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Bioactive secondary metabolites and Antioxidant activity of *Spirulina*

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Abstract

Algae are abundant in bioactive substances that could find use in a variety of sectors, such as functional foods, nutraceuticals, and pharmaceuticals. The present investigation examined the antioxidant capacity of crude extracts derived from two distinct strains of algae, namely *Spirulina* and *Pediastrum*. The selection of these algae was based on their specific morphologies: *Pediastrum* is made up of many individual cells arranged in a flat, disk-like shape; and *Spirulina* has a filamentous, spiral-shaped structure. The DPPH scavenging assay was used to assess the antioxidant activity of the crude extracts, which were prepared using a variety of solvents, including H_2O, methanol, ethanol, dichloromethane, and ethyl acetate. With a DPPH scavenging capacity of 89.55%, *Spirulina* showed the highest antioxidant activity of the algal strains tested, especially in methanol extracts. This emphasizes *spirulina's* potential as a rich source of bioactive chemicals that have the ability to scavenge free radicals. Methanol extracts were shown to be highly effective in extracting antioxidant components, as evidenced by their consistent display of the highest antioxidant activity among the three algae species. On the other hand, the lowest antioxidant activity was found in water extracts, indicating that the solubility of antioxidant chemicals in water is limited.

Key words: *Spirulina*, *Pediastrum*, bioactive chemicals, antioxidant

1. Introduction

Spirulina, or Arthrospira, is the most abundant microalgae and a commercially significant filamentous cyanobacterium with an annual production surpassing 3,000 tons **[1],[2] .***Spirulina* is a tropical and subtropical lake plant that grows best in warm, high-carbonate and high-bicarbonate lakes with high salinity and pH values. *Spirulina*, widely known for its abundance of nutrients, is a powerful supplier of important fatty acids, minerals, vitamin B12, ß-carotene, proteins, and especially linolenic acid. *Spirulina* satisfies the proportions advised by the Food and Agriculture Organization (FAO) with a protein level of between 60 and 70 percent dry weight **[3], [4].**

A unique class of oxygenic photosynthetic organisms known as cyanobacteria is found in a wide range of terrestrial and aquatic habitats and is remarkably tolerant of harsh conditions **[5].**Their ecological relevance is increased by their involvement in the biogeochemical cycle and the synthesis of bioactive chemicals**[6].**Cyanobacteria, which were formerly thought to be prokaryotic algae, had their taxonomic status reevaluated in light of their morphological and characteristics of the cytology. However, the issue lies in the morphological variety that is impacted by external factors, making precise taxonomic classification more difficult **[7]**. It is predicted that incorrect organism phylogenies result from the misidentification of up to 50% of cyanobacterial strains in culture collections. This highlights the need of having a thorough awareness of the distinctive traits of *Spirulina*, as well as its ecological function and the difficulties involved in accurately classifying the cyanobacteria **[8].**

Beyond its commercial significance, spirulina is a great resource for meeting nutritional demands worldwide because of its unique properties **[9].** *Spirulina* presents a prospective treatment for malnutrition due to its richness of proteins, minerals, and critical elements, particularly in areas with limited access to a variety of nutrient-dense food sources **[10].** The promise of Spirulina as an affordable and sustainable dietary supplement is further highlighted by the fact that its amino acid composition aligns with FAO recommendations **[11].**

Beyond their nutritional value, cyanobacteria of which *Spirulina* is an excellent example are very adaptable. Their ability to flourish in harsh conditions places them in a key role in ecological processes. **[12].**

Through photosynthesis, cyanobacteria contribute to the cycle of nutrients and atmospheric oxygen generation, which is an essential part of the biogeochemical cycle. **[13].** Moreover, their capacity to generate bioactive chemicals contributes to their significance by offering prospects for utilization in biotechnology, medicines, and environmental remediation. Notwithstanding the difficulties associated with taxonomic classification, realizing the ecological significance of *Spirulina* and its cyanobacterial relatives is crucial to maximizing their

potential in a variety of domains, ranging from environmental sustainability to nutrition **[14].**

2.Material and methods

1. Algae sample

The algae samples were purchased from biotechnology unit at the National Research Center, Egypt.

2. Extraction of bioactive secondary metabolites

The extraction process utilized a strategic selection of solvents, namely H2O, Methanol, Ethanol, Dichloromethane, and Ethyl Acetate. Each solvent was chosen based on its specific properties to target a diverse range of bioactive compounds within the algae. The extraction process was meticulously designed to capture a wide array of bioactive compounds. Different solvents were employed to target specific types of molecules, ensuring a comprehensive extraction approach that enhances the overall reliability of subsequent analyses.

3. Evaporation for Crude Extracts:

Following the extraction process, an additional step involved the evaporation of solvents to obtain the crude extracts from the organic solvent-algae mixtures. This step was carried out using a rotary evaporator, a sophisticated instrument known for its efficiency and precision in solvent evaporation from liquid samples.

4. Screening of DPPH antioxidant activity of the different extracts

The DPPH radical scavenging assay was used to screen the different extracts according to their antioxidant activity. 0.1 mM DPPH was dissolved in methanol to form a solution, and 2.4 mL of this solution was then added to 1.6 mL of extracts at a concentration (500 µg/mL). the reaction mixture was kept at room temperature for 30 minutes in the dark. At 517 nm, the mixture's absorbance was determined spectrophotometrically **[15]**. The following equation was used to compute the percentage of DPPH radical scavenging activity:

% DPPH radical scavenging activity = $[(A_0 - A1)/A_0] \times 100$

Where A_0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard.

% DPPH radical scavenging activity = $[(A_0 - A1)/A_0] \times 100$

where A0 represents the control's absorbance and A1 represents the extractives' or standard's absorbance. The % of inhibition was then plotted against concentration.

3. Results

Sample Collection:

Based on earlier morphological examination two algal strains *Spirulina* and *Pediastrum* were taken from the biotechnology unit.

Table (1) Morphology of algae **.**

No	Algae name	Morphology
1	*Spirulina*	blue-green algae, that exhibits a distinctive morphology characterized by its filamentous, spiral-shaped structure. **[16]**
2	*Pediastrum*	Green algae composed of numerous individual cells arranged in a flat, disk-like shape. **[17]**

Extraction of Crude Extracts

To extract bioactive chemicals from powdered materials, various solvents including methanol, ethanol, dichloromethane, ethyl acetate, and H_2O were prepared. 10 g of each sample were dissolved in 100 mL of solvent for the extraction process, which was then followed by an overnight incubation at room temperature on a rotating shaker. After filtering, the mixtures were centrifuged for 15 minutes at 6,000 rpm.

Table (2) Summary of Solvent Extraction Parameters

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Solvent	Sample Amount (g)	Solvent Volume (mL)	Extraction Time	Filtration	Centrifugation Speed (rpm)	Centrifugation Time (min)
H2O	10	100	Overni ght	Yes	6,000	15
Methanol	10	100	Overni ght	Yes	6,000	15
Ethanol	10	100	Overnight	Yes	6,000	15
Ethyl Acetate	10	100	Overnight	Yes	6,000	15

dichloromethane	10	100	Overnight	Yes	6,000	15

Antioxidant Screening:

The antioxidant activity comparison study showed notable differences between the substances that were extracted using various solvents. Particularly, *spirulina* showed remarkable DPPH scavenging capacity, up to 89.55%. Due to its exceptional performance, *spirulina* was chosen for more discussion, indicating that it may be rich in bioactive chemicals that have the ability to scavenge free radicals. The effective extraction of these bioactive chemicals, which in turn contributed to the exceptional antioxidant activity of *spirulina*, was made possible by the selection of methanol as the extraction solvent. (Table 3) lists the antioxidant activity of the algal fractions as determined by their ability to scavenge DPPH when extracted with various solvents (dichloromethane, ethanol, methanol, and H_2O). The findings highlight the superior antioxidant activity of *spirulina* in comparison to another algal sample, highlighting the significance of solvent selection in the extraction of bioactive compounds.

In this work, two distinct algae species *Spirulina* and *Pediastrum* were examined for their antioxidant activity in the dichloromethane, ethyl acetate, ethanol, methanol, and H_2O fractions. The findings are shown in (Table 3)

Methanol extracts showed the highest antioxidant activity of all two algae species among the fractions studied; their values ranged from 81.6% to 89.55%. This implies that methanol is especially effective at removing antioxidant components from these types of algae. On the other hand, with values ranging from 14.33% to 19.03%, water extracts had the least antioxidant activity. This might be because antioxidant chemicals dissolve less readily in water than they do in organic solvents.

Variations are also visible when comparing the antioxidant activity of various algae species in the same solvent. in methanol extracts *Spirulina* showed the highest antioxidant activity (89.55%) and *Pediastrum* (81.6%) antioxidant activity. A possible explanation for these discrepancies is that the beneficial components found in each species of algae, such as vitamins, carotenoids, flavonoids, and phenolic compounds, differ in composition.

The antioxidant activity of the algal fractions was similarly impacted by the solvent selection. Ethyl acetate and ethanol fractions demonstrated moderate antioxidant activity, dichloromethane fractions demonstrated comparatively reduced activity, while methanol consistently displayed excellent antioxidant activity. The observed fluctuations in antioxidant activity may be ascribed to dissimilarities in the solvents' polarity and their capacity to extract particular antioxidant chemicals from the algae.

Table (3) Antioxidant activity of the algal fraction

	H_2O	**Methanol**	**Ethanol**	**Ethyl acetate**	**Dichloromethane**
Spirulina	14.33	89.55	52.01	44.61	62.99
Pediastrum	19.03	81.6	55.2	47.1	60.05

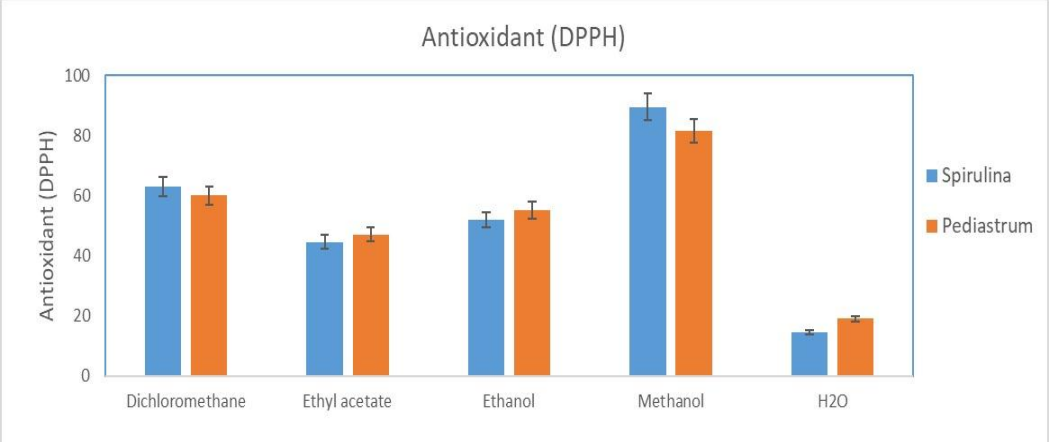

Fig (1) Antioxidant activity of the algal fraction

4. Discussion

There is a lot of potential for using bioactive chemicals extracted from algae in pharmaceuticals, nutraceuticals, and functional foods, among other applications [18]. *Spirulina* and *Pediastrum* are the two algal strains that were chosen for this investigation because to their unique morphologies, and various solvents were used to obtain crude extracts from each of them. Upon evaluating these extracts' antioxidant activity, major differences were found between the types

Spirulina was the most antioxidant-active algal strain that was tested; it was especially effective in methanol extracts, with an 89.55% DPPH scavenging capacity. The noteworthy antioxidant capacity of spirulina highlights its potential abundance in bioactive substances that possess the ability to scavenge free radicals. In order to effectively extract these bioactive chemicals from *Spirulina*, methanol was selected as the extraction solvent. This emphasizes the significance of solvent selection in enhancing antioxidant activity.

Upon comparing antioxidant activity across several solvents and algae species, intriguing tendencies were observed. Across two algae species, methanol extracts consistently displayed the best antioxidant activity, indicating that it is an effective way to extract antioxidant chemicals. On the other hand, water extracts showed the least amount of antioxidant activity, suggesting that the solubility of antioxidant molecules in water is limited.

Variations in antioxidant activity were also noted between the algae species in the same solvent. For example, *Spirulina* (89.55%) showed the highest antioxidant activity in methanol extracts, followed by *Pediastrum* (81.6%). These discrepancies could be explained by variances in the makeup of the bioactive substances found in each type of algae.

Including vitamins, carotenoids, flavonoids, and phenolic substances.
The antioxidant activity of the algal fractions was greatly impacted by the solvent selection. Dichloromethane fractions demonstrated comparatively reduced activity compared to methanol, which consistently displayed excellent antioxidant activity. Ethyl acetate and ethanol fractions displayed moderate activity. These results emphasize how crucial solvent choice is to maximizing the extraction of antioxidant-rich bioactive chemicals from algae.

5. Conclusion

As a result of our investigation, the most antioxidant-active strain of algae that we evaluated was *Spirulina* .This highlights the potential of *spirulina* as a rich supply of bioactive compounds with the capacity to scavenge free radicals. The highest antioxidant-active algal strain that was evaluated was *spirulina*, which exhibited an 89.55% DPPH scavenging capacity in methanol extracts, while *pedipastrum* showed an 81.6%) antioxidant activity. These differences could be accounted for by differences in the composition of the bioactive compounds present in each kind of algae.

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