

Evaluation of Antioxidant Activity of *Chlorella minutissima* Extract

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Abstract

The main characteristic of an antioxidant is its ability to trap free radicals and also reduce the risk of chronic diseases including cancer, central nervous system injury, arthritis and heart diseases. Scientific information on antioxidant properties of various natural sources is still rather scarce. A variety of free radical scavenging antioxidants is found in algae. The purpose of this study was to evaluate the antioxidant activity of ethanolic and acetone extracts of *Chlorella minutissima* by FRAP, Ce (IV) sulphate assays and CuPRAC assays. The results suggested that this algae extract can be a vital source of antioxidant phytochemicals. Further investigation may be carried out in isolation and purification of the compounds responsible for the antioxidant activity.

Keywords: Antioxidant, algae, free radicals, radical scavenging activity.

Introduction

Reactive oxygen species (ROS) as superoxide anion, hydroxyl radicals, hydrogen peroxide & singlet oxygen are derived from normal metabolic activity/ processes in the human body or from external sources such as exposure to radiation, ozone, cigarette smoking, air pollutants & industrial chemicals. (Kalyarat Kruawan and Kaew Kangsadalampai, 2006; T. Sathish Kumar *et al.* 2010).

The ROS formed may cause cellular & sub-cellular damage by peroxidation of membrane lipids, by denaturing cellular proteins, & by breaking DNA strands, disrupting cellular functions. (Patra *et al.*, 2008). The ROS are major cause of human cancer & other diseases. The risk of diseases can be reduced by increased consumption of antioxidants which are abundant in food (Kalyarat *et al.*, 2006; Ramapriya and Usha, 2010).

Another field strongly affected by ROS is the food sector, where the free radical peroxidation of lipids, is the predominant cause of food decay, destruction of vitamins & rancidity during storage & transformation (St. Angelo, 1992).

Antioxidants widely used are mainly of synthetic origins and have recently been suspected to their toxicity & cause lipid alteration as well as carcinogenic effects. [Grillo & Dulout, 1995]. Therefore, attention is focusing on the development of

new, safe & cheap antioxidants of natural origin [Shanab, 2007].

Several studies have investigated the antioxidant activity of natural products in marine & fresh water algae [Fujimoto & Kaneda, 1984, Matsukawa *et al.*, 1997; Lim *et al.*, 2002; Xue *et al.*, 2004]. In longer term, algal species identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage [Vadlapudi *et al.*, 2010].

In the present study, we have evaluated the antioxidant potential of the ethanolic & acetone extracts of *Chlorella minutissima*.

Materials and Methods

Algal Materials and Extraction

Chlorella minutissima was procured from Ecogenics Research Center, U.S.A. The dried samples were weighed [1g] & 100ml each of ethanol & acetone added to sample. The samples were shaken at 37°C for overnight. This was followed by centrifugation (3000g for 5 min., twice), the supernatant as the ethanol extract [AEE] & acetone extract [AAE] were used in this study.

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Solvents & Chemicals Used

All chemicals were purchased from Merck, Qualigens fine chemicals & SD fine chemicals, Mumbai.

Estimation Of Polyphenols [TPC]

Polyphenols was determined by the method developed by Malick and Singh, 1980 with slight modifications. 2ml of the ethanol and acetone extract (100–500 µg/ml) were taken for the experiment. The volume was made up to 2.5ml with water and 0.5 ml of diluted Folin Ciocalteu reagent was added. After 3 min., 2.0 ml of 20% Na₂CO₃ was added and incubated in boiling water bath for exactly 1 min and measured at 650 nm. A standard graph was plotted using catechol as standard with concentration ranging from 50–250 µg.

Ferric Ion Reducing/Antioxidant Power Assay (FRAP)

Antioxidant activity was determined by Ferric ion Reducing antioxidant power assay (FRAP) as described by Oyaizu 1986. 2ml of the ethanol and acetone extract (100–500 µg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 m, pH 6.6) and 0.1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm.

Cerium (iv) Sulphate Reducing/Antioxidant Capacity (CERAC) Assay

A method developed by (Res at Apak *et al.*, 2007) was adopted for the determination of reducing power. About 1.0 ml of the AEE/AEE extracts was made up with 1.0 ml distilled water. To this added 1.0 ml of 0.002 M Ce (IV) sulphate solution. After shaking for a few minutes, the solution was let to stand for 30 min. at room temperature. The absorbance of the reaction mixture was measured at 320 nm against a blank composed of distilled water. The decrease in the absorbance at 320 nm was used indicates an increase in antioxidant power of the sample.

Cupric Ion Reducing/Antioxidant Capacity (CUPRAC) Assay

The method developed by (Res at Apak *et al.*, 2008) was adopted for the determination of reducing power. To 1ml CuCl₂ (0.01M) solution 1ml each of Neocuproine (Nc) solution (0.0075 M) and Ammonium acetate (1M) was added. After proper mixing 0.5 ml of the ethanol and acetone extract (100–500 µg/ml) was added along with 0.6ml distilled water. The mixture was incubated for 30 min. then the absorbance was measured at 450 nm.

Results & Discussion

Total phenolic content in AEE& AAE was found to be 4.46 mg/g tissue and 3.95 mg/g tissue. A positive correlation was observed

between TPC & antioxidant activity $R^2 = 0.993$ for ethanol extract & $R^2 = 0.810$ for acetone extract.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Awika *et al.*, 2003). Fe³⁺/Fe²⁺ transformation was investigated in the presence of samples for the measurements of the reductive ability. Absorbance for 100 µg/ml to 500 µg/ml of extract was presented in fig.1. In the present study effectiveness of the radical scavenging activity appeared to be more pronounced in water as compared to that of the sample in ethanol solution. Moreover, method of the Folin-Ciocalteu reagent for determining total phenols actually measures reducing capacity of a sample and this is no reflected in its name total phenolic assay (Huang *et al.*, 2005). In fact, some studies showed there is excellent linear correlation between the total phenols assay by Folin-Ciocalteu reagent and an electron transfer- based antioxidant activity assay (FRAP) (Huang *et al.*, 2005).

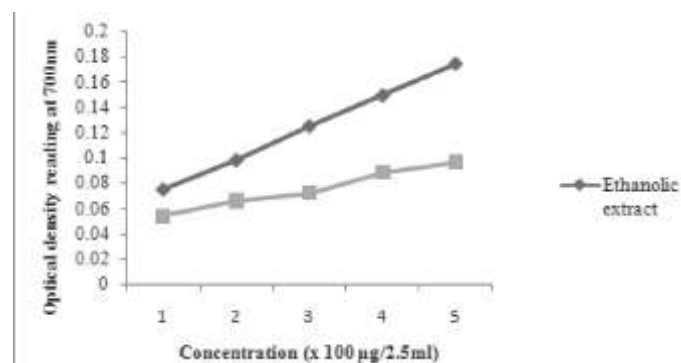


Fig.1. Ferric iron Reducing power of AEE and AAE

The Ce (IV) sulphate assay of AEE & AAE were depicted in fig.2 the absorbance shows inverse relation with the concentration. A decreased absorbance values were noticed as a function of increasing AEE/AEE concentration (100- 500 µg/ml). The one way ANOVA & students t test showed no significant difference at 5% level ($p < 0.05$). These results revealed that the AEE & AAE sample possessed a moderate antioxidant power than Vit. C.

The chromogenic oxidizing reagent of the developed CUPRAC method, i.e., bis-(neocuproine) copper (II) chloride (Cu (II)-Nc) reacts with polyphenolic antioxidants and oxidized them into

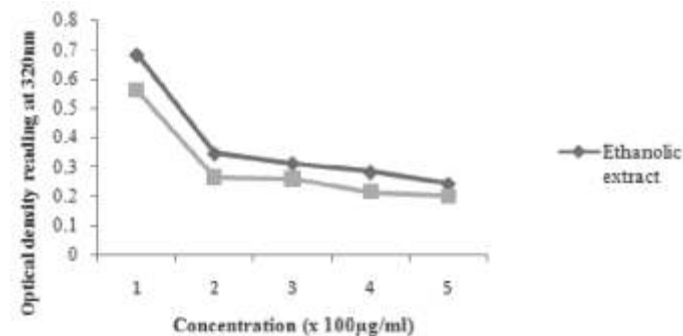


Fig.2. Cerium sulphate scavenging activity of AEE and AAE

the corresponding quinones and Cu(II)-Nc is reduced to the highly colored Cu(I)-Nc chelate showing maximum absorption at 450 nm. Absorbance for 100 µg/ml to 500 µg/ml of extract was presented in fig.3.

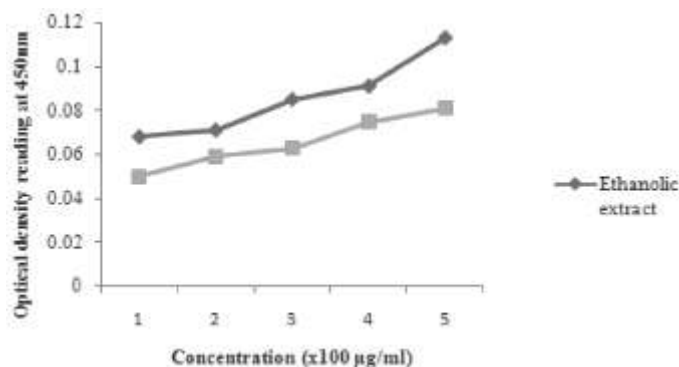


Fig.3. CuPRAC assay of AEE and AAE

The result shows that the ethanol extract possesses a strong antioxidant activity in comparison to AAE but moderate when compared to Vit. C as standard (Fig. 4.). However, the exact mechanism and the compound responsible for the antioxidant activities are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and characterization of the compound.

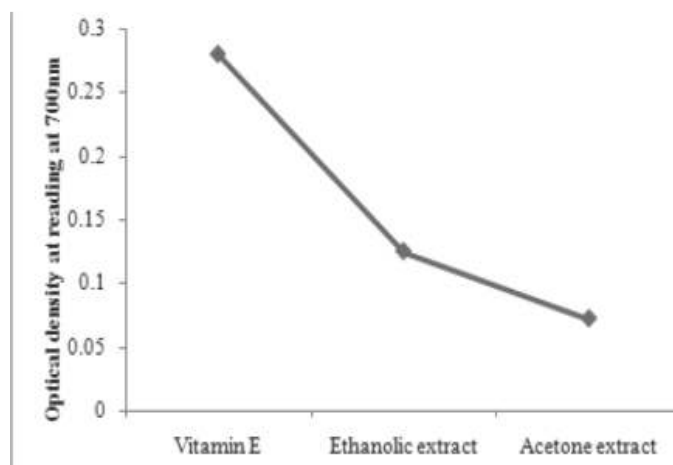


Fig.4 FRAP activity

Conclusion

It is evident from the present study that the ethanol extract of *Chlorella minutissima* could be utilized as a good natural source of antioxidants in pharmaceutical industry. However, the active components responsible for the antioxidant activities need to be evaluated. Therefore, it is suggested that further works may be performed on the isolation and identification of the antioxidant components in *Chlorella minutissima* for its industrial and pharmaceutical application.

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