

ESTIMATION OF MAJOR PIGMENT CONTENT IN SEAWEEDS COLLECTED FROM PONDICHERY COAST

ABSTRACT

The importance of marine algae as sources of natural pigments has been well recognized due to their valuable beneficial effects in food, feed and pharmaceuticals. In the present study, six seaweeds namely *Cheatomorpha antennina*, *Enteromorpha intestinalis*, *Grateloupia lithophila*, *Hypnea valentiae*, *Padina gymnospora*, *Ulva fasciata* were collected from the shores of Pondicherry coast and the major photosynthetic pigment content were estimated. Among the investigated seaweeds *Cheatomorpha antennina* showed maximum total chlorophyll content $0.68 \pm 0.01 \text{ mgg}^{-1}$ and *Padina gymnospora* showed the maximum carotenoid content $0.63 \pm 0.02 \text{ mgg}^{-1}$. Seaweeds with rich pigment content can be as supplementary diet.

Keywords: *Cheatomorpha antennina*, *Enteromorpha intestinalis*, *Grateloupia lithophila*, *Hypnea valentiae*, *Padina gymnospora*, *Ulva fasciata*

INTRODUCTION

Macroalgae is a collective term used for seaweeds and other benthic (attached to the bottom) marine algae that are generally visible to the naked eye. Larger macroalgae are also referred to as seaweeds, although they are not really “weeds”. Macroalgae have been reported to contain more than 2400 natural products of commercial importance in pharmaceutical, biomedical, and nutraceutical industries. They have also been extensively utilized as ingredients in human and animal food preparations owing to their high contents of polyunsaturated fatty acids (PUFAs), carbohydrates, vitamins, minerals, and dietary fibers. Nowadays, algal resources have been studied with renewed interest across the world as an alternative source of renewable energy feedstock that circumvents the controversy of “fuel versus food”.

Seaweeds are classified into green algae (chlorophyta), brown algae (phaeophyta) and red algae (rhodophyta) on the basis of chemical composition. The color in case of green seaweeds is due to the presence of chlorophyll a and b in the same proportions as the ‘higher’ plants; beta-carotene (a yellow pigment) and various characteristic xanthophylls (yellowish or brownish pigments). The dominance of the xanthophylls pigment, fucoxanthin, is responsible for the color of brown seaweeds. This compound masks the other pigments such as Chlorophyll a and c and other xanthophylls. Phycoerythrin and phycocyanin mask the pigments such as Chlorophyll a and beta-carotene and are responsible for the color of red seaweeds. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Gupta and Abu-Ghannam, 2011).

Over the past several decades, seaweeds or their extracts have been studied as novel sources which have been shown to produce a variety of compounds and some of them have been reported to possess biological activity of potential medicinal value (Moore, 1978; Konig et al., 1994; Tutour et al., 1998; Satoru et al., 2003). Seaweeds are considered to be a rich source of antioxidants (Cahyana et al. 1992). Recently, the potential antioxidant compounds were identified as some pigments (e.g. fucoxanthin, astaxanthin, carotenoid) and polyphenols (e.g. phenolic acid, flavonoid, tannins) (Heo et al. 2005).

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Barrow & Shahidi, 2008; Wijesekara & Kim, 2010; Wijesekara, Yoon, & Kim, 2010). Among functional ingredients identified from marine algae, natural pigments have received particular attention. These natural pigments exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. Therefore, various natural pigments isolated from marine algae have attracted much attention in the fields of food, cosmetic and pharmacology (Pangestuti and Kim, 2011).

1.1. Study area: The Union Territory of Pondicherry comprises a coastline of 45 Km. with 675 Sq. Km. of inshore waters, 1347 Ha. of inland water and 800 Ha. of brackish water. Coastal area in and around Pondicherry was selected as the study area. The rocky shores of Pondicherry coast and the muddy shore on the outside border of Pondicherry i.e. marakanam provide two different vegetation and thus enables to study the seaweeds of different growth substratum. Tsunambar estuary is a different kind of platform for seaweeds. Though very few species are available throughout the year these species prove to be different in the basis of their growth environment and all the three types (Chlorophyceae, Phaeophyceae and Rhodophyceae) of seaweeds can be collected from a short coast line itself.

2. Materials and Methods:

2.1. Sample Collection: Six species of marine macroalgae, *Cheatomorpha antennina*, *Enteromorpha intestinalis*, *Ulva fasciata* of Chlorophyceae, *Grateloupia lithophila*, *Hypnea valentiae*, of Rhodophyceae and *Padina gymnospora* of Phaeophyceae, were handpicked from substratum like mud, rocks and concrete surface from Pondicherry coast. The samples were washed thoroughly to remove adhering soil particles and immediately transferred to the laboratory in ice box for analysis of various pigments.

2.2. Estimation of Chlorophyll: Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was stored. The residue were re-extracted with 5 ml of 80% acetone. The extract was utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in the UV-spectrophotometer (Arnon, 1949).

$$\text{Chlorophyll a (mg/g.fr.wt.)} = \frac{(12.7 \times \Delta A_{663} - 2.69 \times \Delta A_{645})}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll b (mg/g.fr.wt.)} = \frac{(22.9 \times \Delta A_{645} - 4.68 \times \Delta A_{663})}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll (mg/g.fr.wt.)} = \frac{(20.2 \times \Delta A_{645} - 8.02 \times \Delta A_{663})}{a \times 1000 \times W} \times V$$

ΔA = Absorbance at respective wavelength

V = Volume of extract (ml)

W = Fresh weight of the sample (g)

2.3. Estimation of Carotenoid: The carotenoid content of seaweeds were determined by the method of Kirk and Allen, 1965. The extract that was used for the chlorophyll estimation was used for carotenoid estimation also. The same chlorophyll extract was measured at 480nm in UV-spectrophotometer to estimate the carotenoid content.

$$\text{Carotenoid } (\mu\text{g/g.fr.wt}) = \Delta A_{480} + (0.114 \times \Delta A_{663}) - (0.638 \times \Delta A_{645})$$

ΔA = Absorbance at respective wavelength

RESULT AND DISCUSSION

The major photosynthetic pigments, total chlorophyll and carotenoid content were estimated from fresh seaweeds. The total chlorophyll ranged from 0.51 ± 0.03 to 0.68 ± 0.01 mgg^{-1} with minimum in the red seaweed *Grateloupia lithophila* and maximum in the

green seaweed *Cheatomorpha antennina*. The carotenoid content ranged from 0.26 ± 0.03 to 0.63 ± 0.02 mgg^{-1} with minimum in the green seaweed *Cheatomorpha antennina* and maximum in the brown seaweed *Padina gymnospora* (Table: 1).

Table: 1. Total chlorophyll and carotenoid content of six seaweeds collected from Pondicherry coast

Seaweeds	Total chlorophyll (a&b) (mgg^{-1})	Carotenoids (mgg^{-1})
<i>Cheatomorpha antennina</i>	0.68 ± 0.01	0.26 ± 0.03
<i>Enteromorpha intestinalis</i>	0.54 ± 0.02	0.38 ± 0.01
<i>Ulva fasciata</i>	0.60 ± 0.02	0.37 ± 0.01
<i>Padina gymnospora</i>	0.66 ± 0.01	0.63 ± 0.02
<i>Grateloupia lithophila</i>	0.51 ± 0.03	0.61 ± 0.03
<i>Hypnea valentiae</i>	0.63 ± 0.03	0.49 ± 0.02

The successful analysis of algal pigments depends on the selection of an appropriate extraction procedure (Leeuwe et al. 2006). Both acetone and methanol are widely applied in the extraction of algal pigments. The highest extraction efficiency generally is achieved by using methanol as an extraction solvent in combination with mechanical disruption (Wright et al., 1997). The stability of pigments is low in methanol. Several papers already have described how methanol promotes the formation of allomers of chlorophyll (Bowles et al., 1985; Brereton et al., 1994). Acetone is known to have a lower extractability of chlorophylls from the protein matrix (Nakamura and Watanabe, 2001). Acetone, on the other hand, provides a stable environment. While acetone and methanol have the same polarity index, acetone has greater eluotropic strength than methanol for carbon-rich substrates (Stock and Rice, 1967).

CONCLUSION

According with the results obtained in this study, chlorophyll content is high in chlorophyceae and carotenoid content was high in phaeophyceae. In particular, chlorophyll was high in *Cheatomorpha antennina* and carotenoid was high in *Padina gymnospora*. The pigment content were influenced by environmental parameters. Seaweeds were used as food ingredients as they are potential source of natural pigments that has nutritional value.

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