

## Assessment of phytochemical components, proximate composition and antioxidant properties of *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis* algae extracts

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### ABSTRACT

In recent decades, natural antioxidant alternatives have become a trending topic for replacing artificial antioxidants. Microalgae have been reported to display interesting bioactive properties and their antioxidant activity is one of them. This study aims to evaluate the antioxidant capacity, phytochemical constituents and proximate composition of three microalgae *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis*. The performed preliminary phytochemical analysis of the algae extracts revealed that they contain good amounts of bioactive compounds. The results showed that the highest total antioxidant activity found was (3720.67 mgAAE/100g) in *Spirulina platensis*, followed by *Chlorella vulgaris* (2794.80 mgAAE/100g). The lowest activity was noticed for *Scenedesmus obliquus* (1718.53 mgAAE/100g). As for total phenolic content, *Spirulina* recorded the highest phenolic content (2238.46 mgGAE/Kg.), followed by *Chlorella* (710.33mg GAE/Kg.), and then *Scenedesmus* (511.20 mgGAE/Kg). The total flavonoids content of algal extracts expressed as mgQE/Kg varied from (548.66) for *Chlorella vulgaris*, (142.23) for *Spirulina platensis* to (66.56) for *Scenedesmus obliquus*. The proximate analysis results indicated high values of protein for *Spirulina platensis* (53.30 % dry wt.) whereas the highest fat, ash and moisture percentages were recorded by *Scenedesmus obliquus* (15.13, 15.07 and 2.57, respectively).

### INTRODUCTION

Due to their various phytometabolic constituents with different chemical structures and biological activities, the use of photosynthetic microorganisms, for instance, microalgae, in life sciences has been growing in recent years. These also have potential beneficial uses as valuable nourishments that can provide important physiological and pharmacological benefits for human well-being. Polyunsaturated fatty acids, sulfated polysaccharides, phycosterols, heat-induced proteins, phenolic compounds and pigments like carotenoids are the active ingredients that have a beneficial effect on human and animal health. A significant number of studies on bioactive microalgal compounds have focused on anti-inflammatory, antiviral, antimicrobial, immunological, antibacterial, medicinal and anticancer compounds (Hemalatha *et al.* 2015; Mostafa *et al.*, 2019; Rashad *et al.* 2019).

The oxidative damage caused by reactive oxygen species on lipids, proteins and nucleic acids that activate various chronic diseases, such as coronary heart disease, some synthetic antioxidants, need to be substituted with natural antioxidants, as they have been found to be toxic and carcinogenic in animal models. It is therefore important to identify new sources of safe and cheap natural antioxidants (Hemalatha *et al.*, 2015).

Oxidative stress is the cause of multiple cellular dysfunctions. The resulting cellular damage can be found in many diseases (diabetes, cancer, atherosclerosis ...). In this respect, natural antioxidants are very advantageous. They protect the body's cells against damage caused by oxidative stress and strengthen the immune system. These natural compounds include vitamins (ascorbic acid and its derivatives, tocopherols of vegetable origin), phenolic compounds and other plant source compounds (Simioni *et al.*, 2019).

Microalgae are abundant in macromolecules and other biologically active substances including amino acids, carotenoids of polysaccharides and fatty acids. Microalgae have therefore been used extensively in food, aquaculture, pharmaceuticals and biofuels (Shao *et al.*, 2018).

*Spirulina platensis* attracts considerable interest among microalgae as a major source of antioxidants, carbohydrates, pigments and proteins, and as a feedstock for biofuels (Chen *et al.*, 2017; Ji *et al.*, 2017; Bauer *et al.*, 2017; Ebaid *et al.*, 2017). *Chlorella*, another microalgae, has also been reported to show antioxidant activity (Ei, 2005). *Scenedesmus sp.* is one of the first successfully grown in vitro microalgae due to its rapid growth and ability to handle. Species of the genus were used for industrial purposes worldwide because of their cultivation facilities and adaptation to environmental conditions (Simioni *et al.*, 2019).

Given the above, the present work has been carried out to evaluate the phytochemical constituents, proximate composition and antioxidant activity of three algae extracts from *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis*.

## MATERIALS AND METHODS

### *Preparation of algae extracts*

The three algae used in the present work were *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis*. These algae were produced under controlled cultivation conditions in the Algal Biotechnology Unit, at the National Research Center, in Egypt. The algae samples were purchased in dry powder form. The samples were then subjected to different chemical analysis during the study.

The algae extracts were obtained by weighing 5 grams of algae powder into 250 ml flask containing 100ml of ethanol (96%). The extraction was done by ultrasonication for 2 hours at 25°C using an ultrasonic water bath. After extraction the samples were filtered and the solvent was evaporated using a rotary evaporator at 40°C

### **Phytochemical screening tests**

Qualitative determination of tannins, saponins, starch, alkaloids, carbohydrates, fats and terpenoids was done following the procedures described by the methods of Harborne, (1998).

### **Determination of the total antioxidant capacity of algae extracts**

The total antioxidant capacity of the extracts was determined by the “phosphomolybdenum” method (Prieto *et al.*, 1999). The results were calculated from

a standard curve using ascorbic acid as a reference antioxidant material and results were expressed as mg ascorbic acid equivalent/100g of extract (mg AAE/100g).

#### Determination of the total phenolic content of algae extracts

The total phenols content of the extracts was determined using the Folin-Ciocalteau method (Turkmen *et al.*, 2006). A calibration curve of gallic acid was prepared and the results were determined from regression equation of the calibration curve and expressed as mg gallic acid equivalents per Kg of the extract (mgGAE/Kg).

#### Determination of the total flavonoids of algae extracts

The total flavonoids content of the extracts was determined by the aluminium chloride test (Mohdaly *et al.*, 2010) using quercetin as standard and the results were calculated as mg quercetin equivalent/Kg of extract (mgQE/Kg).

#### Proximate analysis

Moisture, crude protein, fat and crude fiber were determined according to the methods described by AOAC (2012).

#### Statistical Analysis:

All analysis tests were done in triplicates and the mean values were presented  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

#### Total antioxidant activity

Microalgae are well known for the processing of pigments, fatty acids, proteins and polysaccharides (Borowitzka 2013). Due to the presence of different bioactive components with antioxidative potential in the crude extracts of the samples, total antioxidant activity of three algae is given in Table (1). The highest activities of ( $3720.67 \pm 101.69$  mgAAE/100g) were observed in *Spirulina platensis*, followed by *Chlorella vulgaris* ( $2794.80 \pm 96.69$  mgAAE/100g). The lowest activity was noticed from *Scenedesmus obliquus* ( $1718.53 \pm 86.64$  mgAAE/100g). *Spirulina sp.* has been reported to provide some antioxidant protection both in vitro and in vivo because of the presence of  $\alpha$ -tocopherol,  $\beta$ -carotene, and phenolic acids (Banskota, 2019).

Table 1: Antioxidant properties of algae extracts (mean  $\pm$ SD)

	Total antioxidant capacity mgAAE/100g	Total phenolic content mgGAE/Kg	Total flavonoids mgQE/Kg
<i>Scenedesmus obliquus</i>	1718.53 $\pm$ 86.64	511.20 $\pm$ 22.14	66.56 $\pm$ 2.29
<i>Chlorella vulgaris</i>	2794.80 $\pm$ 96.69	710.33 $\pm$ 39.97	548.66 $\pm$ 13.34
<i>Spirulina platensis</i>	3720.67 $\pm$ 101.69	2238.46 $\pm$ 58.81	142.23 $\pm$ 2.33

#### Total phenolics content

Microalgae contain a variety of phenolic groups but were very distinct from many other species of plants such as herbs, fruits and medicinal plants. Compared to other plants, the microalgae may contain different antioxidants (Manivannan *et al.* 2012). All tested algae extracts contained good amount of phenolic compounds varied from ( $2238.46 \pm 58.81$  to  $511.20 \pm 22.14$  mg GAE/Kg) and *Spirulina* extract recorded the highest phenolic content ( $2238.46 \pm 58.81$  mgGAE/Kg.), followed by *Chlorella sp* ( $710.33 \pm 39.97$  mg GAE/Kg.), and *Scenedesmus sp.* ( $511.20 \pm 22.14$  mg GAE/Kg), respectively. These results are in agreement with the results of Ali and Doumandji, (2017) who reported that the microalgae are more primitive and they are capable of producing relatively complex polyphenols.

### The flavonoid content

As shown in Table 1, the flavonoids content of algal extracts expressed as mgQE/Kg varied from (548.66±13.34) to (66.56±2.29). Klejdus *et al.* (2010) showed that several classes of flavonoids, such as isoflavones, flavanones, flavonols and dihydrochalcones are found in microalgae and cyanobacteria.

### Proximate composition

Microalga biomass is made up of different nutritional components of which the main three are proteins, carbohydrates and lipids (Priyadarshani and Rath, 2012). The proximate nutritional compositions of three algae species are presented in Table 2. The results indicated high values of protein for *Spirulina platensis* (53.30 ± 0.22 % dry wt.) whereas the highest fat, ash and moisture percentages were recorded by *Scenedesmus obliquus* (15.13±0.12, 15.07±0.09 and 2.57±0.06 % dry wt.), respectively.

Table 2: Proximate composition of algae extracts (mean ±SD)

	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
<i>Scenedesmus obliquus</i>	31.07±0.09	15.13±0.12	15.07±0.09	2.57±0.06
<i>Chlorella vulgaris</i>	20.67±0.05	11.90±0.08	8.87±0.05	1.05±0.04
<i>Spirulina platensis</i>	53.30±0.22	12.83±0.17	10.30±0.08	2.03±0.12

### Phytochemical screening for three microalgae strains

Many phytochemicals such as saponins, starch and alkaloids were screened for the three microalgae. The phytochemical active secondary metabolites have been carried out for three microalgae strains, the qualitatively identified phytochemicals have been tabulated in Table 3. The results showed that the three algae extracts contained different types of phytochemicals with several bioactivities. Recently, the importance of alkaloids, saponins and tannins in different antibiotics used in the treatment of common pathogenic strains was demonstrated (Mir *et al.*, 2016).

Table 3: Phytochemical screening of the three microalgae strains

	<i>Scenedesmus obliquus</i>	<i>Chlorella vulgaris</i>	<i>Spirulina platensis</i>
Saponins	-	+	+
Tannins	-	-	-
Terpenoids	-	-	-
Starch	+	+	+
Fat	+	+	+
Carbohydrates	+	+	+
alkaloids	+	+	+

(+): the test result is positive, (-): the test result is negative

## CONCLUSIONS

This study was conducted in order to demonstrate the presence of phytochemical constituents, proximate compositions and antioxidant activities of three algae extracts of *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis*. The results showed that the algae species possess a good quality of phytochemicals, which directly or indirectly can help to maintain the health of living beings. Results suggest also that these microalgae have antioxidant potential that could be considered in the medication, dietary supplements, cosmetics, and food industries for future applications.

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