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## SHORT COMMUNICATION

# Extraction, purification and application study of R-Phycoerythrin from *Gracilaria corticata* (J. Agardh) J. Agardh var. *corticata*

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Red seaweeds, such as *Gracilaria corticata* (J. Agardh) J. Agardh var. *corticata* rich in polysaccharides and found abundantly in Indian waters throughout the seasons, have a considerable amount of phycobiliproteins, especially phycoerythrin which has recently found applications in food and beverage industry as a natural coloring agent. Extraction of phycobiliproteins was carried out using phosphate buffer (0.1M), precipitated in 65 % ammonium sulphate saturation, dialyzed overnight using distilled water and phycoerythrin was separated using DEAE cellulose-52 column chromatography. The purity and yield obtained after purification of R-phycoerythrin was 1.10 and 0.024 % (w/w), respectively where as crude phycobiliproteins yielded 0.078 % (w/w). This could be preserved for a period of 100 days in the presence of 5 % NaCl at 0±5 $^{o}$C with a color loss of only 37.5 %. Dialyzed and lyophilized phycobiliprotein was used for application in commercially available carbonated drinks such as Lehar soda, 7up and TATA mineral water. Among the three drinks, 7up retained the color for more than 30 days at room temperature with 50 % loss where as in other drinks color retention was only for 3 days. The present study concludes that phycoerythrin can be used as a natural colorant in cool, sweetened and carbonated drinks.

**Keywords:** *Gracilaria corticata* var. *corticata*, Phycobiliproteins, DEAE cellulose-52, R-phycoerythrin, Carbonated drinks.

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## Introduction

Seaweeds are macroalgae found abundantly in coastal areas. Among them *Gracilaria corticata* (J. Agardh) J. Agardh var. *corticata*, red seaweed which contains R-phycocyanin, Allophycocyanin and R-phycoerythrin, a phycobiliproteins, can be exploited for pigment extraction and utilization as a natural colorant. The phycobiliproteins are water soluble pigments, pinkish in color and unstable in light, pH and temperature. Extraction of phycoerythrin from this seaweed is a tedious process due to the thick leathery nature of the thallus. The presence of the major water soluble phycocolloid, agar, also interrupts the pigment extraction. Potassium phosphate and sodium phosphate buffers are the mostly used solvents for phycobiliprotein extraction and storage. Step wise extraction procedures are required to obtain high purity of R-phycoerythrin from *G. corticata* var. *corticata* which holds large quantity than phycocyanin and allophycocyanin. There are other red seaweeds which are producing phycobiliproteins but this seaweed is available in plenty during favorable season and their spent biomass can be utilized for other applications such as composting, biofuel and biogas production because it contains feasible amount of phycocolloids. The spent biomass obtained after pigment extraction is also high in quantity when compared to other seaweeds. By this method zero waste discharge can be attained and useful products can be generated.

Among the various chromatographic techniques available, a gradient elution using DEAE Cellulose-52 (an ion-exchange column) is suitable for step wise purification of R-phycoerythrin (negatively charged) from crude phycobiliprotein. Purified phycoerythrin can be used as an alternate coloring agent in food items against synthetic colors[1] since it possess antioxidant property and protects against oxidative stress in target tissue and lipid oxidation in foods[2]. R-phycoerythrin also possesses a yellow fluorescence. It has also been tested in lollipops, dry sugar-drop candies for cake decoration, soft drinks and alcoholic beverages[1]. The present study deals with phycoerythrin extraction, purification, preservation and application in carbonated drinks.

## Materials and Methods

### Pigment extraction

*G. corticata* var. *corticata* was collected from the coastal region of Manapad, Tamil Nadu, India (Plate 1). The samples were cleaned to remove the extraneous matter and phycobiliproteins extracted from 50 g of fresh biomass by grinding in a table top mixer with ice cold potassium phosphate buffer (0.1M) at pH 7.0. The extracts were stored at 0±5 $^{o}$C in amber color bottles after filtration using muslin cloth (450 mesh size) and centrifugation at 10,000 rpm.

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Table 1- Extracted phycobiliprotein yield from *G. corticata* var. *corticata*

| Pigment type | R-phycocyanin mg/g | Allophycocyanin mg/g | R-phycoerythrin mg/g | Purity ratio of R-PE ($A_{565}/A_{280}$) |
|---|---|---|---|---|
| Crude | 0.49 | 0.52 | 0.78 | 0.35 |
| Dialyzed | 0.07 | 0.11 | 0.24 | 0.37 |
| DEAE Cellulose 52 (Ion- exchange chromatography) | - | - | 0.016 (mg/mL) | 1.10 |

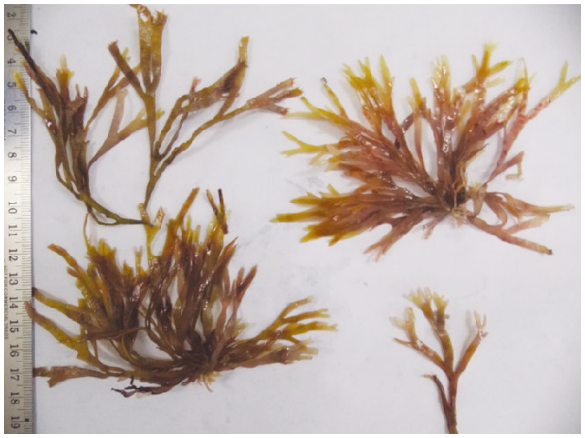

Plate 1- *Gracilaria corticata* var. *corticata* collected from Manapad coastal area

For the estimation of phycocyanin, allophycocyanin and phycoerythrin, the samples were subjected to scan in a UV-Visible Spectrophotometer and quantified using the equations of Bennett & Bogorad[3].

$$PC\ (mg/mL) = [A_{615} - 0.474\ (A_{652})]/5.34 \quad \ldots (1)$$

$$APC\ (mg/mL) = [A_{652} - 0.208\ (A_{615})]/5.09 \quad \ldots (2)$$

$$PE\ (mg/mL) = [A_{562} - 2.41\ (PC) - 0.849\ (APC)]/9.62 \quad \ldots (3)$$

**Purification of phycoerythrin**

The phycobiliproteins were at first precipitated using 65 % ammonium sulphate saturation and dialyzed overnight against distilled water. The phycoerythrin was then separated and purified using DEAE Cellulose 52 column chromatography (Hi-media). Gradient elution using NaCl concentrations of 50 mM to 300 mM was employed to separate phycoerythrin from phycobiliproteins[4].

The purity index was calculated by the following formula:

$$PC = A_{615}/A_{280} \quad \ldots (4)$$

$$APC = A_{652}/A_{280} \quad \ldots (5)$$

$$PE = A_{565}/A_{280} \quad \ldots (6)$$

Where, $A_{615}$ = maximum absorbance of PC, $A_{652}$ = maximum absorbance of APC, $A_{565}$ = maximum absorbance of PE and $A_{280}$ = the absorbance of total proteins.

**Storage of R-phycoerythrin**

Purified phycoerythrin was stored in 5 % NaCl for further use at 0±5 $^{o}$C under dark condition. Color stability was monitored for 100 days.

**Application study**

For application studies lyophilized samples of dialyzed phycobiliprotein were used and 200 mg of lyophilized sample containing 16 µg of phycoerythrin was dissolved in 10 mL of carbonated drinks (7UP and Lehar soda, PEPSICO Ltd., India) and drinking water (TATA). It was kept at room temperature for 30 days under semi-dark condition in order to check the stability nature of pigment.

## Results and Discussion

**Pigment quantification**

Crude phycobiliprotein contains a mixture of phycocyanin, allophycocyanin and phycoerythrin. The contents of these pigments in crude and dialyzed samples are shown in Table 1. Comparable results were reported for PE (0.46 mg/g and 0.54 mg/g) and PC (0.28 mg/g and 0.24 mg/g) in the seaweed *Gracilaria chilensis* by Beer & Eshel[5] and Tello-Ireland *et al*[6], respectively. In fresh samples of *Gracilaria* sp. Gómez *et al*[7] reported higher amount of PE (1.25 mg/g) and a lesser amount of PC (0.11 mg/g) as compared to the present study (PE = 0.78 mg/g and PC = 0.49 mg/g FW). Senthilkumar *et al*[8] reported 0.781 mg/g of R-phycoerythrin from *G. corticata* which is found to be similar to the result of present study. Through this study the yield of R-phycoerythrin obtained was 0.078 % (w/w) in crude extract.

**Phycoerythrin and purity ratio**

In the present study the R-phycoerythrin content was found to be 0.016 mg/mL or 0.24 mg/g and the purity obtained was 1.10. Though the quantity obtained was comparatively less, the purity is

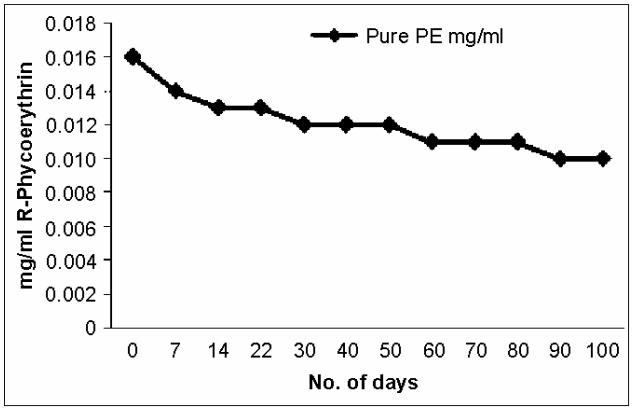


Fig. 1- Loss of R-phycoerythrin in 5% NaCl at 0±5°C

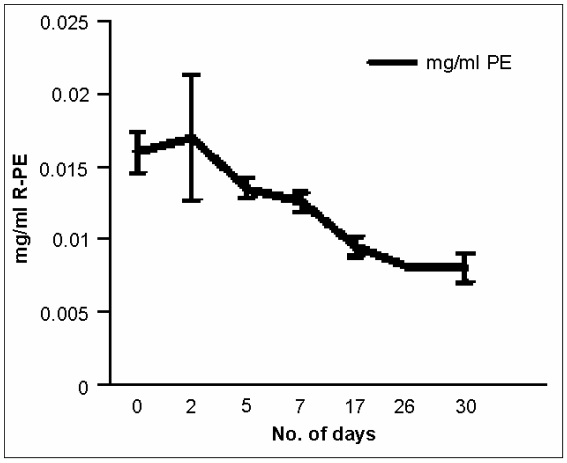


Fig 2- Loss of R-phycoerythrin in 7UP. Values are expressed as the mean ± SD (n=2)

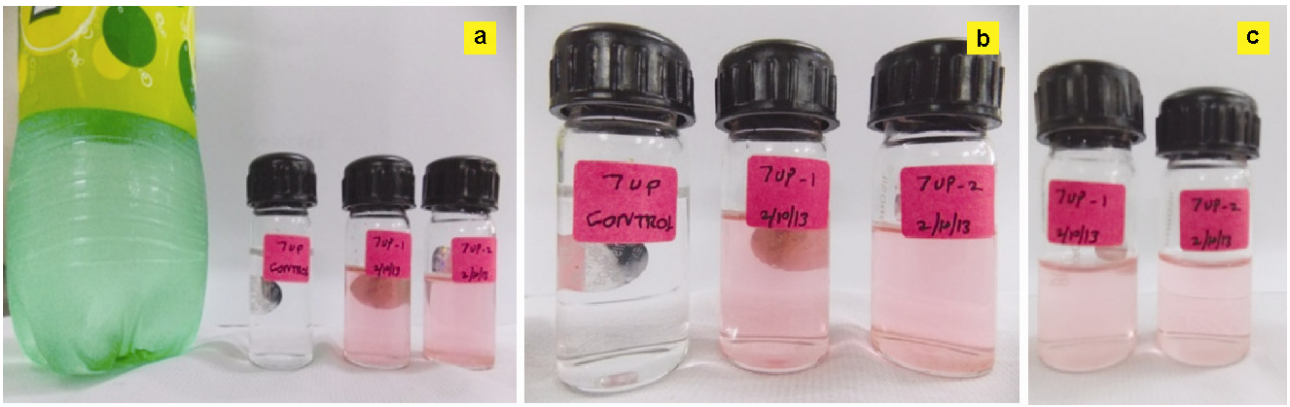


Plate 2- Phycobiliproteins (200 mg) in 7UP. A- 1st day, B- 10th day and C- 30th day

adequate to be used as food grade[4, 9]. The yield of R-phycoerythrin after purification was 0.024% (w/w).

**Preservative study**

The R-phycoerythrin obtained from *G. corticata* var. *corticata* preserved in 5 % NaCl as preservative showed 37.5 % loss of pigment (Fig. 1) on the hundredth day of storage at 0±5°C. Senthilkumar *et al*[10] reported 39.2 % of relative activity in 0.5 M NaCl on 30 days of study at 0±5 °C, however, when compared to earlier study, the present investigation retains the relative activity of 62.5 % of R-PE.

**Stability of phycoerythrin in drinks**

Dialyzed phycobiliprotein added to 7UP retained its color in the drink, whereas drinking water and soda could not retain the color even for a shorter period of three days. The sugar present in 7UP would have probably acted as a preservative and retained the color for longer period (Fig. 2 & Plate 2) as compared to water and soda which do not contain any additives. Similar studies conducted earlier shown that sugar is accepted as a preservative for phycobiliproteins and could retain the color for longer periods[9, 11-16]. However, Dufosse *et al* reported that shelf life of fluorescent color pigment in alcoholic beverages are short[17].

## Conclusion

The present study reveals that R-phycoerythrin either in pure or crude form as phycobiliproteins from *G. corticata* var. *corticata* can be used as an alternative source for natural colorants in drinks due to its antioxidant property and other nutraceutical benefits. R-phycoerythrin stability on carbonated drinks showed favorable outcome by this study. Moreover, this work presents the basic information on stability and application of R-phycoerythrin. Further studies will be needed to check the extent of compatibility and acceptability of the product and to utilize it in various other food applications.

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