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Antioxidant Activity of the Freshwater Microalga *Chlamydomonas* sp.

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ABSTRACT

In recent years, there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. Attempts are being made to discover novel antioxidants from natural sources that can prevent the oxidative damage. Microalgae are well known for their numerous applications. The present study was undertaken to isolate a species of Chlamydomonas from a local pond and culture it. A crude extract was prepared using ethyl acetate as the solvent and tested for its antioxidative potential. Results were highly encouraging as the extracts demonstrated antioxidative properties.

Key words: *Algae, freshwater, antioxidant, solvent, oxidative damage*

INTRODUCTION

Microalgae are photosynthetic microorganisms that are found in all over the earth in diverse habitats. They are unique as they display diverse phytometabolic contents with various chemical structures and biological activities. A perusal of literature indicates that extracts prepared from microalgae have been found to exhibit antibacterial, antimicrobial, anti-inflammatory, antiviral, cytotoxic and antioxidant activities and therefore are getting an increased attention by the scientific community in order to explore novel metabolites[1-3].

Sonawale (2016) recently reported a strong antibacterial activity of the crude ethyl acetate extract antioxidant activity of the extract prepared from this alga.

MATERIALS AND METHODS

Collection of the microalgae

The prepared from the freshwater microalgae *Chlamydomonas* sp.[4]. However, the researcher did not investigate antioxidative potential of this extract. In view of this, an attempt was made in the present study to isolate and culture a freshwater species of *Chlamydomonas* from local pond water and then test the *Chlamydomonas* sp. was collected and cultured from a local pond in Devgad, Maharashtra state. After bringing the sample to the laboratory, methodology described by Sonawale (2016) was followed to culture the alga and prepare its crude extract[4].

Preparation of Seed and Mass Culture

Sterilized Bristol medium was used to culture *Chlamydomonas sp.*

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The flask was incubated at stationary condition for 10 days with 12 hrs light and dark condition respectively. After 10 days, the flask was opened and the culture medium was drained out and the biofilm formed at the bottom of the flask was washed with sterile sea water (physical washing). After washing biofilm was scraped using sterile paint brush and transferred to 2 sterile centrifuge tubes (100 ml), 60 ml of sterile distilled water was added to the centrifuge tubes and centrifuged at 3000 rpm for 10 min process is repeated for 2-3 times, obtained cell mass was treated with antibiotics (Penicillin (1mg/ml), Streptomycin (0.5 mg/ml) and Chloramphenicol (0.2mg/ml)) and incubated for 24 hrs at room temperature. After the antibiotic treatment the culture was inoculated to 5 L flask containing 3L BBM media and incubated at stationary condition for 15 days with 12 hrs light and dark condition respectively, temperature 25°C.

Preparation of the crude extract

Ethyl acetate (1L) was added to 3L of culture media and kept on shaker for 4 hrs and then fractionated with ethyl acetate (EA) in a separating funnel where it forms two layers; the aqueous layer and the clear organic (EA) layer. The aqueous layer was removed from the bottom and EA layer was removed from the top. The organic ethyl acetate layer was collected separately and the same extraction procedure was repeated three times. After extraction with ethyl acetate, n-Butanol was added to aqueous layer and shaken vigorously, same extraction procedure repeated three times. Respective organic solvents were then pooled together and concentrated in a separate Round Bottom Flask (RBF) on a rotary evaporator at 32°C, 150 rpm to obtain crude ethyl acetate extract.

In *vitro* assay for antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of ethyl acetate extract was checked on the basis of scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity. The standard antioxidant ascorbic acid was taken for comparison. A procedure of Rahman *et al*., (2010) was followed for this purpose [5]. Here standard ascorbic acid solution (1 ml) and different concentrations of 1ml of ethyl acetate extract were mixed with 3 ml of 0.4 mM DPPH solution. This mixtures were kept in dark for 30 minutes and after that the absorbance was measured at 517 nm using UV-Visible Spectrophotometer. In this assay ascorbic acid was used as a positive control. As mentioned by Rahman *et al*., lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

Hydroxyl radical scavenging activity

A method reported by Klein et al. (1981) was followed to check hydroxyl radical scavenging activity of the extract [6]. A reaction mixture was prepared by adding 1 ml of different concentration of extract (2–10 mg/ml), 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1 ml of DMSO (0.85% in 0.1 mol/l phosphate buffer pH 7.4) and 0.5 ml of 0.22% ascorbic acid. The experimental tubes were heated at 80 - 90°C. in a water bath for 20 minutes. After 20 mins, 1 ml of ice-cold TCA (17.5%) was added. Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) 3 ml was added and incubated at room temperature for 15 minutes. This incubation was for colour development. The yellow colour intensity was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards. Each concentration was tested in triplicate and mean as well as standard deviation was calculated. Test with standard was compared in order to calculate percentage inhibition.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Antioxidative activity of microalgal ethyl acetate extract was measured by DPPH free radical scavenging method and their scavenging activity was compared with the standard antioxidant ascorbic acid. Both ascorbic acid and ethyl acetate extract showed dose dependent activity. In this assay crude ethyl acetate extract showed significant

amount of DPPH free radical scavenging effect. Among seven different concentrations (10 to 800 µg/ml), 800 µg/ml showed the highest (43%) scavenging activity. On the other hand, ascorbic acid showed 40%, 60%, 62%, 73%, 81%, 88% and 88% activity at 10, 25, 50,100, 200, 500, 800 µg/ml, respectively (Table 1).

Table 1 Comparative % inhibition of DPPH showed by standard antioxidant (ascorbic acid) and crude ethyl acetate extract

Test material	Concentration (µg/ml)	% Scavenging activity
Ascorbic acid	10	40
	25	60
	50	62
	100	69
	200	73
	500	81
	800	88
Ethyl acetate extract	10	-
	25	-
	50	8
	100	11
	200	19
	500	31
	800	43

Hydroxyl radical scavenging activity

The microalgal extract showed hydroxyl radical scavenging activity. The percentage inhibition increased according to the concentration of the extract. At 10 mg/ml concentration of extract, 72% inhibition was observed. The results are given in the table 2.

Table 2 Hydroxyl radical scavenging activity of microalgal extract

Extract mg/ml	% inhibition
2	11±1
4	29±2
6	41±2
8	58±2
10	72±3

Reactive oxygen species (ROS) such as hydrogen peroxide, singlet oxygen and hydroxyl radicals initiate oxidative stress. Low concentrations of these ROS are required for normal physiological conditions but their higher concentrations can lead to toxicity and over hundred of diseases. Antioxidants reduce the oxidative damage by ROS and help the body. Antioxidants can be both natural and synthetic. However, in recent years, synthetic antioxidants were found to have many side effects owing to which novel antioxidants from natural sources are being explored.

The results in the present study clearly support the importance of microalgae in discovering new therapeutic agents like antioxidants. There are already many reports available which have demonstrated antioxidant properties of the extracts prepared from numerous microalgae [7-9]. However, there are only a couple of studies

attempted to study antioxidants from *Chlamydomonas* sp. making this investigation important.

In the present study, ethyl acetate was found to be useful in extracting the antioxidants from the microalga. Renukadevi *et al*., (2011) reported antimicrobial and antioxidant activities of the extracts of *Chlamydomonas reinhardtii* sp. a marine microalga [10]. Researchers employed methanol, chloroform and water as the solvents to prepare the extracts. Annamalai and Nallamuthu (2014) reported the use of methanol to extract antioxidants from *Chlorella vulgaris* and *Chlamydomonas reinhardtii* [11]. These reports suggest that more studies can be carried out using these solvents as well for a comparative analysis.

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