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Bioactivity Assessment of *Chlorella* Algae as Antioxidant Potential

Basma N. Gamal[1], Ahmed A. Hamed[2], Mohamed E. El awady[3], Mohamed G. Battah[1] and Mervat G. Hassan[1]
[1]Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Egypt.
[2]Microbial Chemistry Department, National Research Centre, El-Buhouth Street, Dokki, Cairo, Egypt.
[3]Microbial Biotechnology Department, National Research Centre, El- Buhouth Street, Dokki, Cairo, Egypt.
E-mail: bgamal2011@gmail.com

Abstract

The study sought to determine the antioxidant capacity of two types of green algae: *Nannochloropsis*, a single-celled microalga, and *Chlorella*, a colonial green alga by examining their crude extracts made with different solvents. The morphological characterization of *Nannochloropsis*, which has a single-celled ovoid or spherical form, and *Chlorella*, which is characterized by flat, star-shaped collections of cells, revealed their distinguishing characteristics. Following the preparation of crude extracts with solvents like methanol, ethanol, dichloromethane, ethyl acetate, and water, the antioxidant activity of the extracts was assessed.

The significant antioxidant activity shown by the ethyl acetate fractions of *Nannochloropsis* and *Chlorella* suggests that ethyl acetate is a useful solvent for extracting antioxidant chemicals. Methanol fractions also showed increased antioxidant activity for *Nannochloropsis* than for *Chlorella*, indicating that methanol is a useful solvent for obtaining antioxidants from *Nanochloropsis* algae. These results highlight how distinct solvent fractions of *Nannochloropsis* and *Chlorella* algae exhibit varied degrees of antioxidant activity, highlighting the significance of solvent selection in the extraction of antioxidant chemicals. Overall, the findings point to *chlorella*'s potential as a natural antioxidant source with a range of uses in the food, pharmaceutical, and cosmetics industries. This calls for more research into the specific antioxidant compounds found in *chlorella* fractions and their possible health benefits.

Keywords: Algae, *Chlorella*, antioxidant, extraction, DPPH.

Introduction

Of the nannoplanktonic coccoid green algae, *Chlorella* is a classic example [1,2]. Its importance goes beyond simple taxonomy; it plays a crucial part in many biological systems and human endeavors [3, 4]. *Chlorella*, with its distinctive coccoid shape and green coloring, is a prime example of the variety and flexibility of green algae in aquatic settings [5,6]. *Chlorella* is a taxonomic group representative that makes a significant contribution to the ecological balance of aquatic environments [7, 8]. It is a vital component of primary productivity in both freshwater and marine environments due to its exceptional photosynthetic abilities and capacity to use solar radiation for carbon fixation [9, 10]. Furthermore, *Chlorella* forms the foundation of intricate food webs and supports biodiversity by providing an essential food supply for a wide variety of aquatic creatures [11,12].

Beyond its ecological importance, *Chlorella* has a great deal of potential for use in human applications, especially in the fields of nutrition, biotechnology, and health. Its diverse range of nutrients, including proteins, carbs, fats, vitamins, and minerals, makes it a possible superfood with numerous health advantages for humans [4,13]. Moreover, *Chlorella*'s ability to produce biofuel, clean wastewater, and sequester carbon highlights its importance in sustainable development projects [14,15]. *Chlorella*, however small in size, is a major player in scientific research and industrial applications. It is used as a model organism for studies ranging from cell biology and photosynthesis to bioremediation and the development of biopharmaceuticals [16]. Its fast growth rate, genetic tractability, and suitability for laboratory cultivation make it a priceless instrument for deciphering basic biological processes and maximizing nature's potential to benefit humanity [17, 18].

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

A deliberate choice of solvents, including H2O, Methanol, Ethanol, Dichloromethane, and Ethyl Acetate, was used in the extraction procedure. Based on its unique characteristics, each solvent was selected to target a variety of bioactive chemicals found in the algae. The extraction procedure was carefully planned to extract a variety of bioactive substances. To target different kinds of molecules, different solvents were used; this resulted in a thorough extraction process that improves the overall dependability of the studies that follow.

3. Evaporation for Crude Extracts:

To produce the crude extracts from the organic solvent-algae combinations, evaporating solvents was a step that was added after the extraction process. A rotary evaporator, a high-tech device renowned for its accuracy and efficiency in solvent evaporation from liquid samples, was used for this stage.

4. **Screening of DPPH antioxidant activity of the different extracts**

The assay for DPPH radical scavenging was employed to screen the various extracts based on their antioxidant activity, as documented by Desmarchelier et al., 1997) [19]. A solution of 0.1 mM DPPH was prepared by dissolving it in methanol; 2.4 mL of this solution was then combined with 1.6 mL of extracts to achieve a concentration of 500 µg/mL. For thirty minutes, the reaction mixture was left at room temperature in the absence of light. The mixture's absorbance was measured spectrophotometrically at 517 nm. To calculate the percentage of DPPH radical scavenging activity, the following equation was used:
(A0− A1)/A0] × 100 = ¬ [% DPPH radical scavenging activity]
where A0 represents the control's absorbance and A1 represents the extractives' or standard's absorbance.

Activities of the methanolic extract as antioxidants DPPH radical scavenging assay

A range of doses (100–1000 µg/mL) of extract in methanol were used for the DPPH radical scavenging experiment. The reaction mixture was allowed to sit at room temperature in the dark for 30 minutes after it had finished vertexing. Using spectrophotometry, the mixture's absorbance was determined at 517 nm. The vitamin C was mentioned. The following formula was applied to determine the percentage of DPPH radical scavenging activity:
(A0− A1)/A0] × 100 = ¬ [% DPPH radical scavenging activity]
where A0 represents the control's absorbance and A1 represents the extractives' or standard's absorbance. The % of inhibition was then plotted against concentration, and the IC50 was determined by looking at the curve. At each concentration, the experiment was conducted three times.

1. **Sample collection**

From the National Research Center's biotechnology division, two strains of algae have been gathered. After morphologically characterizing the isolated algae, it was determined that *Chlorella* and *Nannochloropsis* were the two types of algae. Flat, star-shaped cell aggregates are the defining feature of *chlorella*. The single-celled ovoid or spherical structure of *Nannochloropsis* is visible. These results shed important light on the variety of algae strains and their prospective uses in biotechnology, such as the synthesis of biofuel and medicinals.

Table (1) Morphological identification of collected algae.

Serial	Algae	Morphology
1	***Chlorella***	colonial morphology with flat, star-shaped aggregates of cells, showcased a unique cellular arrangement within its colonies
2	***Nanochloropsis***	a single-celled microalga with a spherical or ovoid shape, demonstrated small cell sizes, and ultrastructural insights were gained through electron microscopy, providing a glimpse into its cellular organelles

Extraction of Crude Extracts

Various solvents were developed to extract bioactive chemicals from powdered materials, including water, methanol, ethanol, dichloromethane, and ethyl acetate. After dissolving each 20 g sample in 200 mL of solvent, it was let to sit at room temperature for the whole night on a revolving shaker. The mixtures were filtered and then centrifuged for 15 minutes at 6,000 rpm (Table 2).

Table (2) Summary of Solvent Extraction Parameters

Solvent	Sample Amount (g)	Solvent Volume (mL)	Extraction Time	Centrifugation Speed (rpm)	Centrifugation Time (min)
H2O	20	200	24 h		
Methanol	20	200	24 h		
Ethanol	20	200	24 h	6,000	15
Ethyl Acetate	20	200	24 h		
dichloromethane	20	200	24 h		

Antioxidant activity of algal fractions

The results of the antioxidant activity analysis of the different solvent fractions of *Nannochloropsis* and *Chlorella* algae offer important new information about their possible health advantages as well as industrial uses. fractions of ethyl acetate of *Chlorella* and *Nannochloropsis* showed a remarkable 82.73% and 87.9%, respectively, of antioxidant activity. This demonstrates how well ethyl acetate extracts antioxidant chemicals from certain types of algae. Significant antioxidant activity was also shown by dichloromethane fractions for *Nannochloropsis* (69.78%) and *Chlorella* (67.98%). This suggests that ethyl acetate is a more effective solvent than dichloromethane for extracting antioxidants from *Chlorella* and *Nannochloropsis*. Compared to the dichloromethane and ethyl acetate fractions, their relative efficacy was comparatively lower, as seen by their respective percentages of 40.06% and 41.19%. These results imply that the antioxidant activity of the *Chlorella* and *Nannochloropsis* algae varies among their solvent fractions. The findings highlight the potential of these algae species as sources of natural antioxidants for a variety of uses in the food, pharmaceutical, and cosmetic industries as well as the significance of solvent selection in the extraction of antioxidant chemicals from algae. To fully understand the potential health advantages and industrial applications of the particular antioxidant chemicals found in these algal fractions, as well as to identify and define them, more research is necessary (Table 3, Figure 1).

On the other hand, for both Nannochloropsis and Chlorella, the methanol and ethanol fractions demonstrated modest antioxidant activity (38.41% and 49.4%, respectively).

Title (3) Antioxidant activity of the two algal fractions.

Sample	H_2O	Methanol	Ethanol	Ethyl acetate	Di chloro methane
Cho	22.10 ± 0.92	38.41 ± 1.36	49.40 ± 1.02	87.90 ± 0.81	67.98 ± 1.04
N.C.	17.74 ± 1.11	40.06 ± 0.87	41.19 ± 1.04	82.73 ± 0.90	69.78 ± 0.99

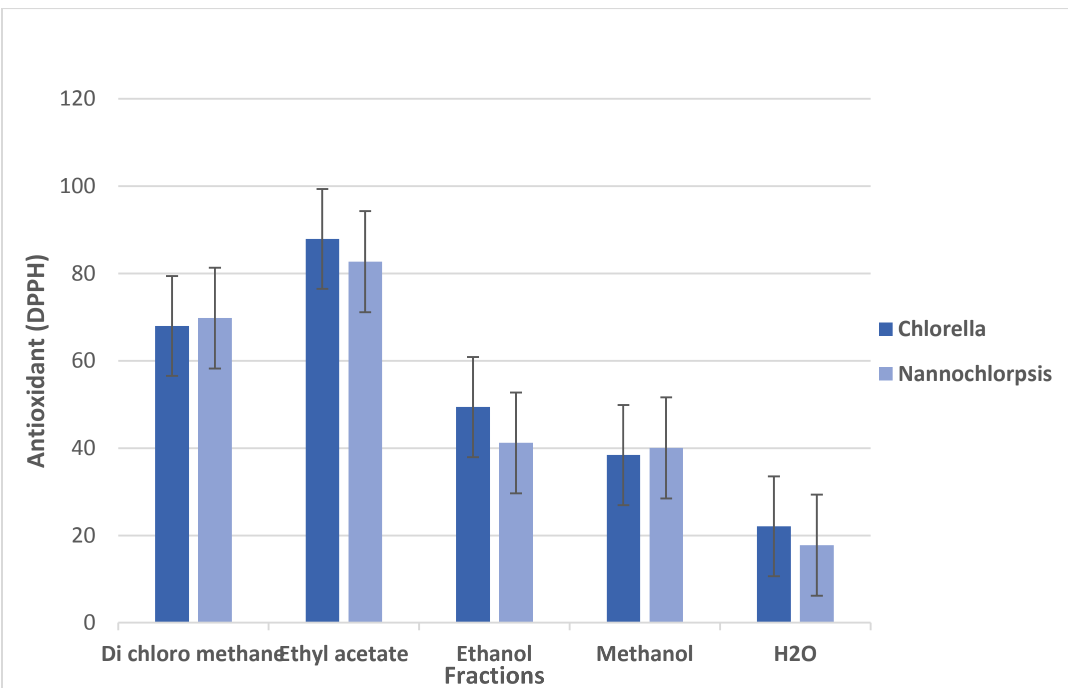

Fig. (1) Antioxidant activity of the two algal fractions.

Discussion

Algae, sometimes known as "nature's powerhouse," are gaining popularity due to their wide range of uses in numerous industries [20]. Algae have a wealth of possibilities for biotechnological, pharmacological, agricultural, and environmental uses due to their extraordinary adaptability and capacity to flourish in a variety of settings [21, 22]. Algae are essential to solving global issues including energy sustainability, food security, and environmental pollution, as they are used in the manufacture of biofuel, food supplements, wastewater treatment, and pharmaceutical formulations [23, 24]. They are desirable candidates for environmentally friendly and sustainable solutions due to their distinct biochemical makeup, which contains beneficial substances such pigments, lipids, proteins, and carbohydrates [20,25,26]. The potential uses of algae are set to grow as our knowledge of them deepens and technologies progress, providing creative answers to various

Two types of algae, *Chlorella* and *Nannochloropsis*, were taken from the National Research Center's biotechnology unit. The selection of these algae was based on their established capacity for a range of biotechnological purposes, such as the synthesis of biofuel and medicinal applications. The isolated strains were identified and distinguished using morphological analysis, which revealed distinctive characteristics that offer important insights into their taxonomic identity and possible uses.

One of the isolated strains, *Chlorella*, was distinguished by the flat, star-shaped cell aggregates that made up its colonial morphology. Its colonies are distinguished by a unique cellular structure that suggests an ecological niche and possible adaptation. Conversely, *Nannochloropsis* had an ovoid or spherical single-celled microalga structure. The examination of *Nannochloropsis'* ultrastructure by electron microscopy gave more light on the plant's cellular organelles and deepened our understanding of its biological traits.

The powdered algal materials were subjected to a systematic extraction process utilizing a range of solvents, including water, methanol, ethanol, dichloromethane, and ethyl acetate. Based on its polarity and capacity to dissolve particular chemicals of interest, each solvent was carefully chosen. To enable effective extraction, each algal sample was dissolved in 200 milliliters of solvent, and each sample was then incubated at room temperature for the whole night on a rotating shaker. The mixes were then filtered and centrifuged to produce crude extracts that were high in bioactive substances. The potential health benefits and industrial applications of the algae fractions were assessed by evaluating their antioxidant activity. Surprisingly, the ethyl acetate fractions of *Nannochloropsis* and *Chlorella* both showed notable antioxidant activity, suggesting their potential

These results emphasize the significance of choosing the right solvent for removing antioxidant components from algae and show how beneficial *Chlorella* and *Nannochloropsis* algae may be as natural sources of antioxidants for a range of industrial uses. To fully understand the possible medical and industrial uses of the particular antioxidant chemicals found in these algal fractions, as well as to identify and define them, more research is necessary.

Conclusion

In summary, the study effectively examined the antioxidant potential of solvent-extracted fractions of *Chlorella* and *Nannochloropsis* algae. The findings showed that different solvent fractions had differing levels of antioxidant potential, with the ethyl acetate and methanol fractions showing particularly strong antioxidant activity. These results demonstrate the potential of *Chlorella* as an important natural antioxidant source with a wide range of industrial uses. To fully understand the potential health benefits and industrial applications of the particular antioxidant chemicals found in *Chlorella* fractions, more research is necessary to identify and define them.

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