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The Source of DPPH Radical Scavenging Activity: Insights from Freshwater Streams Isolated in Giresun

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Abstract Microalgae produce bioactive compounds, specifically antioxidants, that play a central role in fighting oxidative stress. It is a major factor in the development of aging, cancer and cardiovascular diseases. In this study five freshwater microalgal species from Giresun, Türkiye, namely *Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia*, and *Desmodesmus opoliensis* were isolated. They were stringently screened for antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was the method followed in evaluating antioxidant capacity. *Chlorococcum hypnosporum* showed higher antioxidant activity among the other species. However, the other species showed lower antioxidant activity under the experimental conditions compared to *C. hypnosporum*. The results of the our study show that microalgae are a good source of high-potential antioxidant compounds and they can be used in therapeutic and health-related fields as eco-friendly alternatives compared to the currently globally used synthetic derivatives.

Keywords Green Microalgae · Antioxidant Activity · DPPH Radical Scavenging · Health Applications

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INTRODUCTION

Antioxidants are also essential components of defense mechanisms that protect living organisms against oxidative stress, causing numerous damages to cells and molecules. Oxidative stress is a term used to describe the imbalance between the production of reactive oxygen species (ROS) and the availability of antioxidants in the body to neutralize the reactive intermediates or fix their damage (Pizzino et al.,2017). Reactive oxygen species (ROS), which include free radicals such as superoxide anion and hydroxyl radical, and non-radicals, such as hydrogen peroxide, are produced on a daily basis as an ordinary course of oxygen metabolism and contribute to cellular signaling processes and homeostasis preservation (Phaniendra et al., 2015).Even though the organism contains antioxidant defense mechanisms, excessive production of ROS can lead to oxidative damage to proteins, lipids, and DNA (Finkel and Holbrook, 2000; Kaminski et al., 2002; Sharifi-Rad et al., 2020). This damage is associated with the aging process and with the pathology of most diseases, including diabetes, cancer, cardiovascular pathologies, and neurodegenerative diseases like Parkinson's disease and Alzheimer's disease (Valko et al., 2007).Therefore, the elucidation of mechanisms of action involved in antioxidation represents one of the gravest aspects for modern medical research into both physiological senescence processes and pathological states.Enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, but also exogenous antioxidants: Vitamin C, vitamin E, carotenoids and polyphenols (Ratnam et al., 2006; André et al., 2010; Bouayed and Bohn, 2010; Biehler and Bohn, 2010).

Food antioxidants greatly contribute to increasing the coping ability of the body to oxidative stress. They are rich in good quality antioxidants that have been proved to reduce chronic diseases by providing scavengers to free radicals and are found in fruits, vegetables, nuts and some beverages (Okarter and Liu, 2010; Zhu and Sang, 2017; Wallace et al., 2020; Ponnampalam et al., 2022). These include polyphenols, flavonoids, and carotenoids which are powerful antioxidants present in plant foods that account for their beneficial health effects (Vasanthi et al., 2012; Pawase et al., 2024). Consumption of antioxidant active food on a daily basis is associated with better general health and lower disease risk (Caple et al., 2010).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay has become a validated technique for the assessment of antioxidant activity of different compounds (Sharma and Bhat, 2009). The DPPH method is based on Blois's work and later a modification of the method by Brand-Williams et al., which is the most commonly used procedure in literature today (Blois, 1958; Brand-Williams et al., 1995; Akar et al., 2017). The assay relies on direct reduction of the stable free radical DPPH, which has a deep violet color, to a yellow, diphenyl-picrylhydrazine, by an antioxidant, indicating radical-scavenging activity.

The color intensity of the colour change (being directly proportional to the antioxidant concentration) can then be quantified by measuring the 517 nm absorbance using spectrophotometer. The DPPH assay is more preferable because it is simple, quicker, and also reproducible. Except for such complex mixtures as foodstuffs and plant extracts, the test also serves to give a rapid evaluation of the pure compound's antioxidant potential (Baliyan et al., 2022). There is the need to memorize that DPPH test is in vitro and was unable to demonstrate the antioxidant activity in vivo, where metabolism and bioavailability are crucial factors.

Because of its simplicity, velocity, and replicability, DPPH test is ideal. With the exception of complex mixtures such as food and plant extracts, it is a fast antioxidant potential estimation of pure substances (Baliyan et al., 2022). It should be noted that the DPPH assay, despite its widespread use, emerges from the validation of antioxidant compounds, and in fact is an in vitro method that cannot reliably mimic the properties of in vivo, where factors such as metabolism or bioavailability can heavily influence the activity observed.

Microalgae are being considered as significant sources of natural antioxidants owing to their versatile and rich biochemical composition. For centuries, diverse communities have used these photosynthetic organisms that can exist in fresh water and marine environments for their medicinal and nutritive attributes. The antioxidant capacity of microalgae is linked to the high content of bioactive compounds such as phycobiliproteins, carotenoids, polyphenols and vitamins (Ng and Chew et al 2020; Pereira et al., 2024).

In contrast, secondary metabolites like polyphenols have strong antioxidant activity and neutralize free radicals via transfer of an electron or hydrogen atom (Hassanpour and Doroudi, 2023). Likewise, microalgae-meditated antioxidants, especially polyphenols, have exhibited anti-carcinogenic properties in a wide variety of experimental methods, both in vivo and in vitro, mainly by diminishing oxidative stress and inhibiting tumor cell proliferation. (Avila- Roman et al., 2021). In addition to imparting colour to microalgae, carotenoids such as beta-carotene, lutein and zeaxanthin protect microalgae against oxidative damage through singlet oxygen quenching, and free radical scavenging (Black et al., 2020; Gülçin, 2020; Swapnil et al., 2021). Repeating experiments consistently indicate that carotenoids cause

the majority of microalgae species to possess a significant antioxidant ability (Jahnke, 1999; Takaichi, 2011). Although the most used supplemented carotenoid is beta-carotene, the red xanthophyll pigment astaxanthin comes second in place (Novoveská, et al., 2019) It has shown potent antioxidant activity (Bouissiba and Vanshak, 1991; Boussiba, 2000; Lorenz and Cysewski, 2000). Among the reasons making astaxanthin so valuable is its very high free radical absorption capacity; experiments have found that it has antioxidant activity about 10 times greater than in other frequently encountered carotenoids (Borowitzka, 1995; Hamed, 2016; Berton et al., 2017; Mourelle et al., 2017). Some microalgae species such as *Chlorella zofingiensis*, certain *Chlorococcum sp*. and *Scenedesmus sp.* are presently being used to produce astaxanthin by way of biosynthesis. (Yaakob et al., 2014, Odjadjare et al., 2017; Bhalamurugan et al., 2018; Mavrommatis, 2023).

Microalgae also have other valuable antioxidants such as vitamin E (tocopherol) and vitamin C (ascorbic acid), which contribute notably to the antioxidative power of microalgae (Goiris et al., 2015). Microalgae hold a very heterogeneous mixture of antioxidant molecules, including various carotenoids (β-carotene, lutein, and astaxanthin), tocopherols (vitamin E), and phycobiliproteins in red algae such as *Porphyridium*. (Rhodophyta). These pigments are synthesized by such species as *Dunaliella, Chlorella, Haematococcus, Scenedesmus*, and *Trentepohlia* (Chlorophyta), and *Euglena* (Euglenophyceae).

Researches have found that several microalgal species, such as *Chlorella vulgaris, Spirulina platensis* and *Haematococcus pluvialis*, boast amazing antioxidant properties with a significant activity detected against DPPH radical scavenging assays (Rodríguez-García and Guil-Guerrero, 2008; Demorois et al., 2015; Takyar et al., 2019). Research on antioxidants enhances both functional foods and nutritional supplements. It can enable us to find environmentally friendly and sustainable sources of antioxidants. 43 % of the world's arable land goes on feed for livestock. Microalgae can be culturing non-arable land to make feed for animals out of waste water. It further proves to be environmentally friendly and offers a giant ambition: that means even larger at scale production of bioactive chems (Paek et al., 2014).

Our aim in this research study is to determine, at different concentrations, the antioxidative potential of both rutin and some microalgal species that are grown with DPPH radical scavenging assays as a method for assessing antioxidant ability. Rutin is a flavonoid compound studied widely in biochemical research, and based on its known free radical scavenging ability we believe it to be a classic antioxidant. This study will also make it possible for readers to compare the antioxidant levels of different microalgal extracts with the results for rutin and to see exactly how these natural compounds stand up against an established gold standard antioxidant. The study also aimed at comparing the antioxidant potential of different microalgal species and trace which one showed the highest antioxidative activities. This comparative study will draw attention to those candidates that need more in-depth examination and may be marketed for commercial use. An investigation into the potential implications of microalgal antioxidant extracts for health and wellness is also necessary. The specific populations affected by these extracts will become clearer, along with the potential for their incorporation as core ingredients in future health-related products.The identification of effective natural antioxidants from microalgae can help to design new health products that promote human health and disease prevention.

MATERIAL AND METHOD

Chemicals and Reagents

High-quality chemicals and reagents were used to ensure the accuracy of the experimental results. 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\cdot}$) and rutin were acquired from Sigma Chemical Co. Methanol and other reagents were of analytical grade, sourced from Merck.

Microalgal Species

Water samples were collected in September 2021 from the benthic and pelagic zones of the Aksu, Batlama, and Büyük Güre Streams, located in the central district of Giresun Province, using 1 L plastic bottles and transported to the laboratory. Water temperatures and pH values of the sampling sites are provided in Table 1. Each water sample (1 mL) was inoculated onto BG11 and Allen media solidified with 1% agar (Allen, 1968; Allen and Stanier, 1968). The culture plates were incubated at 26°C in a SANYO MLR 351 incubator under a light intensity of approximately 155 µmol/m²/s with a 12:12 light-dark photoperiod.

Table 1.
Physicochemical Parameters of Water Samples

Stream Name	Water Temperature (°C)	pH
Batlama Stream	22.4	7.85
Aksu Stream	21.7	7.40
Büyükgüre Stream	23.0	7.10

After one month of incubation, distinct colonies that developed on the agar plates were carefully transferred to fresh solid media using an inoculating loop. This procedure was repeated multiple times until single-species isolates were

obtained (Demiriz, 2008). The purified isolates were then transferred to liquid media and cultured under controlled incubation conditions to promote growth. Samples were aseptically collected from cultures grown in liquid media for further analysis. Species identification was performed using light microscopy and inverted microscopy, with measurements conducted using a micrometric eyepiece. Identification was based on established references, including *Freshwater Algae of North America* and the AlgaeBase database (Wehr and Sheath, 2003; Guiry and Guiry, 2023). DNA isolation and molecular identification of the algal species were carried out by BM Software Consulting and Laboratory Limited Company. The obtained sequences were analyzed and evaluated using the NCBI-BLAST program for species confirmation. The isolated species, which were identified through both morphological and molecular characterization, include *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis*.

This study's objective is to measure the antioxidant activity of isolated microalgae species and contrast the results with established antioxidant values.

Preparation of Microalgal Extracts

Microalgal biomass was harvested from cultures were centrifuged at 8000 rpm for 5 minutes. The biomass was washed with distilled water, dried at 65°C, and ground into a fine powder. For extraction, 1 grams of the dried biomass was mixed with 20 mL of methanol and and extracted at 50°C for 48 hours (Vehapi et al., 2018). After that, the mixture was centrifuged for 10 minutes at 4000 rpm. After centrifugation, the supernatant was filtered, and the methanol in the supernatant was evaporated. The resulting dry biomass was then dissolved in methanol (Gürlek et al., 2020).

DPPH Radical Scavenging Assay

The scavenging ability of the microalgal extracts was determined according to the method of Brand-Williams et al (Gürlek et al., 2020) using 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical. The DPPH working solution (20 µg/mL) was prepared in methanol, and 1.5 mL of this solution was added to 0.75 mL of microalgal extracts at their respective concentrations (250, 500, 750, 1000 µg/mL). Mixtures of DPPH solution and microalgal extracts were incubated for 30 min in the dark. The mixtures' absorbance was measured at 517 nm using a spectrophotometer after incubation. (Aydın, 2012). The DPPH radical scavenging activity percentage can be determined using the following equation

DPPH Scavenging Effect (%)= [(A0-A1) / A0] x 100

A0: Absorbance value of the control

A1: Absorbance value of the sample or standard

DPPH• + Antioxidant → DPPH-H + Antioxidant

(purple color) (yellow color)

At varying doses (250, 500, 750, and 1000 µg/mL), the free radical scavenging activity of microalgal extracts was assessed, and activity comparisons were performed using rutin as the reference.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's test. Results were presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant Activity of Rutin

The highest antioxidant capacity in all the samples examined was seen in rutin. At the maximum of 1000 µg/mL, rutin's activity of DPPH radical scavenging was 92.0% (Figure 1, Table 2). As the concentration increased, the rutin activity also increased and justified its status as a good antioxidant. Such a high activity is a proof of rutin's high ability to scavenge free radicals, a perfect test of antioxidant potency. This is in line with previous reports (Yang et al., 2008), which supported the high antioxidant capacity of rutin.

The scavenging activity demonstrated is in line with previous work showing the capability of rutin to abate oxidative stress and its potential therapeutic implications (Butchi et al., 2011; Patil et al., 2013; Kamel et al., 2014; Ganeshpurkar and Saluja, 2016). With its higher antioxidant activity, Rutin may be greatly effective in numerous applications, such as application as the basis for supplements or functional foods intended to counteract oxidative damage.

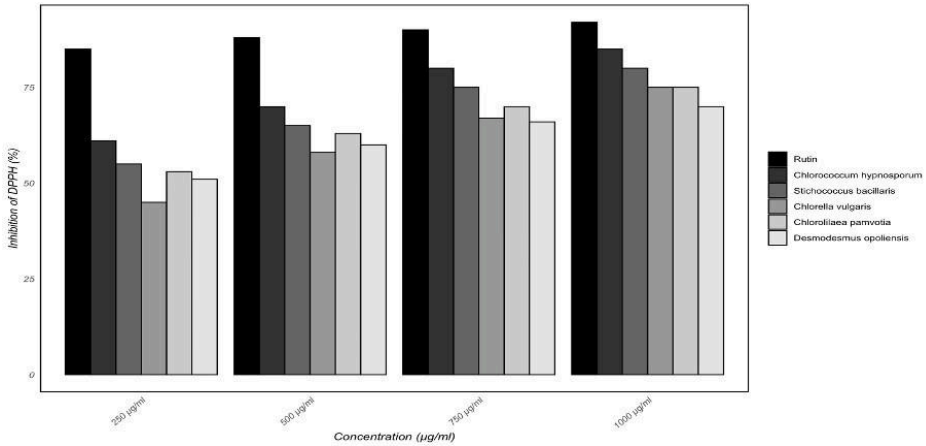
Antioxidant Activity of Microalgal Species

Microalgal extracts showed differential antioxidant activity depending on the species and concentration. *Chlorococcum hypnosporum* was found to have the highest scavenging activity of 90% at a concentration of 1000 µg/mL (Figure 1). Antioxidant activities of *Stichococcus bacillaris, Chlorella vulgaris* and *Chlorolilaea pamvotia* (80.00%, 75.00% scavenging activity at 1000 µg/mL, respectively) were purified.The lowest antioxidant activity was observed for *Desmodesmus opoliensis* (70.00% maximum scavenging at 1000 µg/mL) (Figure 1). Table 2 summarizes the DPPH radical scavenging activities (%) of rutin and microalgal extracts at various concentrations (µg/mL).

Table 2.
DPPH Radical Scavenging Activity (%) of Rutin and Microalgal Extracts (µg/mL)

Concentration (µg/mL)	Rutin	C.hypnosporum	S. bacillaris	C.vulgaris	C. pamvotia	D.opoliensis
250 µg/mL	85	61	55	58	51	51
500 µg/mL	88	70	65	67	63	60
750 µg/mL	90	80	75	72	70	66
1000 µg/mL	92	90	80	75	75	70

Figure 1.
DPPH Radical Scavenging Activity (%) of Rutin and Microalgal Extracts (µg/mL)



Among the microalgae, scavenging activity of *Chlorococcum hypnosporum* was highest, i.e., 90% at concentration of 1000 µg/mL (Figure 1). Thus, *Chlorococcum hypnosporum* is rich in antioxidant potential and can be ascribed to a high content of antioxidant compounds carotenoids, chlorophylls, and other phytochemicals with excellent radical-scavenging activity (Hajialyani et al., 2019). The *Chlorococcum hypnosporum* of the present study may have activities that can effectively perform the role of natural antioxidants as a source of and application in food, pharmaceuticals, and cosmetics. Also, research by Sassi and co-workers (2019) demonstrated that several microalgal species, *Chlorella cf. minutissima* D101Z, *Chlorococcum sp. (cf. hypnosporum) strains* D28Z, D37Z, D65Z, and D76Z, *Pediastrum tetras* D121WC, *Planktothrix isothrix* D39Z, and *Scenedesmus acuminatus* D115WC, are rich in polyunsaturated fatty acids (PUFAs), comprising ω-3, ω-6, and ω-9 types, besides carotenoids and chlorophylls that are relevant to maintaining the human physiological state. These microbes have established themselves as feasible alternative supplies of metabolites for the food industry. *Chlorococcum hypnosporum* exhibited greater antioxidant potential than other species which is primarily a result of biochemical constellations and adaptation-based environmental responses by this organism (Chlumsky et al. 2019). The organism is rich in comprising antioxidant flavonoids, polyphenols, chlorophyll, and carotenoids that are centrally involved in free radical neutralization and oxidative stress. Furthermore, the high content of PUFAs with high levels of ω-6 and ω-3 fatty acids helps in cell membrane structure maintenance and hence promote antioxidant activity.Its genomic composition supports the active synthesis of antioxidant enzymes such as superoxide dis-mutase, catalase, and peroxidase and thereby maintain oxidative homeostasis. Additionally, adaptation mechanism in *Chlorococcum hypnosporum* in response to environmental stresses leads to heightened synthesis of antioxidant compounds.Additionally, Goiris et al. (2012) screened the antioxidant capacity of 32 microalgal biomasses and estimated their phenolic and carotenoid content. Their findings showed that both carotenoid and phenolic compounds contributed to the antioxidant capacity of microalga, but the antioxidative property was species-dependent, growth condition-dependent and extraction solvent-dependent.

The antioxidant capacity of *Chlorolilaea pamvotia* has been evaluated for the first time in the literature. Similarly, *Chlorolilaea pamvotia* was demonstrated to possess strong antioxidant properties reaching a scavenging percentage of 75.0% at the same concentration. Notably, *Chlorolilaea pamvotia* showed lower activity but suggests its potential as a source of antioxidants. *Chlorolilaea pamvotia* has different compounds which may lead to antioxidant activity which makes it a potentialcandidate in this area with potential use as natural sources of antioxidant. Lortou and Gkelis (2023)

isolated a high level of valuable metabolics from *Chlorolilaea pamvotia*, isolated from Greece.

A rutin, a well-known antioxidant standard, was used to compare the antioxidant activity of *Stichococcus bacillaris*. Data revealed an appropriate antioxidant capacity of *Stichococcus bacillaris*, even if not as high as that of rutin. According to Gürlek et al. (2019), they investigated the antioxidant capacity of *Stichococcus bacillaris* for the first time and presented its results in literature. The RSA value of this species was found to be 89 ±0.1% for both methanolic and hot water extracts. Such experiment explained that *Stichococcus bacillaris* has considerable application potential in the pharmaceutical, food, and cosmetic industries. These findings are similar with the findings of our study.

On the other hand, both *Chlorella vulgaris* and *Desmodesmus opoliensis* demonstrated antioxidant activities, as reflected by scavenging percentages of 75% and 70%, respectively, at 1000 µg/mL. Despite showing good antioxidant activity, these species performed less well than *Chlorococcum hypnosporum*, whereas *Chlorolilaea pamvotia* showed similar activity. These species reported moderate activities which could be attributed to their chemical compositions or to the concentration of antioxidant compounds. However, despite this, these microalgae still have potential for efforts toward antioxidant applications. Following Abdel-Karim et al. (2020) revealed that Chlorella *vulgaris* contained an extensive composition of biologically active metabolites and exhibited considerable antioxidant activity based on a cascade of assays. Acetone extract had maximum antioxidant activity with 50.81% scavenging activity at 50 mL of 2.58 mg AAE/g DW antioxidant capacity and 1.95 mg AAE/g DW of reducing power. Acetone extract had a high total phenolic content 3.17 mg GAE/g DW. High antioxidant activities of *Chlorella vulgaris* were found earlier to be present by other researchers. For instance, Yu et al. (2019) investigated antioxidant functions of *Chlorella vulgaris* polysaccharides in vitro and in vivo.Their study found that *Chlorella vulgaris* possesses high antioxidant activity and high biological activity metabolites. Altogether, these researches provide the basis for the potential application of *Chlorella vulgaris* in food, cosmetic and pharmaceutical applications. Stoica et al. (2013) *Scenedesmus opoliensis* maximum radical scavenging activity with ethanol concentrations and DPPH assays is 54.9%. The finding is in line with the outcome of our study.findings.

The results obtained exhibit concentration-dependent and species-specific antioxidant activity of microalgal extracts, with *Chlorococcum hypnosporum* and rutin showing higher potential. Percentages of DPPH inhibition by different microalgae species varied significantly ($p< 0.001$). The remark accounts for the heterogeneity of the antioxidant activity of microalgae species. Additionally, concentration levels exhibited a staggering effect on inhibition of DPPH ($p<0.001$), indicating the significance of the effect of concentration on the antioxidant activity.

Apart from analysis, by applying Tukey's test, rutin's DPPH scavenging activity was considerably higher compared to *Chlorella vulgaris* and *Chlorococcum hypnosporum* ($p<0.001$), which reveals that rutin is significantly more effective than the microalgal genera of *Chlorella vulgaris* and *Chlorococcum hypnosporum* in quenching DPPH radicals. Relatively significant differences also appeared between *Desmodesmus opoliensis* and *Chlorococcum hypnosporum* ($p<0.01$), and between *Chlorolilaea pamvotia* and *Chlorococcum hypnosporum*. All these results illustrate the variable antioxidant activities of *Chlorococcum hypnosporum* and *Chlorolilaea pamvotia*, established by the two species showing different levels of antioxidant activity. Nevertheless, statistically significant differences could not be indicated between *Desmodesmus opoliensis* and *Chlorella vulgaris* ($p>0.05$), but it means the difference in antioxidant activity between these two species is less pronounced as that depicted among rutin and the microalgae.

The research considered the IC50 behavior of rutin and five different microalgae species, namely *Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia,* and *Desmodesmus opoliensis*.The got information uncovered critical contrasts among the tried mixes and microalgae types, as appeared in Table 3.

Table 3.

IC50 Values (µg/mL) of Rutin and Microalgae Species for Antioxidant Activity

Sample	IC50 (µg/mL)
Rutin	54.94 ± 68.8
Chlorococcum hypnosporum	567.0 ± 124.1
Stichococcus bacillaris	780.2 ± 86.9
Chlorella vulgaris	952.0 ± 87.3
Chlorolilaea pamvotia	955.6 ± 171.5
Desmodesmus opoliensis	1211 ± 142.7

The IC50 value of the control compound in this study, rutin, was determined to be 54.94 µM, the lowest IC50 value for all algae tested in this experiment and a figure indicative of its high biological activity. The low IC50 values for rutin indicate that it is functioning as an effective bioactive compound (Ayaz Seyhan, 2019). The IC50 value of the control compound in this experiment, rutin, was 54.94 µM, the lowest IC50 value for all the algae examined in this experiment and a

value that reflects its very high biological activity. The low IC50 values for rutin indicate that it is functioning as an effective bioactive compound (Ayaz Seyhan, 2019). The IC50 value of *Chlorococcum hypnosporum* was 567.0 µM. IC50 values of its extracts, as indicated by the study carried out by Olasehinde et al. (2020), ranged from 13.83 to 493.90 µg/mL, depending on the solvent applied. On the other hand, IC50 values of *Chlorococcum hypnosporum*, indicated a widely diverse range of more than 500 µg/mL for some extracts. Moreover, this difference is due to differences in the chemical composition, metabolic activities, and bioactive compounds between these two members. Variation in the percentage composition of phenolic compounds, alkaloids, and other phytochemicals, and also a difference in the efficiency of solvent extraction, can influence the various results for the cholinesterase inhibitory and antioxidant activities of the two species at various concentrations. The results show that different species, even from the same genus, may have the ability to exhibit considerably different patterns of bioactivity. Therefore, in looking for the pharmacological potential of microalgae, a relevant question is how these differences affect different types from the same genus. The IC50 value of Stichococcus bacillaris was 780.2 µM. Gürlek et al. (2019) examined the antioxidant activity of crude *Galdieria sulphuraria, Ettlia carotinosa, Neochloris texensis, Chlorella minutissima, Stichococcus bacillaris, Schizochytrium limacinum, Crypthecodinium cohnii,* and *Chlorella vulgaris* extracts (DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate radical) as well as their total phenol content (Folin-Ciocalteu). They assumed that in references of theirs Stichococcus bacillaris had an IC_{50} value of 372.5 µg/mL, whereas in our study put the figure decidedly higher at 780.2 µg/mL. The reason behind this difference may be due to various extraction methods, solvents used in the process, culture conditions, varied genetic variant lines between microalgal strains, or analysis methods used. All these would impact the quality and concentration of bioactive compounds and hence finally the antioxidant activity.

Chlorella vulgaris isolated from Giresun exhibited a worse IC50 value of 952 µg/mL compared to the 325 µg/mL reported for the Diyarbakır isolate by Çakmak et al. (2024). This could be due to the chemical structure of algae, which differs based on the conditions in the environment. Physical and biogeochemical characteristics of the water, such as nutrients and stress in their habitat, can affect the production of secondary metabolites, leading to variation in the biological activities of the compounds. *Chlorolilaea pamvotia* yielded an IC50 value of 955.6 µM. *Desmodesmus opoliensis* yielded the highest IC50 value of all the microalgae analyzed (1211 µM), approximately 22 times higher than rutin.

CONCLUSION

Comparative study of antioxidant activities of rutin and five different types microalgae (*Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia, Desmodesmus opoliensis*) by DPPH scavenging method revealed. Rutin showed the highest antioxidant effects, involving a 92% scavenging ratio and IC50 value of 54.94 µg/mL. Of these microalgae species, *Chlorococcum hypnosporum* had best characteristics, exhibiting 90% radical scavenging capacity and an IC50 value 567.0 µg/mL. Amongst the five species, *Chlorolilaea pamvotia* exhibited promising potential (75% scavenging) tested for the first time in this paper: IC50 was 955.6 µg/mL. However, *Desmodesmus opoliensis* was the least active of all algae species (70% scavenging, IC50: 1211 µg/mL), in fact showing that its biological activity was about 22 times lower than that of rutin.

These results show particularly that *Chlorococcum hypnosporum* has a high antioxidant potential, with potential application in such different industrial sectors as dietetic and functional foods, cosmetics or health products of virtually any description. In the long term, antioxidants from microalgae can bring benefits to our health. They include guarding cells against oxidation stress, delaying the signs of aging, being good for the immune system or probably even help to prevent chronic diseases altogether. Thus, these algae species could be of use for potential anti-aging cosmetics, dietetic foods, functional beverages, food preservatives or pharmaceutical preparations with both anti-inflammatory and immunity-enhancing effects.

Based on these findings, conclusion can be drawn that *Chlorococcum hypnosporum* has significant potential as a natural source of antioxidants for use in the food, pharmaceutical and cosmetic industries. Additionally, *Chlorolilaea pamvotia*, evaluated for the first time, has been identified as a promising candidate for future research.

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