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CHLORELLA VULGARIS AS A MICROALGAL SOURCE OF ANTIOXIDANTS: A BIOCHEMICAL ASSESSMENT

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Abstract

Original scientific paper

The objective of this study is to assess the total antioxidant capacity of the biomass derived from the freshwater microalga *Chlorella vulgaris* (*C. vulgaris*), cultivated under static aquarium conditions. *C. vulgaris* is known for its high protein content, essential fatty acids, vitamins, and bioactive compounds such as carotenoids, chlorophyll, phenolics, and flavonoids, which grant it significant antioxidant properties and make it valuable for nutritional and pharmaceutical applications. The biomass was dried and powdered, and its antioxidant profile was analyzed using various assays. These included Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Antioxidant Capacity (TAC), DPPH and ABTS radical scavenging activity, and FRAP (ferric reducing antioxidant power). Results showed that the sample contained 20.90 mg GAE/g phenolics, 29.32 mg QE/g flavonoids, and exhibited a high TAC value of 44.07 mg AAE/g. Additionally, it demonstrated significant antioxidant activity in DPPH (18.21 mg AAE/g) and FRAP (16.75 mg $FeSO_4$E/g) tests. However, the ABTS scavenging value was relatively low (3.05 mg TE/g), possibly due to the specific radical affinity of dominant antioxidant compounds or experimental limitations. In conclusion, the rich phenolic and flavonoid content of *C. vulgaris* biomass has shown remarkable antioxidant activity. The results support its potential use as a natural antioxidant source in dietary supplements, functional foods, cosmetics, and pharmaceutical formulations. This research highlights the promising role of algal-derived compounds in promoting health and combating oxidative stress-related aging processes.

Keywords: *ABTS, antioxidant, chlorella vulgaris, flavonoid, phenolic.*

MİKROALGAL ANTİOKSİDAN KAYNAĞI OLARAK *CHLORELLA VULGARIS*: BİYOKİMYASAL DEĞERLENDİRME

Özet

Orijinal bilimsel makale

Bu çalışma, statik akvaryum koşullarında kültürlenen tatlı su mikroalge *Chlorella vulgaris* (*C. vulgaris*) 'den elde edilen biyokütlenin toplam antioksidan kapasitesini değerlendirmek amacıyla yapılmıştır. *C. vulgaris*, yüksek protein içeriği, esansiyel yağ asitleri, vitaminler ve karotenoidler, klorofil, fenolikler ve flavonoidler gibi biyoaktif bileşiklerle bilinir. Bu bileşikler, ona önemli antioksidan özellikler kazandırır ve beslenme ve farmasötik uygulamalar için değerli kılar. Biyokütle kurutulup toz haline getirilmiş ve çeşitli testler kullanılarak antioksidan profili analiz edilmiştir. Bu testler arasında Toplam Fenolik İçerik (TPC), Toplam Flavonoid İçerik (TFC), Toplam Antioksidan Kapasite (TAC), DPPH ve ABTS radikal temizleme aktivitesi ve FRAP (ferrik indirgeyici antioksidan gücü) yer almaktadır. Sonuçlar, numunenin 20,90 mg GAE/g fenolik, 29,32 mg QE/g flavonoid içerdiğini ve 44,07 mg AAE/g gibi yüksek bir TAC değeri sergilediğini göstermiştir. Ayrıca, DPPH (18,21 mg AAE/g) ve FRAP (16,75 mg $FeSO_4$E/g) testlerinde önemli antioksidan aktivite göstermiştir. Ancak, ABTS temizleme değeri nispeten düşüktür (3,05 mg TE/g), bu durum muhtemelen baskın antioksidan bileşiklerin spesifik radikal afinitesi veya deney sınırlamalarından kaynaklanmaktadır. Sonuç olarak, *C. vulgaris* biyokütlesinin zengin fenolik ve flavonoid içeriği, dikkate değer bir antioksidan aktivite göstermiştir. Bu bulgular, besin takviyeleri, fonksiyonel gıdalar, kozmetikler ve farmasötik formülasyonlarda doğal bir antioksidan kaynağı olarak potansiyel kullanımını desteklemektedir. Bu araştırma, alglerden elde edilen bileşiklerin sağlığı destekleme ve oksidatif stresle ilişkili yaşlanma süreçleriyle mücadelede umut verici rolünü vurgulamaktadır.

Anahtar Kelimeler: *ABTS, antioksidan, chlorella vulgaris, fenolik, flavonoid.*

1 Introduction

Chlorella vulgaris is a microalgae that has received increasing attention due to its nutritional profile and therapeutic potential. Microalgae have gained increasing attention as promising sources of natural bioactive compounds with significant antioxidant potential. Among them, *C. vulgaris* merits particular attention due to its

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balanced biochemical composition and high adaptability to various cultivation conditions. This microalgae is notable for its high protein, essential fatty acid, and vitamin content, as well as its well-documented antioxidant properties. The antioxidant potential of *C. vulgaris* biomass extracts has garnered significant attention in recent research due to their rich composition of phenolic compounds and associated health benefits. Various studies have demonstrated that extracts of *C. vulgaris* exhibit substantial antioxidant activity, primarily attributed to their phenolic and flavonoid content.

One remarkable feature of *C. vulgaris* is its strong antioxidant capacity, linked to the presence of carotenoids, chlorophyll, ascorbic acid, flavonoids, and tocopherols [1]. These compounds play essential roles in the neutralization of free radicals. Literature reports highlight the contribution of carotenoids like lutein and astaxanthin to antioxidant defense mechanisms, showing their efficacy in reducing oxidative damage through both in vitro and in vivo studies [2].

The biochemical composition and antioxidant potential of *C. vulgaris* are highly dependent on its cultivation conditions. Variations in these parameters can significantly influence the synthesis and accumulation of bioactive metabolites. For instance, exposure to moderate light stress has been shown to enhance carotenoid biosynthesis as a protective response against photooxidative damage, while nutrient limitation, particularly nitrogen or phosphorus deficiency, often triggers an increase in total phenolic content and antioxidant enzyme activity. Antioxidant activity has been linked to the modulation of enzymes, including catalase and superoxide dismutase, thereby enhancing cellular protection [3].

Clinical studies further support the safe use of *C. vulgaris* in modulating oxidative stress in humans, showing increased glutathione levels and decreased malondialdehyde (MDA) levels in smokers [4]. Literature also supports its use in aquaculture, improving immune response and intestinal antioxidant status in fish [5].

Beyond its antioxidant potential, *C. vulgaris* is being integrated into food and health products. The substance has been utilized to augment the sensory and antioxidant characteristics of beer [6] and marketed as a dietary supplement, reflecting the rising consumer demand for natural antioxidants [7].

Literature indicates that combining *C. vulgaris* with compounds like tocotrienols and vitamins enhances both its antioxidant and therapeutic effects [8]. This synergistic approach supports the development of innovative health-promoting products. This microalga is known to accumulate a wide range of antioxidant molecules, including phenolic acids, flavonoids, carotenoids, and chlorophyll derivatives, which collectively contribute to its strong free radical–scavenging and metal-reducing properties.

A wide variety of phenolic compounds have been identified in *C. vulgaris*, including chlorogenic acid, catechin, and gallic acid, which have been extensively documented for their robust antioxidant properties. Almutairi [9] noted that the phenolic and flavonoid content in *C. vulgaris* dietary supplements plays a crucial role in mediating its antioxidant effects, a finding corroborated by observations that these compounds effectively scavenge free radicals.

Similarly, Abdel-Karim et al. [10] demonstrated a significant association between the antioxidant activity of *C. vulgaris* and its total phenolic content, underscoring its potential effectiveness in mitigating oxidative stress.

In addition to its biochemical richness, *C. vulgaris* demonstrates broad-spectrum antioxidant activity, as evidenced by its performance in various in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric Reducing Antioxidant Power Assay (FRAP), and Total Antioxidant Capacity (TAC). The correlation observed between total phenolic content and overall antioxidant capacity underscores the pivotal role of phenolic compounds in its redox behavior. Moreover, the presence of additional metabolites such as tocopherols, ascorbic acid, and unsaturated fatty acids further enhances its capacity to stabilize reactive oxygen species. Owing to these characteristics, *C. vulgaris* has emerged as a valuable candidate for applications in food, pharmaceutical, and environmental biotechnology aimed at combating oxidative stress and promoting sustainable bioresource utilization.

Studies have also focused on optimizing cultivation and extraction methods to increase the concentration of antioxidant compounds, such as lutein and phenolics [11,12]. For example, changes in nutrient conditions during cultivation significantly influence biomass yield and antioxidant content.

The present study aims to investigate the antioxidant potential and bioactive compound profile of the freshwater microalga *C. vulgaris* cultivated under static aquarium conditions. While *C. vulgaris* has been extensively studied under controlled photobioreactor and optimized cultivation systems, limited attention has been given to its biochemical characteristics when grown in low-energy, static environments that may better reflect cost-effective and scalable production strategies. Therefore, this study focuses on the comprehensive evaluation of the dried algal biomass obtained at the end of the cultivation period, with particular emphasis on its total phenolic content, total flavonoid content, and overall antioxidant capacity. By situating the findings within the framework of existing literature, this research seeks to contribute to a deeper understanding of the functional potential of *C. vulgaris* biomass and to support its prospective application as a natural source of antioxidants in food, nutraceutical, cosmetic, and pharmaceutical industries.

2 Material and Method

2.1 Microalgae Cultivation Method

The *C. vulgaris* microalgae used in this study were obtained from cells that had been cultured for 14 days. The cells in question was stored at a temperature of -80°C, with the intention of its future utilization. The frozen cells were thawed at room temperature, transferred to a suitable growth medium, and incubated. During incubation, their growth was observed at room temperature and under light intensity of 9000 lux. In addition to these conditions, the

cultures were cultivated under a light cycle of 8/16 hours photoperiod.

Initially, a 10-day pre-incubation was performed in 200 mL of growth medium. During the 10-day pre-culture period, cultures grown in 500 mL Erlenmeyer flasks were shaken once every 24 hours. Samples taken from the pre-culture were screened using a microscope to ensure that no other species was growing in the culture. The cells were then transferred to 30 L glass tanks containing pre-sterilised culture medium to grow the culture. These tanks were maintained at room temperature, with a light intensity of 9000 lux and a light cycle of 8/16 hours photoperiod (Figure 1).

A basal medium supplemented with 5 mL of micronutrient solution was used as the growth medium. The glass tanks and all solutions used were sterilised by autoclaving at 121 °C for 15 minutes. Each tank was inoculated with 200 mL of microalgal culture and then supplemented with basal medium to a final volume of 30 L. The cultures were incubated in glass tanks for 28 days.

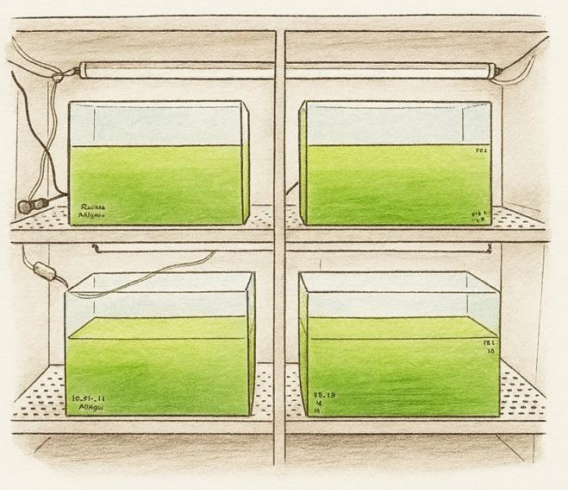

Figure 1. Schematic diagram of algae cultivation process.

The upper surface of the glass tanks was covered with stretch film. It is important to note that meticulous efforts were made to maintain a sterile environment throughout the incubation period. As a static culture growth was applied, it was essential to ensure the circulation of the culture medium and algal cells within the tank. The culture medium in the tank was shaken daily to enable the cells to utilise the culture medium more efficiently. The purpose of this procedure was to agitate the microalgae, prevent them from accumulating at the bottom of the tank, and ensure the optimal use of the culture medium.

The harvested microalgae were subsequently subjected to a centrifugation process at 5,000 rpm for 10 minutes. Biomass underwent a drying process at a temperature of 55°C for a duration of 48 hours. Thereafter, biomass was ground into a powder. The powdered algae were stored in glass containers with septum caps at ambient temperature.

2.2 Method for Determining the Total Phenolic Content (TPC) of Algae Extracts

TPC of the algal samples obtained in the study was determined based on the procedure described by Ahmed et al. [13]. This method utilised a modified Folin–Ciocalteu method. According to the method, 300 µL of algal sample or standard was mixed with 3,400 µL of distilled water, 500 µL of methanol, and 200 µL of Folin–Ciocalteu reagent. After mixing, vortexing was performed to ensure homogenisation. The mixture was then incubated for 10 minutes in a dark environment. Following incubation, 600 µL of a 10% Na_2CO_3 (sodium carbonate) solution was added. After this addition, the mixture was left for 120 minutes in an environment that did not allow light to pass through from outside. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. A blank solution containing deionised water, reagent and Na_2CO_3 was used in the measurement of absorbance.

2.3 Method for Determining the Total Flavonoid Content (TFC) of Algae Extracts

The TFC of the algal samples obtained in the study was determined according to the procedure described by Ahmed et al. [13]. According to the mentioned method, 500 µL of algal sample or standard was mixed with 3,200 µL of 30% methanol. Subsequently, vortexing was performed to ensure homogenisation. Following the vortexing process, 150 µL of 0.5 M $NaNO_2$ (sodium nitrite) and 150 µL of 0.3 M $AlCl_3$ (aluminium chloride) were added sequentially. After a five-minute waiting period, 1 mL of 1 M sodium hydroxide was added. The mixture was then vortexed once more. This final mixture was incubated for 10 minutes in a light-protected environment. Absorbance was measured at 506 nm using a UV-Vis spectrophotometer. The preparation of the blank solution entailed the replacement of the sample with 500 µL of deionized water.

2.4 Method for Determining the Total Antioxidant Capacity (TAC) of Algae Extracts

TAC analysis was performed using a modified method reported by Kobya et al. [14]. A total of 500 µL of test sample or standard was added to the test tubes, followed by 1,000 µL of phosphomolybdenum reagent (prepared with 0.6 M H_2SO_4 (sulphuric acid), 28 mM NaH_2PO_4 (sodium phosphate) and 4 mM ammonium molybdate). The mixture was vortexed and then incubated in a 95 °C water bath for 90 minutes. The sample removed from the water bath was allowed to cool at 25 °C for 30 minutes. Absorbance was measured at 695 nm using a UV-Vis spectrophotometer. For absorbance measurements, 500 µL of deionised water was used as a blank.

2.5 Method for DPPH Radical Scavenging Activity Analysis of Algae Extracts

In conducting this analysis, a modified method developed by Ahmed et al. [13] was employed. According to the method, 39.5 mg of DPPH was dissolved in 10 mL of methanol to prepare a stock solution. This stock solution was diluted to obtain a working solution with an absorbance value of 0.98 ± 0.02 at 517 nm. In addition, in a test tube, 100 µL of algae sample or standard was mixed with 3,000 µL of DPPH solution. The mixture was

vortexed to ensure homogenisation. Following the vortexing step, the tubes were incubated in the dark for 30 minutes. Subsequently, the absorbances were measured at a wavelength of 517 nanometres. A blank was obtained by adding 100 µL of methanol instead of the sample and was used in the measurement of the absorbances.

2.6 Method for ABTS Radical Scavenging Analysis of Algae Extracts

The ABTS method was determined using a method reported by Ahmed et al. [13]. According to this modified method, initially, 38.4 mg of ABTS and 13.4 mg of potassium persulphate were dissolved in 10 mL of deionised water to prepare a stock solution. The solution was then kept in the dark for 12-16 hours for stabilisation. The solution was subsequently diluted with methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. In the test tubes, 150 µL of sample or standard was mixed with 2,850 µL of ABTS solution. Following vortexing, the mixtures were incubated for 120 minutes in a light-free environment. Absorbances were measured at 734 nm. A blank, carefully prepared using 150 µL of methanol, was used in the absorbance measurements.

2.7 Method for Determination of FRAP (Ferric Reducing Antioxidant Power) Activity of Algae Extracts

The method developed by Sethi et al. [15] was used to determine the iron reduction capacity of the algal samples obtained. The results of the relevant analysis were determined using a modified version of this method. The FRAP reagent was prepared by adding 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3$, and 10 mM TPTZ solution in a 10:1:1 (v/v/v) ratio. Prior to use, the reagent was pre-incubated at 37 °C for 15 minutes. 250 µL of algal sample or standard was added to the test tubes, followed by 2,750 µL of FRAP reagent. Vortexing was performed to ensure homogenisation. After vortexing, the mixture was incubated for 30 minutes in the dark. The absorbance of the resulting mixtures was measured at 593 nm using a UV-Vis spectrophotometer. Methanol was used as a blank for absorbance measurements.

3 Results and Discussion

The in vitro antioxidant capacity of the sample coded as CV was evaluated using different methods, and the analysis results are presented in Tables 1 and 2. The TPC, TFC, and TAC values of the sample were quantitatively assessed and summarized in Table 1. The sample exhibited a TPC of 20.90 ± 0.21 mg GAE/g and a TFC of 29.32 ± 1.56 mg QE/g. These values indicate a moderate presence of phenolic and flavonoid compounds, which are widely recognized for contributing to antioxidant activity through mechanisms such as radical scavenging, metal chelation, and inhibition of oxidative enzymes. The TAC value was found to be 44.07 ± 0.96 mg AAE/g, reflecting a strong overall antioxidant potential of the extract.

Table 1. TPC, TFC, and TAC Values of *C. vulgaris**

Method	Results (mean ± SD)	Unit
TPC	20.90 ± 0.21	mg GAE/g
TFC	29.32 ± 1.56	mg QE/g
TAC	44.07 ± 0.96	mg AAE/g

*The results of the experiment are expressed as the arithmetic mean of three replicate measurements (n = 3) ± standard deviation (SD).

As part of the analyses, the results of the DPPH and ABTS, along with the FRAP test, are presented in Table 2. In line with these findings, the sample exhibited significant DPPH radical scavenging activity (18.21 ± 5.41 mg AAE/g) and ferric reducing power (FRAP) of 16.75 ± 0.19 mg $FeSO_4$E/g. These values indicate that the sample possesses notable electron-donating and free radical-neutralizing capabilities, largely attributed to its phenolic and flavonoid content.

However, despite the high concentrations of phenolic and flavonoid compounds, the ABTS assay yielded a relatively low value (3.05 ± 0.23 mg TE/g). This inconsistency may be explained by several factors. Firstly, the dominant antioxidant compounds may exhibit varying degrees of reactivity toward the ABTS radical in comparison to other radicals. For instance, lipophilic compounds often interact minimally with the ABTS radical, which is more responsive to hydrophilic and amphipathic antioxidants. Secondly, experimental conditions such as solvent compatibility, radical generation, and wavelength interference can affect the sensitivity and accuracy of the assay.

Table 2. Antioxidant Capacity of *C. vulgaris**

Method	Result (mean ± SD)	Unit
DPPH	18.21 ± 5.41	mg AAE/g
ABTS	3.05 ± 0.23	mg TE/g
FRAP	16.75 ± 0.19	mg $FeSO_4$E/g

*The results of the experiment are expressed as the arithmetic mean of three replicate measurements (n = 3) ± standard deviation (SD).

In summary, the sample exhibited significant antioxidant activity, particularly in the DPPH, FRAP, and TAC assays, showing a correlation with its phenolic and flavonoid content. Although the low ABTS value is noteworthy, it may be attributed to compound-specific radical affinity or methodological limitations rather than an actual deficiency in antioxidant potential. The present findings lend support to the hypothesis that the CV sample has potential as a natural antioxidant source and indicate the need for further investigation into its specific phytochemical components and biological efficacy.

Moreover, the type of solvent and extraction parameters directly influence the yield of antioxidant compounds. Future studies should expand the polarity range by employing different solvent combinations (e.g., methanol–ethyl acetate, acetone–water) to better target specific bioactive compounds. The levels of lutein, carotenoids, and phenolic compounds vary depending on light, temperature, and nutrient composition in the culture medium. Antioxidant content may be enriched by introducing stress factors such as CO_2 supplementation, increased light intensity, or nitrogen limitation during cultivation.

To evaluate the in vitro findings in vivo, cell culture and animal model tests should be conducted. These should include analyses of intracellular ROS (reactive oxygen species) levels, lipid peroxidation, and antioxidant enzyme activities. The powdered *C. vulgaris* extracts obtained in this study should be tested in functional foods, dietary supplements, natural cosmetic products, or pharmaceutical formulations to assess their industrial potential through practical applications. The characterization of individual phenolic constituents underlying the antioxidant effect using LC-MS/MS or HPLC is expected to advance the development of more tailored product strategies.

Overall, the sample was found to be rich in phenolic and flavonoid compounds that support diverse antioxidant mechanisms. The results of this study suggest the potential utilization of the extract as a natural antioxidant source in dietary supplements, functional foods, cosmetic products, or pharmaceutical formulations. The literature reports that plant extracts with similar compositions exhibit anti-aging, cytoprotective, and antimicrobial properties [16]. Based on the current findings, with improved cultivation conditions and advanced bioactivity analysis, this microalga species holds great potential at both academic and commercial levels.

A substantial body of research has demonstrated that *C. vulgaris* extracts exhibit considerable antioxidant capacity. A study revealed that the methanol extract of *C. vulgaris* exhibited potent inhibition of free radicals in the ABTS assay, with an IC_{50} value of 81.693 ppm [17]. Notably, it demonstrated remarkable efficacy in the DPPH assay, exhibiting corresponding IC_{50} values. This finding suggests that *C. vulgaris* possesses the capacity to neutralize free radicals, thereby enhancing its reputation as a significant source of antioxidants.

Moreover, the reported results of the DPPH assay for *C. vulgaris* suggest that its antioxidant efficacy is comparable to that of high-performance antioxidants. This is a critical aspect to consider when assessing the potential of these extracts in addressing oxidative stress. The antioxidant capacity measured by the FRAP assay reflects the ability of the extracts to reduce ferric ions to ferrous ions, thereby demonstrating their antioxidant activity [17].

A comprehensive analysis of *C. vulgaris* indicates that its phenolic content exerts a substantial influence on its antioxidant properties, exhibiting a correlation with outcomes from both the DPPH and ABTS assays [18]. A substantial body of research has previously demonstrated a notable correlation between total phenolic content and antioxidant capacity in diverse samples. These findings underscore the pivotal role of phenolic compounds in determining the antioxidant potential of *C. vulgaris* extracts. [19].

Other phytochemicals, including flavonoids, which are abundant in microalgae, have been identified as significant contributors to the antioxidant capacity of extracts, as evidenced by findings in diverse plant studies [20]. The interaction among these phytochemicals not only enhances their free radical scavenging ability but also suggests a synergistic effect, amplifying the overall antioxidant activity of *C. vulgaris* extracts [21]. The variability in extraction methods has been demonstrated to have a significant impact on the results of antioxidant activity tests. The efficacy of various solvent extraction techniques in yielding extracts with distinct compositions and antioxidant abilities has been demonstrated. Methanol, in particular, has been shown to be particularly effective in extracting polyphenolic compounds, which have been found to be closely linked to antioxidant activity in various substances. This property of methanol renders it suitable for use in the extraction of *C. vulgaris* [22].

Furthermore, researchers have examined the stability of antioxidant compounds under various conditions, revealing that factors such as light, temperature, and extraction time can significantly impact measured antioxidant activity [23,24]. A comprehensive understanding of these aspects is imperative for successfully translating laboratory findings to potential industrial applications, such as the development of food supplements or functional foods utilizing *C. vulgaris*.

Numerous studies indicate that *C. vulgaris* has a high value due to its unique composition rich in vitamins A, E, and various B vitamins, along with high protein content, constituting approximately 51%-58% of its dry weight [25,26]. The high levels of amino acids and lipid content (ranging from 5% to 58% depending on growth conditions) also suggest its potential as a functional food and ingredient in pharmaceuticals [27,28]. Furthermore, its role in improving gut microbiota and modulating immunological responses has positioned it as a promising candidate for nutritional therapy, particularly in managing autoimmune conditions [29,30].

The commercial cultivation of *C. vulgaris* is favorable due to its rapid growth and efficient resource use. *C. vulgaris* exhibits significant antioxidant properties, leading to its use in dietary supplements aimed at enhancing overall health and managing specific health issues, such as pulmonary diseases and oxidative stress [31]. Its potential for incorporation into food products as a natural pigment and nutrient additive has prompted extensive research into its usability in functional foods [26,30]. The incorporation of *C. vulgaris* into products for athletes and health-conscious individuals highlights the growing consumer interest in superfoods rich in bioactive compounds [26]. It can be produced in controlled environments, which require considerably less land and water compared to traditional crops [30]. This ability to thrive under various environmental conditions makes it an ideal candidate for sustainable agricultural practices and bioremediation efforts, where it can be used to purify wastewater and remove pollutants [32,33]. The high growth rate and adaptability also facilitate its application within aquaculture, serving as a growth enhancer and immunostimulant [34].

The findings of this study also reveal that the antioxidant potential and overall biochemical composition of *C. vulgaris* are influenced by the cultivation environment. It has been reported that changes in light exposure, temperature, and nutrient availability modulate the metabolic pathways responsible for the synthesis of phenolic compounds and other antioxidant molecules. Therefore, the precise and systematic control of environmental parameters can be considered a key strategy for increasing the yield of bioactive compounds in *C. vulgaris* and ultimately supporting its wider use in

pharmaceutical, nutraceutical, and environmental applications.

It should be noted that the variability in reported antioxidant activities across studies may also result from differences in the specific strain of *C. vulgaris* used, growth conditions, and the timing of extraction [35]. This underscores the necessity for standardization in research methodologies, which can aid in comparative assessments of antioxidant efficacy and establish a reliable profile for *C. vulgaris* in therapeutic and dietary contexts. In this context, the biological activity profile of the sample can be strengthened through more comprehensive biological assays, paving the way for advanced.

4 Conclusion

This study demonstrated that *C. vulgaris* biomass cultivated under static aquarium conditions possesses a notable antioxidant capacity, primarily associated with its phenolic and flavonoid content. The ability to obtain bioactive-rich biomass using a low-energy and minimally controlled cultivation system highlights the potential of this approach as a sustainable alternative to conventional, energy-intensive microalgal production methods. The antioxidant performance observed across multiple assays indicates that such systems can generate functionally valuable biomass without compromising bioactivity.

From a bioeconomic and sustainability perspective, static cultivation of *C. vulgaris* aligns well with circular economy principles by reducing energy demand, operational complexity, and production costs. This cultivation strategy supports the scalable production of natural antioxidants for use in functional foods, nutraceuticals, cosmetics, and pharmaceutical formulations, contributing to the development of environmentally responsible bioproducts. Further integration of optimized cultivation, green extraction techniques, and life-cycle assessments will be critical to enhancing industrial feasibility and maximizing the contribution of *C. vulgaris* to sustainable bio-based industries.

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Declaration

Ethics committee approval is not required.

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