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Antioxidant Activity of a Freshwater Alga *Euglena* Sp. Using Methanol Solution

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Research Article

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ABSTRACT

Euglena a green flagellated freshwater alga is used as a model organism for its adaptive abilities and metabolic flexibilities in many research activities. In the present study, antioxidant properties of the *Euglena* sp. was investigated using methanol solution and free radical scavenger that is DPPH. The result obtained in this study showed that with the increase in the concentration of the extract the percentage of antioxidant activity was increased. The present work furnishes useful and important information in order to increase the studies about antioxidant capacity and functional value of *Euglena* sp. for pharmaceutical research.

INTRODUCTION

Oxidation is a chemical reaction involving transfer of an electron from electron rich to electron deficient entity. The electron deficient molecule is termed an oxidizer or oxidizing agent. An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen [1]. Antioxidants are chemicals both naturally occurring and man- made that can prevent or slow cell damage. An antioxidant is actually not a substance, it is a behavior of any compound that can donate electrons and counteract free radicals. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons [2]. Although this definition does not specify exactly, where the unpaired electron is present, still it is preferred because it allows us to classify most of the transition metal ions as free radicals and thus better understand the close interrelation between oxygen and reactive metal ions. Due to presence of this unpaired electron, these radicals confer a considerable degree of reactivity. Oxidation reactions can produce free radicals; these radicals can start chain reactions like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA or the cell membrane. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Carotenoids and fatty acids are two non-enzymatic classes of substances which are able to protect the organism from oxidative damage [3]. The use of methanol as extract solvent restricts the cellular compounds to scavenge the DPPH radical. Only methanol soluble substances like carotenoids, fatty acids are involved in this scavenging process. Free radicals contribute more than hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [4,5]. The synthetic antioxidants e.g., BHA, BHT, gallic acid esters etc., have been suspected to cause negative health effects so, restrictions have been placed on their application [6,7]. Considering the harmful effects of synthetic products, in recent years much attention has been given to natural antioxidant and their association with health benefits [8]. There are several methods available to assess antioxidant activity of several compounds. An easy, popular,

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rapid and sensitive method for the antioxidant scavenging of plant extracts is free radical scavenging assay using DPPH stable radical spectrophotometry.

Algae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected that they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature [9]. *Euglena* is a popular flagellated laboratory microorganism found in freshwater environments [10]. It represents one of the simplest and earliest derived eukaryotic cells. Different species of *Euglena* has been screen for their production of more than a single antioxidant compound like ß-carotene; vitamin C and vitamin E, for providing promising dietary supplement [11]. Recent research has demonstrated that a number of algae like Dunaliella, Chlorella, Chlamydomonas, Ochromonas, Spirulina and *Euglena* have attracted immense attention for their significant antioxidant properties [12,13]. Considering the immense medicinal potential of algae, the present study was carried out to analyses the preliminary free radical scavenging activity of green flagellate algae that is *Euglena* species.

MATERIALS AND METHODS

Collection of Euglena species

The *Euglena* sp. was collected from Dargakona area **(Figure 1)** which situated near the Assam University campus, Cachar district of Assam. The algal sample was authenticated as *Euglena* species by the standard keys [14-16].

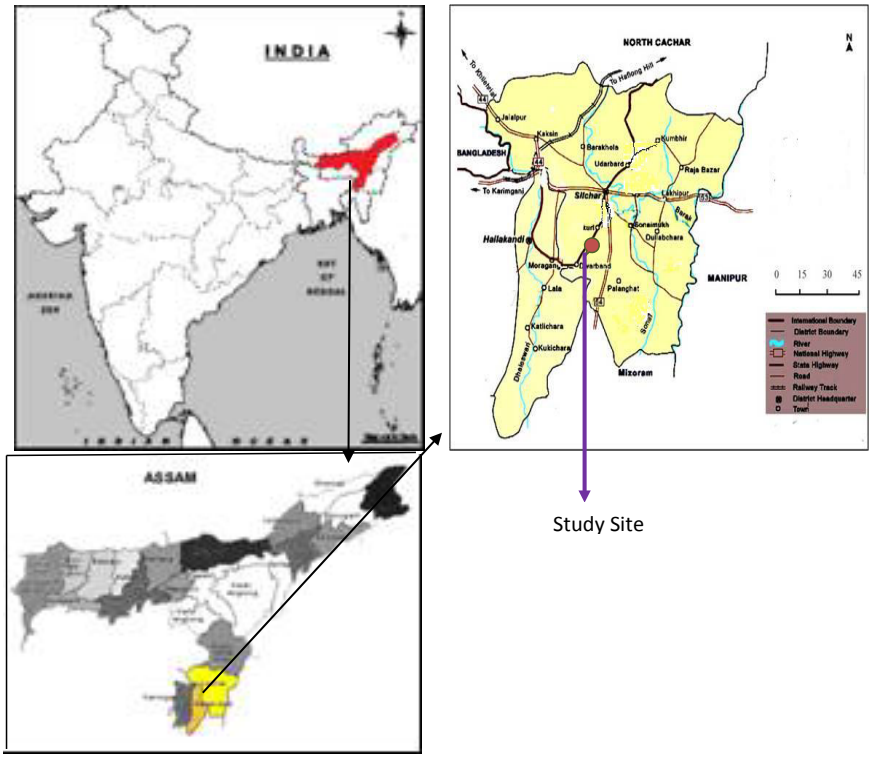

Figure1. Map showing study site at Dargakona area in Cachar District of Assam.

Preparation of the sample

The algae were washed in fresh water thoroughly to remove other contamination. Then the sample was immediately transferred into polyethylene bag with a small hole to leak out excess of water drops and brought to the laboratory were the sample were shade dried at room temperature.

Preparation of organic extract of the sample

The powder (100 g) was mixed with 1000 ml methanol: water (7:3) using a magnetic stirrer for 15 h and then centrifuged at 2850×g for obtaining the supernatant. The process was repeated by mixing the precipitated pellet with 1000 ml fresh solvent. The supernatants from both the phases were mixed and concentrated under reduced pressure in a rotary evaporator, followed by lyophilisation. The lyophilized 70% methanol extract of *Euglena* sp. was kept at -20°C for future use.

Assay of free radical scavenging activity (DPPH)

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extract. DPPH free radical is commonly used to determine radical scavenging activity of natural compounds due to its stable nature. DPPH solution with methanol and ascorbic acid was used as a standard whereas DPPH with methanol and *Euglena* sp. extract used as sample to calculate the percentage of inhibition. The complementary study for the antioxidant capacity of the extract was confirmed by the DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay according to Mahakunakorn et al., [17]

with slight modification. Different concentrations (0-100 µg/ml) of the extract and the standard ascorbic acid were mixed with equal volume of methanol. Then 50 µl of DPPH solution (1 mM) was added into the mixture and stirred thoroughly. The resulting solution was kept standing for 2 min before the OD was measured at 517 nm. The measurement was repeated with six sets. The percentage of scavenging was calculated from the values of the control and the test samples.

The efficacies of antioxidants are associated with their ability to scavenge stable free radicals. Thus, the DPPH radical scavenging activity of methanol extract demonstrated its oxygen radical absorbance capacity and indicated its potent antioxidant nature.

RESULT AND DISCUSSION

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources [18]. Antioxidants are capable of neutralizing free radicals prior to their detrimental physiological effect. **Figure 2** Shows the *Euglena* species in natural condition B. in microscopic scale and C. shows extracted samples. Several studies have been made on biological activities of algae and their substance which could be the potential rich sources of natural antioxidant [19]. The preliminary antioxidant investigation showed the presence of antioxidant property in *Euglena* sp. **(Figure 3).** The result shows that with the increase in concentration the percentage of antioxidant property of *Euglena* species increases. Minimum inhibition was found in 10 µl which was 11.55% and maximum scavenging activity was achieved when concentration was 70 µl where percentage of scavenging was 66.89%. DPPH in its radical form absorbs at 517 nm but upon reduction with an antioxidant its absorption decreases due to the formation of its non-radical form, DPPH–H [20]. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. *Euglena* species showed excellent dose-dependent scavenging activity of DPPH radical **(Figure 4)** but lower than the standard ascorbic acid. The IC_{50} values of the *Euglena* extract and standard ascorbic acid were 147.05 ± 1.81 mg/ml and 5.29 ± 0.28 81 mg/ml respectively **(Table 1).** The high level of antioxidant property in *Euglena* species is contributed by the fact that it contains carotenoid pigment. **Table 2** shows the concentration of carotenoid pigment in *Euglena* sp. Highest concentration of carotenoid was recorded in 100 µg/ml i.e. 12.21 µg/ml. Carotenoid pigment is a potent radical scavenger and singlet oxygen quencher [21]. Carotenoids are known to possess antioxidative properties [22-24]. The spectrum of carotenoid pigment in *Euglena* sp. is given in **Figure 5.** The antioxidant activity of carotenoids arises primarily as a consequence of the ability of the conjugated double-bonded structure to delocalize unpaired electrons [25]. This is primarily responsible for the excellent ability of α-carotene to physically quench singlet oxygen without degradation and for the chemical reactivity of α-carotene with free radicals such as the peroxyl, hydroxyl and superoxide radicals. In the presence of an antioxidant, DPPH radical obtain one or more electron and the absorbance decreases [26]. The antioxidant activity of *Euglena* species may be due to the presence of various groups of phytochemicals like carbohydrates, fatty acids, amino acids, etc. in it. DPPH has been used extensively as a stable free radical to evaluate reducing substances and is a useful reagent for investigating free radical scavenging activity of the components [27-29]. The obtained results in this study suggested that this unicellular *Euglena* is proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit.

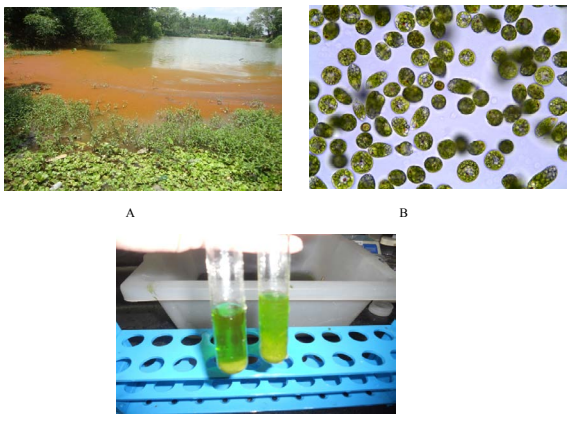

Figure 2. (A) shows the field photo of *Euglena* sp., (B). shows the microscopic photo of *Euglena* sp. and (C) shows the sample extraction.

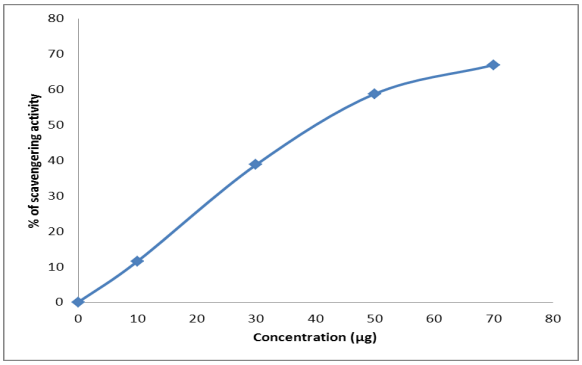

Figure 3. DPPH radical scavenging activity of the extract of *Euglena* sp.

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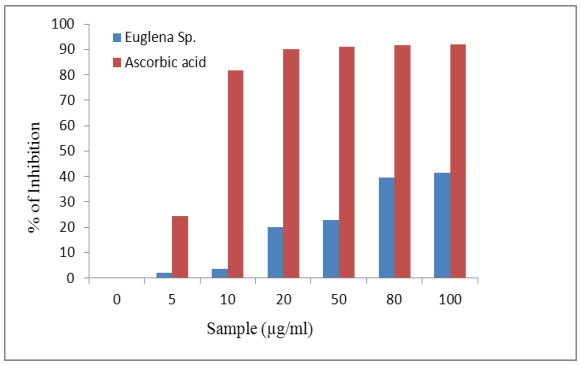

Figure 4. DPPH radical scavenging activity of *Euglena* sp. and standard ascorbic acid. Each value represents mean ± SD. (n=6).

Table 1. Comparison of the free radical scavenging capacities of 70% methanol extract of *Euglena* sp. and Standard reference compound where values are expressed in mg/ml.

Name of the assay	*Euglena* sample	Standard	Values of standard compound
DPPH	147.05 ± 1.81	Ascorbic acid	5.29 ± 0.28

Table 2. Concentration of carotenoid in *Euglena* sp. at different doses, all values is expressed in µg/ml.

Concentration	0	5	10	20	50	80	100
Carotenoid pigment	0	3.11	5.78	7.21	8./87	11.57	12.21

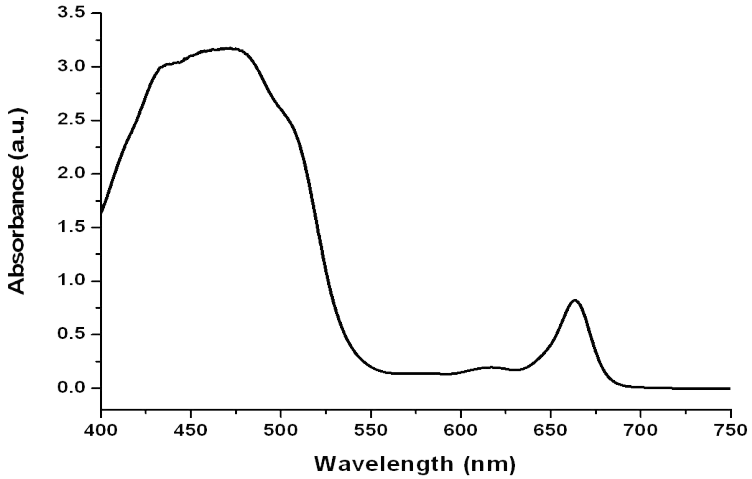

Figure 5. Spectrum of carotenoid pigment of *Euglena* sp.

CONCLUSION

The present information can be useful to closely scrutinize the strategy of pharmaceutical research for selecting *Euglena* sp. for targeted medical science. *In vitro* assays indicate that *Euglena* extract is a promising source of natural antioxidant can be helpful in preventing the progress of various oxidative stresses which is again benefitted in preventing various human diseases.

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