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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigation of *Spirogyra daedaleoides* Czurda in terms of bioactive components

Spirogyra daedaleoides Czurda'nın biyoaktif bileşenler açısından incelenmesi

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Abstract: Algae stand out as suitable sources for use in the cosmetic, food and pharmaceutical industries due to their high content of components such as protein, polysaccharide, lipid, vitamin, mineral, amino acid, fatty acid, and carotenoid and due to the bioactive components that they produce. In this study, the bioactive properties of *Spirogyra daedaleoides* Czurda, located in the Charopyhta phylum of the algae, were examined. This species was isolated from the benthic habitats of the Yeşilırmak River and cultured. Antioxidant properties of *Spirogyra daedaleoides* was determined with the methods of free radical removal activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl), iron (III) ion reduction power activity (FRAP, fluorescence recovery after photobleaching) and cation radical removal activity (ABTS, 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). The fatty acids of *Spirogyra daedaleoides* was determined by gas chromatography analysis, and vitamins were determined by high-performance liquid chromatography (HPLC) analysis. According to the results of antioxidant analysis, the free radical removal activity of *Spirogyra daedaleoides*, iron ion reduction power activity and cation radical removal activity were found to be high. When the fatty acid percentages of *Spirogyra daedaleoides* were considered, the ratios of C20:0 Arachidic acid, C16:0 Palmitic acid, C18:3n3 Alpha linoleic acid, C10:0 Caprinic acid and C18:2n6c Linoleic acid were found to be high. *Spirogyra daedaleoides* was found to be high in Vitamin A and Vitamin E. *Spirogyra daedaleoides* could be used as natural resources in the cosmetics, food and pharmaceutical industries according to the results.

Keywords: Charophyta, bioactive component, antioxidant, fatty acid, vitamin

INTRODUCTION

Algae are potential sources of bioactive secondary metabolites used in the development of new pharmaceutical substances. They are photosynthetic organisms found in both marine and freshwater environments**.**

Algae have been used in many different fields for many years. Due to the protein, carbohydrate, fatty acids, vitamins, minerals, pigments and many other important metabolites that accumulate in the cell, they are used by humans as the main nutritional support (Bulut, 2009). In addition to healthy nutrition, it is important for preventive treatment and the prevention of degenerative diseases with the help of antioxidants obtained from algae. Tocopherols, ascorbic acid, carotenoids, flavonoids and retinoids are the main antioxidants that can be found in algae (Baytaşoğlu and Başusta, 2015).

Bioactive sources of natural products have been used in the fight against diseases for hundreds of years, and the active ingredient of more than half of the drugs used today is formed from these sources (Rice-Evans et al., 1996).

Bioactive components are secondary metabolites that have positive effects on health by affecting physiological activities. Bioactive components, carbohydrates, proteins and fats which are known as primary metabolites, are not the main sources of essential nutrients for the growth and development of a living being. However, they are components that increase the ability of a living being to withstand harsh living conditions. Although bioactive components are usually found in small amounts in foods, they have serious health effects (Gupta et al., 2017). Microalgae produce bioactive components that are valuable products with applications in the cosmetics, food and pharmaceutical industries.

This study aimed to investigate the bioactive properties of *Spirogyra daedaleoides* included in the Charopyhta phylum. Since there are no previous studies on this species whose bioactive properties have been investigated and there is a trend towards natural sources of bioactive substances today, it was decided to conduct studies with this species.

MATERIALS AND METHODS

Isolating and culturing algae

Spirogyra daedaleoides Czurda 1932

Spirogyra: It is generally common in freshwater and benthic habitats. It can also be found in planktonic habitat. Thal structure can be found in the form of nested colonies. Tal structure consists of unbranched filaments. The cells are in single-row arrays. The cylindrical cells are 10 to >200 µm in

diameter and most are 20 to 60 µm long. The interior of the cells is cellulose and has a two-layered cell wall. There is a mucilage layer on the outside of the cells. Basal cells are rarely suitable for rhizoidal attachment. Cells are mononuclear. Chloroplasts are spiral shaped. Spiral fold is important in diagnosis. Akinetes and aplanospores are common; parthenospores are fewer. Asexual and sexual reproduction occurs. Sexual reproduction usually occurs through conjugation, typically in late spring and summer. The Phylum Charophyta is included in the Clade Zygnematophyceae. *Spirogyra daedaleoides* Czurda's synonym is *Spirogyra daedalea* f. *daedaloides* (Czurda) V. Poljansky. (Guiry and Guiry, 2024).

Empire: Eukaryota

Kingdom: Plantae

Phylum: Charophyta

Class: Zygnematophyceae

Subclass: Zygnematophycidae

Order: Spirogyrales

Family: Spirogyraceae

Genus: *Spirogyra*

Spirogyra daedaleoides was transported to the Laboratory and isolated by mechanical isolation method from water samples taken from Yeşilırmak River benthic habitats with plastic containers. The sampling point from Yeşilırmak is located in the city center of Tokat, at latitude 40˚ 21' 33.88" N and longitude 36˚38' 37.59" E. The samples were taken during the summer. The samples were transferred to eppendorf tubes under an inverted microscope, cultured and incubated at 26 °C (155 µmol/m2/h, L:D period) in Sanyo MLR 351 climate cabinet under Allen, BG11 liquid growth culture (Lobban et al., 1988; Andersen, 2005). After reaching a certain volumetric density, it was harvested and stored in a -86 °C freezer in a culture collection for research purposes.

For the identification of algae, several taxonomic literatures (Prescott, 1979; Canter-Lund & Lund, 1995; John et al., 2002) were used. In addition, the synonymous status and categories of the identified species were classified by checking the Algaebase database (Guiry and Guiry, 2024).

Fatty acid, vitamin analyses and antioxidant activity tests were applied after taking 5 g of the algae species extracted from the culture collection and extracting them in 150 mL solvent (1:1methanol+methylene chloride).

Antioxidant activity tests

In order to determine the antioxidant activity of the algae, three different methods have been applied.

Determination of DPPH free radical removal activity (DPPH-2,2-diphenyl-1-picrylhydrazyl)

The DPPH free radical removal activity of algal extracts and standard antioxidant substances was determined using the DPPH radical according to the Brand-Williams method (Brand-Williams et al., 1995). The solution of 20 mg/L DPPH was diluted with methanol and prepared on a regular basis. The 1.5 mL solution was taken and 0.75 ml of algae extract made in a range from 250 to 1000 mg per litre has been introduced into the solution. It was measured on a blind spectrophotometer per minute at an absorbance of 30 min. 517 nm. 0.75 mL methanol and 1.5 mL DPPH solution were used for control purposes. 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) were used as standard. Antioxidant analyses are based on the measurement of DPPH color loss at 517 nm following the reaction with test compounds, and the reaction was monitored with a spectrometer. A high free radical elimination activity is indicated by any decrease in the absorbance of the reaction mixture.

Determination of iron (III) ion reduction power activity (FRAP- fluorescence recovery after photobleaching

The total reduction power of algae extracts was determined using the Oyaizu method (Oyaizu, 1986). 2.5 mL phosphate (KH_2PO_4) buffer (0.2 M pH: 6.6) and 2.5 mL potassium ferricyanide $K_3Fe(CN)_6$ (1%) solutions were added to the tubes after pipetting Algae extracts and standards in different concentrations. Butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were used as standard. After being thoroughly vortexed, it was incubated in a 50°C water bath for 20 minutes. Then, 2.5mL of 10% trichloroacetic acid (TCA) solution was added to this mixture and centrifuged at 3000 rpm for 10 minutes. After taking 2.5 mL of the centrifuged mixture and adding 0.5 mL of 1% iron (III) chloride ($FeCl_3$) solution to it and vortexing, their absorbance against void was read at 700 nm using UV-Vis spectroscopy.

Determination of ABTS cation radical removal activity (ABTS-2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ABTS is based on the inhibition of the absorbance of the radical cation by antioxidants. The Cation Radical (ABTS) Removal Activity was performed according to the method proposed by Re et al. (1999). 0.1 M pH: 7.4 PO_4^{3-} buffer, 2 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) solution were prepared for free radical (ABTS) removal activity. $ABTS^{\cdot+}$ and $K_2S_2O_8$ solutions (1:2) were mixed to become $ABTS^{+}$ - $K_2S_2O_8$ and incubated in the dark for 6 hours. At different concentrations (2,5-5-10 µg/mL) samples and standard solutions were taken and 1 ml of $ABTS^{\cdot+}$ - $K_2S_2O_8$ solution was added to it. A phosphate buffer was added so that the total volume was 4 ml. The mixture was vortexed and incubated for 30 minutes and spectrophotometric measurement was performed at 734 nm under room conditions. The decreased absorbance gives the amount of ABTS radicals removed from the environment. As standard, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) was used (Re et al., 1999).

Determination of fatty acid composition

In order to determine fatty acids, dried algae samples were crushed into powder by crushing in a press and 1 gram of each sample was used.

To determine fatty acids, saponification, methylation and extraction processes were performed. Fatty acids in algal species were determined using a gas chromatography (GC) method.

Gas chromatography analyzes were performed with HP (Hewlett Packard) Agilent brand gas chromatography with FID (Flame Ionization Detector: Flame ionization detector) detector and automatic injector. Capillary column was used and the injector block temperature was set to 210˚C and the detector temperature to 230˚C. The temperature program was applied to the column and the initial temperature of the column was set as 120˚C. Afterwards, it reached 185˚C with an increase of 4˚C per minute, and then 230˚C with an increase of 1˚C per minute. It was kept at this temperature for 5 minutes. Gas flow rates were adjusted as 30 ml min-1 for hydrogen, 300 ml min-1 for dry air and 1 ml min-1 for helium used as carrier gas, respectively (IUPAC, 1979).

Vitamin analysis

The algae samples were extracted with hexane: chloroform (3:1) after taking out of the deep freezer for study in HPLC. Then, the solvent was removed under vacuum and injected into the C18 column (150x4.6 mm ID, 5µm Wakosil) and the column temperature was kept constant at 50°C. Acetonitrile: methanol (1:1) was used as the mobile phase. The flow rate was programmed as 1ml/minute and a DAD detector was used as the detector. Calibration graphs were drawn using α-tocopherol and β-carotene as standard and vitamin amounts were calculated (Moreno and Salvadó, 2000).

Statistical analysis

All studies were performed in three repetition-format and the mean (±) was given as standard deviation (SD). Statistical analyses were performed using Microsoft Excel.

RESULTS

Results regarding antioxidant activity

According to the data obtained from the analysis of diphenyl-1-picrylhydrazyl (DPPH) free radical removal activity, the free radical removal activity of *Spirogyra daedaleoides* was measured as 17.56±0.45 µg (extract)/mL. The free radical removal activities of algal extracts were compared with the standards of Trolox, BHA and BHT (Table1).

The iron (III) ion reduction power (FRAP) activity of *Spirogyra daedaleoides* was measured as 2.65±0.35 µmol/mg (extract) in the results of the analysis. The free radical removal activities of algal extracts were compared with the standards of BHA and BHT (Table1).

According to the obtained data regarding the cation radical removal activity (ABTS), *Spirogyra daedaleoides* was measured as 8.89±0.37 µg (extract)/mL in the analyses. The free radical removal activities of algal extracts were compared with the standards of Trolox, BHA and BHT (Table1).

Table 1. Results of antioxidant analysis of *Spirogyra daedaleoides* extract

Antioxidant activity	DPPH µg/mL	FRAP µmol/mg	ABTS µg/mL
Spirogyra daedaleoides	17.56 ±0.45	2.65±0.35	8.89±0.37
BHA	5.78 ±0.23	4.35±0.18	5.48±0.12
BHT	7.67 ±0.21	3.87±0.17	6.89±0.18
Trolox	5.68 ±0.24	-	5.38±0.06

DPPH-Free Radical Removal Activity
FRAP-Iron (III) Ion Reduction Power Activity
ABTS-Cation Radical Removal Activity

Fatty acids

In the fatty acid analyses of *Spirogyra daedaleoides*, the ratios of C20:0 arachidic acid, C16:0 palmitic acid, C18:3n3 alpha-linoleic acid, C10:0 caprinic acid and C18:2n6c linoleic acid were found to be higher than the others, respectively, when ranked according to their % densities (Table 2).

Table 2. Fatty acid ratios of *Spirogyra daedaleoides* extract

Fatty acids	Value (%)
C10:0 Caprinic acid	**10.80**
C12:0 Lauric acid	3.93
C14:0 Myristic acid	1.00
C14:1 Myristoleic acid	0.06
C15:0 Pentadecanoic acid	0.06
C16:0 Palmitic acid	**18.76**
C16:1 Palmitoleic acid	1.83
C17:0 Heptadecanoic acid	0.37
C18:0 Stearic acid	2.52
C18:1 n9c Oleic acid	2.73
C18:2n6t Linolelaidic acid	0.16
C18:2n6c Linoleic acid	**5.23**
C18:3n6 gama Linoleic acid	1.36
C20:0 Arachidic acid	**25.78**
C18:3n3 alfa Linoleic acid	**18.01**
C20:2 cis11,14-eicosadienoic acid	0.17
C20:3n6 Dihomo-gamma-linolenic acid	1.20
C22:0 Behenic acid	0.31
C22:1n9 Erucic acid	0.38
C20:3n3 Eicosatrienoic acid	0.14
C20:5n3 Eicosapentaenoic acid	2.72
C24:0 Lignoceric acid	1.89
C24:1 Nervonic acid	0.13
C22:6n3 Docosahexaenoic acid	0.48

Vitamins

In the vitamin analyses of *Spirogyra daedaleoides*, the ratios of vitamins C, E and A were examined. According to the data obtained; vitamin C of *Spirogyra daedaleoides* was found to be 21.74 mg/kg, vitamin E was found to be 24.78 mg/kg and vitamin A was found to be 475.16 mg/kg and the results are given in Figure 1.

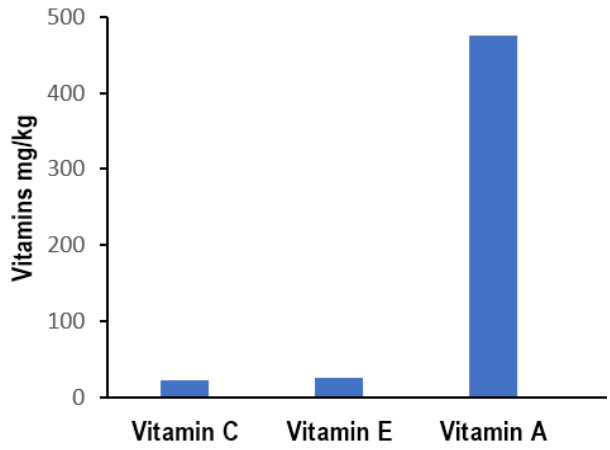

Figure 1. Vitamin C, E, A values of *Spirogyra daedaleoides* extract

DISCUSSION

Algae accumulate specific secondary metabolites, which are valuable products (such as pigments and vitamins) that are used in the cosmetics, food or pharmaceutical industry. In the environment in which they are growing, algae can be subjected to stress and extreme conditions such as changes in salinity, temperature or nutrient levels. To survive, these organisms must adapt to the new environment conditions and thus produce a large number of biologically active secondary metabolites that are not found in any other organism (Demorais et al., 2015).

Algae, which are very rich in nutritional value content, are also used in the health sector. *Spirulina*, a blue-green alga which is rich in protein content, has become a health-promoting food worldwide. It has been recognized as a rich source of protein, vitamins and minerals. While the protein content of Spirulina varies from 50 % to 70 % in its dried weight, the best source of vegetable protein is half that level (Kapoor and Mehta, 1993).

There are many different uses for algae today. Animal feed, vegetable fertilizer, water treatment process, dyes and additives used in food production are the prominent ones among these areas. It has also become an important part of nutrition in recent years. Algae are an important producer link of the food chain. Generally, their use as a food source in Southeast Asian and island countries is increasing the popularity of algae day by day (Ünver Alçay et al., 2017).

Antioxidants are molecules that are used to inhibit or prevent oxidation in living organisms. These molecules can eliminate free radicals. Thus, it delays lipid peroxidation as well as the progression of many chronic diseases (Gülçin, 2012).

According to the antioxidant analysis results of *Spirogyra daedaleoides*, free radical removal activity (DPPH) was found to be 17.56 ±0.45 µg (extract)/mL, iron (III) ion reduction power activity (FRAP) was found to be 2.65±0.35 µmol/mg (extract) and cation radical removal activity (ABTS) was found to be 8.89±0.37 µg (extract)/mL (Table 1).

In the study conducted with *Spirogyra porticalis* (Muell.) Cleve, water, methanol, acetonitrile and n-hexane were used as solvents. Antioxidant activity was determined by ferric reducing antioxidant power (FRAP), ABTS radical scavenging activity, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), β-carotene-linoleic acid bleaching, Nitric oxide (NO) scavenging and lipid peroxidation methods. They noted that methanol extracts had the highest antioxidant activity (Kumar et al., 2015). In this study, antioxidant activity was analyzed with the DPPH, FRAP and ABTS methods using methanol for extraction. The antioxidant activity of *Spirogyra daedaleoides* extracts was found to be high in all three methods.

In the study investigating the antioxidant properties of *Spirogyra neglecta* (Hassall) Kützing species), as a result of the analysis, the DPPH activity of *Spirogyra neglecta* was found to be 48.67 ± 3.06 µg/ml (Thumvijit et al. (2013). In this study, the free radical removal activity (DPPH) of *Spirogyra daedaleoides* was found to be 17.56 ± 0.45 µg (extract)/mL. The result obtained with the free radical removal activity (DPPH) analysis of *Spirogyra daedaleoides* is more effective than the results in the study of Thumvijit et al. (2013).

The fat content of algal species varies between 1-5%. Nevertheless, it contains much more essential fatty acids than other land plants. Due to their role as building blocks for fats in the organism and as building blocks for cell membranes, fatty acids play an important role in human and animal nutrition (Demirel and Özpınar, 2003).

In the fatty acid analysis of *Spirogyra daedaleoides*, it was found to have contained many types of fatty acids. Among them, C10:0 caprinic acid with 10.80%, C16:0 palmitic acid with 18.76%, C18:2n6c linoleic acid with 5.23%, C20:0 arachidic acid with 25.78%, C18:3n3 alpha linoleic acid with 18.01% were found to be higher than the others (Table 2).

In the study investigating the fatty acids of *Spirogyra* species, their fatty acid ratios were determined. C10:0 caprinic acid was 0.05-0.10%, C16:0 palmitic acid was 21.25-20.78%, C18:2n6c linoleic acid was 5.17-5.99%, C20:0 arachidic acid was 0.60-0.51%, C18:3n3 alpha linoleic acid was 0.10-0.09% in the study conducted with *Spirogyra* species (Erkaya and Yalcin, 2021). In this study, C20:0 arachidic acid, C16:0 palmitic acid, C18:3n3 alpha-linoleic acid fatty acid varieties were found to be at a higher rate.

Algae have critical functions in the energy cycle of the ecosystem. Algal biomass is used in the extraction of phycocolloids (alginate, carrageen and agar), as a source of pharmaceutical substances and as a food additive in different regions of the world (Ramaraj et al., 2014).

In the vitamin analysis of *Spirogyra daedaleoides*, vitamin C was found to be 21.74 mg/kg, vitamin E was found to be 24.78 mg/kg, vitamin A was found to be 475.16 (Figure 1).

Aaronson et al. (1977) researched *Chlamydomonas reinhardii*, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Anabaena cyclindrica* and determined the amounts of Vitamin

A-E-C in their study. Vitamin A was found to be 105 ng in the *Chlamydomonas reinhardii*, vitamin E was 4 µg in the *Anabaena cyclindrica*, and vitamin C was 15 µg in the *Chlorella vulgaris*. In the study, it was determined that vitamins A and E were higher in the *Spirogyra daedaleoides*.

Ulva rigida, Gracilaria gracilis, Sargassum vulgare, Cystoseira barbata and Dictyopteris membranacea species were used in the study investigating the vitamin values of algae species. The results of vitamin analysis revealed that algae are rich in β-carotene (provitamin A), ascorbic acid (vitamin C) and α-tocopherol (vitamin E). The richest in β-carotene (provitamin A) is *Gracilaria gracilis with* 3.25 ± 0.41 mg while *Ulva rigida* was found to be the richest in vitamin C (ascorbic acid) with an amount of 17.42 ± 0.67 mg.100 g-1 *Dictyopteris membranacea* was found to be the richest in the tocopherol (vitamin E) with an amount of 5.03 ± 0.12 mg.100 g-1(Turan and Cirik, 2018). In this study, the Vitamin A, E and C levels of *Spirogyra daedaleoides* were investigated and higher values were found in all three vitamins. With these characteristics, the species have the characteristics to be used as a natural resource in the food industry.

CONCLUSIONS

Algae are naturally rich sources of biologically active compounds such as antibiotics, antivirals, anticancer and antioxidants. In addition, these microorganisms can improve health and reduce the risk of developing degenerative diseases. In the development of new pharmaceutical substances, the research of these biologically active compounds is of great importance to ensure chemical and pharmacological innovation and diversity.

Considering the positive results regarding the antioxidant, fatty acid and vitamin contents of *Spirogyra daedaleoides* used in the research, it is planned to isolate the active substances and contribute the information to the literature in future studies.

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CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest on this manuscript.

ETHICAL APPROVAL

Ethical approval is not required for this study.

DATA AVAILABILITY

All relevant data is inside the article.

REFERENCES

Aaronson, S., Dhawale, S.W., Patni, N.J., Deangelis, B., Frank, O., & Baker, H. (1977). The cell content and secretion of water-soluble vitamins by several freshwater algae. *Archives of microbiology*, *112*, 57-59. https://doi.org/10.1007/BF00446654

Andersen, R.A. (2005). *Algal Culturing Techniques.* Elsevier Academic Press.

Baytaşoğlu, H., & Başusta, N. (2015). The usage of marine organisms in medicine and pharmaceutics fields. *Yunus Araştırma Bülteni B*(2),71-80. https://doi.org/10.17693/yunusae.v15i21956.235767 (in Turkish with English abstract)

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie/Food Science and Technology*, *28*, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bulut, Y. (2009). *The investigations on the possibility of increase lipid content of Chlorella (Chlorophyceae).* Master's Thesis, Çukurova University Institute of Science and Technology, Türkiye. (in Turkish with English abstract)

Canter-Lund H., & Lund J.W.G. (1995). Freshwater Algae. Their Microscopic World Explored. Biopress, *Bristol*. 360 p.

Demirel, G., & Özpınar, H. (2003). Algae and their use in animal nutrition. *Uludağ University Journal of Faculty of Veterinary Medicine, 22*(1-2-3), 103-108. (in Turkish with English abstract)

Demorais, M.G., Silvavaz, B.D., Morais, E.G., & Vieira Costa, J.A. (2015). Biologically active metabolites synthesized by microalgae, laboratory of microbiology and biochemistry. *BioMed Research International*, *75*(3), 311–335. https://doi.org/10.1155/2015/835761

Erkaya, I.A., & Yalcin, D. (2021). Determination of nutritional evaluation fatty acid composition and growth kinetics of *Spirogyra* strains. *Fresenius Environmental Bulletin*, 8021.

Guiry, M.D. & Guiry, G.M. (2024). 'AlgaeBase', World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org

Gupta, S., Gupta, C., Garg, A.P., & Prakash, D. (2017). Probiotic efficiency of blue green algae on probiotics microorganisms. *Journal of Microbiology and Experimentation*, *4*(4), 00120. https://doi.org/10.15406/jmen.2017.04.00120

Gülçin, İ. (2012). Antioxidant activity of food constituents: an overview. *Archives of Toxicology*, *86*(3), 345-391. https://doi.org/10.1007/s00204-011-0774-2

IUPAC (1979). Standarts methods for analysis of oils, fats and derivatives (6th ed.). New York: Pergamon Press.

Kapoor, R., & Mehta, U. (1993). Effect of supplementation of blue green alga (*Spirulina*) on outcome of pregnancy in rats. *Plant Foods and Human Nutrition*, *43*(1), 29-35. https://doi.org/10.1007/BF01088093

Kumar J., Dhar P., Tayade A.B., Gupta D., Chaurasia O.P., & Upreti D.K. (2015). Chemical composition and biological activities of Trans-Himalayan alga *Spirogyra porticalis* (Muell.) Cleve. *PLoS ONE*, *10*(2), e0118255. https://doi.org/10.1371/journal.pone.0118255

Lobban, C.S., Chapman, D.J., & Kremer, B.P. (1988). Experimental Phycology A Laboratory Manual. *Chambridge University Press*, London.

John, D.M., Whitton, B.A., & Brook, A.J. (2002). The freshwater algal flora of the British Isles, *Cambridge University Press*, Cambridge, 702 p.

Moreno, P., & Salvadó, V. (2000). Determination of eight water- and fat-soluble vitamins in multi-vitamin pharmaceutical formulations by high-performance liquid chromatography. *Journal of Chromatography A*, *870*(1–2), 207–215. https://doi.org/10.1016/s0021-9673(99)01021-3

Oyaizu, M. (1986). Studies on products of browing reaction-antioxidative activities of products browing reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Prescott, G.W. (1979). Freshwater Algae. *Brown Comp. Pub*., 293, Dubugue, Lawa.

Ramaraj, R., Tsai, D.D.W., & Chen, P.H. (2014). An exploration of the relationships between microalgae biomass growth and related environmental variables. *Journal of Photochemistry and Photobiology B.*, *135*, 44-47. https://doi.org/10.1016/j.jphotobiol.2014.04.001

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

Thumvijit T., Inboot W., Peerapornpisal Y., Amornlerdpison D., & Wongpoomchai, R. (2013). The antimutagenic and antioxidant properties of *Spirogyra neglecta* (Hassall) Kützing. *Journal of Medicinal Plants Research*, *7*, 2494-2500. https://doi.org/10.5897/JMPR2013.4427

Turan, G., & Cirik, S. (2018). Determination of vitamin composition of cultured macroalgae for Thalassotherapy applications. *Ege Journal of Fisheries and Aquatic Sciences*, *35* (2), 151-156. https://doi.org/10.12714/egejfas.2018.35.2.07 (in Turkish with English abstract)

Ünver Alçay, A., Bostan, K., Dinçel, E., & Varlık, C. (2017). Algae as a food source for humans. *Aydın Gastronomy*, *1*(1), 47-59. (in Turkish with English abstract)