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Quantitative Analysis and First Report of *Euglena tuba* from Himachal Pradesh, India

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Abstract: ***Euglena tuba, a fresh water unicellular microalga has been reported first time from the Masran-Ka-Talab in Baroh area of village Dhalwara in district Kangra of Himachal Pradesh, India forming a reddish brown colour bloom in a pond due to the presence of astaxanthin pigment. Quantitative analysis of the sample shows that Euglena tubaexhibits high antioxidant and free radical scavenging activities with high reducing power capacity.***

Keywords: *Euglena tuba*, Kangra, Himachal Pradesh, India

1. Introduction

Himachal Pradesh, one of the North Indian hilly state, situated in north-west Himalayas, lies between 28° 22′ to 33° 12′ N and 75° 47′ to 79° 04′E Kangra is the most populated district which lies between 31° 40′–32° 25′ East longitudes and 70° 35′–77° 5′ North latitudes with the geographical area of 5,739 km, constitutes 10.31% of the state (Plate-1). The district has considerable diversity in its soils, and water bodies, which include fast and slow flowing rivers, ponds, puddles and lakes.*Euglena tuba* is a fresh waterunicellular microalgadistributed in most aquatic bodies with nutrient rich conditionsforming the prominent seasonal algal bloom and representing one of the earliest derived eukaryotic protist with both plant and animal like features. *E. tuba* requires H, C, N, O, Mg, P, S, Cl, K, Ca, Mn, Co, Zn and some other elements at very low levels (Wolken, J.J. 1961). It produces oxygen at a high rate, reduce carbon dioxide and breaks down organic matter (Chae, S.R. 2006).*E. tuba* possess elongated cell with one nucleus that contain pigmented chloroplast which helps in photosynthesis, a contractile vacuole for excretion, an eye spot to spot sunlight and flagella for movement. Chlorophyll a, chlorophyll b, carotenoids like xanthophyll, astaxanthin (euglenorhodone), zeaxanthin, and carotene mainly beta-carotene are the common pigments in *Euglena* (Deb, S. 2015). *E. tuba* that are grown at different light intensities show remarkable changes in their chemical composition, pigment content and photosynthetic activity (Guschina, I.A. and Harwood, J.L. 2005).Euglenophytes (*Euglena*) made up more chlorophyll a than diatoms, chlorophytes and especially cyanobacteria (Pereira, E. 2001 and Reynolds, C.S. 1984).*E. tuba* showed high biochemical properties which can be utilized for the benefit of mankind.In some cases red coloration of water occurs due to the presence of increase in the xanthophyll pigment called astaxanthin or euglenorhodone or hematochrome.

2. Study Area

Algal samples were collected in the month of October, 2015 from a pond named‘Masran-Ka-Talab’ in Baroh area of village Dhalwara which is 1.5 Km from Raja KaTalab towards Jawali and then 400m left side road, in district Kangra of the state of Himachal Pradesh.

Map of District Kangra showing collection site

Day view of collection site Evening view of collection site

3. Methodology

The collected samples were preserved in formalin (4%)and observed using LeicaDM 500light microscope attached with Leica EC3model digital camera with computerized image analysis system in Phycology laboratory atC.S.I.R.-N.B.R.I. Lucknow.Algal samples were deposited at Herbarium of C.S.I.R.-N.B.R.I., Lucknow.Also, the collected pellet as biomass of *Euglena* was dried in sun light for two days, finely powdered for extraction purpose (Plate-1). Quantitative phytochemical analysis of sample was carried out (Table-1) at Phycology Laboratory, C.S.I.R.-N.B.R.I. Lucknow.

Sample Collection Liquid sample from study area Powder form of sample

a) Phenolics analysis:TPC (Total phenolic content)
Dried powder (50 g) were crushed using a laboratory mill. Ground dry plant material (500 mg) was weighed into a test tube and 2 ml of a mixture of enzymes (5 mg of each enzyme: b-glucosidase, b-xylosidase, b-galactosidase, and b-hesperidinase) and 0.5 ml Sulfatase type H-2 diluted in citrate buffer at pH 5.5 were added. Moreover, SO2 (from $NaHSO_3$), in order to prevent oxidative losses of phenolics, was added. The tested sample with enzyme was hydrolyzed in a water bath for 1 h at 37°C. Then, samples were chilled to 20° C and kept in this condition for 24 h. Then 2 ml of methanol were added to each vial and sonificatedfor 10 min by shaking occasionally (BAS-10, Poland).Then, samples were centrifuged (5 min, 19000g; MPW- 250, Poland) and the clear supernatant was injected into the HPLC equipment.

b) Free radical-scavenging ability by the use of a stable DPPH radical
The DPPH radical-scavenging activity was determined using the method proposed by Yen and Chen (1995). DPPH (100 lM) was dissolved in pure ethanol (96%). The radical stock solution was prepared fresh daily. The DPPH solution (1 ml) was added to 1 ml of polyphenol extracts with 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in thedark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were corrected for dilution and expressed in µMtroloxper 100 g dry weight (dw). All determinations were performed in triplicate.

c) Ferric reducing/antioxidant power (FRAP) assay:
The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain (1996)as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe3+) to the ferrous ion (Fe2+); the latter forms a blue complex (Fe2+/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent wasprepared by mixing acetate buffer (300 lM, pH 3.6), a solutionof 10 lM TPTZ in 40 lMHCl, and 20 lM FeCl3 at 10:1:1 (v/v/v). The reagent (300 ll) and sample solutions (10 ll) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of trolox. All solutions were used on the day of preparation. The results were corrected for dilution (e.g. to 1000 ml) and expressed in lMtrolox per 100 g dry weight (dw). All determinations were performed in triplicates.

d) Determination of flavonoid content.(TFC)
Flavonoidcontents were determined as described by Santasetet al. (2008). The absorbance was measured at430 nm against a sample blank without reactants.Values were determined from a calibration curve prepared with rutin

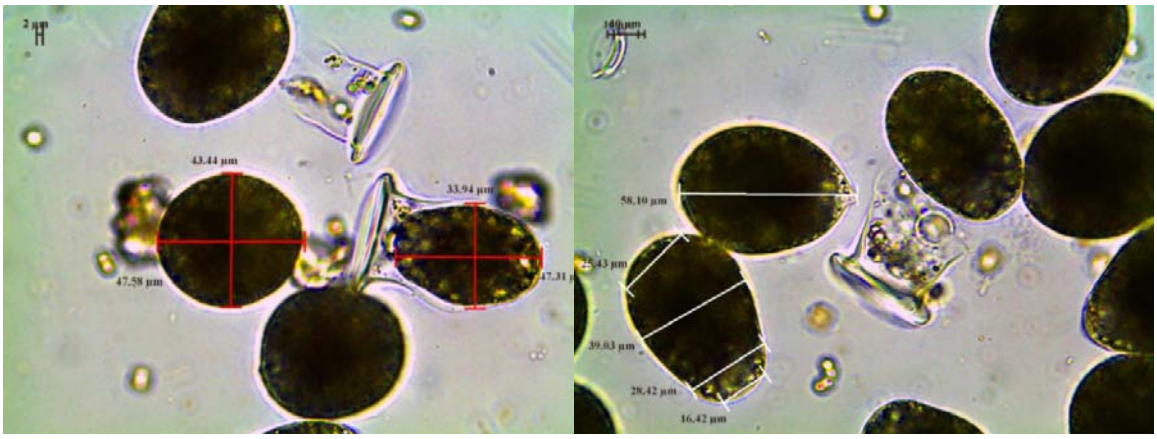
(ranging from 2 to 100 mg/lfinal concentration, R2 = 0.992). Flavonoid content was expressed as rutin equivalent (RE) in mg/g DW.

e) IC_{50} (β-carotene assay)

The antioxidant activity of the alga was determined by β-carotene-linoleic acid system developed by Kolevaet al. (2002) (emulsion preparation) and by Zhang et al. (2007). This method is based on the loss of β-carotene yellow color due to its reaction with the radicals formed by linoleic acid oxidation. Presence of antioxidants slows down the bleach of β-carotene.

4. Results and Conclusions

Cells normally green (if red, only temporarily so and then brick – rather than blood red), larger, longer than their diameter; elongate-fusiform or sub-cylindric, abruptly tapering posteriorly, forming a blunt tip. The size of elongated structure was about 55-105 µm long and 20-40 µm broad, oval form was the size of 60.086 µm long and 43.174 µm broad and the spherical structure was 47.58 µm long and 43.44 µm broad (Plate-1). *E. tuba* changes its body structure continuously and it was irregular and spontaneously pulsating, i.e. exhibiting a rhythmic motion of expansion and contraction. The whole structure of *E. tuba* was localized in a dark brown granular mass.



Photographs showing dimensions of *Euglena tuba*

Table 1: Quantitative phytochemical analysis of *Euglena tuba* extract:

Sr. No.	TPC (Total phenolic content) µg/mg	TFC (Total flavanoid content) µg/mg	EC_{50} DPPH (Free radical scavenging activity)	IC_{50} (β-carotene assay)	FRAP (Ferric reducing antioxidant power)
1.	56.67678	0.063762	0.20953	4.044919	14.19463

5. Conclusions and Discussions

Euglena that grows at different light intensities show remarkable changes in their chemical composition, pigment content and photosynthetic activity (Guschina and Harwood, 2006). Extract of *Euglena tuba*, which contains large amounts of bioactive phytocompounds, exhibits high antioxidant and free radical scavenging activities with high reducing power capacity (Chaudhuri, D. et al.). Scavenging abilities of the extract was observed mainly on superoxide, hydroxyl and hypochlorous acid radicals. Different species of *Euglena* has been screened for their simultaneous production of more than a single antioxidant compound like β-carotene, vitamin C and vitamin E, rendering it a promising dietary supplement (Takeyama, H. et al.). *Euglena* sp. are known to reduce significantly other groups of algae (Hosmani, 1988). Since *Euglena* being non-toxic and does not pose any threat to the ecosystem, it presents an interesting arena for exploring the beneficial potential of this alga. These *in vitro* assays indicate that this algal extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses which is also beneficial in prevention of "various other human diseases" (Goodman et al.). However, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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