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PURIFICATION AND THERAPEUTIC POTENTIALITIES OF PHYCOCYANIN IN SPIRULINA PLATENSIS

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ABSTRACT

The aim of the present study is to purify and to find out the therapeutic potentialities of Phycocyanin(pc) present in *Spirulinaplatensis.* Novel method for purifying the phycocyanin of *S.platensis*was developed by freezing and thawing method. Antimicrobial assay was studied by agar well diffusion assay. The stability of the extracted pc was also confirmed by comparing its antioxidants using Ferric Reducing Antioxidant Potential (FRAP) and 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. Effect of aqueous extract of *S. platensis*was investigated on blood platelet aggregation in terms of prothrombin time (PT) and activated partial thromboplastin time (APTT).Two subunits of pc namely α-pc (15.6 kDa) and β-pc (23.4 kDa) were found to remain from the original mixtures based on SDS-PAGE. The results showed that all *S. platensis* extracts exhibited great potential antibacterial activities against four bacterial and fungal strains. The study revealed that *S. platensis* has potent bio compatibilities. The results of the study confirm that the pc can be used as drug, with lot of therapeutic ventures. All these studies confirm the therapeutic ventures of this compound as potent agent.

KEYWORD: ***SDS-PAGE; Therapeutic ventures: Phycocyanin***

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INTRODUCTION

Cyanobacteria with high levels of specific phycobiliproteins are of commercial interest. The primary potential of these molecules is used as natural dyes. Pc(phycocyanin) is a photosynthetic pigment of cyanobacteria. Pure pc is a widely used as natural colouring agent in food and cosmetics[1] due to the antioxidant and anti-inflammatory properties and also acts as potential therapeutic agents.[2,3] Pc is a major light harvesting pigment of cyanobacteria. It has gained importance in many biotechnological applications in food sciences, chemotherapy, immunodiagnostics, cosmetics and pharmacological processes. Therapeutic benefits on an array of diseased conditions including hypercholesterolemia, hyperglycaemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections are to be highlighted by several researchers. The cardiovascular benefits of *Spirulina*are main source of hypolipidemic, antioxidant and anti-inflammatory activities. A number of investigations have shown their health-promoting properties and pharmaceutical applications.[4]The pigment has a single visible absorption ranging between 615 and 620 nm and a fluorescence emission at the maximum of ~650 nm. Its molecular weight is between 70,000 and 110,000 Daltons.[5]The C-pc is composed of two dissimilar α and β protein subunits of 17000 and 19500 Da respectively, with one bilinchromophore attached to α subunit (α 84) and two to the β subunit (β 84, β 155).[3] The C-pc exists as a complex interacting mixture of trimer, hexamer and decamer aggregates.[6] In the present study, the culture filtrates and C-pc were obtained from filamentous freshwater cyanobacterium*S. platensis* were tested for their antibacterial and antifungal activity against four different bacterial and fungal cultures. The antioxidant capacity of compounds related to the prevention of several diseases including cancer, coronary heart diseases, inflammatory disorders, neurological degeneration, and aging [7,8]also prevent cell damage caused due to free radicals. The effects of different factors on the antioxidant activity of pc were studied. Reactive oxygen species (ROS) are involved in a diversity of vital processes like inflammation, atherosclerosis, cancer, reperfusion injury. Studies have demonstrated its antioxidant property.[9] Blood platelets are involved in haemostasis. The normal haemostatic system limits blood loss by precisely regulating the interactions between the components of vessel wall, circulating blood platelets and plasma proteins. Platelets can adhere to the walls of the blood vessels and release bio-reactive compounds. They then aggregate to each other. These properties increase to a well-established level in conditions of arterial thrombosis and thermogenesis which may cause life-threatening disorders such as unstable angina, heart attack and reclusion's after angioplasty.[10] Therefore, inhibition of platelet aggregation is important in the prevention and treatment of cardiovascular diseases.[11,12] Several drugs have been developed to block the different steps in platelet activation pathways. Inhibition of platelet function by Aspirin has been very well established.[13] Besides using various anticoagulant drugs like heparins, vitamin K-antagonists and their derivatives, they evince deleterious and life-threatening side effects.[14,15] Thus the focus is being laid on potent photo-origin anticoagulants to reduce the risks of thromboembolism.[16]

MATERIALS AND METHODS

In Zarrouk media[17] we used these components ($g \cdot L^{-1}$) are as Sodium bicarbonate, Potassium hydrogen phosphate, Sodium nitrate, Potassium sulphate, Sodium chloride, Magnesium sulphate heptahydrate, Calcium chloride, Iron (ii) sulphate, 2,2,'2",2"'-(Ethane-1,2-diyldinitrilo)tetra acetic acid and pH- 10.2.In SDS PAGE[18] we used these components for two gels (resolving and stacking) are as Acyle/Bisacyle(30:1), Lower buffer gel (Tris HCL-1.5M and 0.5M, P^H- 8.8 and 6.8), Distilled water, Sodium dodecyl sulphate (10%) TEMED and Ammonium per sulphate.

Organism and culture conditions

S. platensis is an African species which develops in middle high temperatures (25-26 °C), alkaline pH (9.5 to 11), and high salt concentrations ranging from 8.5 to 200 g/L; therefore, their production is relatively simple and does not require a complex culture medium. The initial optical density of the culture suspension was maintained as 0.3 at 750 nm and was allowed to grow in light intensity provided by cool-white fluorescent tubes of 50 μ mol photons$m^{-2}s^{-1}$ following 12:12 h, light and dark regime at 30 ^oC ± 1 ^oC.[4] Culture flasks were shaken at every 2 h manually to allow air and nutrients circulation.

Extraction and quantification of phycobiliproteins[19]

*S. platensis*cells were harvested by centrifugation (10,000 g for 15 min). Adhering salts were removed by washing with double distilled water and then fresh biomass was homogenized with phosphate buffer and repeated freezing and thawing cycle (Table 1) till the yield of pc was optimized. The mixture was subsequently centrifuged (10,000 g for 20 min, 4 ºC) to separate the phycobiliprotein containing clear supernatant. Absorbance of supernatants was measured at wavelengths 620 nm (Varian CARY 500 Scan UV–VIS spectrophotometer) for C-pc.[19]the correlation between freezings and thawing cycles (number of cycle 1 to 4, 1 h for each cycle, freezing and thawing -20 and 4 ^oC) on pc extraction was found out.

Purification and characterization of pc[20]

1g of *S. platensis* fresh biomass was crushed with liquid nitrogen in mortar and pestle till the whole biomass resulted in frozen powder and rapidly thawed by adding 1 ml sodium phosphate buffer resulting leaching out of intracellular proteins including pc. The process of freezing and thawing was repeated till blue coloured supernatant was obtained. Cell debris was removed by centrifugation and labelled as crude extract. Crude extract of pc was fractionally precipitated by ammonium sulphate first at 25 % and then 50 % saturation.[20]Then, ammonium sulphate was added gradually into the crude extract by continuously stirring it. The resulting solution was kept overnight and centrifuged (17,000 x g for 20 min). The precipitate obtained from 25% saturation was discarded. The supernatant was further brought to 50% saturation in a manner similar to that of 30% saturation. The precipitate obtained from 50% saturation containing

mainly pc was dissolved in the small quantity of sodium phosphate buffer (pH-7.0, 0.005 mol) and subjected to dialysis overnight against 1000 times volume of the same buffer. The dialyzed solution after centrifuge was passed through Sephadex G-25 column (1.5 x 20 cm) pre-equilibrated and eluted with same buffer. The column was developed at a flow rate of 0.5 ml/min and elutions were collected in 1 ml fraction tubes. All those fractions having purity ratio of A615/A280 > 3.0 were pooled together and again passed through Sephadex G-100 column (2.5 x 20 cm) pre-equilibrated and eluted with 0.005 mol sodium phosphate buffer, pH-7 at 1 ml/min. The products obtained at every step of purification were analysed for total protein, total phycobiliprotein and pc content and subjected to both absorbance and fluorescence spectral scanningreference of the method followed is missing.

SDS-PAGE analysis

SDS–PAGE was performed using 15% polyacrylamide gel run at 100 V[18]. The standard of Proteins which was of medium range between 16 –209 kDa was used as molecular weight markers and visualized by staining with coomassie brilliant blue G-250.

Antimicrobial activity

Antimicrobial activities of the C-pc and culture filtrate from *S. platensis* were measured by the well diffusion method. Antibacterial and Antifungal activity of pc were studied for their potent activities. Dried and stored 10mg pc was dissolved in 1ml of DMSO (Dimethyl sulfoxide).Clinical laboratory bacterial isolates of *Bacillus subtilis (MTCC No 10619), Staphylococcus aureus* (MTCC No 3381*), Pseudomonas aeruginosa*(MTCC No 2143), *Escherichia coli*(MTCC No 443),*Klebsiella pneumonia* (MTCC No 3384) and *Streptomyces grisveus* (MTCC No 4734) and fungal isolates viz. *Aspergillusniger* (ATCC No 9029), *Trichodermareesei* (ATCC No 13631), *Fusariumoxysporum (*ATCC No 62506*), Penicilliumfuniculosum* (ATCC No 11797), *Rhizopusstolonifera* (ATCC No 3313) and *Aspergillusflavus* (ATCC No 2197) were collected from the stock cultures of Microbiology Laboratory, SMS Medical College, Jaipur India.

Culture and maintenance of bacteria

Pure cultures obtained from S.M.S. (SawaiMansingh Medical College), Jaipur, India were used as indicator organisms. These bacteria were grown in Nutrient agar medium and incubated at 37 °C for 48 h. Each bacterial culture was further maintained on the same medium after every 48 h of transferring. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.[21]

Culture and maintenance of fungus

Anti-fungal activity of the pc was investigated by agar well diffusion method.[22] Fungus colonies were sub-cultured onto Potatoes Dextrose Agar (PDA) media and incubated respectively at 37 °C for 24 h and 25 °C for 2 - 5 d. The 20 ml of sterilized Muller Hinton Agar was poured into sterile petri plate after solidification and 30 µl of fresh culture of bacteria and fungus was swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher and various concentrations (20, 40, 60 and 80 µg/ml) of pc extract were added to the wells. The antibiotics such as streptomycin and ketoconazole were added b/w the petri plates. The plates were incubated for 24 to 48 h at 37 ^{o}C and after incubation the diameter of the inhibition zones were measured in mm and recorded which method used for measuring the diameter of zone.

Antioxidant activity ABTS assay

This technique involves the reaction between ABTS and radical caution a blue green chromogen. In the presence of the antioxidant reluctant, the colour radical caution is converted back to colourless ABTS the absorbance of which is measured at 734 nm. The objective of this study was to investigate the antioxidant-potencies of pc. The ABTS assay is based on the ability of the antioxidants to scavenge the long-life radical caution ABTS. This scavenging produces a decrease in the absorbance at 734 nm. ABTS (54.2 mg) was dissolved in phosphate buffer (pH 7,0.5 m mol) and activated to $ABTS^{+}$ radical by addition of 1 g MnO_2 with occasional stirring and time of activation 30 min.[23] Then it is diluted with buffer. Sample addition was 10, 15 and 20 µg/ml, time of reaction 20 min. Absorbance of the solution was measured at a wavelength of 734 nm.[24]

$$\text{Inhibition \%} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

FRAP assay

A simple, automated test measuring the Ferric Reducing Ability of Plasma, the FRAP assay, is presented as a novel method for assessing ‘‘antioxidant power.’’ Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. This assay is based on the reduction of Fe^{3+} to Fe^{2+} due to the action of antioxidant activity. The Fe^{2+} interacts with TPTZ providing a strong absorbance at 593 nm.[25] The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. The method was performed by the established protocol.[25]

Reagents

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x H_2O and 16 mL conc. Acetic acid per 1 mL of buffer Solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$ x 6 H_2O in distilled water

FRAP working solution: 25 mL acetate buffer (1), 2.5mL TPTZ solution and 2.5mL $FeCl_3$ x 6 H_2O solutions. The working solution must be always freshly prepared.

Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100-1000µ mol/L).

Assay
Blank: FRAP reagent
Sample: FRAP reagent -1.5mL, plant extract- 50mL
Calculation: The relative activities of samples were assessed by comparing their activities standard curve of ferrous sulphate.

Antiplatelet activity Preparation of extract
At the outset, the pc was extracted by freezing and thawing method. The extract was obtained by dissolving pc (20 mg/ml) in 0.005 mol phosphate buffer (20 ml). Blood samples were collected from SMS Medical College, Jaipur and subjected to centrifugation at 10,000 rpm for 5.5 min. 0.2 ml platelet rich plasma was taken from the sample, dissolved in isotonic $CaCl_2$. Various haemostatic parameters; PT and APTT were measured.[26] Amelung/Sigma Amax CS400 Coagulant Analyser, New York. USA was used for the experimental work.

PT assay[26]
Principle
The coagulation process gets triggered by the incubation of plasma with the optimum amount of thromboplastin and calcium. The time duration for the formation of a fibrin clot is then measured.

Reagents used
- Fresh Standard Human Plasma for determining the reaction time
- PT Reagent (Thromborel)
- Sodium Citrate solution

Procedure
One part of sodium citrate (0.11 mol L^{-1}) with 9 parts of venous blood was mixed with care and formation of foam was avoided. The blood specimen was centrifuged at 1500 rpm for 15 min at room temperature and stored in a capped tube at room temperature for not more than 5 min. Plasma was tested within 24 h of blood collection. 100 µl of citrated plasma samples were pipetted in pre sterilized test tube at room temperature with different concentrations of pc (100 to 2000 µg ml^{-1}) added. Stop watch was used on the coagulation analyser to determine the coagulation time.

Activated Partial thromboplastin Time[26]
Principle
The deficiency of one or the more clotting factors of the intrinsic pathway and also the presence of heparin (coagulation inhibitor) prolongs the APTT of the plasma. The time taken for the clot formation is measured and used to determine the anticoagulant status.

Reagents used
- APTT Reagent (Activated Cephaloplastin)
- Calcium Chloride (CaCl2)
- TSC solution (3.2% buffered Tri-Sodium Citrate solution)

Procedure
Fresh plasma was used for the entire tests because it performed best when tested immediately after collection as compared to stored plasma. 9 parts of the whole blood from veins was poured in a clean test tube containing 1 part of TSC solution (3.2% buffered tri-sodium citrate solution) and mixed immediately with an anticoagulant to avoid foam formation. Later, it was centrifuged for 15 min at 3000 rpm and the plasma was collected in a separate test tube. Haemolysed, lipaemic turbid samples were avoided. APTT reagent was added gently before use. 100 µl each of APTT reagent and 0.025 mol L^{-1} $CaCl_2$ reagent was used as standard in separate test tubes that was pipetted and incubated on water bath at 37 ^{0}C for 3 min. After 3 min, 100 µl of well mixed $CaCl_2$ reagent was added to the 37 ^{0}C maintained temperature test tube containing APTT reagent and plasma and simultaneously the stopwatch was used to for observation. The contents of the tube were mixed and the stopwatch was closed as soon as fibrin strand became visible through high powered lens in coagulation analyser that initiated gel clot formation. The time taken for clot formation was measured to the nearest 0.1 s. Thus the APTT value was calculated in s. The control was also run on parallel where pc in different concentrations (100 to 2000 µg ml^{-1}) were added replacing the APTT reagent to evaluate the APTT activity of the test samples.

STATISTICAL ANALYSIS

The results obtained were statistically evaluated through appropriate statistical methods viz. mean, standard deviation and two ways ANOVA. The statistical analysis was conducted on PASW SPSS 18.0 trial version and MINITAB 15.0 trial version software**.** The statistical error of mean was calculated by the following formula

$$S.E. = \frac{\sigma}{\sqrt{n}}$$

Where,
σ = standard deviation
n = number of observations

The test of significance (t-test) was calculated by the following formula

$$t = \frac{m_1 - m_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$$

Where,

m_1 = mean of one set of values.
m_2 = mean of second set of values.
SEM_1 = standard error of the first set of values.
SEM_2 = standard error of the second set of values.

The probability 'p' for obtaining 't' value of at least as great as the calculated one for a given number for the degree of freedom was found in the Fisher's table. The p - values were signified according to the following conventions.

P<0.05 = difference was almost significant.
P<0.01 = difference was significant.
P<0.001 = difference was highly significant.

RESULTS

Freezing and thawing process optimization

In the process of isolation of pc method using freezing and thawing, we followed 4 steps, then maximum yield (137mg/g) was observe in 3rd step with time interval of 3 h and 3 cycles. (Table 1).

Table 1
Evaluation of extraction efficiency at different time interval of freezing and thawing

Sample no	Numberof cycle Freezing and Thawing	Freezing and Thawing		Time Duration Freezing and thawing		Extraction efficiency of pc(mg/g)
01	1	-20^{0}C	4^{0}C	1h	3 min	102.00±2.31
02	2	-20^{0}C	4^{0}C	2h	4 min	107.00±2.44
03	3	-20^{0}C	4^{0}C	3h	5 min	137.00±2.56
04	4	-20^{0}C	4^{0}C	4h	6 min	115.00±2.38

SDS-PAGE analysis of pc

In the present research, the β and α polypeptide subunits of the pc were isolated and their molecular weights were 23.4 kDa and 15.6 kDa respectively. These weights were consistent with those of previous reports.[4]This finding suggests that the freezing and thawing process enabled isolation of a stable and highly purified pc with a short treatment 3cycle (-20 ^{o}C with time interval 3 h) as 137 mg/g (Table number and Fig. 1)whose value was 10% higher than the case from other conventional separation method using phosphate buffer. The isolated pc from this process also showed the highest purity of 2.500 based on absorbance of 0.026 at 280 nm and 0.065 at 615 nm. Two subunits of pc namely α-pc (15.6 kDa) and β -pc (23.4 kDa) were found to remain from the original mixtures after being extracted based on SDS-PAGE analysis.

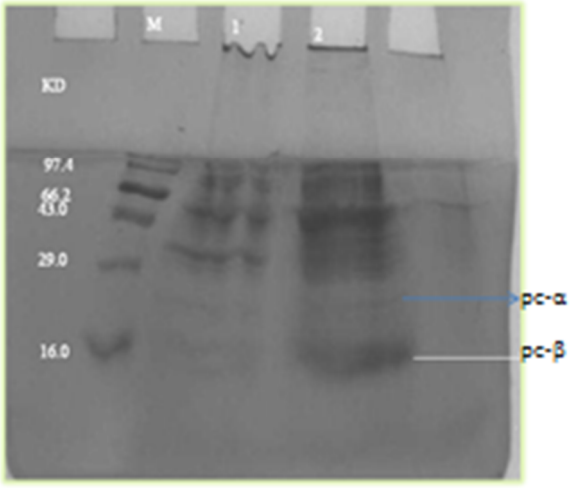

Figure 1
SDS-PAGE of pc M (Marker, 16 to 209 kDa) 1 (Crude extract) 2 (After passed through Sephadex G-25 column) using freezing and thawing process

Antimicrobial activity

The stock solution was 10mg/ml for antimicrobial activity in the initial phase and different dilutions were prepared. Pc showed good antibacterial activity against the *B. subtilis, P. aeruginosa, and E.coli* and *S. aureus* was found to be resistant. The maximum zone was on *B. subtilis* at 80 µl was 18 mm. Against *P. aeruginosa*, pc gave maximum zone of 20 mm at 20 µl and 18mm against *E. coli.* Against*S. Griseus*the sample showed maximum activities at all diluted concentration as

compared to other strainswhile in K. Pneumoniathe samples were slightly potent. In antifungal activity, the maximum zone of inhibition was observed against *T. reesei,* (14 mm) and against *F. oxysporum, P. funiculosum* activity was reduced but *A. niger* was found to be resistant.(Table 2 and 3; Fig. 2-8).

Table 2
Anti-bacterial activity of pc

S.NO.	Bacterial Isolates	Zone of inhibition (in mm) (IZ)				(st)-20 mm
		20 µl	40 µl	60 µl	80 µl	
1	Bacillus subtilis	NA	NA	12±0.13 AI- 0.6	18±0.18 AI- 0.9	
2	Staphylococcus aureus	NA	NA	NA	NA	
3	Pseudomonas aeruginosa	20±0.6 AI- 1.0	NA	NA	12±0.11 AI- 0.6	
4	Escherichia coli	18±014 AI- 0.9	16±0.11 AI- 0.8	14±0.10 AI- 0.7	16±0.12 AI- 0.8	
5	Streptomyces griseus	10±0.09 AI- 0.5	8±0.06 AI- 0.4	4±0.03 AI- 0.2	12±0.11 AI- 0.6	
6	Klebsiella pneumonia	NA	NA	4±0.03 AI- 0.2	8±0.07 AI- 0.4	

IZ- inhibition zone, * AI- Activity index, NA-No activity
**** As standard zone of streptomycin is 20 mm***

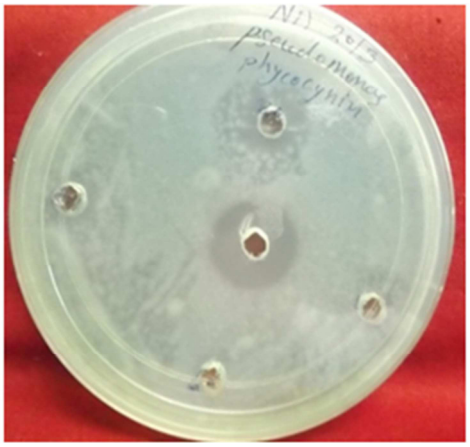

Figure 2
Pseudomonas aeruginosa

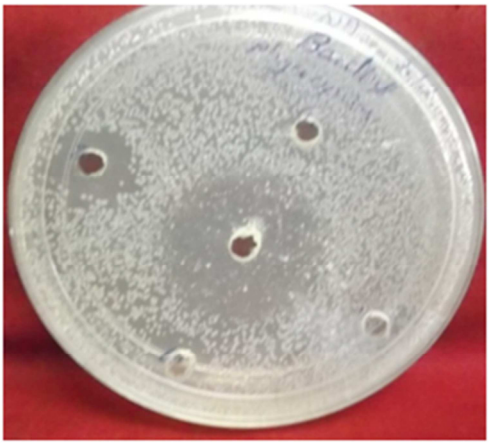

Figure 3
Bacillus subtilis

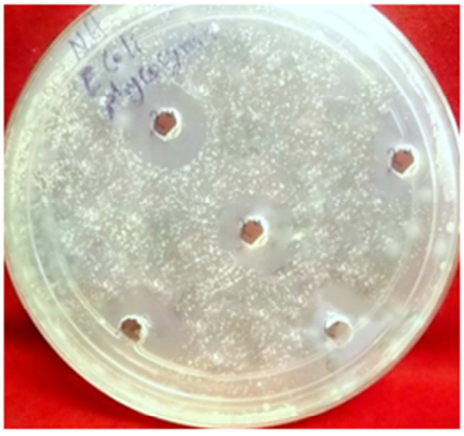

Figure 4
Escherichia coli

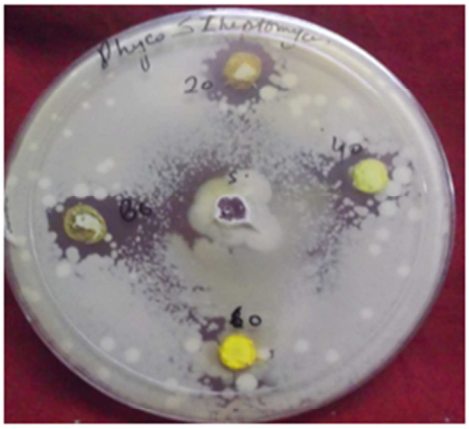

Figure 5
Streptomyces griseus

Table 3
Anti-fungal activity of pc

S.NO.	Fungal Isolates	Zone of inhibition (in mm) (IZ)				(st)-20 mm
		20 µl	40 µl	60 µl	80µl	
1	Aspergillusniger	NA	NA	NA	NA	
2	Trichodermareesei	NA	NA	10±0.08 AI- 0.5	14±0.12 AI- 0.7	
3	Fusariumoxysporum	NA	NA	NA	12±0.11 AI- 0.6	
4	Penicilliumfuniculosum	NA	NA	10±0.08 AI-0.5	12±0.11 AI- 0.6	
5	Rhizopusstolonifer	NA	NA	10±0.06 AI-0.5	14±0.09 AI- 0.8	
6	Aspergillusflavus	NA	2±0.009 AI-0.1	4±0.02 AI- 0.2	6±0.04 AI- 0.3	

***IZ- inhibition zone, * AI- Activity index and* *as standard (st)**
Use here Ketoconazole and zone is 20 mm

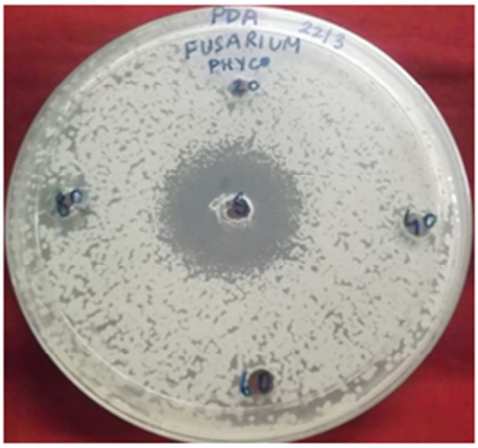

Figure 6
Fusariumoxysporium

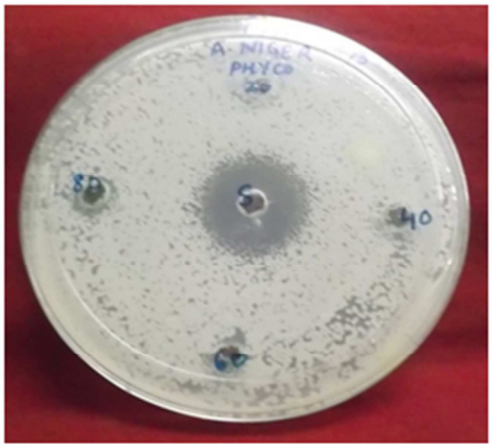

Figure 7
Aspergillusniger

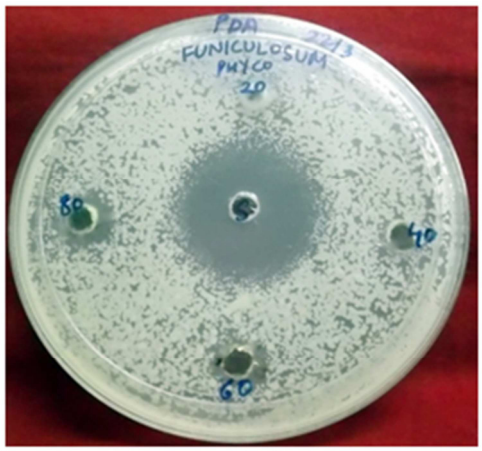

Figure 8
Penicillium funiculosum

Antioxidant activity
FRAP assay

A significant linear correlation was found between antioxidant activity and concentration of test compounds in reducing power assay. The FRAP value was calculated using the standard curve and the value was recorded as 130 µ mol /l. (Fig 9)

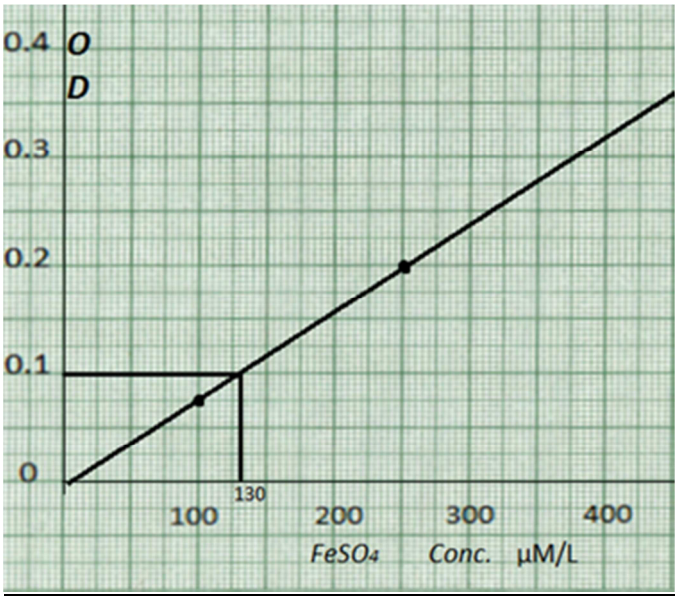

Figure 9
FRAP activity of Phycocyanin as calculated by regression curve

ABTS radical scavenging assay
ABTS radical caution decolourisation assay is applicable for both lipophilic and hydrophilic antioxidants. C-pc failed to scavenge the ABTS radical significantly (Fig. 10) after calculation from the curve, ABTS activity was found to be 3.6 µg/ml.

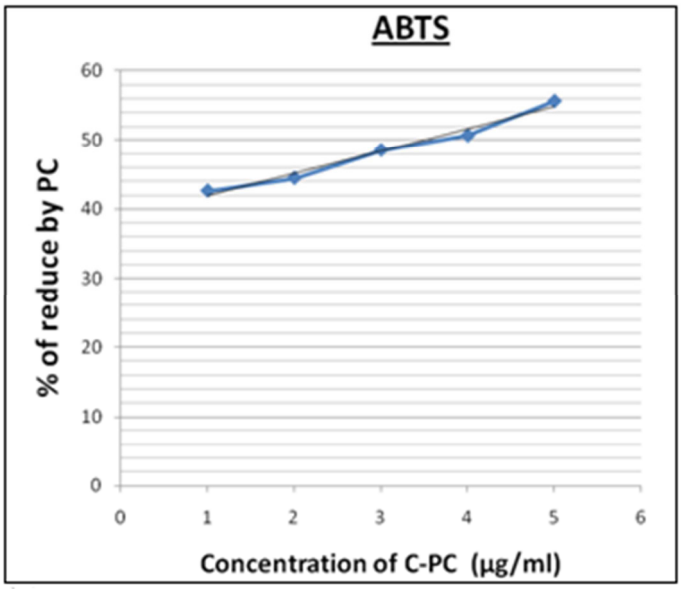

Figure 10
ABTS radical scavenging curve

Antiplatelet activity PT Assay
Pc was found to be potent antiplatelet agent as it enhanced the clotting time as compared to control. Significant activity was observed at 2 µl which was 90 s and the highest at 10 µl around 1965 s. On 10 µl concentration, the sample showed 53.10 times more potency than the control and 131.00 time more potency than the standard. (Table 4)

Table 4
Thrombin time in Pc

S.NO	Volume	Time in s	Standard (a*)	Control(b*)
1	1 µl	120±3.21	08.00	03.24
2	2µl	90±2.21	06.00	02.42
3	3 µl	125±3.49	08.33	03.37
4	4 µl	225±4.02	15.00	06.08
5	5 µl	185±3.58	12.33	05.00

6	6 µl	280±5.19	18.66	07.56
7	7 µl	1080±3.28	72.00	29.18
8	8 µl	1000±3.24	66.66	27.18
9	9 µl	1570±3.31	104.66	42.43
10	10 µl	1965±3.81	131.00	53.10

a*- value compared with standard, b*- value compared with control

APTT assay

Pc prolonged the clotting time in terms of APTT as compared to control ($CaCl_2$). Pc shows the highest potent retention time at 3 µl which was 1440 s and it showed 30.63 times more potency than the control and 36.00 time more potency than the standard. Minimum clotting (potency) time was 130 s for the concentration at 1 µl. This concentration showed least potency as compared to control and standard and other dilutions of pc. (Table 5)

Table 5
Activated partial thromboplastin time in pc

S.NO	Volume	Time in s	Standard (a*)	Control(b*)
1	1 µl	0130±3.48	03.25	02.76
2	2µl	0540±4.89	13.50	11.48
3	3 µl	1440±5.21	36.00	30.63
4	4 µl	0392±3.67	09.80	08.34
5	5 µl	1260±4.31	31.50	26.80
6	6 µl	1060±4.08	26.50	22.53
7	7 µl	1284±4.56	32.60	27.31
8	8 µl	0638±3.91	17.45	14.85
9	9 µl	0795±4.02	19.87	16.91
10	10 µl	0685±3.99	17.12	14.57

Standard value for APTT pl2 asma+activatedcephaloplastin (APTT Reagent) +CaCl2; 1:1:1] - 40 s; Control A- (plasma+50 % EtOH+CaCl2); Control B- (plasma+distill water+CaCl2) *s times higher than Standard;; ** c times higher than controla*- value compared with standard, b*- value compared with control

DISCUSSION

The isolated pc, by using the freezing and thawing process, had two subunits α and β polypeptide that were separated on SDS- PAGE and possessed the corresponding molecular weights as described in previous reports[27]of *Spirulina*[20]. Pc is a water-soluble, highly fluorescent protein derived from cyanobacteria (blue-green algae) used as food colour[1], cosmetics[28] and biomedical research[29]. It has been demonstrated that pc derived from *Spirulina* had the significant antioxidant property and it also has potent radical scavenging properties both *in vivo* and *in-vitro* conditions[30, 31, 32]which is becoming a potential therapeutic agent in oxidative stress-induced diseases. Various organic and aqueous extracts of *S.platensis* were screened for antibacterial activities by agar well diffusion method against human pathogens.[33]Water extract showed maximum activity (18 mm) against *K. pneumoniae*. This result could be due to the water

Soluble pigment C-pc from *Westiellopsissps.* (Fresh water Cyanobacterium) tested for their antibacterial activity against *B. subtilis, Pseudomonas sps. And Xanthomonassps.* all were inhibited by pc (50 µg/disc). Oxidative stress which is induced by oxygen radicals promote damage to essential biomolecules such as carbohydrates, proteins, amino acids, lipids, nucleic acids among other oxidative substances thus contributing to aging and installation of chronic-degeneration diseases such as cancer, cardiovascular diseases, arthritis, gastric ulcer, among others.[34]It has been proved that antioxidant activity which advocated a new approach to a study binding of drug receptors under in vitro conditions.[35]Therefore, the inhibition of aggregation of platelet formation and anticoagulants using phytoceuticals and nutraceuticals can be a promising approach for the prevention of thrombosis.[36] During the initial stage of thrombosis, damage in the blood vessels causes the production of adhesive proteins and soluble agonists at the injury site. This act then stimulates platelet adhesion, activation and aggregation resulting in the formation of a platelet-rich thrombus.[36]Thrombin is not only responsible for the formation of fibrin but also acts an extreme platelet activator. The growing aggregation of activated platelets is eventually stabilized by cross-linked fibrin and results in the formation of a platelet-rich thrombus.[37]Increased platelet aggregation as a result of increased platelet sensitivity to agonist *in vivo,* contributes to the initiation and progression of atherosclerosis and occurrence of thrombotic events.[38,39] Platelet aggregation which is associated with an increased release of reactive oxidative species[40] and platelet-vessel wall interactions[41] results in damage to the vascular endothelium.[42]

CONCLUSION

In the present investigation, an attempt has been made to improve the yield of pc with reduction in the cycle of time. Further, the isolated pc exhibited significant antimicrobial, antioxidants and antiplatelet activities. The results of the present study concluded that pc can be used as drug, natural antioxidants as well as antiplatelet agents. These studies confirm the therapeutic ventures of this compound as potent agent for future endeavours. In conclusion, it was first demonstrated that C-pc is an inhibitor of platelet aggregation. Previous studies have

reported that during platelet aggregation, ROS including superoxide anion and hydrogen peroxide are released by platelets which can induce platelet activation. However, other reports on various biological parameters are mentioned above in several reports.

AUTHOR CONTRIBUTION STATEMENT

Ms. Neha Puri conducted the present research work. Dr.Manas assisted the work of Antimicrobial, antioxidant and antiplatelet activity Dr.Ajit conducted the isolation and purification of phycocyanin along with protein profiling using SDSMr. Ashish Sharma formatted the manuscript as per journal Mrs. Ankita Anand did the editing in language of the manuscript

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CONFLICT OF INTERESTS

Conflict of interest declared none.

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