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Research Article

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Efficacy of different extraction methods of phycocyanin from *Spirulina platensis*

Pandian Prabakaran*, A. David Ravindran

Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram-624 302, Tamilnadu, India.
**E-mail: psspvm@gmail.com*

ABSTRACT

Phycocyanin is a major light harvesting pigment of cyanobacteria, it has gained importance in many biotechnological applications in food sciences, therapy, immunodiagnostics, cosmetics and pharmacological processes. Screening of cyanobacterial strain showed maximum phycobiliprotein in *Spirulina platensis*. A number of drying methods studied for the processing of *Spirulina* (oven dried and sun dried) resulted in loss of phycocyanin. Therefore phycocyanin extraction from fresh biomass was studied. Of the extraction methods tested, homogenisation using a mortar and pestle yielded 18.11% phycocyanin while freezing and thawing extraction yielded 17.68%. Acid treatment also resulted in phycocyanin leaching. Effective phycocyanin extraction was achieved only with 8–10 N hydrochloric acid and yielded phycocyanin ranging from 5% to 46%. Phycocyanin sample was analysed by Fourier transform infrared spectroscopy.

Key words: Phycocyanin, *Spirulina platensis*, Cyanobacteria, Drying methods, Acid treatment

INTRODUCTION

Cyanobacteria with high levels of specific phycobiliproteins are of commercial interest. The primary potential of these molecules are as natural dyes in food industry. A number of investigations have shown their health-promoting properties and pharmaceutical applications. Among different phycobiliproteins phycocyanin, is of greater importance because of its various biological and pharmacological properties. Studies have demonstrated its antioxidant property (Miranda *et al*., 1998) antimutagenic (Chamorro *et al*., 1996), antiviral (Ayehunie *et al*., 1998), anticancer (Chen *et al*., 1995; Schwartz *et al*., 1988), anti-allergic (Kim *et al*., 1998), immune enhancing (Qureshi *et al*., 1996), hepato-protective (Gonzalez *et al*., 1993), blood vessel relaxing (Paredes-Carbajal *et al*., 1997), neuro-protective (Romay *et al*., 1999), antitumor (Li *et al*., 2005), radical scavenging (Vadiraja and Madyastha, 2000) and anti inflammatory properties (Romay *et al*., 1999). Alzheimer's and Parkinson's can also be treated with phycocyanin (Rimbau *et al*., 2003). Blood lipid-lowering effects of phycocyanin also need special attention (Iwata *et al*., 1990). Owing to its fluorescence properties it has gained importance in the development of phycofluor probes for immunodiagnostics (Kronik and Grossman, 1983). As of now the annual market of phycocyanin is around 5-10 million US dollars (Sekar & Chandramohan, 2007). During screening of cyanobacteria for phycobiliproteins *Spirulina platensis* produced maximum phycobiliproteins (Sarada *et al*., 1999) and thus it was selected for extraction. In view of the multiple uses of phycocyanin were investigated different extraction procedures on phycocyanin yields from *Spirulina platensis*.

MATERIALS AND METHODS

Isolation and identification of microalgae

Water samples for microalgae isolation were collected from fresh water bodies (in and around Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India.) that appeared to contain algal growth. All samples were collected at about the time ranging between 0800 to 1100 hrs. Surface water and water at a depth of 0.50 meter were collected at each location. Water samples were taken from the sites to laboratories in bottles cooled in ice. Ten ml of water samples were transferred to a 500 ml conical flask containing 200 ml of sterilized Bold's Basal Medium (BBM) [Kanz and Bold, 1969] and then incubated on a rotary shaker at 27°C and 100 rpm under continuous illumination using white fluorescent light (maximum 2500 lux) for three weeks. BBM was composed of (mg/L) $NaNO_3$, 250; K_2HPO_4, 75; KH_2PO_4, 175; $CaCl_2$, 25; NaCl, 25; $MgSO_4$, 75; $FeCl_3$, 0.3; $MnSO_4$. $7H_2O$, 0.3; $ZnSO_4$. $7H_2O$, 0.2; H_3BO_3, 0.2; $CuSO_4$. $5H_2O$, 0.06. Every two days, the flasks were examined for algal growth using optical microscope, with serial dilutions being made in BBM from flasks showing growth. Subcultures were made by inoculation 50 µl culture solution onto petri plates containing BBM solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. Petri plates were incubated at 27°C under continuous illumination for two weeks. The purity of the cultures was confirmed by repeated plating and by regular observation under a microscope. The freshwater microalgae were identified and authenticated based on the guidelines of the standard manual (Presscott, 1959).

Culture conditions and growth

At first, cells of identified microalgae were cultivated in 2 L flask using modified Zarrouks medium and incubated batchwisely at 30±2°C. The cultures were bubbled with a sterile air and illuminated (3500 lux maximum). The cultures were grown for a period of 18 days and growth was monitored spectrophotometrically at 560 nm. Every three days the microalgal cells were harvested by centrifugation and washed twice with deionized water. Microalgal pellets were dried at 60°C for dry weight measurements (Sarada *et al*., 1999).

Drying method

The harvested biomass of *Spirulina platensis* was subjected to sun drying and oven drying methods. Phycocyanin was estimated from these samples and was compared with the content of phycocyanin extracted from wet biomass.

Extraction procedures

Phycocyanin was extracted from the wet biomass of *Spirulina platensis* using the following methods.

Method 1 Water extraction: *Spirulina* biomass was suspended in distilled water and the phycocyanin leached out was estimated spectrophotometrically (Siegelman and Kycia, 1978).

Method 2 Homogenisation of cells in a mortar and pestle: Biomass was homogenised in a mortar and pestle in the presence of acid washed neutral sand using 50 mM sodium phosphate buffer pH 6.8. The extract was centrifuged and the supernatant contained phycocyanin. The pellet was re-extracted with buffer to ensure complete recovery of phycocyanin.
Method 3 Freezing and thawing: Phycocyanin was extracted by repeated freezing and thawing of cells in 50 mM phosphate buffer pH 6.8.
Method 4 Acid extraction: The wet biomass was treated with different concentrations of hydrochloric acid (2, 4, 6, 8 and 10 N) at room temperature. At different time intervals (2, 4, 24 h) samples were centrifuged and supernatants taken for phycocyanin and estimated by the method of Sigelman and Kycia (1978) and the amount of phycocyanin was calculated as mg phycocyanin per ml using the equation O.D at 615 nm–0.474 (O.D at 652 nm)/5.34. The same method was followed for estimation of phycocyanin extracted by other procedures.

FTIR Spectra Collection

FTIR spectroscopy is used investigate the structure and chemical bonding of the phycocyanin, especially to indentify the functional groups in the same. FTIR attenuated total reflectance spectra were collected on a PerkinElmer Spectrum 400 FTIR instrument using a diamond smart iTR reflectance cell with a DTGS detector. *Spirulina platensis* phycocyanin did not require any preparation and was pressed against the diamond cell prior to scanning. Phycocyanin from *Spirulina platensis* was taken for each spectrum and the spectra were collected in the range of 4000 to 500 cm^{-1} (at 4 cm^{-1} resolution) and data were exported using Orgin 6.0 Microcal ™ Software Inc.

RESULTS AND DISCUSSION

In this study, from the six different water bodies were collected and 16 microalgal cultures were isolated. Only one isolates (*Spirulina platensis*) of the 7 microalgal cultures was selected based on the literature (Table 1).

Table 1. Isolation of microalgae from different locations

S.No	Location	Latitude	Longitude	Name of the microalgae
1	Kamarajar dam	10°17’43.44” N	77°48’44.06” E	*Chlorella* sp.
2	Palar dam	10°24’30.61” N	77°29’38.39” E	*Haematococcus* sp.
3	Palani pond	10°26’12.59” N	77°30’52.27” E	*Ulothrix* sp., *Chlorococcus* sp.
4	Manjalar dam	10°11’37.15” N	77°37’55.86” E	*Scenedesmus* sp.
5	Nanganji dam	10°35’35.34” N	77°29’38.39” E	*Spirulina platensis*.
6	Anaippatti dam	10°05’20.15” N	77°51’10.28” E	*Scytonema* sp.

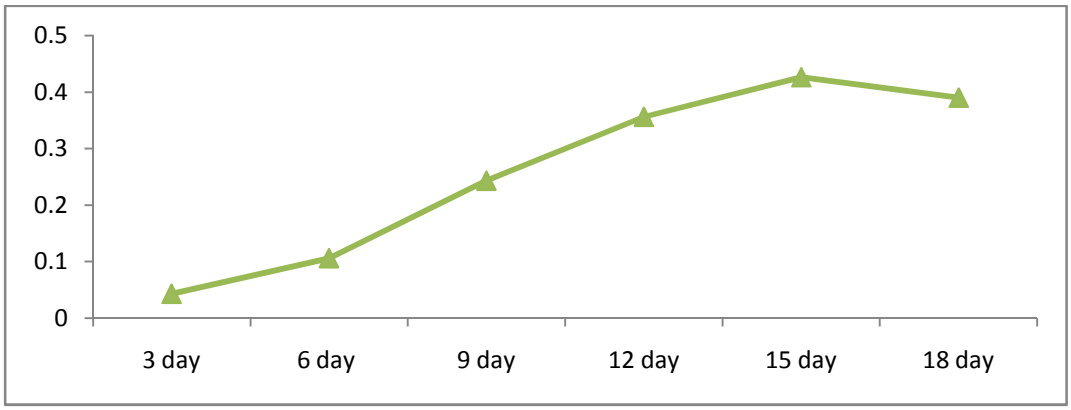

Fig.1. Growth analysis of *Spirulina platensis*

Under suitable conditions and sufficient nutrients, microalgae can grow profusely (Chisti, 2007). Fig.1 shows the growth rate of *S. platensis* indicating the enhanced growth rate corresponding with enhanced incubation times. The growth rate of the *S. platensis* reached its peak in 15 days of incubation. The biomass productivity was expressed as dry cell weight per litre indicating *S. platensis* (0.42±0.01 g l^{-1} dw) good growth rate.

Drying method	Temperature (°C)	Drying time (h)	% Phycocyanin recovery
Wet biomass (control)	-	-	100
Oven dried	60	8	67
Sun dried	33*	78	82

***Range between 30°C to 35°C during the experiment period**
Table.2. Phycocyanin content in *Spirulina platensis* dried by different drying methods

The harvested biomass of *Spirulina* was subjected to various drying methods (Table 2). The phycocyanin content extracted from fresh biomass was considered as 100%. In oven dried, the phycocyanin content was 67% and sun dried 82%. There was considerable loss of phycocyanin in dried samples. The significant loss of phycocyanin in dried samples could be due to its peripheral position in phycobilisomes on the thylakoid membrane and attributable to its sensitivity to temperature (Gantt, 1981).

S. No	Methods	Phycocyanin (mg/100 mg)
1	Water extraction	17.25
2	Homogenization (mortar and pestle)	18.11
3	Freezing and thawing	17.68

Table 3. Comparision of different methods for phycocyanin extraction from *Spirulina*

In the extraction methods, water extraction method is the slowest and takes 2-3 days for comparable yields of phycocyanin while homogenization either by mortar and pestle or freezing and thawing takes 8-10 h. In Table 3 homogenization method showed higher extraction of phycocyanin than freezing and thawing method. In the water extraction process, phycocyanin leaching was very slow and observed only in cells harvested in late exponential phase.

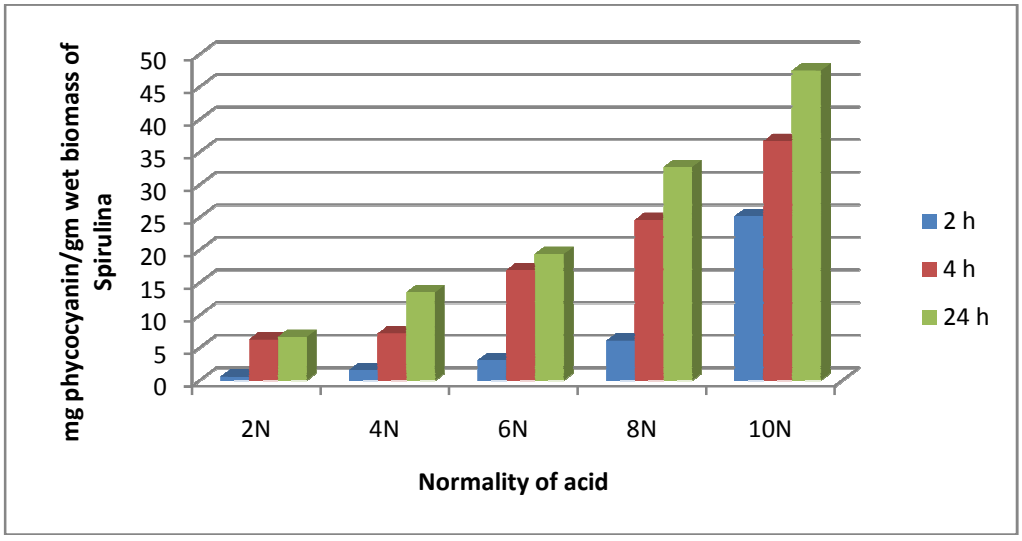

Fig. 2. Phycocyanin extraction from *Spirulina platensis* wet biomass with different concentrations of HCl

The results obtained for the effect of increasing concentration of hydrochloric acid on extraction of phycocyanin with time are shown in Fig. 2. Higher amount of phycocyanin extraction was achieved only with 8–10 N

hydrochloric acid. The microscopic observation of cells indicated complete disintegration of cells at these concentrations and also resulted in separation of the pigment phycocyanobilin from phycobiliprotein, phycocyanin (O'hEocha, 1963). Hydrochloric acid concentration of 2N, 4N and 6N of were not good enough for phycocyanin extraction.

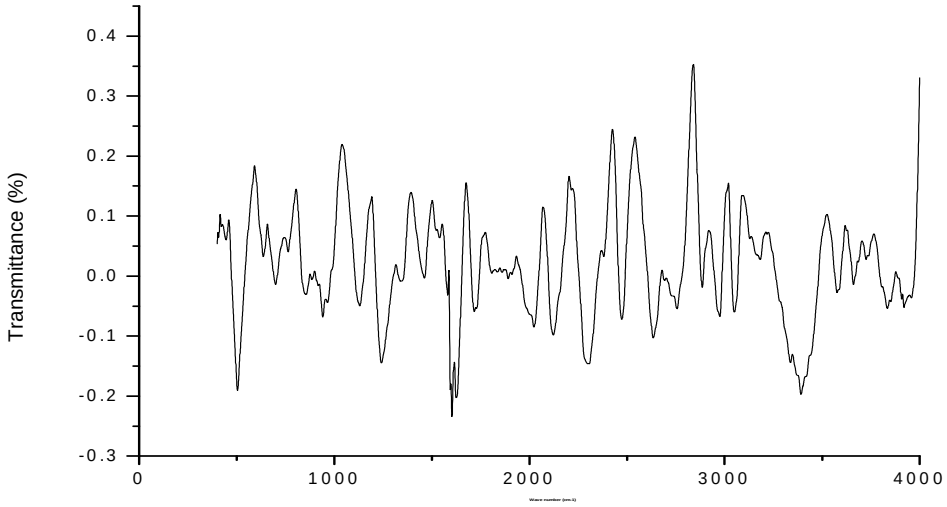

Fig.3. FTIR transmittance spectra of the phycocyanin from *Spirulina platensis*

FTIR technique was used for evaluation the type of organic and inorganic complexes. The FTIR analyzes of *Spirulina platensis* represent the (Fig. 3) functional groups. The infra red spectrum shows a frequency ranges from 3860-3700 cm^{-1} representing the O-H stretching vibration, presence of carbohydrate and amino acid. The frequency ranges from, 3395-3187 cm^{-1} peaks are representing in the N-H stretching vibration presence of secondary amines (protein, lipid) and frequency ranges from 3051-2978 cm^{-1} peak are representing aliphatic C-H stretching vibration. The frequency ranges from 1638-1593 cm^{-1} peak are representing C=O stretching vibration (ester and amino acid). The following peaks 1650-1580 cm^{-1} are present in the N-H bending vibration present in the carbonyl β unsaturated ketone amide. The frequency ranges from 1435-1405 cm^{-1} peak are present in the CH_2 bending vibration. The particular frequency ranges from 1350-1260 cm^{-1} C-O stretching, O-H bending vibration presence of alcohol. The following frequency ranges from 1300-1250 cm^{-1}, presence of C-O asymmetric C-O-C stretching presence of esters, the peak range 1120-1030 cm^{-1} present in symmetric C-H stretching, presence of antioxidant enzymes, the peak value representing 1050-1010 cm^{-1} present in of SO_3 symmetric stretching vibration, presence of acids, RSO_3 and ionic sulphonates. The frequency ranges from 700-620 cm^{-1} peaks are representing the S-O stretching vibration of sulphonic components. The frequency ranges from 620-490 cm^{-1} peaks are representing in C-I stretching vibration presence of iodo compounds. From these individual lipid spectra, it is clear that characteristic and distinct fingerprints for phycocyanin exist in the FTIR spectrum of *Spirulina platensis*. This result has been in agreed with many others (Laurens and Wolfrum, 2011; Patil *et al*., 2011; Venkatesan, 2012)

CONCLUSION

This study investigated the effective extract methods of phycocyanin from *Spirulina platensis.* Water samples were collected from the six different water bodies and 7 microalgal cultures were isolated. Only one isolates (*Spirulina platensis*) of the 7 microalgal cultures was selected based on their purity and phycocyanin producing ability. The selected *Spirulina platensis* reached highest growth rates (0.42±0.01 g l^{-1} dw) on 15 day of incubation. In drying methods, fresh biomass is best for phycocyanin extraction to avoid loss in pigment.

The homogenization method is more effective than freezing and thawing, while acid extraction (8-10 N) would be useful for direct extraction of phycocyanin. The presences of phycocyanin compounds from *Spirulina platensis* were substantiated by the findings of FTIR.

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