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***SHORT COMMUNICATION***

# Biochemical profiling of chlorophylls, carotenoids, proteins and lipids of *Trentepohlia aurea* (l) c. Martius, *C*hlorophyta

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**ABSTRACT:** The northeastern region of India is a biodiversity hot spot rich in flora and fauna including green microalgae. The present communication deals with the synthesis of photosynthetic pigments (chlorophyll-*a* & *b* and carotenoids) along with other value-added products such as total proteins and total lipids from the dominant sub-aerial, branched filamentous green microalga *Trentepohlia aurea* (L.) C. Martius belongs to the family Trentepohliaceae (Ulvophyceae, Chlorophyta). The microalga was found growing naturally on the cemented walls near Bimal Singha stadium, Kailashahar, Unakoti Tripura, India. Fresh biomass was collected and evaluated for biochemical characterization. In light of our findings, the mean chlorophyll a:b ratio was 2.69 ± 0.03, while the chlorophyll: the carotenoid ratio was observed 1.99 ±0.08. The green microalga *T. aurea* could be a good source of biofuel, natural pigments, and other bioactive compounds which could be a boon for the food and pharmaceutical industries.

**Keywords**: *Trentepohlia aurea*, biochemical, carotenoids, chlorophyll, green algae, lipids, proteins, India

## INTRODUCTION

Plants including algae are considered potential source of various natural products and they have been used since long back as good sources of food, fodder and fuel (Sarma et al., 2024). Algae are a diverse group of eukaryotic organisms having very competent biological system for harvesting solar energy for the production of organic compounds through the process of photosynthesis (Ahmad et al., 2016). A large number of researchers in India have worked on different groups of algae on various aspects (Kant et al., 2004a-c, 2005a-c, 2008-a-c, 2020a-b; 2021; Kant, 2011a-b, 2012a-b; Singh et al. 2007a-b; Tiwari et al., 2004, 2009). Many species of algae can produce very high amounts of valuable compounds such as chlorophylls, carotenoids, lipids, proteins, etc (Markou and Nerantzis, 2013; Barba et al., 2015; Benavente-Valdés et al., 2016; Doli et al., 2023). The potential of algae for the fabrication of priceless compounds or energetic exercise is widely recognized due to their more efficient utilization of solar energy as compared with higher plants (Ayres, 1998). They also play a crucial role in aquaculture, bioremediation of waste water and can also be incorporated into cosmetics (Joshi et al., 2018; Mishra et al., 2022; Sarma et al., 2023).

Recently microalgae gained much attention due to their numerous commercial applications such as enhancing the nutritional value of food and animal feed (Kant et al., 2006a). Microalgae cultivation process requires water, light, $CO_2$ and other nutrients which facilitate the growth (Kant et al., 2006b). Microalgae promotion captures greenhouse gas $CO_2$ while concurrently produces biomass containing high-value consumer products (Pires et al., 2012).

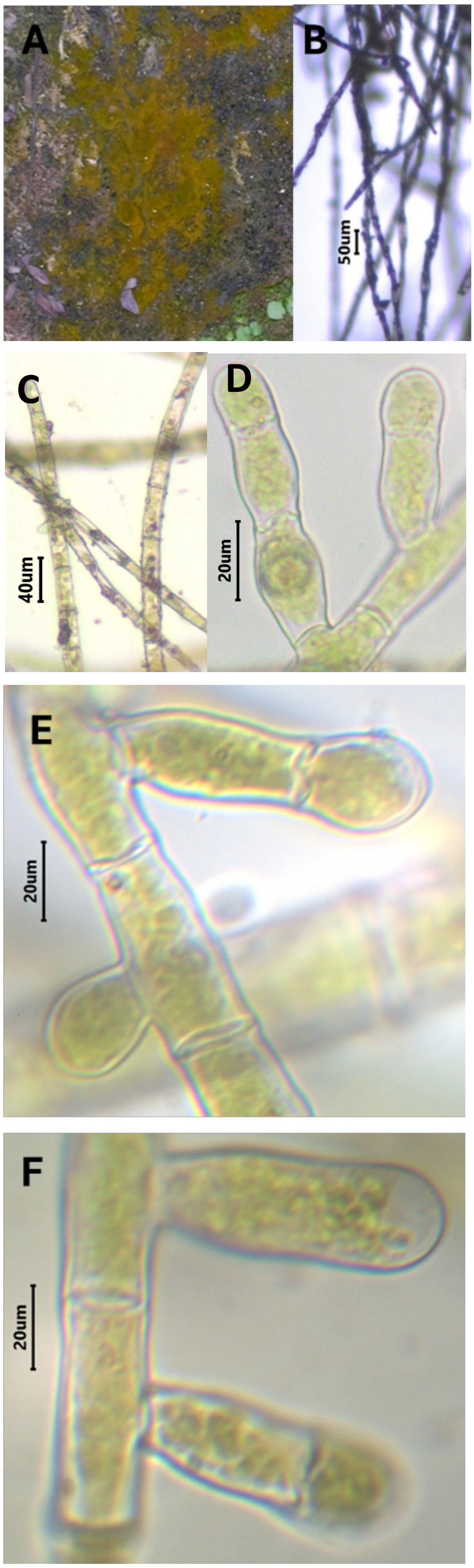
The genus Trentepohlia C. Martius belongs to the family Trentepohliaceae, order Trentepohliales which includes subaerial, branched filamentous microalgae widespread in regions with humid climates and growing on rocks, buildings, tree bark, leaves, stems, and fruits (Printz, 1939; Lopez-Bautista et al., 2006). Though they are present in temperate regions, they are most abundant and diverse in tropical zones (Liu et al., 2012). Due to the high production of carotenoid pigments, they form yellow, orange, or red coatings on the surfaces on which they occur (Rindi et al., 2005).

The present work focused on the evaluation of photosynthetic pigments such as chlorophyll a & b, carotenoids along with value-added products such as total proteins and total lipids from naturally growing green microalga Trentepohlia aurea.

## MATERIALS AND METHODS

### Sample collection and identification

Fresh algal biomass was collected in sterile polybags on 25th October 2023 from naturally growing colonies on the cemented walls near Bimal Singha Stadium (24.31°N, 91.99°E), Kailashahar, Unakoti, Tripura, India. Morphological observations were conducted with the help of Trinocular Research Microscope (Olympus, CH20i microscope) and digital camera (Magnus, Magcam DC 10), and the alga was identified as Trentepohlia aurea (Martius, 1817) (Figure 1A-E).




Figures (A) Growth of Trentepohlia aurea on cemented wall; (B) 4× observation; (C) 10×

observation; (D-F) 40× observations of naturally growing T. aurea.

**Estimation of Chlorophyll**

The estimation of Chlorophyll-a and b was done by the method of Tuba et al. (1994) taking 1g of fresh algal biomass using a spectrophotometer (SYSTRONICS 118) under dark conditions to avoid photoreaction and loss of pigments.

**Estimation of Carotenoid**

Carotenoid content in the freshly growing T. aurea was determined by the method described by Jensen (1978) taking 1gm of freshly growing biomass. The absorbance for carotenoid is measured at 453nm in a microprocessor UV-VIS spectrophotometer (Systronics 118) against acetone blank.

**Estimation of Total Protein**

Total protein was extracted using the method followed by Lowry et al. (1951) taking 100mg of fresh biomass. Total protein was calculated by reading the absorbance at 750nm in a microprocessor UV-VIS spectrophotometer (Systronics 118) against the blank and a standard graph was prepared against BSA (Bovine Serum Albumin).

**Estimation of Total Lipid**

Total lipid content was determined using the method described by Bligh and Dyer method (Bligh and Dyer, 1959). The total lipid was extracted by taking 1g of fresh biomass using a mixture of Chloroform: Methanol (2:1 v/v).

**Analysis of Variance**

The data obtained was subjected to analysis of variance (ANOVA) using Microsoft Office Excel 2007 (Armstrong and Hilton, 2010).

## RESULTS AND DISCUSSION

**Results and Discussion**

The experimental organism used in the present study was freshly growing *T. aurea* Martius collected from cemented walls near Bimal Singha Stadium, Kailashahar, Unakoti Tripura, India. Freshly collected biomass was analyzed for Chlorophyll-*a*&*b*, Carotenoid, Total protein, and Lipid. In the determination of chlorophyll-a from the freshly growing *T. aurea* it was observed that *T. aurea* can synthesize chlorophyll-*a* 220-260 mg/g with an average synthesis of 239.34mg/g which is almost 23.93% of their body weight. Similarly chlorophyll-*b* content was observed 86-98mg/g with an average synthesis of 89mg/g which is almost 8.9% of their body weight. The mean chlorophyll a:b ratio was observed 2.69 ± 0.03. Abe *et al*. (2008) cultured *T. aurea* on a glass fiber filter under a light intensity of 20µmol Photon/$m^2$/s and observed that it can synthesize 15-20 mg/g dry weight chlorophyll which is very low compared to fresh growth. According to Ong *et al*. (1992), the mean chlorophyll a:b ratio was observed 1.76 ±0.09 which is lower than our observation. A detailed result on the synthesis of chlorophyll a & b by naturally growing *T. aurea* is given in Figure 1(A).

In the synthesis of photoprotective pigment carotenoid by freshly growing *T. aurea* it was observed that it can synthesize carotenoid pigment 114-129mg/g dry weight with an average synthesis of about 120mg/g dry weight which is almost 12% of their body weight. Abe *et al*. (2008) reported that when *T. aurea* was cultured at 25°C and 43 µmol photon/$m^2$/s in modified BBM buffered with HEPES under 5% $CO_2$ the culture contained 1.3 mg/g dry weight β-carotene, 0.3 mg/g dry weight L-ascorbic acid and 2.0 mg/g dry weight tocopherols. From our results obtained on synthesis of carotenoid pigment, it was observed that naturally growing *T. aurea* could synthesis more carotenoid pigment than that of controlled laboratory condition. Mean chlorophyll: carotenoid ratio observed from our present investigation was 1.99±0.08 which is almost similar to the observation of Ho *et al*. (1983) containing 2.2 mg/g in *T. odorata* and very less than that of the observation done by Ong *et al*. (1992) which was 18.90±2.40 in *T. aurea* in laboratory condition. Detailed result on the synthesis of total carotenoid pigment by naturally growing *T. aurea* is given in Figure 2.

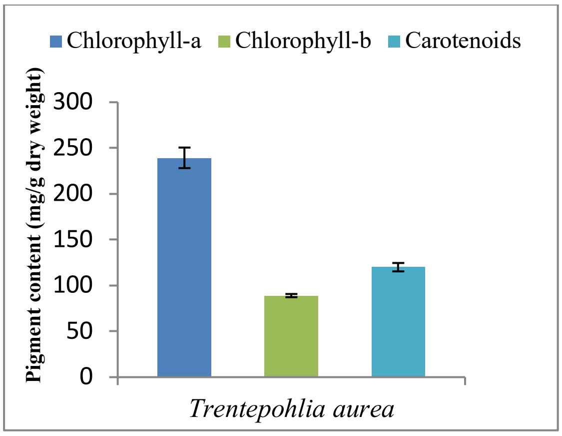



Figure 2. Analysis of chlorophyll a & b and carotenoid of naturally growing *T. aurea.*

Analysis of total protein content in the freshly growing *T. aurea* was also observed and it was observed that total protein content in the freshly growing cells can range from 183-226mg/g with an average synthesis of 202mg/g protein which is 20.2% of their body weight. *T. orodata* when cultured by Ho *et al*. (1983) under light intensity of 700 lux for 15 days showed a protein content 235 mg/g while cultured under dark or high intensity of light showed low protein content. A detailed result on the synthesis of total protein by naturally growing *T. aurea* is given in Figure 3.

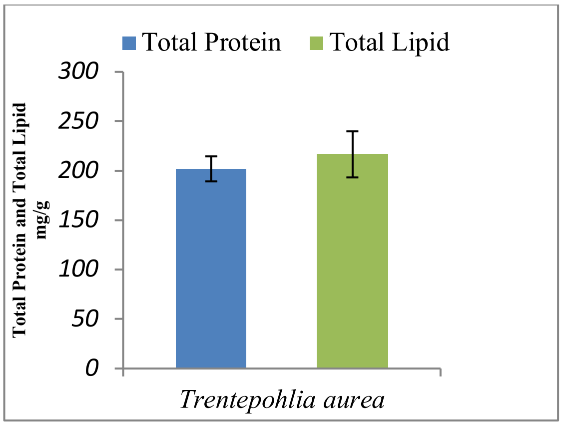



Figure 3. Total Protein and Total Lipid from naturally growing *T. aurea*.

Lipids are organic compounds that are fatty acids or derivatives of fatty acids which have important role in cell structure and also responsible for energy storage (Sarma *et al*., 2020). In synthesis of total lipid content it was observed that *T. aurea* can synthesis lipid ranging from 180-260mg/g with an average synthesis of 216.67mg/g which is almost 21.67% of their body weight. Chen *et al*. (2016) worked on lipid content in *T. arborum* under different ammonium concentrations and different light intensity and reported that *T. arborum* under 0mmol/L ammonium and 35µmol/m$^2$/s light can synthesize a maximum amount of lipid. Detailed result on the synthesis of total lipid content by naturally growing *T. aurea* is given in Figure 1(B).

## Conclusion

From the present study on biochemical characterization of *T. aurea* it is concluded that the microalga could be a rich source of photosynthetic pigments including chlorophyll-*a* & *b*; carotenoids and other value-added compounds including proteins; lipids. In near future attempts should be taken on large scale production and harnessing of natural compounds from *T. aurea* for health, food and other commercial applications.

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