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Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and antiinflammatory effect

Gopal Prabakaran [a], Pitchai Sampathkumar [a], M. Kavisri [b], Meivelu Moovendhan [a,c,*]

[a] Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India
[b] Department of Civil Engineering, School of Building and Environment, Sathyabama Institute of Science and Technology, Chennai 600119, Tamil Nadu, India
[c] Department of Biotechnology, Bhupat Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras (IIT-M), Chennai 600 036, Tamil Nadu, India

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ABSTRACT

The phycocyanin was purified by Sephadex- G-100 and RP-HPLC and protein content was found to be 52.82% and the high purity fraction was collected and RP-HPLC analysis of fractionated phycocyanin, the α-subunit and β-subunit were detected in 4.9 and 11.1(mAU). The frequency of peak 1456.26 cm^{-1} has showed the CH_2 bending vibration and the protein amide II band was detected at 1539.20 cm^{-1} (C═O stretching) and 2358.94 cm^{-1}. In ^{1}H NMR analysis, 14 chemical shifts (δ) were observed and signals confirmed namely alkyl halide, alkene, aldehyde proton and carboxylic acid. The *in vivo* anticancer effect was assessed by MTT assay against HepG-2 cell lines and *in vivo* antidiabetic effect was carried out through α-amylase and β-glucosidase enzyme inhibition methods. The promising anticancer effect 68% was noticed at the concentration of 500 μg/ml and lower anticancer effect was noticed at the concentration of 100 μg/ml against Hep-G2 cell lines. The α-amylase and β-glucosidase enzyme inhibition of phycocyanin showed dose dependent and maximum inhibition effect at 250 μg/ml. Phycocyanin anti-inflammatory effect such as inhibition of albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities have been recorded maximum level at 500 μg/ml. Phycocyanin have complex structure and high molecular weight with more biomedical applications for drug development.

1. Introduction

Spirulina is a symbiotic, multicellular and filamentous blue-green microalgae with high capability of fix nitrogen from air. It is recognizable by the arrangement of the multicellular cylindrical trichomes in an open left-hand helix with the entire length [1]. The body surface of *Spirulina* is smooth and without covering, so it can easily digestible through simple enzymatic systems and the main photosynthetic pigment is phycocyanin, which is blue in color [2]. *Spirulina* have been used for over 1000 years as a food source, some of which have rich protein content as 55% to 70% of the total dry weight [3].

Phycocyanins are commonly well-known as proteins (Phycobiliproteins) and it's a large, high water soluble with supramolecular protein clusters. In a microalga phycocyanins was present in high level and the composition was reported 40–60% of the total soluble proteins in cells of microalgae [1]. Generally, microalgae derived phycocyanins are majorly classified into 3 types namely phycoerythrin, phycocyanin and allophycocyanin. These phycocyanin pigments are composed of two polypetides, such as low molecular weight α-unit (12–19 kDa), another one is large molecular weight β-unit (14–21 kDa) and commonly found in equimolar amounts [4,5]. Phycobiliproteins are accessory photosynthetic pigments that involved in an extremely potent energy transfer chain in photosynthesis process, dependable for about 50% of light capitation from cyanobacteria and red algae [6].

Phycocyanin have been widely used as nutritional ingredients, natural dyes, florescent markers, pharmaceuticals such as antioxidants and anti-inflammatory reagents also it was applied in colorant in food (chewing gums, dairy products, gellies *etc.*) cosmetics namely lipstick and eye liners applications in worldwide [3,4,7]. It also has high therapeutic values such as immune modulatory activity and anticancer activity and also a dense blue pigment and it having food and biotechnology applications because of their color, fluorescence and antioxidant properties [7]. Cyanobacteria, as a source of phycocyanin are being exploited for a long time. However, most of researchers have been focused on production and purification of phycocyanin from *Spirulina platensis* [8,9]. The above facts keeping in mind, the present study was undertaken to investigate the production purification and chemicals characteristics of

* Corresponding author at: Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India.
E-mail addresses: prabakaranbio@gmail.com (G. Prabakaran), kavisrimanikannan1985@gmail.com (M. Kavisri), moovendhan85@gmail.com (M. Moovendhan).

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phycocyanin, additionally the biomedical applications namely anticancer, antidiabetic and anti-inflammatory potential was screened by *in vitro* assays.

2. Materials and methods

2.1. Extraction and estimation of pigment (C-Phycocyanin)

The marine microalgae *Spirulina platensis* was isolated from Vellar estuary, Parangipettai, Tamil Nadu, India and identified through morphological key characters by microscopic observation and the *S. platensis* was cultured mass scale in Zarrouk medium under optimum condition in laboratory. The matured *S. platensis* was harvested and processed for pigment extraction. The homogenized log phase *S. platensis* culture was centrifuged at 4000 rpm and to obtain pellet in a clean tube. The pellet was mixed with 100 ml of 20 mM acetate buffer containing 50 mM sodium chloride ($NaCl_2$) and 0.002 M sodium azide (NaN_3) (pH -5.10). The C-Phycocyanin was extracted by repeated freezing (−20 °C) and thawing at room temperature until the blue color becomes in acetate buffer. The cell debris was discarded by centrifugation at 5000 rpm for 10 mins and the extract therefore obtained was termed as crude extract. The amount of C-Phycocyanin was estimated as described by Bennett and Bogard [10] and the purity was determined by using the standard formulae:

$$\text{Purity} = A_{620}/A_{280}$$

2.2. Purification of C-phycocyanin

The crude phycocyanin from *S. platensis* was subjected to precipitation by using 65% $(NH_4)_2\,SO_4$ (Sigma Aldrich, USA) and kept overnight at 4 °C. The pellet was obtained by centrifugation at 27,000 rpm for 15 min at 4 °C and dissolved in 10 ml of the same extraction buffer solution and named as ammonium sulfate extract (ASE). The 10 ml of ASE was dialyzed against the extraction buffer solution by dialyses membrane (HiMedia, Mumbai, 12–14 kDa). Dialyses was carried out twice against 1000 ml extraction buffer, first at room temperature and again dialyzed against 1000 ml of extraction buffer at 4 °C overnight. The resultant extract was recovered from the dialyses membrane and filtered through 0.45 mm filter membrane [11]. The dialyzed phycocyanin was passed through Sephadex-G-100 column (2.5′ 20 cm) pre-equilibrated and eluted with 0.005 M Na-phosphate buffer (pH 7) at 1 ml/min^{-1}. The purity of obtained fractions was analyzed for every step of purification by estimating the total protein [12].

2.3. Reverse Phase High Performance Liquid Chromatography analysis (RP-HPLC)

The fraction purity was selected based on the high protein content. The high purity fraction of phycocyanin from *S. platensis* was performed by HPLC using a reversed phase (Supelco, Sigma Aldrich) column (250 × 4.6 mm i.d.) packed with 5 μm porous silica particles. This column was run at the flow rate of 1 ml min

5 $^{-1}$ for optimum separation efficiency. The entire solution and sample matrix were filtered through 0.5 μm membrane filter. The Discovery BIO Widepore C5 column was pre-equilibrated with 20% (*v*/v) aqueous acetonitrile (ACN) solution matrix containing 0.1% (*v*/v) Trifluoroacetic acid (TFA). The 20 μl of sample (200 μg/ml) was introduced and the elution was done by using a linear gradient from 20 to 100% (v/v) aqueous ACN (containing 0.1% TFA) in 45 min. Both PDA and fluorescence detector were connected in series for the detection of biliprotein subunits.

2.4. FT-IR spectral analysis

The functional groups profile of the purified phycocyanin from *S. platensis* was done by FT-IR spectral analysis. Infrared spectral analysis was performed using Shimadzu FT-IR 8300 instrument. The KBr pellet was prepared by mixing 1 mg of sample with 100 mg of anhydrous potassium bromide. The spectra were recorded from 500 to 4500 cm-1 and 30 scans at a resolution of 4 cm were averaged and referenced against air.

2.5. ^{1}H NMR spectral analysis

The structural feature of the purified phycocyanin from *S. platensis* was evaluated by ^{1}H NMR spectra by following the method of Schanda and Brutscher [13]. Approximately 30 mg of sample was dissolved in 0.5 ml of D_2O (99.9%) in a NMR tube (5 mm diameter). The ^{1}H NMR spectra were taken at 27 °C and the chemical shift was expressed in parts per million (ppm).

2.6. Anticancer activity

2.6.1. Cell lines and MTT assay

The Vero and Hep-G2 were obtained from National Centre for Cell Sciences (NCBS), Pune, India. The cells were grown in 96-well tissue culture (TC) plate in Dulbecco's Minimum Essential Medium (MEM) and essential solutions and incubated in CO_2. The MTT assay was done using the methodology of Siddiqui [14]. The monolayer of cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using growth medium. The 100 μl of purified phycocyanin from *S. platensis* with different concentrations (100, 200, 300, 400 and 500 μg/ml) was seeded to each well respectively. The absorbance was measured using a microplate reader at the wavelength of 540 nm. The percentage of cell growth inhibition was determined by the following standard formula:

% of Cytotoxicity = (1-Abs test /Abs Control) × 100.

2.7. In vitro antidiabetic activity

2.7.1. α-Amylase inhibitory assay

The *in vitro* α-amylase inhibitory activity of purified phycocyanin from

S. platensis was done by following the method of Apostolidis and Lee [15]. Briefly, the starch solution (1% *w*/*v*) was prepared by stirring with 1 g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7 mM of sodium chloride solution. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase α-amylase (PPA) in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7 mM of sodium chloride. The 100 μl of (50, 100, 150, 200 and 250 μg/ml) purified phycocyanin and the same concentrations of Acarbose (Standard drug) was taken for comparison, then 200 μl porcine pancreatic amylase was added and the mixture was incubated at 37 °C for 20 min. To the reaction mixture, 100 μl (1%) starch solution was added and incubated at 37 °C for 10 min. The reaction was stopped by adding 200 μl DNSA (1 g of 3,5 di nitro salicylic acid, 30 g of sodium potassium tartarate and 20 ml of 2 N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 min. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 μl in distilled water. The control, representing 100% enzyme activity was prepared in a similar manner without extract.

2.7.2. β-Glucosidase inhibitory assay

The *in vitro* β-glucosidase inhibitory activity of purified phycocyanin from *S. platensis* was done by following the method of Kim et al. [16]. 1 mg of glucosidase was dissolved in 100 ml of phosphate buffer

(pH 6.8). To 100 μl of (50, 100, 150, 200 and 250 μg/ml), 200 μl glucosidase were added and the mixture was incubated at 37 °C for 20 min and the same concentrations of Acarbose (Standard drug) was taken for comparison. To the reaction mixture, 100 μl 3mMNitrophenyl p-Dglucopyranoside (p-NPG) was added and incubated at 37 °C for 10 min. The reaction was terminated by the addition of 2 ml Na_2CO_3 0.1 M and the β-glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of nitrophenol released from p-NPG. Acarbose was used as positive control of amylase and β-glucosidase inhibitor. The IC_{50} value of the purified phycocyanin was calculated by Probit analysis software (EPA, USA).

2.8. In vitro anti-inflammatory assays

The *in vitro* anti-inflammatory activity of purified phycocyanin from *S. platensis* was estimated by inhibition of albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities. The albumin denaturation inhibition assay was performed spectroscopically by Mizushima [17]. The antiproteinase activity was determined spectroscopically following by the method of Oyedepo [18]. The hypotonicity-induced haemolysis assay was determined by spectroscopic method following the method of Azeem [19] and the anti-lipoxinase activity was carried out by Shinde et al. [20].

2.9. Statistical analysis

The experiments were carried out by triplicate and the statistical analysis was performed using one-way analysis of variance (ANOVA) using SPSS Software. The results were expressed as mean ± S.D. *P* values at <0.05 level were considered as significant.

3. Results and discussion

3.1. Phycocyanin yield

Several methods were employed to extract the pigments (phycocyanin) from microalgae but some are multiple steps, long time process and most cost effective [11]. In the present study, the phycocyanin was extracted by standard procedure and the yield was estimated by 52.82%. The present study is in agreement with Moraes et al. [21] who extracted the phycocyanin from *S. platensis* wet biomass by following six extraction procedures and reported the maximum yield of 56% in the ultrasonication method [22] reported the, similar amount of phycocyanin presented in *Spirulina platensis* by following the High pressure extraction process. Correspondingly, Kumar et al.[11]described the, similar level of phycocyanin content in *S. platensis* extracted by ammonium sulphate precipitation methods. The present study and previous reports clearly showed that the sonication method have been more efficient than the other methods.

3.2. Purification of phycocyanin

Purification is an important step and techniques for separating the complex molecules. Researchers widely are being followed several purification methods *viz* Ion exchange, column, HPLC chromatography *etc.* [11]. In this study, the crude phycocynin was purified by column chromatography with Sephadex- G-100 resins and the each fractions were determined the protein concentration to check the purity. The results of the present study have been showed 92.11% of purity was recorded in 4th fraction (Table 1). Seo et al. [22] purified the phycocyanin from *S. platensis* by hexane separation method and the high-pressure process who reported the lowest purity of fractionated phycocyanin. Additionally, the phycocyanin purity separated by ion exchange chromatography with DEAE cellulose resins and reported moderate purity of phycocyanin [11]. From the results of the present study and literature survey revealed that the DEAE column chromatography is an effective method for purification of the phycocyanin from *S. platensis* compared with the other previously followed methods.

Table 1
Purification of phycocyanin from *S. platensis.*

Column type	Fractions	Purity (%)
Sephadex-G-100	1	29.14
	2	53.18
	3	74.32
	4	92.11
	5	61.24
	6	56.17

3.3. RP-HPLC analysis of purified phycocyanin

Nowadays, RP-HPLC is widely used by researchers instead of spectroscopic methods for purification and identification of the pigments from plant and microbial sources [23,24]. In the present investigation, the fractionated phycocycnin pigment was purified through RP-HPLC system and the results have been displayed in Fig. 1**.** This study, the purified phycocyanin has showed 4 peaks and two major peaks 4.9 (mAU) and 11.1 (mAU) were detected at the time of 17.5 and 19.1 respectively. The peak 4.9 (mAU) was considered as α-subunit and 11.1 (mAU) was considered as β-subunit of purified phycocyanin. In the present study results in correlated with Kumar et al. [11] who confirmed α and β sub units of the purified phycocyanin derived *S. platensis* through HPLC analysis and reported the molecular weight 16 and 17 kDa respectively. Kissoudi et al. [25] studied the purified food grade

C-phycocyanin from *Arthrospira platensis* and reported 98.14% of purity and different sub units of phycocyanin. Similarly, Kasinak et al. [26] reported the quantity and identified the phycocyanin and β-carotein from cyanobacteria, *Microcystis aeruginosa* by HPLC analysis. The present study revealed that the HPLC is a most suitable technique to identify and quantify the microalgae derived pigments.

3.4. FT-IR spectral analysis

The FT-IR spectroscopy is a major tool for analysis rapid and non-destructive of <1 mg, sized samples. In this technique, the chemical bonds vibrate at a characteristic frequency envoy of its structure, bond angle and length. Hence, separate molecules include interacting with incident radiation through absorbing the radiation at precise wavelength. Generally researchers have been used the FT-IR spectra used to evaluate the functional groups and structure of the molecules [27,28]. In the present study, the *S. platensis* phycocyanin has showed functional groups, in peak frequency of 673.86 and 794.67 cm^{-1} represents the

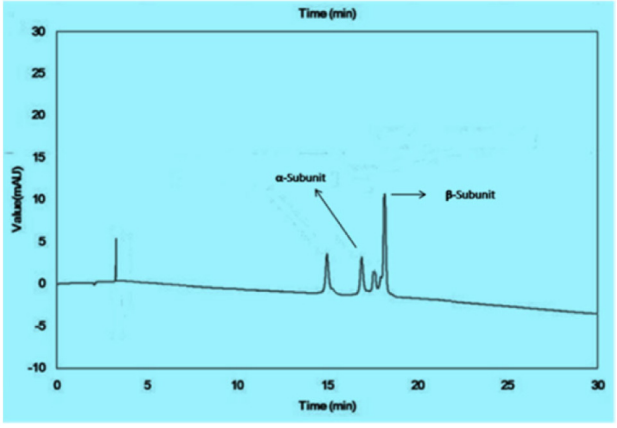

Fig. 1. RP-HPLC profile of purified phycocyanin from *S. platensis.*

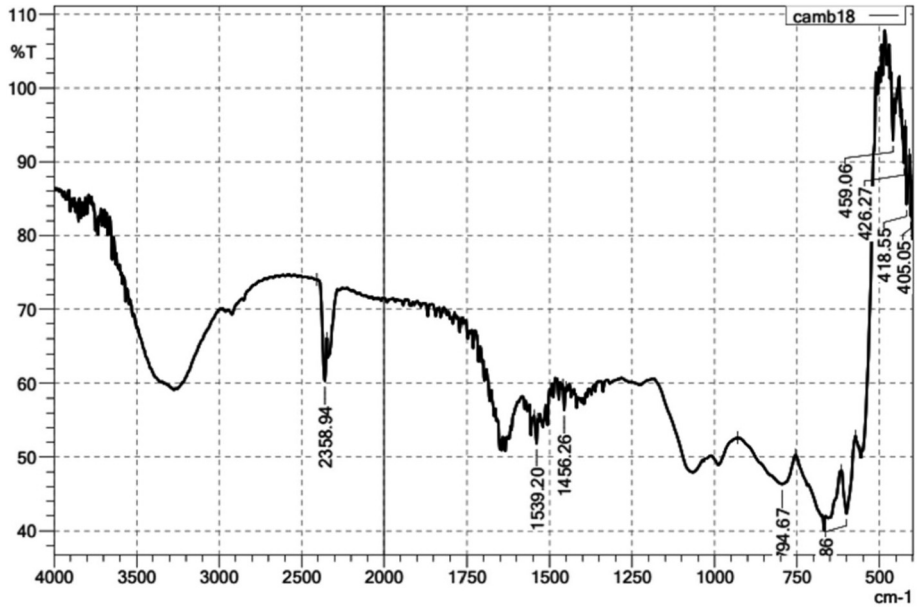

Fig. 2. FT-IR spectrum of purified phycocyanin from *S. platensis.*

S—O stretching vibration. The frequency of peak 1456.26 cm^{-1} has showed the CH_2 bending vibration and the protein amide II band was detected at 1539.20 cm^{-1} (C═O stretching) and 2358.94 cm^{-1} revealed that the presence of carboxylic acids (Fig. 2). Duygu et al. [29] recorded eleven active functional groups with different bond stretching at different wave numbers in marine microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* respectively. Similarly, Domenighini and Giordano [30] have been reported different active functional groups *viz* Nitro, amine, amide and halides in 14 microalgal species through FT-IR spectroscopy respectively. Additionally, Venkatesan et al. [31] have studied the functional groups profile of *S. platensis* by FT-IR spectral analysis and results has showed 12 different active functional groups with different bonds stretching at frequency range 620–3560 cm^{-1}. Likewise, Vidyadharani and Dhandapani [32] have recorded the functional groups such as -NH2, –OH, –C-H, –C═C and alkyl stretching in *Chlorella vulgarisviz*, FT-IR spectral analysis. The present study confirmed that the *S. platensis* have been found more active functional groups than the other algae and especially the phycocyanin showed more active functional groups.

3.5. ^{1}H NMR spectral analysis

Nuclear Magnetic Resonance (NMR) spectroscopy played an important role in chemistry and drug industry *etc.* Several unknown compounds structure was identified, functional groups stretching and known compounds structure was confirmed by this analysis [33]. In this analysis, 14 chemical shifts (δ) were detected at different ppm range between 2 and 13 ppm in ^{1}H NMR spectrum. From the proton chemical shifts of phycocyanin, the signals namely 2.52 (δ), 2.88 (δ), 3.11 (δ) and 3.20 (δ) chemical shifts revealed that the presence of Alkyne (C ≡ C-H) type protons. The chemical shift 3.81 and 3.99 (δ) indicated the Alkyl halide with CH-X proton and the chemical shifts 6.12 (δ), 6.28 (δ), 6.51 (δ), 6.79 (δ), 6.85 (δ) and 6.93 (δ) confirmed that the occurrence of Alkene with C—H type protons and 12.60 (δ) chemical shift revealed that the aldehyde proton RC(═O)-H. The chemical shift 12.60 (δ) indicated the presence of the carboxylic acid (RCO_2H) (Fig. 3). Nuzzo et al. (2013) have been studied the structural features of lipids molecules ^{1}H NMR from microalgae species *viz Thalassiosira weissflogii*, *Cyclotella cryptica* and *Nannochloropsis salina* and 7 chemical

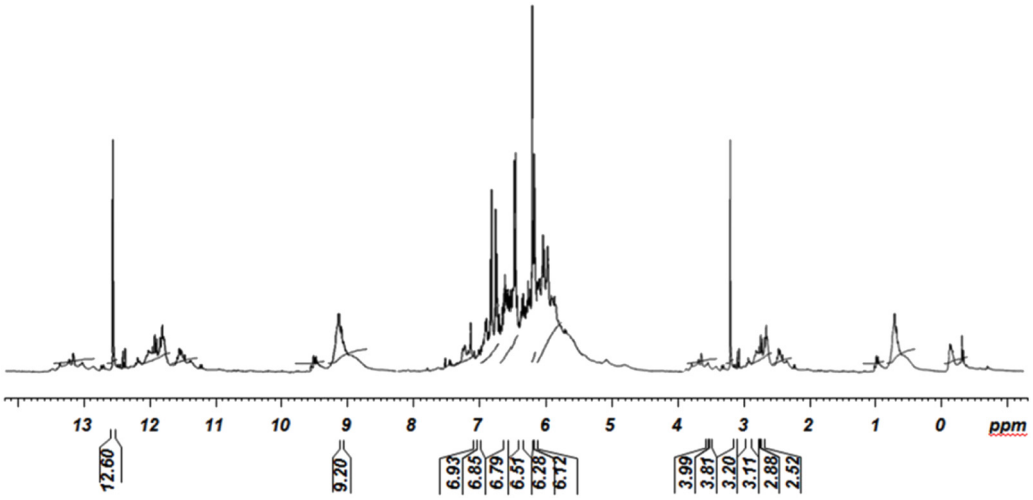

Fig. 3. ^{1}H NMR spectrum of purified phycocyanin from *S. platensis.*

shifts were recorded between 2.06 and 4.34 ppm respectively. Correspondingly, Wiegand et al. [34] investigated the structural characters of the α-phycoerythrocyanin peptides from Thermophilic cyanobacterium *M. laminosus* and *Fischerella* sp. through NMR spectroscopy and reported the different functional groups with different proton types at various ppm and chemical shifts respectively. Mangoni et al. [35] recorded 19 and 6 chemical shifts with different ppm of pigments extracted from *Chattonella verruculosa.* Similarly, Schra and Kroe [36] have been confirmed the Phycocyanobilin (Phycocyanin) from the microalgae by using ^{1}H NMR spectral technique. The NMR spectra result of the present study is confirmed that the *S. platensis* purified phycocyanin has similar protons bond stretching of other microalgae species.

3.6. Anticancer activity

The anti-liver cancer ability of *S. platensis* phycocyanin was carried out by MTT assay and to access the cancer cell growth inhibition. The anticancer effect was noted increased with increasing of phycocyanin concentrations and cell growth was inhibited for 18, 26, 32, 55 and 68% at the concentrations of 100, 200, 300, 400 and 500 μg/ml against Hep-G2 (liver cancer) cell lines and did not show any cell growth inhibition against Vero cell lines. The maximum anticancer effect was noticed in 68% at the concentration of 500 μg/ml followed by 400 μg/ml concentration for 55% and the minimum anticancer effect was noticed at the concentration of 100 μg/ml (Table 2) (Fig. 4). The anticancer potential of phycocyanin is intermediated by BCR-ABL signaling and inactivation of the downstream PI3K/Akt pathway. Furthermore, phycocyanin can modify the mitochondrial membrane potential (MMP), which can encourage the release of cytochrome *c* and stimulate the formation of reactive oxygen species (ROS), eventually lead to cancer cell apoptosis. Shanab et al. [37] investigated the anticancer potency of *Oscillatoria* sp. and *Nostoc muscorum* species pigments on Hep-G2 and EACC cell lines and reported the maximum activity in both cell lines. At the same time, Abu Zaid et al. [38] have been studied the anticancer activities of *S. platensis* pigments extracted by polar solvents on HCT116, HEPG2 cell lines through MTT assay and the result showed the maximum cancer cell growth in inhibition. Sujatha et al. [39] evaluated the anticancer effect of Fucoxanthin pigment from marine macroalgae on Hep-G2 cancer cell lines by MTT assay and noticed concentration dependent activity and the maximum activity was recorded in 83.64% of cancer cell growth at the concentration of 1000 μg/ml. Similarly, Lauritano et al. [40] tested the cell growth inhibition on human melanoma A2058 cancer cell line of microalgal pigment extracts of *Skeletonema marinoi*, *Alexandrium andersoni*, *Alexandrium tamutum* and *Alexandrium minutum* at different concentrations between 2.5 and 100 μg/ml by MTT assay and reported the maximum anticancer effect of microalgal species respectively. Correspondingly, Gardeva et al. [41] pointed out the anticancer ability of phycocyanin from microalgae *Arthronema africanum* against bone marrow extracted Graffi tumor cells and the results were found to be higher Transplant ability (%), lower Tumor size (mm), concentration dependent Mortality (%) and survival (days) in tumor bearing hamster. The result of the present study clearly showed that the phycocyanin from microlalgae have more potent against different cancer cell lines inhibitions.

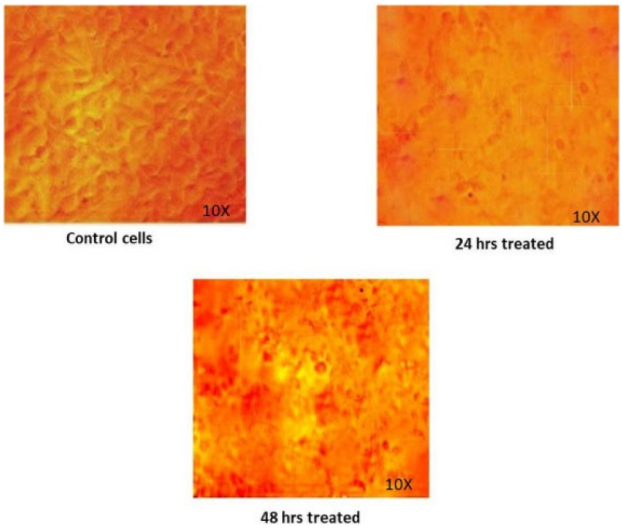

Fig. 4. Anticancer effect of purified phycocyanin from *S. platensis.*

3.7. Antidiabetic activity

The *in vitro* antidiabetic activity of *S. platensis* phycocyanin was estimated by α-amylase and β-glucosidase enzyme inhibition through spectroscopy method. In the present investigation, the α-amylase activity was recorded as concentration dependent and increasing of activity with increasing concentration of phycocyanin. The maximum α-amylase enzyme inhibition activity was recorded in phycocyanin and acarbose at the concentration of 250 μg/ml for 72 and 88% followed by 200 μg/ml for 56 and 61% respectively and the standard Acarbose have showed maximum inhibition in a lower concentration than the phycocyanin (Table 3). Ghosh et al. (2016) studied the *in vitro* antidiabetic activity of pigments from cyanobacteria species *viz*, *Lyngbya*, *Microcoleus*, and *Synechocystis* sp. by α-amylase inhibition method and reported the lesser enzyme inhibition effect than the present study. Likewise, Xu et al. [42] described lowest α-amylase enzyme inhibition activity of Phlorotannins pigments extracted from *Ecklonia kurome.* At the same time, Hwang et al. [43] studied the α-amylase enzyme inhibition activity of Fucoxanthin extracted microalgae *Sargassum hemiphyllum* and reported the maximum enzyme effect at the concentration of 500 μg/ml.

In this study, the β-glucosidase enzyme inhibition activity of phycocyanin from *S. platensis* was screened by spectroscopical method. The β-glucosidase enzyme inhibition action was recorded as concentration

Table 2
MTT assay of purified phycocyanin from *S. platensis.*

S. No	Concentration (μg/ml)	Cell inhibition (%)	CTC_{50} (μg/ml)
Vero cell lines			
1	100	–	–
2	200	–	
3	300	–	
4	400	–	
5	500	–	
Hep-G2 Cell lines			
6	100	18 ± 0.1	387.12 ± 0.34
7	200	26 ± 0.34	
8	300	32 ± 0.10	
9	400	55 ± 0.30	
10	500	68 ± 0.05	

Table 3
α-Amylase inhibition activity of purified phycocyanin from *S. platensis.*

Name of the sample	Sl. No.	Concentrations μg/ml	Percentage of α-amylase inhibition (%)	IC_{50} (μg/ml)
S. platensis purified phycocyanin	1	50	13 ± 0.1	231.45 ± 0.47
	2	100	21 ± 0.30	
	3	150	37 ± 0.21	
	4	200	56 ± 0.47	
	5	250	72 ± 0.31	
Acarbose (Standard)	1	50	18 ± 0.30	151.96 ± 0.57
	2	100	34 ± 0.15	
	3	150	49 ± 0.25	
	4	200	61 ± 0.35	
	5	250	88 ± 0.15	

dependent and increasing of activity with increasing of concentration of phycocyanin. The maximum activity of phycocyanin was found to be 65 and 80% at the concentration of 250 μg/ml followed by 51 and 67% at the dose of 200 μg/ml respectively. The minimum inhibition effect was noticed in 50 μg/ml and it was found to be 10 and 19% respectively and the standard Acarbose have showed maximum inhibition in a lower concentration than the phycocyanin (Table 4). The macroalgae derived pigments (Butyl-isobutyl-phthalate) have showed moderate β-glucosidase enzyme inhibition action (Bu et al., 2010). Similarly, Ghosh et al. [44] screened the *in vitro* antidiabetic effect of purified pigments from three cyanobacterial species namely, *Lyngbya*, *Microcoleus* and *Synechocystis* and reported the maximum enzyme inhibition (96.62%) in Purified C-phycocyanin and C-phycoerethrin from all species and lowest activity was noticed in crude solvent extracted compounds. Correspondingly, Priatni et al. [45] pointed out the α-glucosidase enzyme inhibition potency of marine cyanobacteria crude pigments and recorded the lesser enzyme inhibition even in higher concentration. Based on the facts, this investigation have exposed that the algae purified pigments has showed better α-amylase and β-glucosidase enzyme inhibition property.

3.8. In vitro anti-inflammatory activity

The protein inhibitory effect of purified phycocyanin has showed dose dependent and the maximum protein inhibitory activity was recorded in 500 μg/ml, it was found to be 47% in purified phycocyanin followed by 35% inhibition in 400 μg/ml and the maximum inhibition of aspirin (Standard) was recorded for 100% in 300, 400 and 500 μg/ml concentrations respectively and IC_{50} of phycocyanin was not recorded (Fig. 5). The proteinase enzyme inhibitory effect of purified phycocyanin and aspirin was noticed concentration dependent and the results was found to be 5, 11, 17, 29 and 36% at the concentrations of 100, 200, 300, 400 and 500 μg/ml of phycocyanin and the proteinase enzyme inhibition effect of commercial drug aspirin was recorded for 60, 88, 97, 100 and 100% in the concentrations of 100, 200, 300, 400 and 500 μg/ml respectively (Fig. 5).

In the present study, highest proteinase enzyme inhibition of purified phycocyanin was noticed in 500 μg/ml and it was found to be 36% followed by 29% inhibition in 400 μg/ml and the maximum enzyme inhibition of aspirin was recorded for 100% in 400 and 500 μg/ml concentrations. Further the hemolysis inhibitory potential of purified phycocyanin was recorded concentration dependent and found to be 17, 29, 42, 56 and 64% at the doses of 100, 200, 300, 400 and 500 μg/ml and the Diclofenac (Standard) was recorded for 55, 82, 96, 100 and 100% at the doses of 100, 200, 300, 400 and 500 μg/ml respectively (Fig. 5). The higher hemolysis inhibition was displayed in50 μg/ml and it was found to be 64% in phycocyanin followed by 56% inhibition in 400 μg/ml with the IC50 value of 427 μg/ml and the higher hemolysis inhibition of diclofenac was recorded for 100% in 400 and 500 μg/ml doses respectively (Fig. 5). The anti-lipoxygenase activity of purified phycocyanin and indomethacin was recorded dose dependent and results was found to be 21, 38, 49, 63 and 80% at the doses of 100, 200, 300, 400 and 500 μg/ml in purified phycocyanin and the commercial drug indomethacin was recorded in 91, 100, 100, 100 and 100% at the doses of 100, 200, 300, 400 and 500 μg/ml respectively (Fig. 5). The strong anti-liopoxygenase effect was displayed in 500 μg/ml with IC50 value of 307.41 μg/ml and it was found to be 80% followed by 63% inhibition in 400 μg/ml of purified phycocyanin and the strong anti-liopoxygenase effect of indomethacin was noticed in 100% at the doses of 300, 400 and 500 μg/ml respectively (Fig. 5).

Similar *in vitro* anti-inflammatory effects were recorded by Leelaprakash and Mohandass [46], who investigated the anti-inflammatory action of microalgae *Enicostemma axillare* derived pigments y through enzymes inhibition and proteins denaturation assays and reported correspondingly to that of present study. Additionally, Radhika et al. [47] have been studied the anti-inflammatory effect of marine macroalgae (Seaweeds) such as *Padinater tastomatica*, *Sargassum wightii*, *Gracilaria edulis* and *Caulerpa racemosa* and explored the minimum anti-inflammatory response of all seaweeds than the *S. platensis*. Correspondingly, Ramos et al. [48] reported the potential anti-inflammatory response of β-Carotene from microalgae *Dunaliella salina* and *Haematococcus* sp. and results has showed maximum anti-inflammatory potency such as anti-lipoxygenase and preotein and proteinase denaturation effect. Liu et al. [49] demonstrated the anti-inflammatory ability of pigment (Astaxanthin) extracted from microalgae species *viz Haematococcus pluvialis*, *Chlorella zofigiensis* and *Chlorococcum* sp. and results have displayed the maximum inhibition of anti-inflammatory enzymes inhibition respectively. Soontornchaiboon et al. [50] recorded the similar anti-inflammatory effects of microalgae pigments. Violaxanthin from *Dunaliella tertiolecta* and *Chlorella ellipsoidea* respectively. Singh et al. [51] have been recorded the potent anti-inflammatory effects of Zeaxanthin from *Chlorella saccharophila*. Researchers have been clearly reported previously for the mechanism of action of phycocyanin, the pigment phycocyanin can down-regulate the expression of pro-inflammatory cytokines such as IL-1β, IL-2, interferon-γ and tumor necrosis factor-α, transcription factors namely Janus kinase 3, signal transducers and activators of transcription 3 (stat3) and also it induce the expression of anti-inflammatory cytokines IL-4 [52]. From the results of the present study and literature survey revealed that the microalgae derived pigments has showed maximum anti-inflammatory response compared with the macroalgae and other marine species.

Table 4
β-glucosidase inhibition activity of purified phycocyanin from *S. platensis*.

Name of the sample	Sl. No.	Concentrations μg/ml	Percentage of β-glucosidase inhibition (%)	IC_{50} (μg/ml)
S. platensis purified phycocyanin	1	50	10 ± 0.20	198.11 ± 0.25
	2	100	18 ± 0.20	
	3	150	35 ± 0.45	
	4	200	51 ± 0.20	
	5	250	65 ± 0.26	
Acarbose (Standard)	1	50	19 ± 0.26	141.33 0.34
	2	100	31 ± 0.30	
	3	150	55 ± 0.29	
	4	200	67 ± 0.26	
	5	250	80 ± 0.35	

4. Conclusion

In the present study, the nutritional composition, chemical profiles were investigated and the phycocynin structural features and biological activities were studied. From the results of the present study, the *S. platensis* has showed more nutritional components and higher potent active metabolites. The *S. platensis* derived phycocyanin pigments have more active functional groups with potential anticancer, anti-diabetic and anti-inflammatory action and it could be considered as an alternate functional foods for food and drug industry.

Author contributions

Gopal Prabakaran - Conducting a research and performing the experiments, **Pitchai Sampathkumar** - Research activity planning and execution, formulation of overarching research goals and aims, **M. Kavisri** - Application of statistical and computational works, **Meivelu Moovendhan** - Oversight and leadership responsibility for the research activity planning and execution, writing the draft.

Declaration of competing interest

The authors have declared that no competing interests exist.

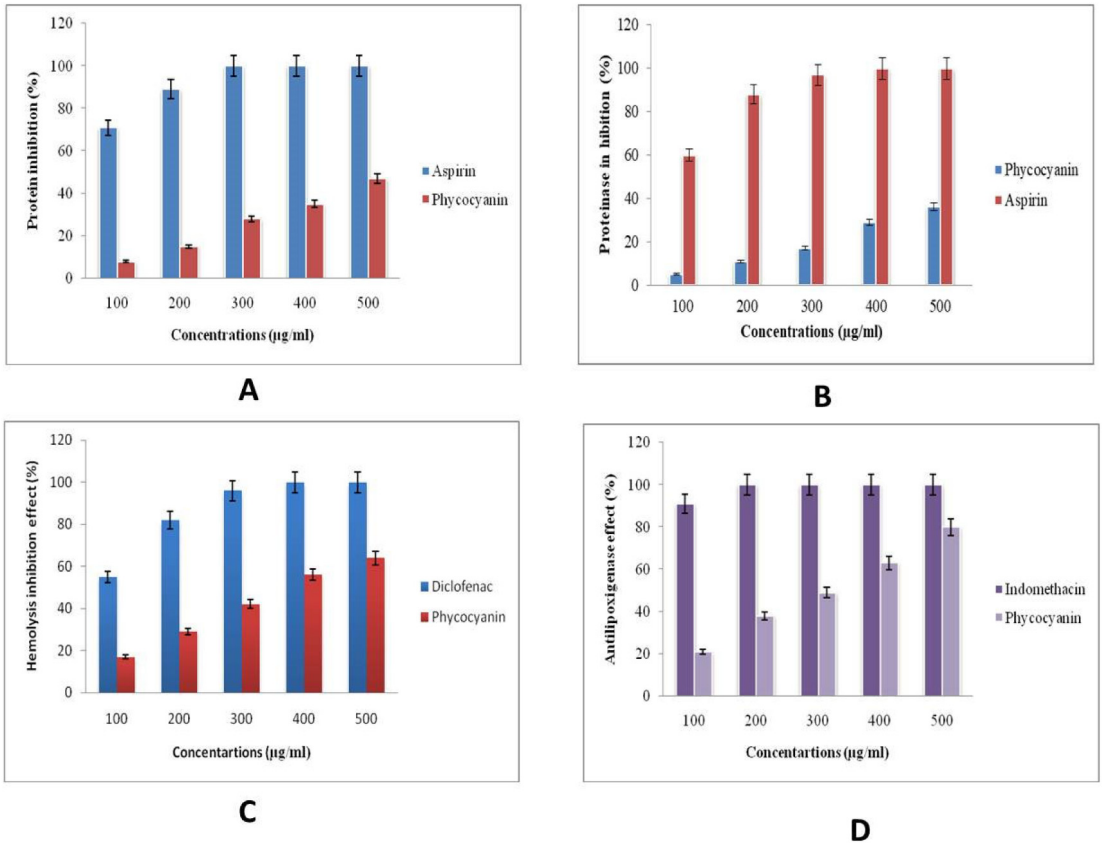

Fig. 5. *In vitro* anti-inflammatory effect of purified phycocyanin. A: Protein inhibition activity, B: Proteinase inhibition activity, C: Hypotonicity-induced haemolysis effect, D: Anti-lipoxigenase effect.

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