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Contents of Fatty Acid in *Gomphonema olivaceum*

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ABSTRACT: Algae are seen as an important source of long-chain polyunsaturated fatty acids (PUFA), especially γ-linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is thought that the fatty acids obtained from these organisms can be used in the treatment of hypertension, heart and autoimmune diseases and in the prevention of cancer. High valence compounds obtained from algae; Oils, fatty acids, proteins, carbohydrates, pigments, minerals, vitamins, sterols, antioxidants and some other bioactive polyphenols are used in many fields in the pharmaceutical, cosmetic, food and health sectors. Species belonging to the Bacillariophyta division are single-celled siliceous algae that live cosmopolitanly in all aquatic and humid environments on earth. They contain high levels of silicon. With these properties and specific compounds such as levosine, it has valuable use in pharmaceutical, food and biomedical sciences. Diatoms are remarkable organisms with their highly ornamented morphological structures.In this study, fatty acids of *Gomphonema olivaceum*, which was isolated and cultured from the benthic habitats of the Tokat Yeşilırmak River, were investigated. As a result of the chromatographic analysis, a total of 24 essential fatty acids were determined (some in trace amounts and some in high proportion). Of these, C16:0 Palmitic acid (32.04%) and C16:1 Palmitoleic acid (30.97%) were measured at high rates. C18:0 Stearic acid (6.93%), C14:0 Myristic acid (6.17%) and C20:0 Arachidic acid (5.03%) were recorded as relatively higher than the others.

Keywords – *Bacillariophyta, Fatty acid, Gomphonema olivaceum*

Gomphonema olivaceum'da Yağ Asidi İçerikleri

ÖZET: Algler, özellikle γ-linolenik asit, araşidonik asit, eikosapentaenoik asit (EPA) ve dokosaheksaenoik asit (DHA) gibi uzun zincirli çoklu doymamış yağ asitlerinin (PUFA) önemli bir kaynağı olarak görülmektedir. Bu organizmalardan elde edilen yağ asitlerinin hipertansiyon, kalp ve otoimmün hastalıkların tedavisi ile kanserin önlenmesinde kullanılabileceği düşünülmektedir. Alglerden elde edilen yüksek değerlikli bileşikler; yağlar, yağ asitleri, proteinler, karbonhidratlar, pigmentler, mineraller, vitaminler, steroller, antioksidanlar ve diğer bazı biyoaktif polifenoller eczacılık, kozmetik, gıda ve sağlık sektöründeki birçok alanda kullanılmaktadır. Bacillariophyta bölümüne mensup türler, yeryüzündeki tüm sucul ve nemli ortamlarda kozmopolit olarak yaşayan, tek hücreli silisli alglerdir. Bünyelerinde yüksek oranda silisyum bulundururlar. Bu özellikleri ve levkosin gibi spesifik bileşikleriyle farmasötik, gıda ve biyomedikal bilimlerde değerli kullanıma sahiptir. Diyatomeler, son derece süslü morfolojik yapılarıyla da dikkat çekici organizmalardır. Bu çalışmada, Tokat Yeşilırmak Nehri bentik habitatlarından izole edilerek kültürü yapılan *Gomphonema olivaceum*'un yağ asitleri incelenmiştir. Yapılan kromatografik analizler sonucunda, (bir kısmı eser miktarda bir kısmı ise yüksek oranda) toplamda 24 esansiyel yağ asidi tespit edilmiştir. Bunlardan C16:0 Palmitic asit (%32.04) ve C16:1 Palmitoleic asit (%30.97) yüksek oranlarda ölçülmüştür. C18:0 Stearic asit (%6.93), C14:0 Myristic asit (%6.17) ve C20:0 Arachidic asit (%5.03) ise diğerlerine oranla nispeten yüksek olarak kaydedilmiştir.

Anahtar Kelimeler – *Bacillariophyta, Yağ asidi, Gomphonema olivaceum*

1. Introduction

Algae, which are divided into microalgae and macroalgae, are oil producers that very effectively convert carbon dioxide and sunlight into energy through photosynthesis. One of the other important contributions of algae other than photosynthesis is that they greatly increase the organic matter of the water they are in. For this reason, the waters in which algae are found are extremely productive and are a source of food for other living things, and with these aspects, algae form the basis of the food chain in the ocean environment (Brady et al., 1994).

Algae cells convert solar energy into chemical energy through photosynthesis. Chemical energy is stored in the form of chemical compounds with specific biological activities, called “biologically active compounds” (Ariede et al., 2017).

Polyunsaturated fatty acids, PUFAs obtained from microalgae are important bioactive components with health benefits. Polyunsaturated fatty acids such as omega 3 and omega 6 in PUFAs attract attention. Because these fatty acids are essential fatty acids and cannot be synthesized in the human body (Ward and Singh, 2005).

In addition to its health benefits, its high PUFA content also allows algae to be used as a potential raw material in the cosmetic field (Servel et al., 1994).

In this study, it was aimed to investigate the fatty acid properties of a species included in the Bacillariophyta branch.

2. Material and Methods

2.1 Isolation and Culture of Algae

The algal species Gomphonema olivaceum, which is included in the Bacillariophyta branch, was brought to the Laboratory in plastic containers in water samples taken from the benthic habitats of the Yeşilırmak River and isolated there by mechanical isolation method. Then, it was transferred to eppendorf tubes under an inverted microscope and transferred to liquid culture and incubated in Allen, BG11 broth media, in a Sanyo MLR 351 brand climate cabinet at 26 °C (155 µmol / m2 /h, L: D period) (Lobban et al., 1988; Andersen, 2005). It was harvested after reaching a certain volumetric density and stored in the culture collection in a deep freezer at -86 °C to be used in the examination. Relevant sources were used in the diagnosis of algae (Lund, 2002; Prescott, 1979; Guiry and Guiry, 2021).

After extracting 5 g of algae species from the culture collection in 150 mL solvent (1:1 methanol + methylene chloride), fatty acid analysis was applied.

2.2. Determination of Fatty Acid Composition

2.2.1 Preliminary preparation of the algae sample

Dried algae samples were crushed into powder and 1 gram of each sample was used for fatty acid analysis.

In the determination of fatty acids, saponification, methylation and extraction processes were carried out. Fatty acids of algal species were determined by gas chromatography (GC) method.

The process steps are as follows;

In Step 1, 1 ml of NaOH + CH_30H, purified water solution was added to each tube to lyse the cells and rinsed for 5-10 seconds. Then, the test tubes were kept in boiling water at 100 °C for 5 minutes, rinsed in warm water for 10 seconds and incubated again in boiling water at 100 °C for 25 minutes to release the fatty acids.

In Step 2, 2 ml of the second solution (6N HCl+CH_3OH for methylation) was added to the test tubes and rinsed for 5-10 seconds. It was then incubated in an 80ºC water bath for 10 minutes and cooled in ice for 2 minutes.

In step 3, 1.25 ml of the third solution (methyletherhexane + MFBE) was placed in chilled tubes and rinsed for 10 minutes. Fatty acids were separated from the acidic phase and transferred to the organic phase, with the organic liquid at the top and the other at the bottom.

In Step 4, 3 ml of the fourth solution (NaOH + Deionized distilled water) was added to each tube and rinsed for 5 minutes. After 10 minutes of incubation at room temperature, the samples were analyzed by transferring them to gas chromatography tubes with a pasteur pipette. Methyl esters of fatty acids were carried out by FID (Flame Ionization Detector) and gas chromatograph with auto-injector. A 100-meter HP-88 capillary column was used for analysis. In the gas chromatograph, the injector block temperature was set to 210 °C and the detector block temperature to 230 °C. The flow rate of the carrier gas (helium) was 40mL/min (IUPAC, 1979).

2.2.2. Determination of fatty acids by gas chromatography

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.

2.3. Statistical Analysis

All studies were performed in triplicate and are given as mean (±) standard deviation (SD). Statistical analyzes were made using Microsoft Excel.

3. Results

Gomphonema olivaceum is a siliceous algae. In line with the analyzes made, a total of 24 essential oil components were detected in the algae content, some in trace amounts and some in high proportion. As a result of GC-MS analyzes made with hexane extract of *G. olivaceum*, the ratios of C16:0 Palmitic acid and C16:1 Palmitoleic acid were very high, while C18:0 Stearic acid, C14:0 Myristic acid and C20:0 Arachidic acid were relatively lower rate (Table 1, Figure 1).

Table 1. Fatty acid types and ratios of *Gomphonema olivaceum*

Yağ asitleri	**%**
C10:0 Caprinic acid	0.46±0.07
C14:0 Myristic acid	**6.17**±1.02
C14:1 Myristoleic acid	0.41±0.02
C15:0 Pentadecanoic acid	1.14±0.09
C15:1 Cis-10-Pentadecanoic acid	0.17±0.05
C16:0 Palmitic acid	**32.04**±1.45
C16:1 Palmitoleic acid	**30.97**±1.07
C17:0 Heptadecanoic acid	2.00±0.09
C18:0 Stearic acid	**6.93**±0.67
C18:1 n9c Oleic acid	3.38±0.22
C18:2n6t Linolelaidic acid	0.07±0.04
C18:2n6c Linoleic acid	0.79±0.23
C18:3n6 Gama Linoleic acid	1.03±0.09
C20:0 Arachidic acid	**5.03**±1.02
C18:3n3 Alfa Linoleic acid	1.08±0.76
C20:1 Cis-11-eicosenoic acid	0.15±0.30
C20:3n6 Dihomo-gamma-linolenic acid	1.32±0.07
C21:0 Heneicosanoic acid	0.49±0.15
C22:1n9 Erucic acid	1.05±0.76
C20:3n3 Eicosatrienoic acid	0.11±0.09
C20:5n3 Eicosapentaenoic acid	2.06±0.18
C24:0 Lignoceric acid	2.15±0.22
C24:1 Nervonic acid	0.84±0.14
C22:6n3 Docosahexaenoic acid	0.15±0.09

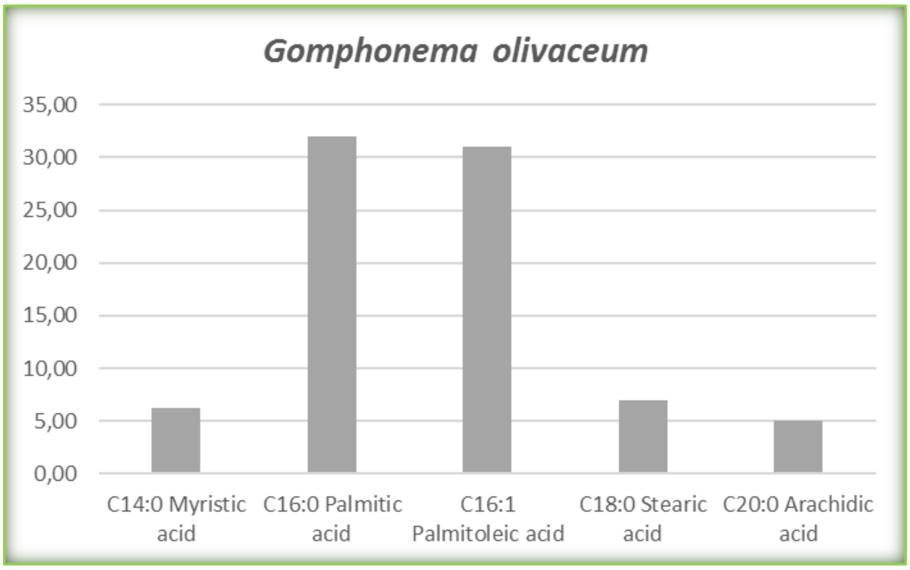

Figure 1. Fatty acid percentages of Gomphonema olivaceum

4. Discussion and Conclusion

The oil content of algae species varies according to the species. However, the essential fatty acids it contains are much more than other land plants. Fatty acids have an important function in human and animal nutrition because they serve as building blocks for fats in the organism and are the building blocks of cell membranes (Demirel and Özpınar, 2003).

According to the analyzes of *Gomphonema olivaceum*, a total of 24 essential oil components were detected, some in trace amounts and some in high proportion. C16:0 Palmitic acid and C16:1 Palmitoleic acid ratios were found to be very high, while C18:0 Stearic acid, C14:0 Myristic acid and C20:0 Arachidic acid were found to be relatively low (Table 1, Figure 1).

Erkaya and Yalçın (2021), in their study with *Spirogyra* species, fatty acid determination was made. In the study with two species, C10:0 Caprinic acid 0.05-0.10%, C16:0 Palmitic acid 21.25-20.78%, C18:2n6c Linoleic acid 5.17-5.99%, C20:0 Arachidic acid 0.60-0.51%, C18:3n3 alpha Linoleic acid is specified as 0.10-0.09%. In our study, C16:0 Palmitic acid was detected at a higher rate.

Algae, which are of great importance for the preservation of the integrity of the ecosystem, are also very rich in high-value bioactive compounds. These compounds are of high valence and are used in the food, pharmaceutical and cosmetic industries. Due to these high-value compounds, algae are used industrially as food raw materials or additives, in cosmetics and commercial areas such as pharmaceutics. High valence compounds obtained from algae; polyunsaturated fatty acids (PUFA), polysaccharide, protein, pigment, sterol, vitamin and other compounds. Polyunsaturated fatty acids obtained from algae, PUFAs are important bioactive components with health benefits. Polyunsaturated fatty acids such as omega 3 and omega 6 in PUFAs attract attention. Because these fatty acids are essential fatty acids and cannot be synthesized in the human body (Akyıl et al., 2016; Ward and Singh, 2005).

Durmaz et al. (2008) determined the fatty acid, alpha tocopherol and total pigment amount of Ulva spp. In the samples, total saturated fatty acids (SAT) were determined as 37.2%, total polyunsaturated fatty acids (PUFA) 32.0%, total monounsaturated fatty acids (MUFA) 23.2%. In the PUFA group, fatty acids such as 18:3(n-3) 5.4%, 18:3(n-6) 1.6%, 18:4 (n-3) 6.5% and 20:5 (n-3) 4.4% was detected. In our study, C16:0 Palmitic acid and C16:1 Palmitoleic acid, C18:0 Stearic acid, C14:0 Myristic acid and C20:0 Arachidic acid were found in high values.

Algae, especially in the food industry, as they contain primarily protein, pigments such as β-glucan, vitamins, minerals, β-carotene, astaxanthin, and important bioactive compounds. are used in many different sectors. According to their dry biomass weight, they can contain 9-50% protein, 7-50% carbohydrates and 7-50% fat. The most important microalgae species used commercially are Isochrysis, Dunaliella, Chaetoceros, Chlorella and Spirulina (Sankaran et al., 2018).

Peksezer et al. (2021), the total fat and fatty acid profile of Ulva rigida was determined. The dominant saturated fatty acids (SFA) are palmitic acid and stearic acid. The highest level of palmitic acid was found to be 40.64% in the 4th station samples, and the lowest level was 27.78% in the 1st station samples. The highest level of stearic acid was found to be 12.49% in the 4th station samples, and the lowest level was 2.71% in the 2nd station samples. Monounsaturated fatty acids (MUFA) are palmitoleic acid and oleic acid. The highest level of palmitoleic acid was found to be 3.69% at the 2nd station, and the lowest level was 1.69% at the 3rd station. The highest level of oleic acid was 14.08% in the 2nd station samples, and the lowest level was 6.44% in the 3rd station samples. The highest level of gamma-linolenic acid, the dominant fatty acids in polyunsaturated fatty acids (PUFA), was 8.21% at the 2nd station and the lowest level was 2.70% at the 4th station. In our study, the amount of C16:1 Palmitoleic acid was determined to be higher as 30.97%.

In addition to consuming seaweed as food, iodine-rich ones are used in thyroid treatment, intestinal disorders, hypocholesterolemic and hypoglycemic agents, malnutrition thanks to rich minerals and vitamins, hydrocolloids such as agar, alginate and carrageenan are used in many fields such as medicine and pharmacy (El Gamal, 2012).

It contains different biochemical compounds, including algae, polysaccharides, proteins and other macro and micro elements. They produce primary metabolites to perform their physiological functions such as growth, development or reproduction. Under different stress conditions such as exposure to UV radiation, salinity, temperature changes or environmental poisons, it produces secondary metabolites. These primary metabolites are; Contains polysaccharides, proteins, amino acids and fatty acids. Secondary metabolites are pigments, phenolic compounds, sterols, vitamins and other bioactive substances. Lipids found in algae include fatty acids, glycol and phospholipids, sterols (Pereira, 2018).

Thanks to these high-value compounds, algae will be used industrially as a food raw material or additive, in cosmetics and in commercial areas such as pharmaceutics.

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