**Journal of Anatolian Environmental and Animal Sciences**
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1095138

**JAES**
Year: 7, No: 2, 2022 (227-234)
**AÇEH**
Yıl: 7, Sayı: 2, 2022 (227-234)

**ARAŞTIRMA MAKALESİ** **RESEARCH PAPER**

# Determination Of Pigment Content And Antioxidant Activities Of Some Chlorophyta Species Isolated From Altınapa Dam Lake (Konya/Turkey)

**Baran AŞIKKUTLU*** **Cengiz AKKÖZ**
*Selçuk University, Faculty of Science, Department of Biology, Konya, Turkey*

***Geliş/Received:*** *31.03.2022* ***Kabul/Accepted:*** *13.06.2022* ***Yayın/Puplished:*** *30.06.2022*

*How to cite:* **Aşıkkutlu, B. & Akköz, C. (2022).** Determination Of Pigment Content And Antioxidant Activities Of Some Chlorophyta Species Isolated From Altınapa Dam Lake (Konya/Turkey). *J. Anatolian Env. and Anim. Sciences*, **7**(2), 227-234.
Atıf yapmak için: **Aşıkkutlu, B. & Akköz, C. (2022).** Altınapa Baraj Gölü'nden (Konya/Türkiye) İzole Edilen Bazı Chlorophyta Türlerinin Pigment İçeriği ve Antioksidan Aktivitelerinin Belirlenmesi. *Anadolu Çev. ve Hay. Dergisi*, **7**(2), 227-234.

*: https://orcid.org/0000-0003-2532-5517
: https://orcid.org/0000-0003-3268-0189

***Corresponding author:**
Baran AŞIKKUTLU
Selçuk University, Faculty of Science, Department of Biology, Konya, Türkiye
✉: baranasikkutlu@selcuk.edu.tr

**Abstract:** It is an important issue to investigate the water resources necessary for the continuation of life and the creatures living in these ecosystems. Algae living in aquatic ecosystems are necessary organisms for the creatures with which they share the same environment to survive, as they provide oxygen to their environment and constitute the first step of the food chain. In addition, algae are creatures that have the potential to be used in the food and pharmaceutical industries due to their pigment content and antioxidant properties. Due to these importance, many studies are carried out on microalgae. In this study, it was aimed to determine the chlorophyll-a, chlorophyll-b, total carotenoids content and antioxidant activities of some green algae species isolated from the benthic algae flora of Altınapa Dam Lake, which is one of the important drinking water sources of the city of Konya. Three Chlorophyta (*Chlorella vulgaris*, *Acutodesmus obliquus*, *Monoraphidium minutum*) species showing high growth potential under culture conditions were selected and studies were carried out with these species. The highest chlorophyll-a and chlorophyll-b values were detected as 62.4±0.8 µg $mL^{-1}$ and 18.5±1.0 µg $mL^{-1}$ in *Acutodesmus obliquus* species, respectively, and the highest total carotenoids value was detected as 40.3±0.4 µg $mL^{-1}$ in *Monoraphidium minutum* species. In addition, $IC_{50}$ values close to the reference antioxidant ascorbic acid were obtained in *Monoraphidium minutum* species. According to the results obtained in this study, the economic usability potentials of the studied microalgae were determined.

**Keywords:** Microalgae, Pigment, Antioxidant Activity, Altınapa Dam Lake.

# Altınapa Baraj Gölü'nden (Konya/Türkiye) İzole Edilen Bazı Chlorophyta Türlerinin Pigment İçeriği ve Antioksidan Aktivitelerinin Belirlenmesi

***Sorumlu yazar:**
Baran AŞIKKUTLU
Selçuk Üniversitesi,Fen Fakültesi,Biyoloji Bölümü, Konya, Türkiye
✉: baranasikkutlu@selcuk.edu.tr

**Öz:** Yaşamın sürmesi için gerekli olan su kaynaklarının ve bu ekosistemlerde yaşayan canlıların araştırılması önemli bir konudur. Sucul ekosistemlerde yaşayan algler, bulundukları ortama oksijen sağlamaları ve besin zincirinin ilk basamağını oluşturmaları nedeniyle aynı ortamı paylaştıkları canlıların yaşamlarını sürdürebilmesi için gerekli organizmalardır. Ayrıca algler pigment içerikleri ve antioksidan özellikleri nedeniyle gıda ve ilaç sektörlerinde kullanım potansiyeli olan canlılardır. Bu önemlerinden dolayı mikroalgler üzerinde birçok çalışma yapılmaktadır. Bu çalışmada, Konya şehrinin önemli içme suyu kaynaklarından biri olan Altınapa Baraj Gölü'nün bentik alg florasından izole edilen bazı yeşil alg türlerinin klorofil-a, klorofil-b, toplam karoten içeriği ve antioksidan aktivitelerinin belirlenmesi amaçlanmıştır. Kültür koşullarında yüksek çoğalma potansiyeli gösteren üç Chlorophyta (*Chlorella vulgaris*, *Acutodesmus obliquus*, *Monoraphidium minutum*) türü seçilmiş ve çalışmalar bu türlerle gerçekleştirilmiştir. En yüksek klorofil-a ve klorofil-b ölçüm değeri *Acutodesmus obliquus* türünde sırasıyla 62.4±0.8 µg $mL^{-1}$ ve 18.5±1.0 µg $mL^{-1}$, en yüksek toplam karoten ölçüm değeri *Monoraphidium minutum* türünde 40.3±0.4 µg $mL^{-1}$ olarak tespit edilmiştir. Ayrıca *Monoraphidium minutum* türünde, referans alınan antioksidan askorbik asite yakın $IC_{50}$ değerleri elde edilmiştir. Bu araştırmada elde edilen sonuçlara göre çalışılan mikroalglerin ekonomik açıdan kullanılabilirlik potansiyelleri belirlenmiştir.

**Anahtar kelimeler:** Mikroalg, Pigment, Antioxidan Aktivite, Altınapa Baraj Gölü.

## INTRODUCTION

The majority of the earth's surface is covered with salty waters, and there are much less wetlands, such as streams and lakes, which are composed of fresh water. These scarce fresh water resources need to be protected (Moss, 2010). Algae often live in the water. These waters can be fresh, salty and brackish. In their habitats, algae are the primary producers in the food chain and produce organic materials using sunlight, carbon dioxide and water. In addition to forming the main food source in the food chain, they produce the required oxygen for consumer organisms (Lee, 2008). Algae are important indicators for monitoring changes in aquatic ecosystems, although they are often ignored (McCormick & Cairns Jr, 1994). It has been shown in studies on microalgae which has been one of the most important bioresources used in bioindustry studies. However, despite the importance of its industrial applications, the main field of research on the long-term preservation of microalgae culture has not received much attention (Yim et al., 2021). The fact that algae produce half of the total oxygen production in the period we live in, that the ancestors of the land plants that make up our nutrition source are green algae, that they have an important potential in the production of biofuels, and that they are a source of active pharmaceutical compounds against drug-resistant bacteria reveal the reason for the importance of researching these living things (Chapman, 2013).

There are many studies about microalgae in the world and in our country. In terms of their seasonal changes, identification, culture, element content, carotenoids found in the structure, studies on the production of algae for food support and for use in the pharmaceutical industry are remarkable (Atıcı & Obalı, 2002, Pulz & Gross, 2004, Atıcı & Çalışkan, 2007, Wijffels et al., 2013, Keskinkaya et al., 2020). In recent years, there has been a significant interest in obtaining antioxidants from natural sources. This source may be algae that synthesize, metabolize, accumulate and secrete a wide variety of primary and secondary metabolites, including carotenoids, phenolic compounds, phycobilins, sulfated compounds and vitamins. All these compounds have applications in the health and food industries. These antioxidant compounds have great potential to be used in the pharmaceutical and other industries. (Munir et al., 2013). Pigments such as chlorophyll, carotenoids and phycobiliprotein cause algae in different amounts to appear in green, yellow, red and brown colors. In recent years, with the awareness of harmful substances in humans, the use of algae containing natural dyestuffs in its structure instead of synthetic ones in food additives has been increasing. Algal pigments are used in the cosmetic and pharmaceutical industries with their natural coloring properties. In addition, these creatures are consumed as food supplements because they provide a healthy life opportunity (Prasanna et al., 2007). Algae are a good source of pigments, minerals and vitamins and available foods can be naturally enriched with their use. (Kraan, 2013). Free radicals and oxidants are both toxic and useful compounds because they can have harmful and beneficial effects on the body. They arise from normal cellular metabolisms or from external sources such as any pollution or drugs. Free radicals cannot be destroyed gradually if overloaded. Their accumulation in the body causes a condition called oxidative stress, which plays an important role in the development of cancer, autoimmune disorders, aging, cardiovascular and neurodegenerative diseases. The human body can resist oxidative stress by producing antioxidants through different mechanisms. These are either naturally produced or supplied through external foods or supplements. (Pham-Huy et al., 2008). Among the tests used to determine the total antioxidant capacity from natural extracts, the 2,2'-diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay is a common test. The results of this test are often expressed as $IC_{50}$ values (Martinez-Morales et al., 2020). Brand-Williams et al. (1995) reported that a low $IC_{50}$ value indicates a high level of antioxidant activity.

Algae, which have an important position as a primary producer in aquatic ecosystems, have the potential to be used as a food supplement or in the drug sector, depending on their pigment content and antioxidant activity. Studies on the systematics of algae that live in aquatic ecosystems are maintained in Turkey for a long time. In addition to these studies, studies on the industrial usability of algae are increasing. For these reasons, it constitutes the necessary conditions for the examination of these living beings. In this study, it was aimed to identify the algae in Altınapa Dam Lake belonging to the benthic Chlorophyta phylum morphologically and to investigate the pigment content and antioxidant activities of the algae that can be grown in single cell culture in order to use them in the food and pharmaceutical industry. As a result, it is aimed to contribute to the studies and literature on these living beings.

## MATERIAL and METHOD

***Research Area:*** The Altınapa Dam Lake in Konya city, which constitutes our study area, is located at the 16th kilometer of Konya-Beyşehir road. The most important stream that feeds the dam is Meram Stream. The study materials were collected from four stations that could characterize the entire lake. Coordinates of sampling stations given in Table 1. Satellite view (Google, 2016) of

Altınapa Dam Lake and sampling stations are given in Figure 1.

**Table 1:** Coordinates of sampling stations.

| Station | Coordinate | |
|---|---|---|
| 1 | 37° 53' 41" N | 32° 17' 13" E |
| 2 | 37° 53' 15" N | 32° 18' 25" E |
| 3 | 37° 52' 42" N | 32° 17' 54" E |
| 4 | 37° 53' 22" N | 32° 17' 20" E |


**Figure 1:** Satellite view of Altınapa Dam Lake and sampling stations.

***Identification of Microalgae:*** Benthic algae samples belonging to Chlorophyta phylum, were collected from 0 to 5 m area in the coastal zones of four stations determined in seasonally conducted field studies in April 2014, July 2014, October 2014 and January 2015.

Benthic algae were examined with a Nikon brand Eclipse 80i model microscope, and morphological characterization was made according to Prescott, (1973), John et al., (2002) and Algaebase site (Guiry & Guiry, 2021).

Scanning electron microscopy (SEM) images of some algae samples in single cell cultures were made by Zeiss brand LS10 model electron microscope in the laboratories of Selçuk University Advanced Technology Research Application Central Directorate.

***Isolations and Cultures of Microalgae:*** In order to make cultures of benthic algae samples, at first a medium has been formed which allows the development of algae under laboratory conditions. For this medium, BG-11 medium was selected as described by Rippka (1988). Bg-11 is a medium that can be used for both Cyanobacteria and Chlorophyta (Atıcı, 2020, Qing et al., 2020).

Benthic algae found on stone, plant or sediment were morphologically detected with microscope then single cell cultures were made by using a pastor pipette with a Prior CM-110 inverted microscope. After that cells were transferred in sterile conditions to 100 mL erlens containing BG-11 media prepared as liquids. Cells were allowed to reproduce at a temperature of 25 $^{o}$C under 3000 lux light, 12 hours light, 12 hours dark cycle (Katırcıoğlu et al., 2008). After 20-25 days of incubation, samples were taken from the media which showed green color and examined in the microscope and studies were continued with the samples which were observed to be single cell culture without contamination.

***Chlorophyll-a, Chlorophyll-b and Total Carotenoids Analysis of Microalgae:*** It has been shown in studies that algae extracts containing carotenoids have antioxidant effects (Abe et al., 2007). The total reducing capacity, which shows the antioxidant effect, was found to be higher in the stationary growth phase at the end of the logarithmic growth phase. (Zamani & Moradshahi, 2014). Algae samples were started to be studied around 20-25 days when the growth rate of the cells was fixed and the maximum biomass was produced (Hamidian & Zamani, 2022). Rapidly growing single-celled species were selected from cultured benthic algal specimens for this study. For each species, a total of 6.5 L cultures were obtained from single cell culture stocks in sterile conditions. Following a 20-25 day incubation period, samples were transferred to balloon slides and lyophilized for 2 days with a Scanvac CoolSafe-110 model lyophilizator. Then the sample mass of each species was measured and 50 mL, acetone (100%) was added to 1 gram of samples. Then it was homogenized on a Bandelin brand UW-2070 homogenizer at a maximum level for 3 minutes. After homogenization of the samples, extractions were carried out at 37 ºC for 30 minutes on a Heidolph brand Unimax 1010 heated mixer. Subsequently, taken samples in 50 mL eppendorf tubes were centrifuged at 4000 rpm for 5 minutes using a model NF-800R model centrifuge. The liquid part of the samples, except for the precipitate in the bottom was taken to the ependorf tubes and measurements were made on a Biochrom brand Libra S22 model spectrophotometer (Dere et al., 1998). Three measurements were made for each sample. The absorbance values and formulas used in the measurements were taken from the work done by Lichtenthaler and Wellburn (1983). The following formulas are given below;

$C_a = 11.75\ A^{662} - 2.35\ A^{645}$
$C_b = 18.61\ A^{645} - 3.96\ A^{662}$
$C_{x+c} = 1000\ A^{470} - 2.27\ C_a - 81.4\ C_b / 227$
($C_a$: Chlorophyll a, $C_b$: Chlorophyll b, $C_{x+c}$: Total carotenoids)

***Antioxidant Activity Analyzes of Microalgae:*** In order to determine the antioxidant activities of benthic algae extracts, DPPH free radical scavenging effect method as described by El-Agbar et al. (2008), has been modified. Acetone was evaporated from benthic algae extracts at 37 $^{o}$C using Heidolph brand Hei-VAP rotary evaporator. Samples were placed in 2 mL eppendorf tubes. 98% methanol was added to the final concentration of each sample as a 5 mg mL$^{-1}$ stock solution. Stock solutions were prepared by diluting in the range of 500 µg mL$^{-1}$ to 3.90625 µg mL$^{-1}$. The ascorbic acid, designated as the standard solution, was diluted at 100 µg mL$^{-1}$ to 0.78125 µg mL$^{-1}$ and placed in 96-well plates. The diluted solutions were

mixed with DPPH free radical (0.003%) and methanol. Then 1 mL of these solutions was taken and mixed with 1 mL of solution containing the samples. The mixture was read in Biotek brand Epoch model microplate reader at 517 nm wavelength. After 30 minutes, the samples kept in the dark were read again on the device and the results were recorded. 1 mL of methanol and 1 mL of DPPH (0.003%) solution were used as blank samples. Concentration inhibition graphs were drawn from the results obtained using benthic algal solubles and standard solutions and $IC_{50}$ (µg mL-1) values, which is the amount of sample required to decrease the absorbance of the DPPH free radical by 50% is calculated from these graphs. Three measurements were made for each sample. % inhibition values were determined by Bors et al. (1992), calculated according to the formula given below:

% of inhibition of DPPH activity = A–B/A × 100

(A: optical density of the blank, B: optical density of the sample)

## RESULTS

***Light Microscope and Scanning Electron Microscope Images of Single-Cell Cultured Microalgae:*** Among the benthic algae samples, light microscope images were taken of the species which showed reproducing with the BG-11 medium. Three taxa belonging to the Chlorophyta phylum were isolated and cultivated. A list of these species is given in the Table 2. Light microscope images of cultured Chlorophyta species were and Figure 2. Scanning electron microscope images of cultured, Chlorophyta species were and Figure 3.

**Table 2:** List of isolated and cultured microalgae species identified by light microscope.

| | | | | |
|---|---|---|---|---|
| **Superregnum** | Eukaryota | | | |
| **Regnum** | Plantae | | | |
| **Divisio** | Chlorophyta | | | |
| **Classis** | Chlorophyceae | | | |
| | **Ordo** | Sphaeropleales | | |
| | | **Familia** | Scenedesmaceae | |
| | | | **Genus** | Acutodesmus |
| | | | | *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata |
| | | **Familia** | Selenastraceae | |
| | | | **Genus** | Monoraphidium |
| | | | | *Monoraphidium minutum* (Nägeli) Komárková-Legnerová |
| **Classis** | Trebouxiophyceae | | | |
| | **Ordo** | Chlorellales | | |
| | | **Familia** | Chlorellaceae | |
| | | | **Genus** | Chlorella |
| | | | | *Chlorella vulgaris* Beyerinck (Beijerinck) |

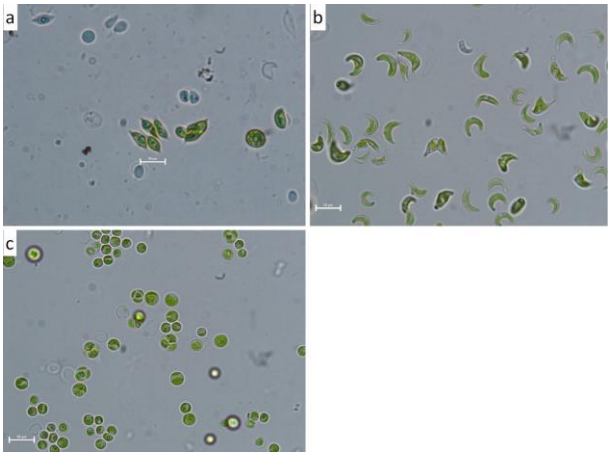


**Figure 2:** Light microscope image of isolated and cultured Chlorophyta species. a- *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata, b- *Monoraphidium minutum* (Nägeli) Komárková-Legnerová, c- *Chlorella vulgaris* Beyerinck (Beijerinck) (Scale: 10 µm).

***Chlorophyll-a, Chlorophyll-b and Total Carotenoids Analysis Results of Microalgae:*** The chlorophyll-a, chlorophyll-b and total carotenoids average values obtained from the algae are given in the Table 3.

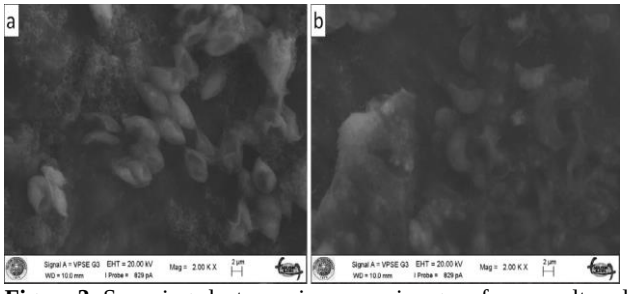


**Figure 3:** Scanning electron microscope images of some cultured Chlorophyta species. a- *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata, b- *Monoraphidium minutum* (Nägeli) Komárková-Legnerová (Scale: 2 µm).

**Table 3:** The chlorophyll-a, chlorophyll-b and total carotenoids average values of cultured benthic algae (µg $mL^{-1}$).

| | **Chlorophyll- a** | **Chlorophyll- b** | **Total Carotenoids** |
|---|---|---|---|
| *Chlorella vulgaris* Beyerinck (Beijerinck) | 11.2±0.2 | 4.9±0.6 | 2.8±0.2 |
| *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata | 62.4±0.8 | 18.5±1.0 | 23.5±0.3 |
| *Monoraphidium minutum* (Nägeli) Komárková-Legnerová | 62.0±0.9 | 12.5±1.2 | 40.3±0.4 |

***Antioxidant Activity Analysis Results of Microlgae:*** The $IC_{50}$ measurement results of reference ascorbic acid and cultured bentic algae species are given in Table 4.

**Table 4:** The $IC_{50}$ measurement results of ascorbic acid and cultured bentic algae species (µg $mL^{-1}$).

| | **$IC_{50}$ Results** |
|---|---|
| Ascorbic Acid | 1.228±0.12 |
| *Chlorella vulgaris* Beyerinck (Beijerinck) | 1.759±0.007 |
| *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata | 1.507±0.012 |
| *Monoraphidium minutum* (Nägeli) Komárková-Legnerová | 1.398±0.017 |

## DISCUSSION and CONCLUSION

In Altınapa Dam Lake, 8 Chlorophyta taxa were isolated from benthic algae and these taxon were cultured. Apart from these taxon, a large number of taxon have been isolated but not reproduced when they have been transferred to the medium and have not been able to obtain successful results from their cultures. Since the obtained taxa conformed to the culture conditions, they showed reproduction and studies continue with these species. Light microscopy images of the reproducing taxa were taken from detailed images by scanning electron microscopy of some of them. When the detection results of similar studies in Turkey are examined, the benthic algae detected in Altınapa Dam Lake showed similarities with the benthic algae belonging to Chlorophyta phylum determined in other studies. In addition, some types of scanning electron microscope images were taken in order to take more detailed pictures. It is envisaged that microscopic identified benthic algae in our study will contribute to the other algal flora studies. As a result of studies to determine the amounts of chlorophyll-a, chlorophyll-b and total carotenoids synthesis, the highest amount of chlorophyll-a was found to be 62.4 ± 0.8 µg $mL^{-1}$ for *Acutodesmus obliquus* species and the lowest amount found 11.2 ± 0.2 µg $mL^{-1}$ for *Chlorella vulgaris* species. The highest amount of chlorophyll-b was determined as 18.5 ± 1.0 µg $mL^{-1}$ for *Acutodesmus obliquus* and the lowest amount found 4.9 ± 0.6 µg $mL^{-1}$ for *Chlorella vulgaris*. The highest total carotenoids amount was found to be 40.3 ± 0.4 µg $mL^{-1}$ for *Monoraphidium minutum* and the lowest amount found 2.8 ± 0.2 µg $mL^{-1}$ for *Chlorella vulgaris*. $IC_{50}$ values were calculated as 1.228 ± 0.12 µg $mL^{-1}$ for ascorbic acid that have strong antioxidant properties, lowest value as 1.398 ± 0.017 µg $mL^{-1}$ for *Monoraphidium minutum* that calculated. According to these results *Monoraphidium minutum* was found to have the highest antioxidant activity.

When the results obtained are compared with the results of other studies, Dere et al. (1998), in the study used methanol solvent, determined the amount of chlorophyll-a in Chlorophyta members macroalgae taken from fresh water for *Cladophora glomerata* as 60.7 µg $g^{-1}$. They determined the amount of chlorophyll-a in Chlorophyta members macroalgae taken from salt water for *Ulva rigita* species as 54.6 µg $g^{-1}$, for *Codium tomentosum* species as 49.0 µg $g^{-1}$, for Phaeophyta member *Cladostephus verticillatus* species as 51.3 µg $g^{-1}$. When the data are compared, the results we obtained are higher than *Chlorella vulgaris* species and similar compared to other species. In the same study, determined the amount of chlorophyll-b in Chlorophyta members macroalgae taken from fresh water for *Cladophora glomerata* as 23.0 µg $g^{-1}$, in Chlorophyta members macroalgae taken from salt water for *Ulva rigita* as 24.1 µg $g^{-1}$, for *Codium tomentosum* as 21.8 µg $g^{-1}$. In the same study used methanol solvent, determined the amount of total carotenoids in Chlorophyta members macroalgae taken from fresh water for *Cladophora glomerata* as 19.2 µg $g^{-1}$, total carotenoids amount in Chlorophyta members macroalgae taken from salt water for *Ulva rigita* as 20.8 µg $g^{-1}$, for *Codium tomentosum* as 24.7 µg $g^{-1}$, for Phaeophyta member *Cladostephus verticillatus* species as 33.8 µg $g^{-1}$. When the data are compared, the results we obtained are higher than *Chlorella vulgaris* species and similar compared to other species.

Seyfabadi et al. (2011) found the highest chlorophyll-a value of 13.25 µg $mL^{-1}$ in different light regimes of the microalgae species *Chlorella vulgaris* the sample we studied, and it has been determined that the result is close to the 11.2 µg $mL^{-1}$ value we obtained.

Fabrowska et al. (2018) determined the amount of chlorophyll-a for *Cladophora glomerata* species as 16.9 µg $mL^{-1}$, for *Cladophora rivularis* species as 5.9 µg $mL^{-1}$, for *Ulva flexuosa* species as 17.6 µg $mL^{-1}$ in different extraction methods in Chlorophyta members macroalgae taken from fresh waters and it was determined that they found lower results than the values we calculated. In the same study, they found the highest amount of chlorophyll-b in Chlorophyta members macroalgae for *Cladophora glomerata* as 9.9 µg $mL^{-1}$, for *Cladophora rivularis* as 2.6 µg $mL^{-1}$, and fot *Ulva flexuosa* as 20.1 µg $mL^{-1}$ species. In the same study, they found the highest total carotenoids amount in Chlorophyta members macroalgae for *Cladophora glomerata* species as 3.0 µg $mL^{-1}$, for *Cladophora rivularis* species as 1.0 µg $mL^{-1}$ and for *Ulva flexuosa* species as 2.2 µg $mL^{-1}$, and they found lower results compared to the values we obtained.

In the study of Durmaz et al. (2008), the amount of chlorophyll-a was found in Chlorophyta members macroalgae for *Ulva* sp. species, as 706.8 µg $g^{-1}$, the total amount of carotenoids was found in the same species as 311.0 µg $g^{-1}$ and when the data were compared, the results we obtained were found to be lower.

In the study of Duygu et al. (2019), the amount of chlorophyll-a was found in Chlorophyta members

macroalgae taken from fresh waters for *Cladophora glomerata* as 5.07 µg $mL^{-1}$, *Mougeotia* sp. as 1.47 µg mL-1, the amount of chlorophyll-b for *Cladophora glomerata* as 3.99 µg $mL^{-1}$, *Mougeotia* sp. as 0.71 µg $mL^{-1}$, the amount of total carotenoids for *Cladophora glomerata* as 756.4 µg $mL^{-1}$, *Mougeotia* sp. as 196.4 µg $mL^{-1}$ and when the data were compared, the results we obtained for chlorophyll-a and chlorophyll-b were higher and total carotenoids was lower.

Yiğitkurt et al. (2020) examined 12 different species taken from salt water and determined the highest amount of chlorophyll-a and total carotenoids as 0.319 mg $g^{-1}$ and 0.532 mg $g^{-1}$, respectively, in the Chlorophyta member *Chaetomorpha linum* and when the data were compared, the results we obtained were found to be lower.

Liu et al. (2017) stated that extracts with low $IC_{50}$ values show stronger antioxidant activity. In the study, in which the antioxidant activity of brown algae was evaluated and two different extraction methods were used, $IC_{50}$ values were determined as 0.0063 mg $mL^{-1}$ for *Ascophyllum nodosum* species, as 0.0038 mg $mL^{-1}$ for *Fucus vesiculosus* species, as 0.74 mg $mL^{-1}$ for *Laminaria digitata* species, as 0.09 mg $mL^{-1}$ for *Alaria esculenta* species and as 0.62 mg $mL^{-1}$ for *Saccharina latissima* species. The $IC_{50}$ value determined as the positive control was calculated as 0.051 mg $mL^{-1}$ for butylated hydroxytoluene and as 0.0063 mg $mL^{-1}$ for ascorbic acid. The studied *Fucus vesiculosus* species gave lower $IC_{50}$ than the reference ascorbic acid, while the *Ascophyllum nodosum* species gave the same $IC_{50}$ value as ascorbic acid.

Souza et al. (2011) evaluated the antioxidant potential of red algae, and the $IC_{50}$ values in the methanol extract were determined as 0.76 mg $mL^{-1}$ for *Gracilaria birdiae* and as 0.86 mg $mL^{-1}$ for *Gracilaria cornea.* The $IC_{50}$ value determined as the positive control was calculated as 0.48 mg $mL^{-1}$ for butylated hydroxytoluene. The studied species gave $IC_{50}$ values close to the reference butylated hydroxytoluene.

Coulombier et al. (2021), according to the data about the table in the review article that which they evaluated many studies on the antioxidant compounds of microalgae, they reported the $IC_{50}$ value of *Chlorella vulgaris* and *Chlamydomonas reinhardtii* species as 55-73 µg $mL^{-1}$, and the $IC_{50}$ value of ascorbic acid, which was determined as positive control, as 127.5 µg $mL^{-1}$. Also, according to the author, the antioxidant activity of the Chlorella genus has been proven by several researchers. According to the study, it was stated that *Chloromonas* sp,, *Botryidiopsidaceae* sp., *Scenedesmus obliquus*, *Haematococcus pluvialis*, *Dunaliella salina*, *Galdieria sulphuraria*, *Ettlia carotinosa*, *Neochloris texensis*, *Chlorella minutissima*, *Chlorella vulgaris*, *Schizochytrium limacinum*, *Stichococcus bacillaris* and *Crypthecodinium cohnii* species gave interesting results. When the data were compared with the results in our study, the $IC_{50}$ value of 1.398 µg $mL^{-1}$ obtained from *Monoraphidium minutum* species gave very close results to the $IC_{50}$ value of 1.228 µg $mL^{-1}$ obtained from the reference antioxidant ascorbic acid.

Consequently, in our study, Chlorophyta species which are studied for the determination of chlorophyll-a, chlorophyll-b and total carotenoids amounts, especially the species *Monoraphidium minutum* and *Acutodesmus obliquus* produced remarkable results. In our study, *Monoraphidium minutum* and *Acutodesmus obliquus* species showed that they have high antioxidant activity by giving approximately the same $IC_{50}$ value due to their high pigment content, which causes high antioxidant activity. Since *Monoraphidium minutum* and *Acutodesmus obliquus* species belonging to the Chlorophyta phylum give $IC_{50}$ values that were close to the highly antioxidant ascorbic acid that is taken as reference, it is concluded that, especially these species from the studied microalgae has a potential to contribute economically when they are produced under suitable conditions for use in the food and pharmaceutical industries.

## ACKNOWLEDGMENT

This work was supported by Selçuk University Scientific Research Project (S.U. BAP) [Project number: 14101009] and TUBITAK-BIDEB (2211). In addition, this work is part of my doctoral thesis.

## REFERENCES

**Abe, K., Hattori, H. & Hirano, M. (2007).** Accumulation and antioxidant activity of secondary carotenoids in the aerial microalga Coelastrella striolata var. multistriata. *Food Chemistry*, ***100***(2), 656-661. DOI: 10.1016/j.foodchem.2005.10.026

**Atıcı, T. (2020).** Production and collection of microalgae isolated from freshwater reserves in Central Anatolia, Turkey. *Türler ve Habitatlar*, ***1***(1), 37-44.

**Atıcı, T. & Çalışkan, H. (2007).** Effects of Some Environmental Variables on the Benthic Shore Algae (Excluding Bacillariophyta) of Asartepe Dam (Ankara). *International Journal of Natural & Engineering Sciences*, ***1***(2).

**Atıcı, T. & Obalı, O. (2002).** Comparison of seasonal variation of phytoplankton and chlorophyll–a values of abant and Yedigöller Lakes. *E.U. Journal of Fisheries & Aquatic Sciences*, ***19***(3-4), 381-389.

**Bors, W., Saran, M. & Elstner, E.F. (1992).** Screening for Plant Antioxidants. In: Linskens, H.F., Jackson, J.F. (eds) Plant Toxin Analysis. Modern Methods of Plant Analysis, vol 13. *Springer*,

Berlin, Heidelberg. DOI: 10.1007/978-3-662-02783-7_11

**Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995).** Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, ***28***(1), 25-30.

**Chapman, R.L. (2013).** Algae: the world's most important "plants"-an introduction. *Mitigation and Adaptation Strategies for Global Change*, ***18***(1), 5-12. DOI: 10.1007/s11027-010-9255-9

**Coulombier, N., Jauffrais, T. & Lebouvier, N. (2021).** Antioxidant compounds from microalgae: A review. *Marine drugs*, ***19***(10), 549. DOI: 10.3390/md19100549

**Dere, Ş., Güneş, T. & Sıvacı, R. (1998).** Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany*, ***22***(1), 13-18.

**Durmaz, Y., Duyar, H.A., Gökpınar, Ş., Öğretmen, Y.Ö. & Bandarra, N.M. (2008).** Research on Fatty Acids, α-tocopherol and Total Pigment Content of the *Ulva* spp. (Sinop-Turkey, Black Sea). *Journal of FisheriesSciences.com*, ***2***(3): 350-356. DOI: 10.3153/jfscom.mug.200723

**Duygu, D. Y., Erkaya, İ.A. & Sızmaz, Ö. (2019).** Biochemical Compositions Of *Cladophora Glomerata* (Linnaeus) Kützing And *Mougeotia* Sp. Species Collected In Bulk From Natural Freshwater Environments. *Aquatic Research*, *2*(1), 24-31. DOI: 10.3153/AR19003

**El-Agbar, Z.A., Shakya, A.K., Khalaf, N.A. & Al-Haroon, M. (2008).** Comparative antioxidant activity of some edible plants. *Turkish Journal of Biology*, ***32***(3), 193-196.

**Fabrowska, J., Messyasz, B., Szyling, J., Walkowiak, J. & Łęska, B. (2018).** Isolation of chlorophylls and carotenoids from freshwater algae using different extraction methods. *Phycological Research*, ***66***(1), 52-57. DOI: 10.1111/pre.12191

**Google. (2016).** Google Earth. https://www.google.com/earth/. https://earth.google.com/web/; searched on 10 December 2016.

**Guiry M.D. & Guiry G.M. (2021).** AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 20 May 2021.

**Hamidian, N. & Zamani, H. (2022).** Biomass production and nutritional properties of Chlorella sorokiniana grown on dairy wastewater. *Journal of Water Process Engineering*, ***47***, 102760.

**John, D.M., Whitton, B.A. & Brook, A.J. (2002).** The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae. *Cambridge University Press*, 26-613.

**Katırcıoğlu, H., Aslım, B., Türker, A. R., Atıcı, T. & Beyatlı, Y. (2008).** Removal of cadmium (II) ion from aqueous system by dry biomass, immobilized live and heat-inactivated Oscillatoria sp. H1 isolated from freshwater (Mogan Lake). *Bioresource Technology*, ***99***(10), 4185-4191. DOI: 10.1016/j.biortech.2007.08.068

**Keskinkaya, H.B., Gümüş, N. E., Aşıkkutlu, B., Akköz, C., Okudan, E.Ş. & Karakurt, S. (2020).** Macro and Trace Element Levels of Green Algae Codium fragile (Suringar) Hariot 1889 From Dardanelles (Çanakkale/Turkey). *Anadolu Orman Araştırmaları Dergisi*, ***6***(2), 55-61.

**Kraan, S. (2013).** Pigments and minor compounds in algae. In *Functional ingredients from algae for foods and nutraceuticals* (pp. 205-251). Woodhead Publishing. DOI: 10.1533/9780857098689.1.205

**Lee, R.E. (2008).** Phycology. *Cambridge University Press*, 614 pp.

**Lichtenthaler, H.K. & Wellburn, A.R. (1983).** Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, ***11***(5), 591-592.

**Liu, X., Yuan, W., Sharma-Shivappa, R. & van Zanten, J. (2017).** Antioxidant activity of phlorotannins from brown algae. *International Journal of Agricultural And Biological Engineering*, ***10***(6), 184-191. DOI: 10.25165/j.ijabe.20171006.2854

**Martinez-Morales, F., Alonso-Castro, A.J., Zapata-Morales, J.R., Carranza-Álvarez, C. & Aragon-Martinez, O.H. (2020).** Use of standardized units for a correct interpretation of IC50 values obtained from the inhibition of the DPPH radical by natural antioxidants. *Chemical Papers*, ***74***(10), 3325-3334. DOI: 10.1007/s11696-020-01161-x

**McCormick, P.V. & Cairns, J. (1994).** Algae as indicators of environmental change. *Journal of Applied Phycology*, ***6***(5), 509-526.

**Moss, B. (2010).** Ecology of fresh waters: a view for the twenty-first century. *John Wiley & Sons*, 480 pp.

**Munir, N., Sharif, N., Naz, S. & Manzoor, F. (2013).** Algae: a potent antioxidant source. *Sky J. Microbiol. Res*, ***1***(3), 22-31.

**Pham-Huy, L.A., He, H. & Pham-Huy, C. (2008).** Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, ***4***(2), 89.

**Prasanna, R., Sood, A., Suresh, A., Nayak, S. & Kaushik, B. (2007).** Potentials and applications of algal pigments in biology and industry. *Acta Botanica Hungarica*, ***49***(1-2), 131-156. DOI: 10.1556/abot.49.2007.1-2.14

**Prescott, G.W. (1973).** Algae of the western Great Lakes Area. *WC Brown Company Dubuque*, 680-974.

**Pulz, O. & Gross, W. (2004).** Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, ***65***, 635-648. DOI: 10.1007/s00253-004-1647-x

**Qing, C., Zhang, H., Chen, A., Lin, Y. & Shao, J. (2020).** Effects and possible mechanisms of sanguinarine on the competition between Raphidiopsis raciborskii (Cyanophyta) and Scenedesmus obliquus (Chlorophyta): A comparative

toxicological study. *Ecotoxicology and Environmental Safety*, ***206***, 111192. DOI: 10.1016/j.ecoenv.2020.111192

**Rippka, R. (1988).** [1] Isolation and purification of cyanobacteria. *Methods in Enzymology*, ***167***, 3-27.

**Seyfabadi, J., Ramezanpour, Z. & Amini Khoeyi, Z. (2011).** Protein, fatty acid, and pigment content of Chlorella vulgaris under different light regimes. *Journal of Applied Phycology*, ***23***(4), 721-726. DOI: 10.1007/s10811-010-9569-8

**Souza, B.W., Cerqueira, M.A., Martins, J.T., Quintas, M.A., Ferreira, A.C., Teixeira, J.A. & Vicente, A.A. (2011).** Antioxidant potential of two red seaweeds from the Brazilian coasts. *Journal of Agricultural and Food Chemistry*, ***59***(10), 5589-5594. DOI: 10.1021/jf200999n

**Wijffels, R.H., Kruse, O. & Hellingwerf, K. J. (2013).** Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Current Opinion in Biotechnology*, ***24***(3), 405-413. DOI: 10.1016/j.copbio.2013.04.004

**Yiğitkurt, S., Kirtik, A., Uğur, S. & Durmaz, Y. (2020).** Pigment Potential of Macroalgae Collected from The Coasts of Izmir Bay. *Menba Journal of Fisheries Faculty*, ***6***(2), 45-52.

**Yim, J.H., Seo, Y.B., Kim, S.M. & Jeon, Y.J. (2021).** Recent Research Trends of Cryopreservation Technology Based on Microalgae Chlorophyta. *Journal of Life Science*, ***31***(10), 960-968. DOI: 10.5352/JLS.2021.31.10.960

**Zamani, H. & Moradshahi, A. (2014).** Evaluation of total reducing capacity in three Dunaliella salina (Dunal) Teodoresco isolates. *Journal of applied phycology*, ***26***(1), 369-376. DOI: 10.1007/s10811-013-0074-8