ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

MORPHOLOGICAL CHARACTERISTICS AND ESTIMATION OF β-CAROTENE OF THE EXPERIMENTAL RED ALGAE

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Received on 09-11-2011 *Accepted on 28-11-2011*

Abstract

Seaweeds have been widely used for human consumption in many parts of the world. Marine algae can serve as a source of minerals, vitamins, free aminoacids and polyunsaturated fatty acids. Macroalgae can be classified as red algae (*Rhodophyta*), brownalgae (*Phaeophyta*) or green algae (*Chlorophyta*) depending on their nutrient and chemical composition. This paper presents the details of morphological studies and estimation of β-carotene of the experimental red algae. From the morphological studies of experimental red alga, the species was identified as *Kappaphycus alvarezii*. The species grow as clusters lobed thalli attached to the rocky substratum. From the studies, it is observed that the ash value and water soluble ash are found to be 54.32% and 24.32%. Quantitative analysis on the amount of β -carotene was performed using high performance liquid chromatography (HPLC) reverse phase column. HPLC result showed that one compound is presented in large besides other impurities which are confirmed as - β carotene.

Keywords: Red algae, *Kappaphycus*, Morphology, β-carotene.

1.0 Introduction

Fresh seaweeds have long been used in food diets, as well as traditional remedies. In Asian countries such as in China, Japan and Korea, seaweeds serve as an important source of bioactive natural substances. Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolities with biological activities, from

macroalgae, has increased significantly in the past three decades, on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Some seaweeds have the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. In the marine ecosystems seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and viruses. Antioxidants are effective in protecting the body against damage by reactive oxygen species. Seaweeds have been widely used for human consumption in many parts of the world. Marine algae can serve as a source of minerals, vitamins, free aminoacids and polyunsaturated fatty acids. Macroalgae can be classified as red algae (*Rhodophyta*), brownalgae (*Phaeophyta*) or green algae (*Chlorophyta*) depending on their nutrient and chemical composition. Red and brown algae are mainly used as human food sources. Seaweed species are rich in beneficial nutrients, in countries such as China, Japan and Korea, they have been commonly utilized in human alimentation. Seaweeds have been consumed in Asia since ancient times. Further, marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical uses [1, 2, 3]. In Europe, there is an increasing interest in marine seaweeds as a food, nevertheless, at present there are no European union specific regulations concerning their utilization for human consumption. Ke Li et al. [4] determined various chemical constituents of the red alga *Grateloupia turuturu*.

Antioxidants are effective in protecting the body against damage by reactive oxygen species. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid containing food [5]. Many natural antioxidants have already been isolated from different kinds of plant, such as oilseeds, cereal crop, vegetables, leaves, roots, species and herbs [6]. Among natural antioxidants, phenolic antioxidants are in the fore

front as they are widely distributed in the plant kingdom. Plants contain diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid datives and flavonoids. Reactive oxygen species (ROS) is generated in living organisms during metabolism [7]. Excess amounts of ROS may be harmful because they can initiate biomolecular oxidants which lead to cell injury and death and create oxidative stress which results in numerous diseases and disorders such as cancer, stroke, mycocardial infraction, diabetes, septic and haemorrhagic shock alzhemer's and parkinsons diseases. The negative effects of oxidative stress may be mitigated by antioxidants. Marine algae extracts have been demonstrated to have strong antioxidant properties [8, 9]. Some of the seaweeds are considered to be a rich source of antioxidants)[10]. New technologies involving the removal of metals ions from waste waters have directed attention to biosorption based on metal binding capacities of various biological materials. Biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solution. Research in the field of biosorption has mostly concerned itself with brown algae [11, 12] and to a less extent with red algae [13]. Literature survey found that the marine red algae belonging to this family are rich sources of phenolic compounds, especially bromophenols [14]. Phenolic compounds play an important role in one antioxidative properties of many plant derived antioxidants. Phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions.

Present investigation aims at the following:

- Morphological studies of experimental alga
- Estimation of total ash value
- Estimation of water soluble ash
- Estimation of β-carotene

2. Materials and Methods

Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Algae samples were cleaned at epiphytes and necrotic parts were removed. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was

ground thoroughly to powder form. The powder was then used for the estimation of chemical constituents such as heavy metals, lipids, phenol, vitamins, carrageen, carbohydrates, antioxidants. This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing. Properties of pharmacognosical significance of the shade dried powdered samples of the experimental alga were determined.

2.1 Morphological studies of experimental alga

Freshly harvested, clean thalli of the experimental alga were studied for their morphology and external characters of the vegetative and reproductive thalli were recorded by free hand sketches and photographed using NIKON – F2 camera and Fuji (Crystal) colour negavtive films (Fig. 1a, b). Free – hand sections of fixed thalli of the alga were taken and stained with methylene blue, safranin and eosin red. Microscopic observation of the stained sections was made in NIKON – OPTIPHOT research microscope. The observations were recorded by camera lucida diagrams and photomicrography using NIKON F – 2 camera and Fuji – crystal 100 ASA negative film under bright field.

2.2 Estimation of total ash value

Powdered material (1.0g) was weighed in a silica crucible, which was previously ignited and cooled. Ignition was repeated until constant weight was obtained. The difference between the initial weight and the final weight was considered as total ash content.

2.3 Estimation of water soluble ash

The ash in the crucible was boiled with 25 ml water and filtered using What man No. 41 filter paper (pre-weighed) followed by a washing with hot water. Then the filter paper was ignited in a silica crucible, cooled and the water insoluble ash was weighed. Water soluble ash was calculated by subtracting the water insoluble ash from the total ash.

2.4 Estimation of β-carotene

Quantitative analysis on the amount of β -carotene was performed using high performance liquid chromatography (HPLC) reverse phase column, waters m-Bondapak C18 column (30cmX3.9 mm i.d.) operated at 30^{o}C. The column was preceded by a Waters Guard-Pak pre-column module housing a disposable Guard-Pak pre-column insert packed with the same material as that in the analytical column. A Waters 510 pump was used to deliver the mobile phase which was a ternary mixture of acetonitrile, methanol, dichloromethane, 75:20:5 v/v/v, containing 0.1% BHT and 0.05% trie-thylamine (TEA), a solvent modifier and prepared fresh daily. The flow rate was 1.0 ml/min. Solvents for liquid chromatography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45 mm cellulose membrane filter and degassed using an ultrasonic bath. β -carotene standard was purchased from Sigma Chemical Company and a concentration 0.2 mg/ml was prepared diluted in the mobile phase and 20 µl injected into HPLC. Peak responses were determined at 450 nm with a variable wavelength programmable photodiode array UV detector (Waters 994) and Waters 520 printer plotter. β-carotene peak was identified by its retention time and compared with that of pure β-carotene standard. Twelve sample extracts were analyzed. Thin layer chromatography (TLC) and UV± vis absorption spectrophotometry were also used to aid in the identification of β -carotene.

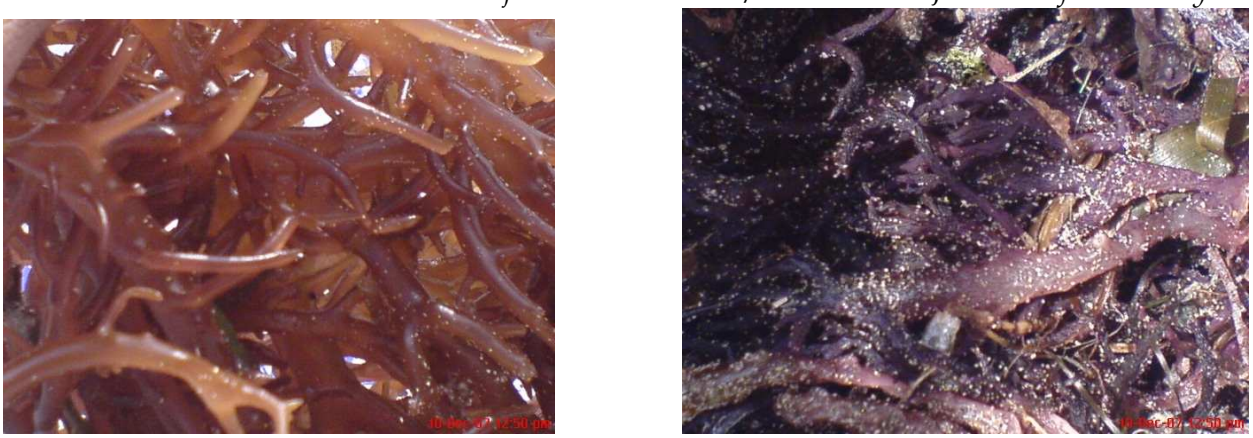
3. Results and Discussion

3.1 Morphological studies of experimental alga

From the morphological studies of experimental red alga, the species was identified as *Kappaphycus alvarezii.* The species grow as clusters lobed thalli attached to the rocky substratum.

3.2 Total ash and water soluble ash

Characteristics of pharmacognosical significance of the experimental algal material (shade dried and powdered algae) were studied in the present investigation as a part of the evaluation of their antimicrobial and other bioactivities. Conventional parameter ash value was determined and the value is 54.32%. Water soluble ash value was found to be 24.32%.

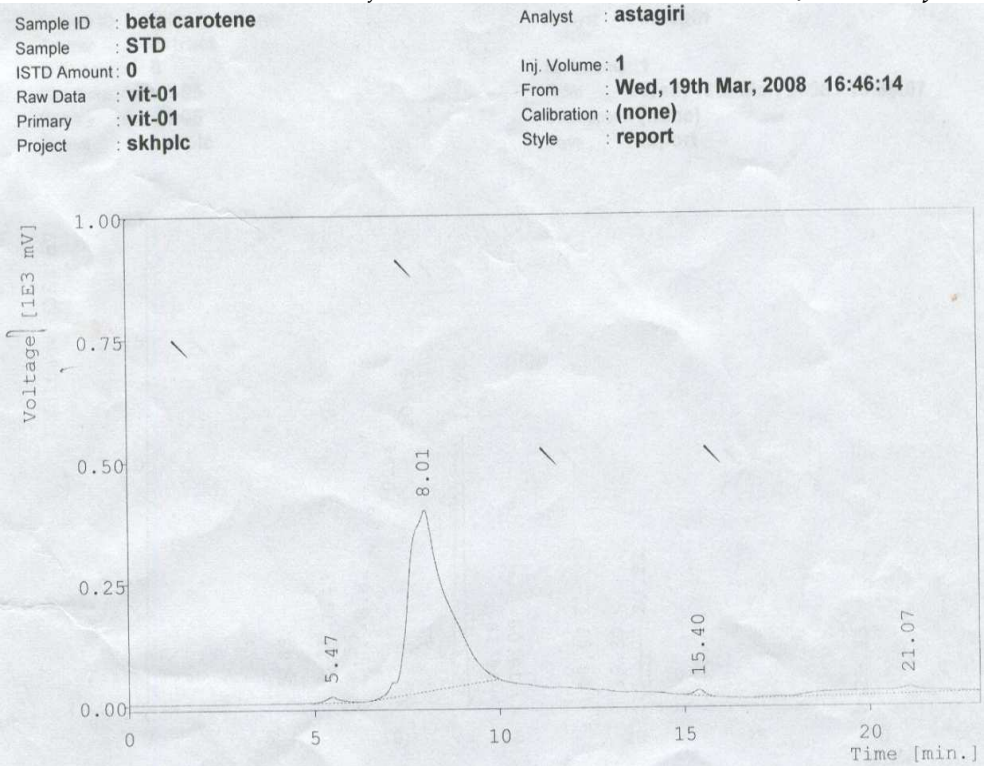


(a) Fresh form (b) Dry form

Fig. 1 Photographed Red algae species in (a) wet form and (b) dry form

3.3 β-Carotene

HPLC result shows that one compound is present in large besides other impurities which is confirmed as β-carotene (Fig. 2). The red colour of these algae results from the pigments phycoerythrin and phycocyanin; this masks the other pigments, Chlorophyll *a* (no Chlorophyll b), β-carotene and a number of unique xanthophylls. The main reserves are typically floridean starch, and floridoside; true starch like that of higher plants and green algae is absent. The walls are made of cellulose and agars and carrageenans, both long-chained polysaccharide in widespread commercial use. There are some unicellular representatives of diverse origin; more complex thalli are built up of filaments. Fayaz et al. [15] determined HPLC chromatogram of β-carotene in extracted sample of *Kappaphycus sp*. It showed the β-carotene peak (RT=9.11/minute). The concentration of β-carotene was 5.26 mg/100 gm sample. In the present study, the β-carotene peak is observed to be 12.94 /minute. The concentration of β-carotene is 2.5 mg/50 gram sample. Difference between the RT values of the above study and in the present investigation may be due to the influence of environmental conditions of the habitat over the physiology and biochemistry of the algae in the marine eco system, which indicates by the seasonal and geographical variations observed in the proximate composition of the algae. Dave et al. [16] have studied 29 genera of red algae from Gujarat coast of India and showed monthly variations in their crude protein levels. Variations may be due to seasonal and biochemical composition of the algae.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	5.473	307.2627	11.6568	0.3733	1.0495	2.8434
2	8.013	26581.4083	372.8526	1.0733	90.7964	90.9491
3	15.400	220.8588	11.6339	0.3067	0.7544	2.8378
4	21.073	2166.3046	13.8144	1.8933	7.3997	3.3697
-	Total	29275.8343	409.9577			

Fig. 2 β-Carotene Estimation (HPLC) in *K.alvarezii*

4.0 Summary and Concluding Remarks

Morphological studies and estimation of β-carotene of the experimental red algae have been carried out. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. From the morphological studies of experimental red algae, the species was identified as *Kappaphycus alvarezii*. The species grow as clusters lobed thalli attached to the rocky substratum. From the studies, it is observed that the ash value and water soluble ash are found to be 54.32% and 24.32%. Quantitative analysis on the amount of β -carotene was performed using high performance liquid chromatography (HPLC) reverse phase column. HPLC result showed that one compound is present in large besides other impurities which is confirmed as - β carotene.

5.0 References

1. Nisizawa, K., Noda, H., Kikuchi, R., Watanabe, T., 1987. The main seaweeds in Japan. Hydrobiologia 151/152, 5-29.

2. Indegaard, M. and Minsaas, J., 1991. Animal and human nutrition. In M.D. Guiry & G. Bluden (Eds.), *Seaweed resources in Europe: uses and potential* (pp. 21-64). Chichester: John Wiley & Sons Ltd.

3. Mabeau, S. and Fleurence, J., 1993. Seaweed in food products: Biochemical and nutritional aspects. Trends in Food Science and Technology 4, 103-107.

4. Ke Li, XiaoMing Li, BinGui Wang, 2008. Chemical constituents of the red alga Grateloupia turuturu. *Journal of* Biotechnology 136, S598-S599.

5. Ito, N., Hirose, M., Fukushima S.W., Tsuda, H., Shirai, T., Tatematsu, M., 1986. Studies on antioxidants: their carcinogic and modifying effects on chemical carcinogenesis. Food and Chemical Toxicology 24, 1071-1082.

6. Ramarathnam, N., Osawa, T., Ochi, H., Kawakishi, S., 1995. The contribution of plant food antioxidants to human health. Trends in Food Science and Technology 6, 75-82.

7. Cavas L and Yurdakoc K., 2005. An investigation on the antioxidant status of the invasive alga *Caulera racemosa var. cylindracea (Sonder) Velaque, Huisman, et Boudoresque (Caulerpales, Chlorophyta*). J. Exp. Mar. Biol. Ecol. 325, 189-200.

8. Kuda, T., Tsunekawaa, M., Goto, H., Araki, Y., 2005. Antioxidant properties of four edible algae harvested in the Noto peninsula. Japan J. Food Composition and Analysis 18, 625-633

9. Yuan, Y.Y. and Walsh, N.A., 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food and Chemical Toxicology 44, 1144-1150

10. Lim, S.N., Cheung, P.C.K, Ooi VEC, Ang, P.O., 2002. Evaluation of antioxidative activity of extracts from a brown seaweed Sargassum siliquastrum. J. Agricultural and Food Chemistry 50, 3862-3866.

11. Holan, Z.R., Volesky, B., Prasetyo, I., 1993. Biosorption of cadmium by biomass of marine algae. Biotech. Bioeng 41, 819-825.

12. Leusch, A., Holan, Z.R., Volesky, B., 1995. Biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by chemically reinforced biomass of marine algae. J. Chemical Technology Biotechnology 62, 279-288.

13. Holan, Z.R. and Volesky, B., 1994. Biosorption of lead and nickel by biomass of marine algae. Biotech. Bioeng 43,1001-1009.

14. Zhao, J., Fan, X., Wang, S., Li, S., Shang, S., Yang, Y., et al. (2004) Bromophenol derivatives from the red alga *Rhodomela confervoides*, Jl. Natural products 67,1032-1035.

c acid. Aquaculture 161, 383-392.

15. Mohamed Fayaz, Namitha, K.K., Chidambara Murthy, K.N., Mahadeva Swamy, M., Sarada, R., Salma Khanam, P.V. Subbarao, Ravishankar, G.A., 2005. Chemical composition, iron bioavailability and antioxidant activity of *kappaphycus alvarezii*(Doty). J. Agricul. Food Chemistry 53, 792-797.

16. Dave M. J., Parekh R. G., Dosh, Y. A. and Chauhan V. D, (1987), 'Protein contents of Red seaweeds from Gujarat coast', Seaweed Res. Utiln, Vol. 10, pp. 17-20.

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