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Research Article

In Vitro Antioxidant Activity and Total Phenolic Content of *Nannochloropsis salina*

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ABSTRACT

Microalgae are a good source of bioactive substances from aquatic origin. Algae have accounted for almost 3,000 natural products, representing ~20% of the chemicals reported from the marine realm. Majority of the compounds extracted from microalgae possess antioxidant and antibacterial properties. Present study was under taken to analyse the total phenolic content and antioxidant properties of the microalgae *Nannochloropsis salina*. There was a positive correlation between the total antioxidant activity and total phenolic content of *N. salina*. The highest phenolic content was detected in the acetone extracts. These acetone extracts also had the maximum antioxidant activity, ferric-reducing power (FRAP), DPPH scavenging activity and hydrogen peroxide radical scavenging activity.

Keywords: *Nannochloropsis salina*, Antioxidant activity, Microalga, Phenolic content.

INTRODUCTION

The ability to utilize oxygen has provided organisms with the benefit of metabolizing carbohydrates, proteins and fats for energy; however, it does not come without a cost. Oxygen is a reactive atom that is capable of potentially damaging biomolecules, functioning as 'free radicals'. Free radicals are electrically charged molecules. They have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Cell damage caused by free radicals is a major contributor to degenerative diseases of aging such as brain dysfunction, cancer, cardiovascular disease, cataracts and immune system decline. Overall, free radicals have been implicated in the pathogenesis of many diseases. Fortunately, free radical formation is controlled naturally by compounds known as antioxidants. An antioxidant is a substrate that significantly delays or prevents certain types of cell damage and oxidation of substrates when present at low concentrations. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Thus antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being[1].

Microalgae are exposed to direct sunlight for photosynthesis; these organisms produce phytochemicals in order to protect their cells from damage due to exposure to sunlight. These compounds could be used by humans as a protective measure from many degenerative diseases. Many pharmacologically active substances are produced by microalgae. Some algae are rich source of natural antioxidants. The normal antioxidant defense mechanisms in biological systems consist of both enzymatic and non-enzymatic reactions. The non-enzymatic antioxidants include nutrient antioxidants such as water- and fat soluble vitamins, carotenoids, α-tocopherol, ascorbic acid, glutathione, flavanoids, uric acid and plasma proteins such as albumin, transferrin, ceruloplasmin, metallothionein, etc[2,3]. The use of synthetic antioxidants has decreased due to their suspected activity as promoter of carcinogenesis. Therefore, natural antioxidants from plant and algal extracts have attracted increasing interest due to safety[4]. Some algae are considered as rich source of natural antioxidants. Phenolic compounds and carotenoids are the naturally occurring phytochemicals which have positive effects on the health of man and animals. The present study aims to evaluate the total phenolic content and antioxidant properties of *Nannochloropsis salina*. Antioxidant activity was analysed by measuring the scavenging activity of algal extracts against free radicals, to access their reducing capacity and protection of biological molecules from reactive oxygen species (ROS) induced damage.

MATERIALS AND METHODS

Sample preparation

0.2 g freeze dried microalgae was extracted with 2 mL of the respective solvents; chloroform, methanol and acetone for 30 min at room temperature (20°C). The tube was centrifuged at 4500 rpm for 10 min and the supernatant was recovered. The extraction was repeated with 2 mL of the solvent and the two supernatants were combined. The residue was subsequently extracted twice for 30 min at room temperature and the supernatants were combined. Further, the residues were extracted twice with water (2

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mL each) for 30 min at 80°C, and the supernatants were combined[5].

Determination of total phenolic content

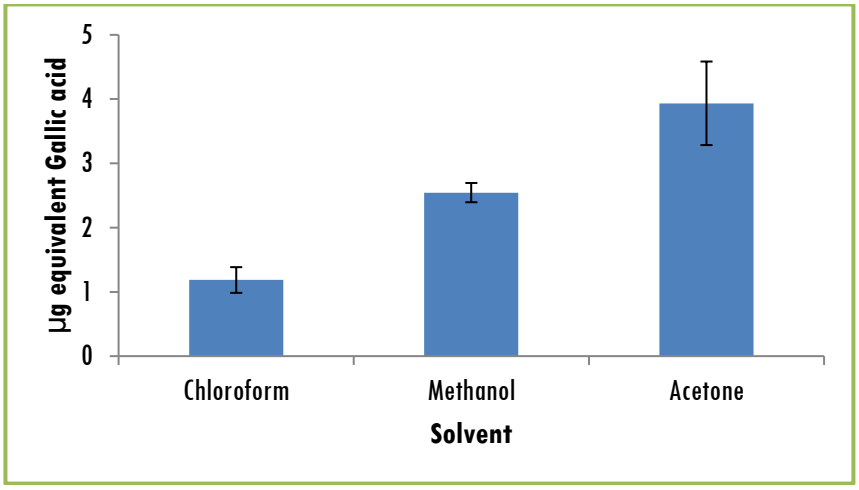

Figure 1: Total phenolic content of *N. salina*

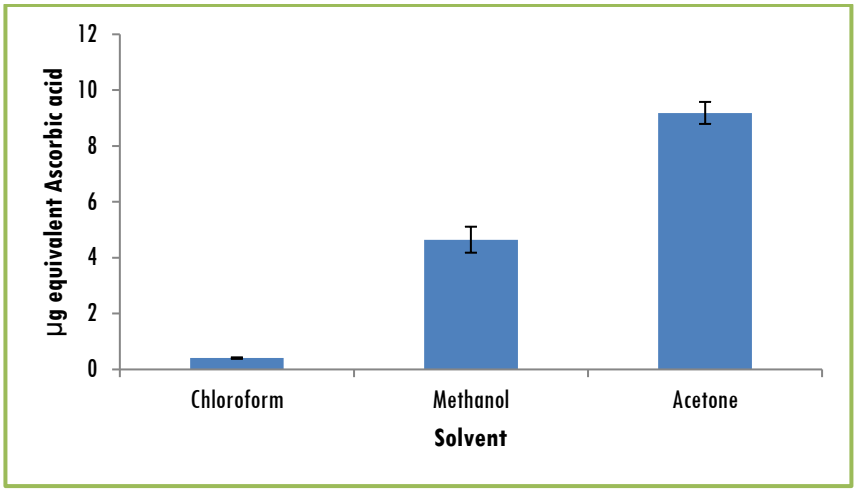

Figure 2: Total antioxidant activity of *N. salina*.

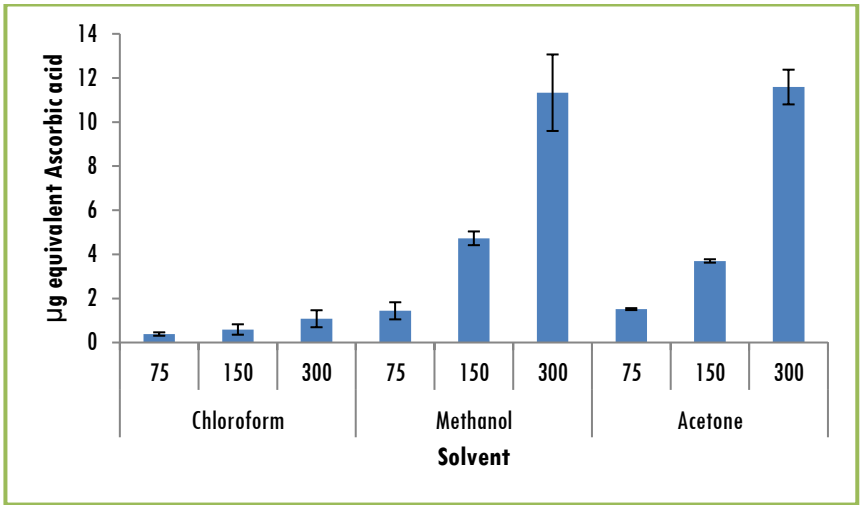

Figure 3: Ferric reducing antioxidant Power (FRAP) of *N. salina.*

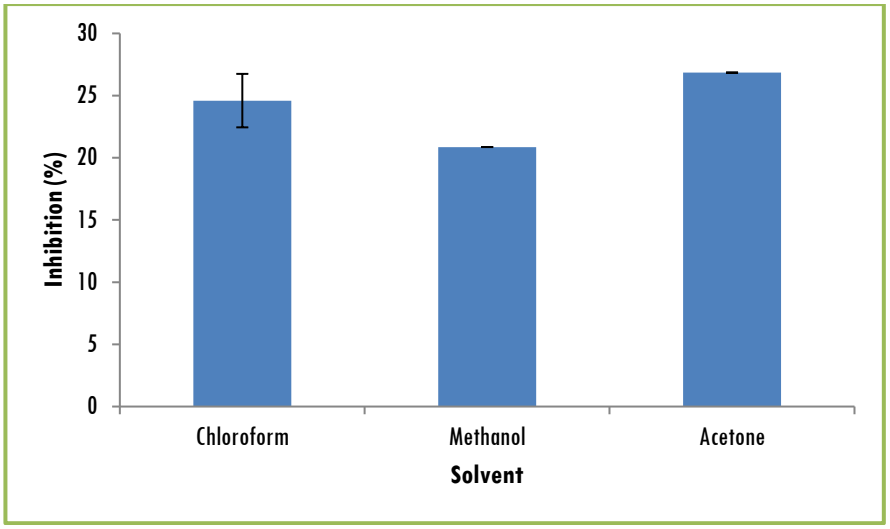

Figure 4: DPPH radical scavenging activity of *N. salina.*

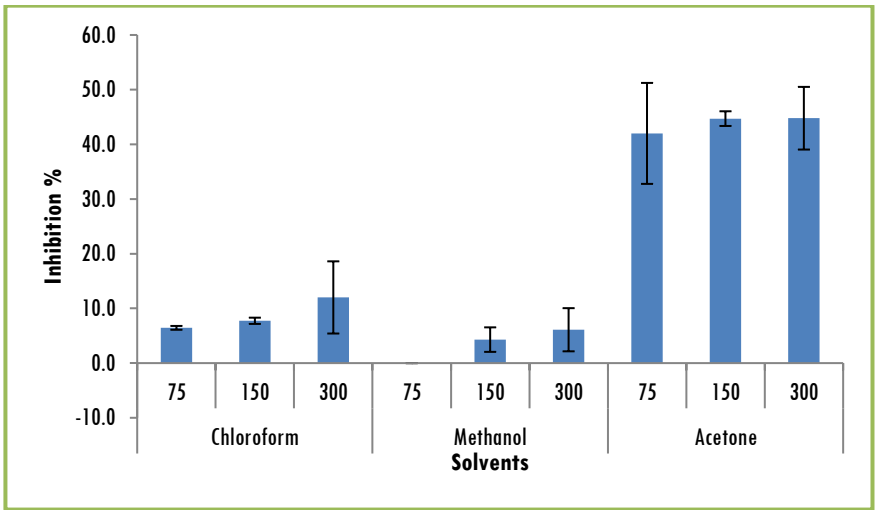

Figure 5: Hydrogen peroxide radical scavenging activity of *N. salina.*

The total phenolic content of the extracts was estimated by the Folin-Ciocalteu method[6]. 200 µL of the diluted sample was added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µL of saturated sodium carbonate (75 g/L) was added. After 2 hr of incubation at room temperature, the absorbance at 765 nm was measured using a UV- Vis Spectrophotometer. The results were expressed as Gallic acid equivalent (GAE)/g dry weight of microalga and calculated as mean value ± SD (n = 3).

Determination of total antioxidant activity

Total antioxidant activity of crude extracts was determined according to the method of Prieto[7]. 300 µL of sample solution was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min in a water bath. Absorbance of the sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid equivalents per gram of sample on a dry weight basis.

Ferric - reducing antioxidant power (FRAP)

Reducing power of crude extracts was determined by the method prescribed by Oyaizu[8]. 100 µL of the extract containing different concentrations of sample was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. From the upper layer, 2.5 mL solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance of the solutions was measured at 700 nm. Increased absorbance indicated increased reducing power. Different concentrations of the sample were analysed.

DPPH radical scavenging assay

The DPPH radical scavenging assay was carried out by the method of Blois[9]. 200 µL aliquot of test samples were added to 3.0 mL of 0.1 mM DPPH ethanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance of solutions was measured at 517 nm. The scavenging effect (%) was calculated using the formula:

Inhibition (%) = [Abs (control) - Abs (sample)]/Abs (control) x100.

Hydrogen peroxide radical scavenging assay

The ability of microalgal crude extract to scavenge hydrogen peroxide was determined by the standard procedure[10]. 10 mM hydrogen peroxide solution was prepared in phosphate buffer of pH 7.4. 500µL of the extract was rapidly mixed with 2.5 mL of hydrogen peroxide solution. The absorbance was measured at 240 nm in the UV spectrophotometer against a reference (without hydrogen peroxide) after 0 min and 60 min at room temperature. Different concentrations of the sample were analysed.

RESULTS

In the present study, total phenolic content, total antioxidant activity, Ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity and Hydrogen peroxide radical scavenging activity of *N. salina* was studied.

Phenolic compounds are the major chemicals with antioxidant potential. In the present study, total phenolic content of *N. salina* sample was measured in chloroform, methanol and acetone extracts. Results were expressed as gallic acid equivalence (GAE) in µg and are presented in Fig. 1. The result showed higher content of phenolic compounds in acetone extract compared to methanol extract. Lowest phenolic content was noted in chloroform extract.

Total antioxidant activity of *N. salina* was also measured using chloroform, methanol and acetone extracts (Fig. 2). The results are presented as ascorbic acid equivalence (AE). It was found that acetone extract showed maximum antioxidant activity (9.185 AE). Chloroform extracts showed the least activity.

Methanol and acetone extracts of *N. salina* showed higher FRAP compared to chloroform extract (Fig. 3). Reducing activity was expressed as µg equivalent ascorbic acid units. Methanol extract showed activity of 11.3 AE at 300 µL concentration while, acetone showed the highest 11.6 AE at 300 µL concentration. Lowest activity was found in chloroform extracts.

DPPH radical scavenging activity was expressed as percentage inhibition. Chloroform, methanol and acetone extracts of *N. salina* were used for DPPH activity studies. Maximum percentage inhibition was noted in acetone extract whereas, methanol extracts showed the least percentage inhibition (Fig. 4).

Hydrogen peroxide radical scavenging activity of *N. salina* extracts were studied using acetone, chloroform and methanol extracts. Results are expressed as inhibition percentage. Acetone extract showed better results compared to chloroform and acetone extracts (Fig. 5).

DISCUSSION

Phenolic compounds serve as an important antioxidant because of their ability to donate hydrogen atom or an electron in order to form stable radical intermediates[5]. The total phenolic content of *N. salina* was determined and expressed as gallic acid equivalent (GAE). Methanol, acetone and chloroform were used as solvents for the extraction of phenolic compounds. The highest concentration of phenolic content was found in acetone extracts. Lowest content of phenolic was noted in chloroform extract. Phenolic content was low in methanol compared to acetone extract. Saranya *et al.* also reported superiority of acetone over methanol in phenolic extraction[11]. Manivannan showed that methanol extract of *Chlorella marina* had highest phenolic content compared to diethyl ether and hexane[5]. Li and co workers[12] found highest content of phenolic in hexane fraction.

Jimenez-Escrig *et al.*[13] found a significant relationship between total antioxidant capacity and total phenolic compounds, whereas, Li *et al.* [12] showed the opposite results. Hence, there is as yet, no definite conclusion on the role of phenolic compounds in antioxidant capacity. In the present study, the total antioxidant activity of extraxts in methanol, acetone and chloroform as was examined. Antioxidant activity was measured as ascorbic acid equivalents. Maximum antioxidant activity was noted in acetone extract when compared to methanol and chloroform extracts. Whereas, Sivakumar and Rajagopal and Hemalatha *et al*. found highest antioxidant activity in methanol extract[14,15]. There was a correlation between total antioxidant activity and total phenolic content of *N. salina*. The results suggest that when microalgal biomass is used as a source of natural antioxidants, both carotenoids and phenolic compounds should be considered. Amount of phenolic content detected in microalgae are very low compared to terrestrial plants[16]. Identification of phenolic substances from microalgae is required to evaluate whether microalgae may contain novel phenolic compounds that are not known from land plants.

Ferric-reducing antioxidant power (FRAP) was also studied using methanol, chloroform and acetone extract of *N. salina*. FRAP was found to be high in acetone and methanol extracts when compared to the chloroform fraction. A good result in methanol or acetone fraction may be due to the presence of carotenoids, polyunsaturated fatty acids etc. Maximum DPPH scavenging activity was found in acetone extract of *N. salina*. The methanol extract showed the minimum DPPH radical scavenging activity. Uma *et al*. reported significant dose-dependent reduction of DPPH in both methanolic and acetone extract[4]. Saranya et *al*. reported maximum DPPH radical scavenging activity in methanol extract of *Isochrysis galbana*[11].

Hydrogen peroxide radical scavenging activity was found maximum in acetone extract of *N. salina*. But, both in chloroform and methanol extract the H_2O_2 radical scavenging activity was minimal. Uma *et al*. found better H_2O_2 scavenging activity in methanol extract of *Desmococcus olivaceous* compared to acetone extract[4]. Hemalatha *et al*. also found high H_2O_2 scavenging activity in methanol fraction compared to acetone fraction[15]. These results suggest that selection of extraction solvents primarily depend on the metabolites present in the species. The present study reveals that both carotenoid and total phenolic content play a vital role in the total antioxidant capacity in *N. salina*.

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