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A STUDY ON DETERMINATION OF TOTAL PHENOLIC AND PROTEIN AMOUNTS OF WASTE GREEN ALGAE OF MAMASIN DAM LAKE (AKSARAY-TURKEY)

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Keywords

Algal Proteins, Phenolic Compounds, Green Protein Sources, Waste Algae Utilization.

Abstract

The excessive proliferation of green algae in aquatic ecosystems threatens aquatic life, leading to oxygen depletion and water pollution. This study investigates two common green algae species, *Ulva sp.* and *Cladophora sp.*, with potential in terms of protein and phenolic compounds. *Cladophora sp.* and *Ulva sp.* extracts were analyzed for total phenolic content using the Folin-Ciocalteau method. Despite lower phenolic content compared to specific plant species, both algae species exhibit various phenolic compounds. GC-MS analysis indicates the presence of major compounds such as limonene in *Cladophora sp.* and Tetradec-1-ene in *Ulva sp.*, suggesting potential applications in the pharmaceutical and cosmetic industries. Despite modest protein amounts, the study emphasizes that algae, aligned with the increasing interest in plant-based nutrition, are a promising source for plant-based protein production. *Ulva sp.* and *Cladophora sp.* algae demonstrate potential as alternative protein sources and reservoirs of bioactive phenolic compounds from waste sources. This study pioneers further research in the food, pharmaceutical, and cosmetic industries to contribute to sustainable water resource utilization.

MAMASIN BARAJI GÖLETİ'NİN ATIK YEŞİL ALGLERİNİN TOPLAM FENOLİK VE PROTEİN MİKTARLARININ BELİRLENMESİ ÜZERİNE BİR ÇALIŞMA (AKSARAY-TÜRKİYE)

Anahtar Kelimeler

Alg Proteinleri, Fenolik Bileşikler, Yeşil Protein Kaynakları, Atık Yosun Kullanımı.

Öz

Su ekosistemlerinde yeşil alglerin aşırı çoğalması sudaki yaşamı tehdit ederek oksijen tükenmesine ve su kirliliğine yol açmaktadır. Bu çalışma, protein ve fenolik bileşikler açısından potansiyeli olan iki yaygın yeşil alg türü olan *Ulva sp.* ve *Cladophora sp.*'yi incelemektedir. *Cladophora sp.* ve *Ulva sp.* ekstraktları, toplam fenolik içeriği Folin-Ciocalteau yöntemi kullanılarak analiz edildi. Fenolik içerik, belirli bitki türleriyle kıyaslandığında düşük olmasına rağmen, her iki alg türü de çeşitli fenolik bileşiklere sahiptir. GC-MS analizi, *Cladophora sp.*'de limonen ve *Ulva sp.*'de Tetradec-1-en gibi ana bileşiklerin tespit edildiğini göstererek, bunların farmasötik ve kozmetik uygulamalarda kullanılabileceğini işaret etmektedir. Çalışma, beslenme alanındaki artan ilgiye paralel olarak, mütevazı protein miktarlarına rağmen, alglerin bitkisel bazlı protein üretimi için umut verici bir kaynak olduğunu vurgular. *Ulva sp.* ve *Cladophora sp.* algleri, atık kaynaklardan elde edilebilecek alternatif protein kaynakları ve biyoaktif fenolik bileşik rezervuarları olarak potansiyel gösterir. Bu çalışma, sürdürülebilir su kaynakları kullanımına katkı sağlamak amacıyla gıda, ilaç ve kozmetik endüstrilerinde daha fazla keşfe öncülük etmektedir.

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Highlights

- Exploring algae as sustainable protein and phenolic sources.
- Protein isolation from *Ulva sp.* and *Cladophora sp.* for potential industrial use.
- Addressing environmental challenges through algae-based solutions.

Purpose and Scope

The primary purpose of this paper is to explore the potential of marine plants, specifically *Ulva sp.* and *Cladophora sp.*, as valuable sources of both protein and phenolic compounds. The research aims to address the excessive proliferation of green algae in aquatic ecosystems, offering insights into their protein and phenolic content. The scope involves investigating the utilization of waste algae for sustainable industrial applications, particularly in the fields of food, pharmaceuticals, and cosmetics.

Design/methodology/approach

The objectives of the research are achieved through a multi-faceted approach. The study involves collecting samples of green filamentous algae from Mamasın Dam Lake, followed by careful processing and analysis. Methodologies include the determination of total phenolic compounds using the Folin-Ciocalteau method, GC-MS analysis for identifying bioactive components, and protein extraction and concentration using the Bradford assay. The theoretical scope encompasses the potential industrial applications of algae in various sectors.

Findings

The research findings reveal that both *Ulva sp.* and *Cladophora sp.* exhibit phenolic compounds and proteins, making them promising candidates for industrial use. GC-MS analysis identifies specific compounds such as limonene in *Cladophora sp.* and Tetradec-1-ene in *Ulva sp.* These findings contribute to the understanding of the bioactive potential of waste algae and highlight their suitability as alternative protein sources.

Originality

The study introduces valuable insights into the diverse applications of waste algae, emphasizing their potential in sustainable industries. This research adds value to the scientific community by providing a detailed analysis of marine plants' bioactive components, paving the way for further investigations into their environmental and industrial significance.

1. Introduction

The excessive proliferation of green algae can form a detrimental cover on the surface of water ecosystems, posing a danger to other organisms. Additionally, the decomposition of algae by bacteria can lead to high oxygen consumption, consequently reducing the oxygen levels on the water surface (Jousson et al., 2000; Mihranyan, 2011). Disposing of these natural green algae in a waste state, which pollutes water masses and threatens the ecosystem, becomes crucial for preserving aquatic ecosystems. Nowadays, there is a growing interest in natural compounds derived from organisms in aquatic ecosystems. Proteins successfully isolated from algae, particularly those that have attracted attention due to their substantial protein content, have been the subject of extensive research and are currently being produced on an industrial scale for integration into human diets (Rasala and Mayfield, 2015; Geada et al., 2021; Ijaola et al., 2023). Thanks to this, seaweed and microalgae are increasingly being recognized as valid protein sources (Bleakley and Hayes, 2017). Certain varieties of seaweed and microalgae have been discovered to possess protein levels akin to those found in conventional protein sources like meat, eggs, soybeans, and milk (Fleurence, 1999; Sousa et al., 2008).

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Algal proteins, encompassing a full spectrum of essential amino acids, emerge as a noteworthy alternative protein source (Lourenço et al., 2004; Barbarino and Lourenço, 2005; Becker, 2007; Safi et al., 2014). Algae stand out for their tendency not to be endemic and their abundance. These characteristics make algae a sustainable raw material source for fuel, food, polymer, and pharmaceutical industries (Viegas et al., 2015). Comprising essential components like lipids, proteins, and carbohydrates, algae offer protein concentrates that find applications across various industries, including food, feed, and bulk chemicals (Chacón-Lee and González-Mariño, 2010). The isolation of bioactive secondary metabolites, specifically phenolic compounds, is also possible from these waste-state algae. Secondary metabolites are highly active compounds that enhance the survival chances of the organism they are present in, primarily plants, allowing them to adapt to their environment (Azmir et al., 2013; Santos et al., 2016). The diversity of phenols leads to a focus on plants; other organisms, such as algae, can also synthesize these compounds. (Jimenez-Lopez et al., 2021). These hydroxyl group-based compounds have numerous positive impacts on human health, attributed to their significant effects such as scavenging free radicals and reactive oxygen species (Chisté et al., 2013; Barbosa-Pereira et al., 2014). The discovery, development, and industrial-scale production of these natural metabolites, common in many algal classes, are crucial. Such natural metabolites have the potential to replace synthetic compounds linked to specific health problems or disorders, not only offering benefits but also addressing concerns related to synthetic alternatives (Liu et al., 2011; Al-Saif et al., 2014). Therefore, the isolation and utilization of both protein and phenolic bioactive compounds from waste-state algae, which occasionally contribute to algal blooms, are feasible.

Ulva sp. is a *Chlorophyta* division member and a disk-shaped, thin, and flat species known as sea lettuce. *Cladophora sp.*, on the other hand, is found in various environments, from seas to freshwater, providing habitat and food for organisms. *Ulva sp.* can undergo proliferation by absorbing chemical compounds containing phosphorus or nitrogen in eutrophic waters and has high protein content (Zhang et al., 2015). *Cladophora sp.* has a high metal binding capacity and complex heteropolysaccharides in its green algal cell wall. Both algal species have environmental significance in various ecosystems; *Ulva sp.* stands out as a potential protein source for human nutrition, while *Cladophora sp.* plays a crucial role in understanding environmental impacts due to its high metal binding capacity (Ito and Hori, 1989; Ramelow et al., 1992; Holan and Volesky, 1994). In recent years, the number of studies on algae processing methods and their use as a protein source has rapidly increased (Ghribi et al., 2015; Waghmare et al., 2016; Vernes et al., 2019). These studies aim to isolate high amounts of proteins from algae in waste status and transform them into high-value products.

This study determined the total phenolic components of *Ulva sp.* and *Cladophora sp.* algae and identified bioactive components using the GC-MS device. Additionally, for the first time in this study, the protein concentration of these algae was determined, and their potential for future industrial use was evaluated.

2. Material and Method

2.1. Material

The proliferation of green filamentous algae *Ulva sp.* and *Cladophora sp.* was observed in Mamasın Dam Lake in Gucunkaya (38°22'58" N, 34°2'17" E). Macroalgal mats formed in the riverbed and on the rocks were collected, washed, and left to dry in a shaded environment. The dried filamentous algae were powdered using a waring commercial blender.

2.2. Analysis of Total Phenolic Content

The total phenolic compound content in algae extracts was assessed using the Folin–Ciocalteu method (Wu et al., 2007). Extracting 0.5 g of dried material was achieved using 20 mL of 75% methanol for 24 h with the VWR Mini Shaker. Each 100 µl of the sample extract was combined with 3000 µl of a stock solution (4% sodium carbonate) and 100 µl of Folin-Ciocalteu reagent (2N), followed by two inversions for thorough mixing. The blank was similarly formulated using 100 µl of methanol (75%). After a 30-minute incubation at 25 °C, absorbance measurements at 760 nm were taken for the samples. Total phenolics were then estimated by applying a rutin calibration curve. The concentration range was 0.1-0.8 mg/mL. Total phenolics were determined in milligrams of rutin equivalent per milliliter of extract.

2.3. GC-MS

For the analysis, samples were prepared utilizing Solid-phase Microextraction (Jarmalavičienė et al., 2008) with a Stableflex (TM) 50/30 micrometers layer PDMS/CAR/DVB fiber (Supelco, USA). The GC-MS analysis was conducted using a GC-2010 model (Shimadzu) gas chromatograph and a mass spectrometer with the GC-MS-QP2010 model detector (Shimadzu). Effective separation was achieved using a Restek RTX-5MS column (30 M x

0.25µm, 0.25 mm). The GC-MS analysis parameters employed are presented in Table 1, and the temperature gradient is provided in Table 2.

Table 1. Parameters for GC-MS analysis

Mobile Phase	Flow Rate (mL/min)	Injector Temperature (°C)	Mode	Split Ratio	Ion Source (°C)	Ionization Energy (eV)	Mass Scanning Range (m/z)
He gas	1.7	280	Split	1:10	220	70	40-600

Table 2. Oven conditions for GC-MS analysis

	Rate (°C/min)	Value (°C)	Hold Time (min)	Run Time (min)
Initial	-	50	2	2
Ramp 1	5	200	2	34
Ramp 2	20	280	15	52

2.4. Protein Extraction

The experiment was conducted with certain adjustments according to the method outlined by Barbarino and Lourenço (2005). Initially, 4ml of ultra-pure water was added to 50 mg of dry material, and left at 4 °C for 12 h. Following this, the material was ground with a homogenizer for 5 min at 4 °C. Subsequently, the mixture underwent centrifugation at 15000g for 20 min at 4 °C, and the supernatant was gathered and kept at 4 °C. 1.0 mL of 0.1N NaOH was added to the sediment and permitted to stand at 20 °C for 1 h with intermittent stirring. Subsequently, it was subjected to centrifugation at 15000g for 20 min at 20 °C, and the resulting supernatant was amalgamated with the initial one while disregarding the pellets. For the precipitation of proteins, 25% TCA was introduced, and the amalgamation underwent a 30-minute ice bath. After centrifugation at 15000g for 20 min at 4 °C, the supernatant was removed, and the residue was rinsed with cold 10% TCA. This was succeeded by a 2-min centrifugation at 15000g at 4 °C, and once again, the supernatant was removed. The protein was dissolved using 5% TCA (5:1) and then centrifuged at 15000g for 20 min at 20 °C. The final supernatant was discarded, completing the preparation of the protein pellet.

2.5. Protein Concentration via Bradford Assay

The analysis was carried out with certain alterations following the description provided by (Giannakos, 2016). The resuspension of precipitated protein was performed in 0.5 mL of 1.0 N NaOH. To create the dye solution, 100 mg of CBB G-250 was solubilized in 50 mL of 95% ethanol, with the subsequent addition of 100 mL of 85% H3PO4. The resulting solution underwent further dilution with ultra-pure water to achieve a final volume of 1.0 L. Using 5 milliliters of the solution for every 0.1 mL sample; the absorbance was gauged at 595 nm, precisely 5 min after instigating the chemical reaction at 20 °C. Calibration curves were generated by employing bovine serum albumin (BSA). The concentration range is 0.1-0.8 mg/mL.

4. Experimental Results

The total phenolic content of methanol extracts from waste green algae species, *Cladophora sp.* and *Ulva sp.* was determined in terms of rutin equivalent using the Folin-Ciocalteau method. The total phenolic content was determined using the rutin standard curve and regression equation shown in Figure 1. As a result of the calculation, the phenolic content for *Cladophora sp.* algae was calculated as 0.35 ± 0.027 RE, while for *Ulva sp.* algae, it was calculated as 0.33 ± 0.018 RE. It has been determined that 0.5 g of *Cladophora sp.* contains approximately 66 mg of total phenolic compounds, and 0.5 g of *Ulva sp.* contains about 7 mg of total phenolic compounds. Total phenolics are lower than those observed in some previously studied plants and microalgae (Tawaha et al., 2007; Saeed et al., 2012; Sirbu et al., 2019). However, we anticipate future potential use for these waste materials. The Folin–Ciocalteu method provides general comparative results, but is not specific for determining different compounds or phenolic classes. Therefore, an attempt was made to determine the phenolic content of methanol extracts of *Cladophora sp.* and *Ulva sp.* algae samples using GC-MS.

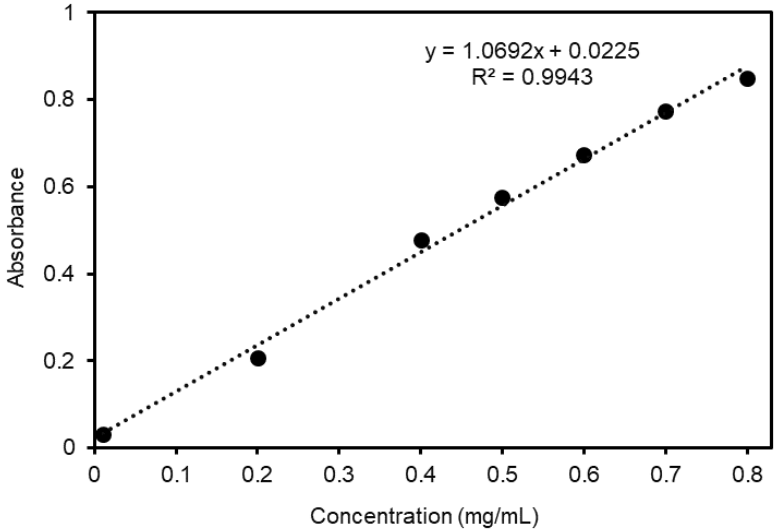

Figure 1. Standard curve using rutin and regression equations for total content of phenolic compounds

Chromatograms of *Cladophora sp.* and *Ulva sp.* extracts are presented in Figure 2. Various phenolic compounds were identified in the extracts, with some notable ones. Majorly, limonene (23.12% Area) was detected in *Cladophora sp. Cladophora sp.* also contained high volumes of Oct-3-en-2-ol (12.87% Area) and Tetradec-1-ene (11.7% Area). In the analysis conducted for *Ulva sp.*, Tetradec-1-ene (71.68% Area) stood out among various phenolic compounds. Particularly, limonene, identified as a major component in *Cladophora sp.*, has been the subject of numerous studies due to its valuable properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Roberto et al., 2010; Santana et al., 2020; Araújo-Filho et al., 2021; Han et al., 2021). It is foreseeable that this valuable component could be extracted from this waste algae species and commercialized in the future.

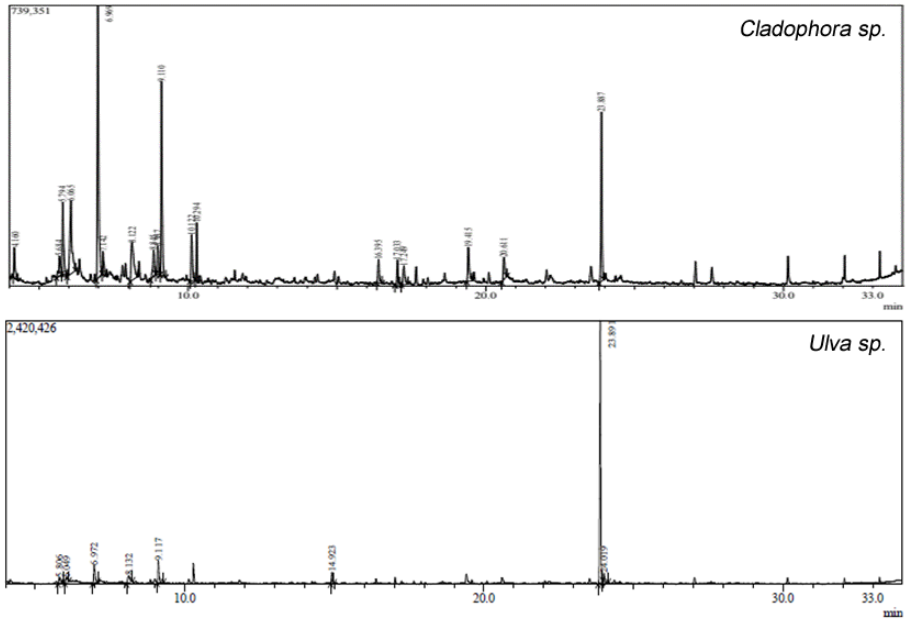

Figure 2. Typical GC-MS chromatogram of *Cladophora sp.* extract and *Ulva sp.* extract

Protein isolation was performed from *Cladophora sp.* and *Ulva sp.* samples, and their concentrations were attempted to be determined using the Bradford method. A calibration curve was established using BSA, and calculations were made using the regression equation. As a result of the analysis, the protein concentration was determined as 0.202 ± 0.04 and 0.265 ± 0.09 mg/ml for *Ulva sp.* and *Cladophora sp.*, respectively. It has been determined that 50 mg of *Cladophora sp.* contains approximately 1.01 mg of protein, and 50 mg of *Ulva sp.* contains about 0.132 mg of protein. The detected protein amounts may seem relatively low compared to many other sources. However, especially in recent years, the increasing concern about climate change and carbon footprint has led to a growing interest in vegan nutrition and plant-based protein products (Heusala et al., 2020; Gaillac and Marbach, 2021). Therefore, proteins produced from sources other than plants, such as algae, are highly valuable. Additionally, algae, particularly compared to plants, have an intriguingly wide range of nutritional qualities,

containing not only proteins but also peptides, carbohydrates, lipids, vitamins, minerals, and other valuable trace elements (Becker, 2007). The waste-state *Cladophora sp.* and *Ulva sp.* algae analyzed in the current study show promise as alternative protein sources.

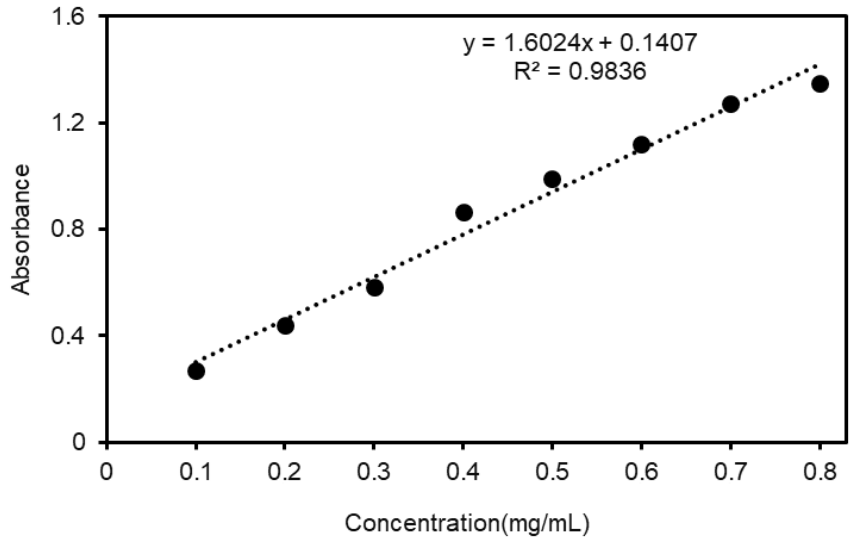

Figure 3. Standard curve using human serum albumin and regression equations for protein concentration

5. Result and Discussion

The excessive proliferation of green algae in aquatic ecosystems poses a significant threat to the overall health and balance of these environments. The detrimental impact includes forming algal covers on water surfaces, leading to oxygen depletion and water pollution. Addressing this issue is crucial for preserving of aquatic ecosystems, and disposing of natural green algae in waste states further exacerbates the problem.

This study focused on two common green algae species, *Ulva sp.* and *Cladophora sp.*, prevalent in the Uluırmak River. It is aimed to investigate the potential of these algae as reservoirs for both protein and phenolic compounds. Despite the relatively lower phenolic content compared to certain plants and microalgae, both *Ulva sp.* and *Cladophora sp.* exhibited various phenolic compounds. GC-MS analysis identified major compounds such as limonene in *Cladophora sp.* and Tetradec-1-ene in *Ulva sp.*, hinting at potential applications in pharmaceuticals and cosmetics.

Protein isolation from *Cladophora sp.* and *Ulva sp.* was carried out, and their concentrations were determined. While the detected protein amounts may appear modest, the study emphasizes the growing interest in plant-based and sustainable nutrition. Algae offer a promising avenue for plant-based protein production with their diverse nutritional qualities. *Ulva sp.* and *Cladophora sp.* algae from waste sources demonstrate potential as alternative protein sources and reservoirs of bioactive phenolic compounds.

This study opens avenues for further exploration of these algae in food, pharmaceuticals, and cosmetics industries, contributing to the sustainable utilization of aquatic resources. The findings encourage ongoing research to unlock the full potential of algae in addressing environmental challenges and meeting the increasing demand for alternative and sustainable resources in various industries.

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Conflict of Interest

No conflict of interest was declared by the authors.

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