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Extraction and Spectral Characterization of R-Phycoerythrin from Macroalgae – Kappaphycus alvarezii

Charu Deepika[1]

[1]*Department of Biotechnology, Shri Andal Alagar College of Engineering, Mamandur - 603111*

Abstract - *R-Phycoerythrin (R-PE) a well-known intensely bright red macromolecular phycobiliprotein which is naturally produced as an accessory pigment in photosynthetic red macroalgal species. The characteristics of this pigment is highly recognized for its use in many analytical and diagnostic techniques such as an antibody conjugate or antibody-labeling, flow cytometry etc. Kappaphycus alvarezii, a red seaweed was selected for extraction of R-Phycoerythrin by continuous freeze-thaw technique incorporating the use of phosphate buffered saline. The pigment was precipitated with 70% ammonium sulphate, dialyzed with higher concentration buffer saline, and lyophilized. The final lyophilized product was characterized against different metal ions, inhibitors, organic solvents, preservatives, and monochromatic irradiances for its stability and reactivity.*

Key Words: *Kappaphycus alvarezii*, macroalgae, R-Phycoerythrin, reactivity

1. INTRODUCTION

A coastline of about 7,500 km is an asset to India with rich marine biome, a sizable exclusive economic zone and a large shelf area. This biogeographically important area harvests variety of natural resources, flora & fauna widely used for fuel, food and feed in human welfare. The Southern Coast of India bears luxuriant growth of seaweeds, globally used for phycocolloids. Many of the seaweeds possess bioactive components which contain low calories, and they are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers [11,13]. Most of these macroalgae-derived compounds are eminent anti-oxidant, Antidiabetic, anticoagulant, anticholinesterase inhibition, anti-tyrosinase, anti-cancer and anti-inflammatory agents [3,4,6].

Kappaphycus alvarezii is a tough, fleshy, firm marine alga up to 6 feet in length and approximately 0.5 inch in diameter. It grows to 2 m long and is green or yellow in colour. It is very fast growing, known to double its biomass in 15 days. Growing attached to calcareous solid materials of detrital origin.

K. alvarezii, a red macroalga, is well known for its commercial utility and is also easy to cultivate. Generally, it is used for the extraction of the phycocolloids, j-Carrageenan [3,5]. Carrageenan, a linear, sulfated polysaccharide, is a major cell wall constituent of K. alvarezii.

Table -1: Scientific Classification of *K.alvarezii*

Domain	Eukaryota
Phylum	Rhodophyta
Class	Rhodophyta
Order	Gigartinales
Family	Solieriaceae
Genus	Kappaphycus

Meinita *et al*., (2012) have recently reported bioethanol production from *Kappaphycus alvarezii* [14]. Application of seaweed extract in horticulture was first practiced in field experiments at Panjabrao Deshmukh Krishi Vidhyapeth (2006), The use of seaweed products as fertilizer and soil conditioner for horticultural crops was proposed by Aitken et al., (1965) [2]. Akola using *Kappaphycus alvarezii* on different crop plants in which the first foliar application was given at the time of initiation of flowering and the yield was calculated [1]. Ferreria and Lourens (2002) studied the liquid fertilizer prepared from seaweeds on Canola plant [7]. Tay et al., (1985) discovered the presence of cytokinins in seaweeds [18].

Phycoerythrins are macromolecular compounds categogorized under the large group of phycobiliproteins. In most of the macroalgal species, these proteins assemble to form a super-molecular protein complex called phycobilisome, as the sub-units attach together using linkers [16,17]. These functional protein molecules participate in photosynthetic reactions by aiding in light harvesting from the sun. The light harvested or captured in phycobilisome through phycobiliprotein antennae is immediately transferred from phycoerythrins to the phycocyanins which in turn transports them to the allophycocyanins and the energy is finally transferred to the chlorophylls [12]. It is believed to occur with an overall quantum efficiency of about 100% accounting to the survival capacity of macroalgae in low-light environments in the ocean [9,10,17].

The principal pigments found in Rhodophyta or red seaweeds are phycoerythrin and phycocyanin [15]. Visually, phycoerythrin appears red and phycocyanin range from purple (R-Phycocyanin) to deep blue (C-Phycocyanin) and allophycocyanins are blue with a hint of green. Marine algae are known to produce a variety of compounds and some of them have been shown to possess biological activity of potential medicinal values in the last

three decades the discovery of metabolites with biological activities from seaweeds has increased significantly.

Against these backdrops, this study analysed R-Phycoerythrin from *Kappaphycus alvarezii* for its various physico-chemical properties namely metal ions, inhibitors, organic solvents and preservatives at different monochromatic irradiances.

2. MATERIALS AND METHODS

2.1 Collection of macroalgal sample

The red seaweed *Kappaphycus alvarezii* was collected from Gulf of Mannar, near Rameswaram coastal region, Tamil Nadu. The *K.alvarezii* samples were cleaned from epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags, and transported to the laboratory. Samples were washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground until a fine powder is obtained.

Fig -1: Red Macroalgae – *Kappaphycus alvarezii*

2.2 Extraction and Estimation of R-Phycoerythrin

100g of algal sample was added with 1000mL of sodium phosphate buffer (pH 7.4; 0.1 M) in a sterile conical flask and was subjected to continuous freezing and thawing.

Fig -2: Initial Stage of *K.alvarezii* processsing

Later the biomass was separated by centrifugation at 8000 rpm for 20 minutes. The proteins in the supernatant were precipitated with 70% ammonium sulphate saturation and the mixture was stirred overnight.

The phycoerythrin pigment was assayed by reading the supernatant at 562nm, 615nm and 652 nm using Beckman-Coulter DU730 UV/Vis spectrophotometer to confirm the presence of R-PE.

2.3 Effect of different metal ions on R-Phycoerythrin

Solutions of different metal ions such as Magnesium chloride (MgCl2), copper sulphate (CuSO4), Ferrous sulphate (FeSO4), Calcium chloride (CaCl2) were prepared in sodium phosphate buffer. 1mg of the purified R-PE was added in the above metal ions and incubated for 1 hour in room temperature and assayed using UV-spectrophotometer at 562 nm.

2.4 Effect of different inhibitors on R-Phycoerythrin

Solutions of different inhibitors such as EDTA, DMSO, β-mercaptoethanol, SDS were prepared in sodium phosphate buffer. One mg of purified R-PE was added to it, incubated for 1 hour at 37˚C and assayed at 562 nm using UV-spectrophotometer.

2.5 Effect of different solvents on R-Phycoerythrin

1 mg of the pigment was dissolved in 3 mL of different organic solvents namely chloroform, acetone, ethanol, ethyl acetate and incubated for 1 hour in room temperature and assayed at 562 nm using Spectrophotometer.

2.6 Effect of edible preservatives on R-Phycoerythrin

Phycoerythrin stability was determined by adding (0.1 g) different preservatives such as sucrose, calcium chloride, sodium chloride and citric acid was added in 25 mL phycoerythrin solution (5 mg of R-PE was dissolved in 25 mL of sodium phosphate buffer 0.1 M, pH 7.2 at 35˚C). The absorbance of the solution was measured after regular interval up to 5 days on UV-spectrophotometer at 562 nm.

2.7 Effect of different monochromatic irradiances on R-Phycoerythrin

10 mg phycoerythrin was dissolved in 25 mL of sodium phosphate buffer kept under different monochromatic irradiances viz., red (650-750 nm), blue (470-500 nm), green (500-560 nm) and control (white light). These monochromatic irradiances were provided by wrapping the conical flasks with the respective cellophane colour transparent papers. The absorbance was measured after regular intervals of 30 minutes up to 3 hours on UV-spectrophotometer.

All the above experimental runs were carried out as triplicates to check the replicability and the values presented in the graphs are the average values with the error bars indicating their standard deviation.

3. RESULT AND DISCUSSION

3.1 Extraction and Estimation of R-Phycoerythrin

Fig -3: Final Product – R-Phycoerythrin

R-Phycoerythrin was extracted from *K.alvarezii* and purified by ammonium sulphate precipitation. The precipitates were freeze dried and the assayed at different wavelengths in which the peak at 562nm confirmed the presence of R-phycoerythrin and the final product purity was calculated to be 89%.

3.2 Effect of different metal ions on R-Phycoerythrin

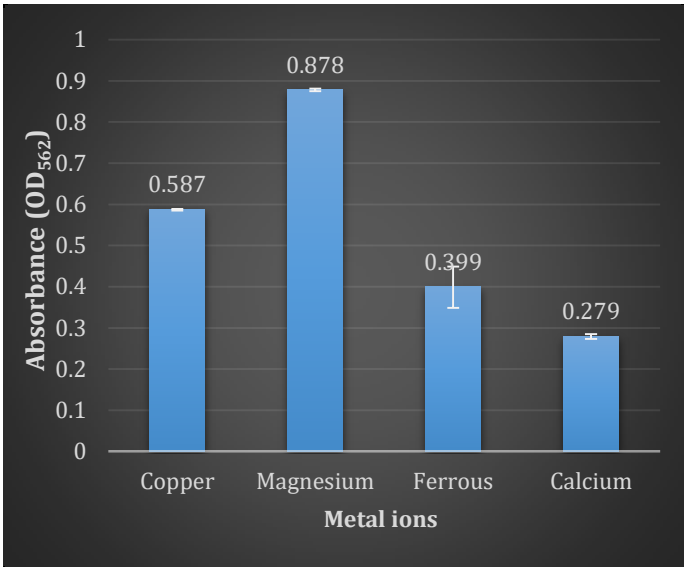

Chart -1: Effect of different metal ions on R-Phycoerythrin

Mg^{2+} showed a consistent effect on R - Phycoerythrin followed by Cu^{2+}, Fe^{2+} and Ca^{2+}. This infers that the R-PE produced from *K.alvarezii* has high affinity to magnesium ions which may be useful deciding its conjugates in a biomedical application.

3.3 Effect of different inhibitors on R-Phycoerythrin

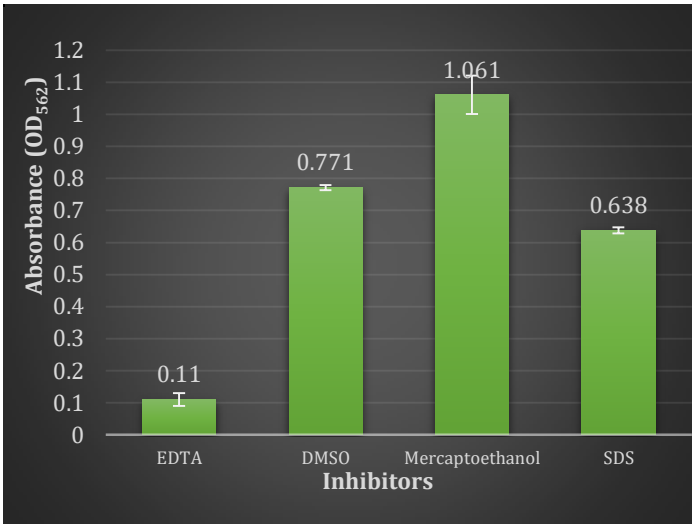

Chart -2: Effect of different inhibitors ions on R-Phycoerythrin

In case of beta-mercaptoethanol, higher activity was observed followed by DMSO and SDS while EDTA being the most widely used preservative for varied compounds represented very small effect on R-Phycoerythrin.

3.4 Effect of different solvents on R-Phycoerythrin

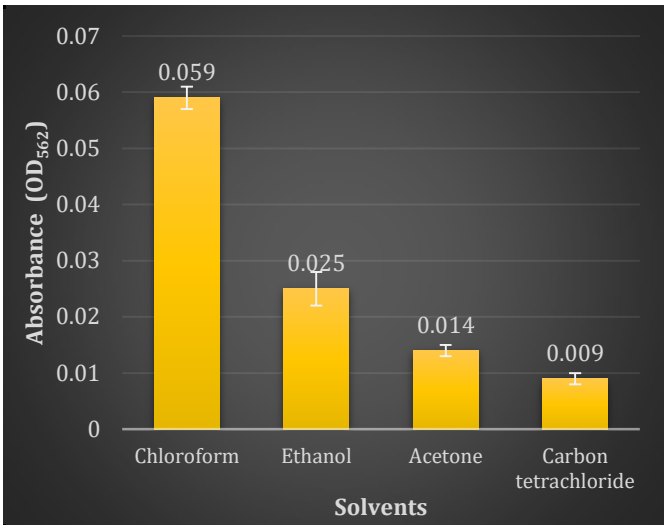

Chart -3: Effect of different solvents on R-Phycoerythrin

When different organic solvents were used for to test their effect on the activity of R-PE, it was observed that chloroform had the best activity followed by ethanol, acetone and carbon tetrachloride. It infers that chemical reactions involving solutions made out of carbon tetrachloride would not affect the biological activity of R-PE.

3.5 Effect of different monochromatic irradiances on R-Phycoerythrin

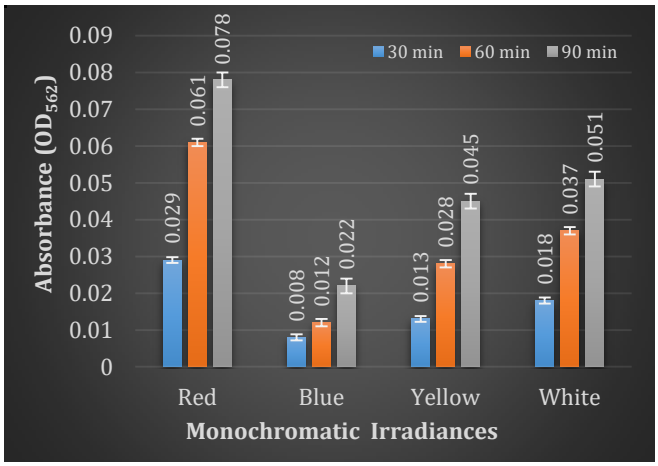

Chart -4: Effect of different monochromatic irradiances on R-Phycoerythrin

When R-PE was exposed to different monochromatic irradiances, the enhanced activity was observed as the incubation time increased. The enhanced effect of monochromatic irradiance was observed in the red irradiance followed by white, yellow and blue irradiances.

3.6 Effect of different Preservatives on R-Phycoerythrin

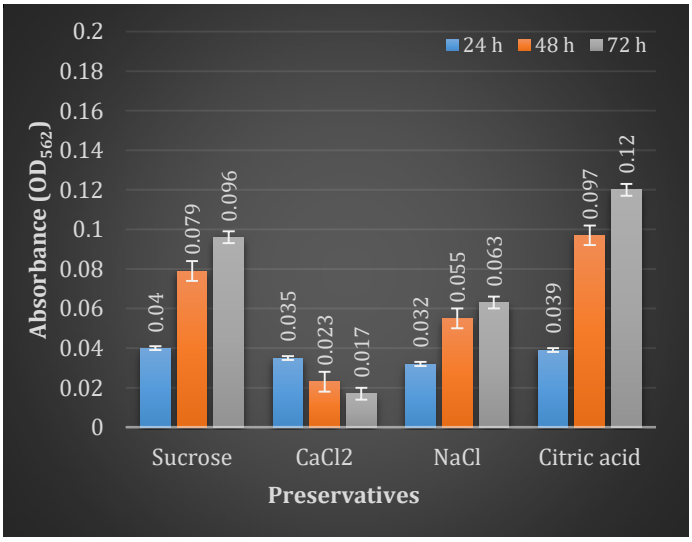

Chart -5: Effect of different Preservatives on R-Phycoerythrin

When R-PE was added to different Preservatives, the enhanced activity was observed as the incubation time increased. The enhanced effect of preservative was observed using citric acid followed by sucrose, sodium chloride and calcium chloride.

4. CONCLUSION

R-phycoerythrin was extracted using simple free-thaw technique being cost-effective and time-effective from an abundantly available natural source *K.alvarezii*. The acquired final product was confirmed by its spectral quality and achieved 89% purity. The results indicate that the R-PE obtained from *K.alvarezii* can act as a good alternative to the commonly used R-PE from *C. elongata* with regards to its higher content which would be the reason accounting for its easy purification by simple precipitation technique. Its affinity over different range of metal ions, inhibitors, preservatives and organic solvents emphasize their applications in food and pharmaceutical industries as coloring agents and indicators respectively.

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