghazeer et al., 2024; Baharfar et al., 2015; Chaabani et al., 2024; Huang et al., 2011; Tungmunthum et al., 2018). Notably, the predominance of compounds such as chlorogenic acid, ferulic derivatives, rosmarinic acid, and quercetin—well-established molecules with radical-scavenging and metal-chelating properties—further supports the mechanistic basis of the observed activity.

Overall, these findings suggest that *C. prolifera* serves as a valuable natural source of bioactive secondary metabolites, and its phenolic-rich extracts may offer promising potential for applications in pharmaceutical, nutraceutical, or functional food development.

In the present study, none of the *C. prolifera* extracts exhibited notable DPPH radical scavenging activity (IC₅₀>800µg/mL), whereas the synthetic standards BHT and BHA showed strong activity with IC₅₀ values of 23.90±0.14µg/mL and 22.80±0.59µg/mL, respectively. In contrast, the ethanolic extract displayed the highest ABTS•+ scavenging capacity (IC₅₀:333.16±0.74µg/mL), although still

weaker than BHT (IC_{50} : $12.05\pm 0.97\mu\text{g/mL}$) and BHA (IC_{50} : $12.75\pm 0.63\mu\text{g/mL}$). The strongest metal chelating activity was recorded for the hexane extract (IC_{50} : $254.45\pm 0.89\mu\text{g/mL}$), compared to the reference chelator EDTA (IC_{50} : $4.29\pm 0.06\mu\text{g/mL}$). The ethanolic extract also exhibited the highest antioxidant capacity in the CUPRAC assay ($A_{0.50}$: 538.5 ± 0.02), while BHT and BHA displayed significantly lower IC_{50} values of $26.54\pm 0.02\mu\text{g/mL}$ and $28.21\pm 0.01\mu\text{g/mL}$, respectively.

Regarding phytochemical composition, the aqueous extract presented the highest total phenolic content (TPC: $18.94\pm 0.68\mu\text{g GAEs/mg}$), while the ethanolic extract contained the highest total flavonoid content (TFC: $51.05\pm 0.54\mu\text{g QEs/mg}$). In this study, the higher TPC shown by the aqueous extract compared to other solvents can be attributed to the more efficient solubility of the highly polar and glycosylated phenolic compounds that are dominant in *C. prolifera* in aqueous (Castillo-Correa et al., 2025). Similarly, Yilmaz et al. (2021) reported that aqueous extracts of *Gongolaria barbata* exhibited higher or comparable TPC values ($2.29\pm 0.01\text{mg GAE/g}$) compared to methanol and ethanol extracts. Likewise, Yucetepe et al. (2022) reported that highly polar environments facilitated the release of phenolic compounds from the matrix during *C. prolifera* protein extraction and could increase TPC values up to ($31.12\text{ g/kg}^{-1}\text{ dw GAEs}$). These observations are consistent with previous reports. For instance, Yucetepe et al. (2022) quantified TPC, CUPRAC, and DPPH values of *C. prolifera* extracts as $31.12\text{ g}\cdot\text{kg}^{-1}\text{ dw}$ (GAEs), $4.45\text{ g}\cdot\text{kg}^{-1}\text{ dw}$ (TEs), and $0.83\text{ g}\cdot\text{kg}^{-1}\text{ dw}$ (TEs), respectively. Similarly, Rosa et al. (2025) evaluated dichloromethane (CP1), acetone (CP2), and ethanol (CP3) extracts of *C. prolifera*, reporting DPPH scavenging activities of $15.3\pm 0.54\%$, $5.6\pm 0.01\%$, and $37.7\pm 0.37\%$, and ABTS activities of $31.5\pm 0.11\%$, $21.9\pm 0.37\%$, and $48.1\pm 1.21\%$, respectively, at the maximum tested concentration. In another study, Yilmaz et al. (2021) reported the highest total flavonoid content ($6.91\pm 0.09\text{ mg/g ext.}$) and total phenolic content ($2.29\pm 0.01\text{ mg GAE/g ext.}$) in extracts from the green algae *G. barbata* using different solvents. The TPC and TFC results of the present study are in line with these previous findings.

Chaabani et al. (2024), also demonstrated comparable findings: TPC and TFC values of sequentially extracted samples were 1.57mg GAE/g and 1.45mg QE/g , whereas maceration extracts yielded 3.67mg GAE/g and 1.93mg QE/g . In agreement with our results, the authors reported no detectable DPPH scavenging activity, a moderate reducing power (IC_{50} : $1.72\text{--}6.97\text{mg/mL}$), and chelation activity ranging from IC_{50} : 6.91 to 3.44mg/mL .

Collectively, when compared with previous studies, our findings confirm that *C. prolifera* possesses measurable antioxidant capacity, although the magnitude of activity depends strongly on the extraction solvent, assay system, and selected standard compounds. Consistent with Chaabani et al. (2024), the absence of DPPH activity further supports the notion that the antioxidant capacity of *C. prolifera* is not

primarily mediated through hydrogen atom transfer but may instead be associated with electron transfer or metal ion chelation-based mechanisms.

In the present study, the methanolic and ethanolic extracts of *C. prolifera* exhibited selective antimicrobial activity, particularly against *Pseudomonas aeruginosa* (MIC: 1.562mg/mL), *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Sarcina lutea* (MIC: 3.125mg/mL). In addition, the hexane extract showed activity against *P. aeruginosa* (MIC: 1.562mg/mL), *S. aureus*, and *Bacillus cereus* (MIC: 3.125mg/mL). No activity was recorded against *Candida albicans*, indicating that the antimicrobial potential of *C. prolifera* is predominantly antibacterial rather than antifungal. The stronger inhibition observed in Gram-negative *P. aeruginosa* compared to several Gram-positive strains is noteworthy, as macroalgae are generally reported to be more active against Gram-positive bacteria. Similar findings have been documented for other *Caulerpa* species, where the antimicrobial activity was predominantly directed toward *Staphylococcus* and *Bacillus* strains rather than Gram-negative bacteria (Chadorshabi et al., 2025; Lomartire and Gonçalves, 2023; Palaniyappan et al., 2023). This unexpected trend suggests that *C. prolifera* may contain non-polar or mid-polar compounds with an affinity toward the outer membrane structure of Gram-negative bacteria an effect previously associated with sulfated polysaccharides, phenolic constituents, and terpenoid derivatives in members of the genus *Caulerpa*. The antimicrobial effects of 18 marine macroalgae collected from the Aegean coast and the region between Çanakkale and Muğla were investigated against seven bacteria, including *S. aureus*, *Enterococcus faecalis*, and *E. coli*. In the study, methanol extracts were found to be effective against *E. faecalis* Çınar (2012) and Karabay-Yavasoglu et al. (2007) reported the antimicrobial activity of *Jania rubens* against 5 gram-positive and 4 gram-negative bacteria and the fungal strain *C. albicans* and revealed that methanol extracts were the most active extracts compared to other extracts tested in the study. The antifungal analyses we conducted in our study are in line with this study. Ravikumar et al. (2011) reported that *Caulerpa taxifolia* and *Caulerpa peltata* showed very high activity compared to their positive controls. They suggested that the effects might be due to their polysaccharide content and that carbohydrate-based drugs should be developed as antiplasmodial agents. Antimicrobial activity of 82 marine algae species was investigated by (Salvador et al., 2007). Summer was recorded as the season with the highest activity for Chlorophyta.

These results are in agreement with Chaabani et al. (2024) who reported that sequentially extracted *C. prolifera* exhibited inhibitory activity against both Gram-negative and Gram-positive bacteria, with inhibition zone diameters ranging from 2 to 9 mm. Similarly, Keskinakaya et al. (2022) demonstrated that green algal species *Codium fragile* and *Codium bursa* displayed antibacterial activity against *P. aeruginosa*, consistent with our findings. Furthermore, Alghazeer et al.

(2024) confirmed that *C. prolifera* possesses broad-spectrum antibacterial effects, although the extent of inhibition depends on both the microbial strain and the extraction solvent, a trend that aligns with observations in the present study.

Taken together, the antimicrobial behavior of *C. prolifera* appears to be strain-dependent and strongly influenced by solvent polarity, with methanol, ethanol, and hexane yielding bioactive fractions, whereas aqueous extracts remained inactive.

CONCLUSION

This study aimed to characterize the phytochemical profile of *C. prolifera* extracts and to evaluate their *in vitro* antioxidant and antibacterial activities. Among the extracts, ethanol and methanol showed the highest bioactivity, indicating their efficiency in recovering biologically active metabolites. The methanolic and ethanolic extracts also exhibited moderate antibacterial activity against selected Gram-positive and Gram-negative strains.

These findings indicate that *C. prolifera* is a valuable source of phenolic compounds with potential uses in food spoilage inhibitors, pharmaceutical formulations and other bioactive product development. Future studies should aim to isolate and characterize individual active metabolites, elucidate their molecular mechanisms of action, and validate their efficacy through *in-vivo* models. Considering Türkiye's exceptionally rich marine biodiversity much of which remains scientifically underexplored this species and other native macroalgae represent valuable resources for the advancement of sustainable blue biotechnology, offering both scientific and economic potential.

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ACKNOWLEDGEMENTS AND FUNDING

This study was supported by the Scientific Research Projects Coordination Unit of Karamanoğlu Mehmetbey University (Project No. 03-M-23).

AUTHORSHIP CONTRIBUTIONS

Hatice Banu Keskinaya: Writing–review & editing, Conceptualization, Software, Data curation, Methodology, Visualization, Resources. Emine Şükran Okudan: Supervision, Writing–original draft, Formal analysis, Data curation, Investigation, Resources. Bahar Yılmaz Altınok: Writing–original draft, Formal analysis, Data curation, Methodology. Merve Koçak: Methodology, Formal analysis, Software. Numan Emre Gümüş: Writing–review & editing, Methodology, Conceptualization, Validation, Software, Data curation, Supervision, Visualization, Project administration, Resources.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

No specific ethical approval was required for this study.

DECLARATION OF AI USE

AI-assisted technologies were not used in the preparation of this article.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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