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Research Article

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Microencapsulated C-Phycocyanin decreases IL-17 gene expression in Peripheral Blood Mononuclear Cells (PBMCs)

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Abstract

Chronic inflammatory diseases are characterized by persistent immune responses in genetically predisposed individuals. Environmental factors trigger these responses and are often accompanied by increased inflammatory factors, such as inflammatory cytokines or oxidative stress. Phycocyanin (PC), a pigment extracted from *Spirulina platensis*, has anti-oxidant and anti-inflammatory properties and has been shown to have beneficial effects in patients with inflammatory disease. In this study, we aimed to evaluate the impact of both encapsulated and non-encapsulated forms of PC on the expression of Catalase, Superoxide dismutase 1 (SOD1), Superoxide dismutase 2 (SOD2), and IL-17 genes in peripheral blood mononuclear cells (PBMCs) obtained from healthy individuals. For the experiment, *Spirulina platensis* (*Arthrospira*) PCC9108 was obtained from the microalgae bank of the Science and Research Branch of Islamic Azad University in Tehran, Iran. The optimal non-toxic concentration of PC was determined using an MTT assay. PBMCs were isolated from the whole blood, cultured, and stimulated with phytohemagglutinin (PHA) (10 µg/ml) in either encapsulated or non-encapsulated PC form. Following 48 hours, the gene expression analysis was assessed using Real-time PCR. Multi-group analysis of Catalase, SOD1, SOD2, and IL-17 gene expressions between unstimulated cells and stimulated cells under different conditions (treated with a high or low concentration of non-encapsulated (NEC-PC) and encapsulated PC (EC-PC)) revealed no significant difference (P=0.06, P=0.5, P=0.3 and P=0.2, respectively). However, the pairwise analysis showed that encapsulated PC (1000µg/ml) significantly decreases the IL-17 level (p=0.05). Our findings indicated that a high concentration of encapsulated PC reduces IL-17 levels. The results may inform future clinical trials for patients with inflammatory and autoimmune diseases that involve inflammatory responses.

Keywords: Spirulina; Phycocyanin; Capsulation; Interleukin-17; Catalase; SOD1; SOD2.

1. Introduction

Persistent inflammatory responses are a hallmark of various chronic inflammatory diseases in humans. These responses typically occur due to inappropriate and excessive immune reactions [1, 2]. Inflammatory responses are associated with releasing inflammatory

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markers, such as inflammatory cytokines. These small, soluble proteins are crucial to both the induction and regulation of the immune response [3, 4]. Moreover, a decrease in anti-oxidant levels and oxidative stress have been observed in some inflammatory diseases [5].

In humans, inflammation and oxidative stress significantly contribute to the development of various disorders, including psoriasis and *multiple sclerosis (MS)* [6, 7]. Anti-oxidants such as catalase and superoxide dismutase 1 and 2 (SOD1 and SOD2) help combat oxidative stress [8]. Interleukin 17 (IL-17) is an inflammatory cytokine associated with the pathogenesis of many inflammatory diseases, such as psoriasis and MS [9].

Pharmacological treatment for inflammation primarily focuses on reducing inflammatory responses [5]. However, while these medications can effectively treat inflammatory disease, they may also cause a variety of side effects, including gastrointestinal issues [10], renal complications [11], and central nervous system effects [12]. Moreover, Immunosuppressive medications used to treat inflammatory diseases carry the risk of infection due to their ability to suppress the immune system [13].

Herbal medicines, including microalgae, exhibit anti-inflammatory and anti-oxidant properties [14]. Arthrospira, commercially known as Spirulina, is a blue-green microalga belonging to the cyanobacteria family. It is the source of a blueish anti-oxidant pigment known as C-Phycocyanin (C-PC) [15, 16]. Besides being used as a source of protein and vitamins [17], Spirulina displays anti-cancer and antimicrobial activities [15]. *The* biological effects of Spirulina are attributed to C-PC. Indeed, studies have demonstrated that C-PC has anti-oxidant [18] and anti-inflammatory [19] properties. However, C-PC is sensitive to environmental stressors, mainly changes in temperature, pH, and light conditions [20]. Hence, C-PC should be encapsulated to prevent degradation and enhance its stability and anti-oxidant activity [21].

C-PC was effective in improving clinical symptoms and redox status and reducing IL-17 expression in a chronic model of experimental autoimmune encephalitis (EAE) in mice [22]. In this study, our objective was to assess the impact of the C-PC in both encapsulated and non-encapsulated forms on the gene expression of Catalase, Superoxide dismutase 1 (SOD1), Superoxide dismutase 2 (SOD2), and IL-17 genes expression in peripheral blood mononuclear cells (PBMCs) from healthy individuals.

2. Materials and methods

2.1. Microalgae cultivation

For the experiment, *Spirulina platensis* (*Arthrospira*) PCC9108 was obtained from the microalgae bank of the Science and Research Branch of Islamic Azad University in Tehran, Iran. The *Spirulina platensis* was then cultivated in the Zarrouk's culture medium [23]. The cultivation started at an initial optical density (OD_{650}) of 0.5, and the measurements were done using HACH (DR 5000) spectrophotometer. It was inoculated in a 2-liter Erlenmeyer flask at a ratio of 1:10. The Erlenmeyer flask was equipped with an air pump capable of generating airflow of 2 $L.min^{-1}$. A constant temperature of 28 ± 2 °C was maintained, and the culture was exposed to a white LED with a light intensity of 150 mmol photons m^{-2} s^{-1}. The OD at 680 nm was measured using a Shimadzu UV-1700 PharmaSpec UV-V spectrophotometer (Japan). Cells' dry weight (DW) was also measured using the method described by Sohani et al. [24].

2.2. C-PC extraction and purity

The freeze-thaw technique is a reliable method for extracting C-PC due to its effectiveness in breaking down intracellular barriers and releasing pigments. The extraction process was conducted using the standard protocol for the freeze-thaw procedure [16]. The absorbance of this bluish pigment was measured at 615 and 652 nm, with its concentration determined by the following Equation: [25]

$$C_{C-PC} = \frac{(A_{615} - 0.474 \times A_{652})}{5.34}$$

C-PC represents the concentration of C-PC ($mg.ml^{-1}$), with A_{615} and A_{625} referring to the extract's absorbance at 615 and 652 nanometers, respectively. Moreover, the purity was determined by using the following formulae:

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

A620 and A280 refer to the extract's absorbance at 620 and 280 nanometers, respectively.

2.3. High-performance liquid chromatography (HPLC)

The chromatographic analysis was conducted using the Agilent Technologies Infinity 1260 HPLC system (USA). A C5 column (Shimadzu, Japan) was used, with an injection volume of 20 µL. The mobile phase consisted of 20% (v v^{-1}) acetonitrile mixed with 0.1% (v v^{-1}) trifluoroacetic acid at 25 °C and a 1.0 $mL.min^{-1}$ flow rate under isocratic conditions for 45 min. Absorbance

was measured with a UV detector at 580 and 640 nm and compared with the peaks of a standard C-PC (Sigma-Aldrich Germany) [26, 27].

2.4. C-PC encapsulation

First, the extracted phycocyanin powder is passed through a 6-mesh sieve and then a 14-mesh sieve. The resulting powder is transferred to a Fluid Bed Dryer Granulating Machine (Pilotech YC-03 spray granulator, Shanghai Pilotech Instrument & Equipment Co., Ltd.), coated with an encapsulating solution. This solution is applied to the phycocyanin pigment to uniformly cover the entire surface of the pigment particles, resulting in a spherical to semi-spherical structure. A solution containing cetyl alcohol (5 grams), Polyvinylpyrrolidone K30 (Pvp-k30) (7 grams), Hydroxypropyl MethylCellulose 6 CPS (HPMC) (2 grams), Polyethylene Glycol 4000 (PEG 4000) (3.1 grams), and ethanol (20 grams of 75% ethanol) is sprayed onto 5 grams of pigment, resulting in a final weight of 5.25 grams to increase the total weight by 5%. As a result, the weight of 5 grams of phycocyanin pigment increases to 5.25 grams after being coated with the encapsulating material. In this process, the diameter of the pores in the microencapsulating layer is adjusted using Polyethylene Glycol 4000. This material promotes the formation of fine pores in the microencapsulated structure when exposed to aqueous environments, which facilitates the release of phycocyanin from the membrane [28].

2.5. Blood sampling and cell culture

Ten ml of whole blood was obtained from three healthy individuals with no inflammatory or autoimmune disease history. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll density gradient sedimentation using Lymphodex (Inno-Train, Germany). The isolated cells (1×10^6 cells/mL) were cultured, and the activated with PHA (10 µg/mL) for 48 hours in RPMI 1640 medium (Cegrogen, E0500370) containing 10% (V/V) FBS (Biosera—France, FB-1001) and 1% Penicillin-Streptomycin Solution 100X (Cegrogen, P0100-790) and incubated at 37°C in a humidified 5% CO2. Different concentrations of PC in both encapsulated and non-encapsulated forms were added. **Figure 1** displays the conditions for cell culture.

2.6. Methyl Thiazol Tetrazolium (MTT) assay

The Methyl Thiazol Tetrazolium (MTT) assay is based on the capacity of metabolically active cells to transform MTT through the enzyme succinate dehydrogenase in the mitochondria. This transformation produces insoluble purple formazan crystals, which can subsequently be dissolved, enabling the evaluation of cell metabolic activity through spectrophotometry [29].

The effect of different concentrations of phycocyanin (200, 600, and 1000 µg/ml) on the viability of PBMC cells was assessed using an MTT kit (MTT Cell Viability Assay Kit, DMA100) . Following the isolation of PBMCs, 1×105 cells/well were cultured in the complete medium and stimulated with PHA (10µg/ml). Three wells were considered for each concentration of phycocyanin. After 48 hours of incubation, MTT solution was added, and following incubation for 4 hours at 37^0C, the production of dark blue formazan by viable cells was assayed. The resulting purple color absorbance was red at 570. The background absorbance of the plate was read at 690 nm and subtracted from the 570 nm measurement.

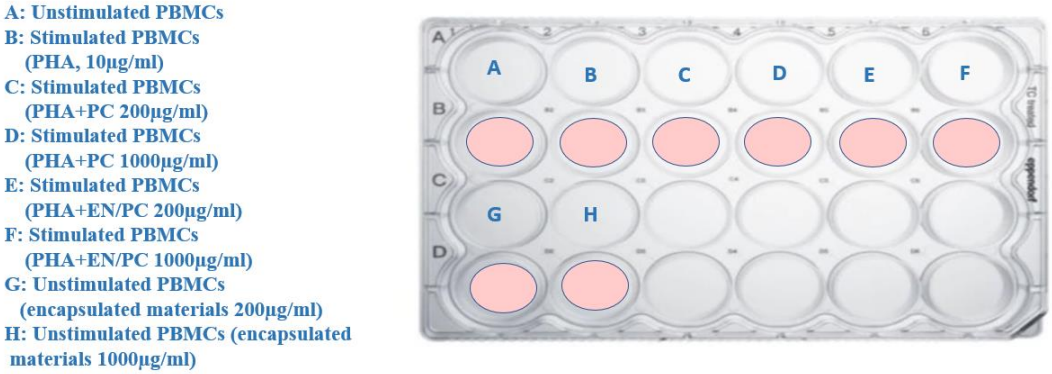

Figure 1. The conditions of the cell culture are represented. PBMCs were cultured at 1×10^6 cells/well in a complete medium and stimulated with PHA (10 µg/mL) with 200 or 1000 µg/mL of phycocyanin (PC) or En-capsulated phycocyanin. Following 48h, cells were harvested. RNA was isolated, and gene expression analysis was performed using real-time PCR—PBMCs, peripheral blood mononuclear cells, and PHA, phytohemagglutinin.

2.7. Relative expression of IL-17 and anti-oxidant genes.

To evaluate the influence of non-encapsulated and encapsulated phycocyanin on Catalase, SOD1, SOD2, and IL-17 gene expression, RNA was isolated from the stimulated cells (for 48 hours) and un-stimulated PBMCs (using RiboEx^{TM} LS GENEALL, 302-001). cDNA was synthesized using Reverse Transcription Kit (SMOBIO, RP1300). Real-time PCR was performed on ABI step one plus real-time PCR system (Applied Biosystem) using RealQ plus 2x Master Mix Green (AMPLIQON, A325402). Primers for target genes are listed in **Table 1** [30, 31]. The relative expression of the genes was determined using the ΔΔCt method [32].

Table 1. Primer sequences for Catalase, SOD1T SOD2, IL-17A and 18S rRNA

Gene	Primer Sequences
Catalase	Forward: TGCTGAATGAGGAACAGAGGAA Reverse: CCTCACAGATTTGCCTTCTCC
SOD1	Forward: AGCGAGTTATGGCGACGAAG Reverse: CAGCCTGCTGTATTATCTCCA
SOD 2	Forward: CTCAGGTTGGGGTTGGCT Reverse: TGAAGGTAGTAAGCGTGCTCC
IL-17	Forward: GTCAACCTGAACATCCATAACCG Reverse: ACTTTGCCTCCCAGATCACAG
18S rRNA	Forward: GTAACCCGTTGAACCCCATT Reverse: CCATCCAATCGGTAGTAGCG

Abbreviations: 18S rRNA, 18S Ribosomal RNA; CAT, catalase; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2, IL-17: Interleukin 17.

2.8. Statistical analysis.

Statistical analysis was performed using SPSS (IBM SPSS statistics version 22, USA). Mann-Whitney U test was used to compare two independent samples, and Kruskal–Wallis test was used to compare between groups. A P value ≤ 0.05 was considered significant.

3. Results and Discussion

3.1. Biomass growth and C-PC biosynthesis

Following the 14-day cultivation, the DW of *Spirulina platensis* reached 1.39 g L^{-1}, while its C-PC concentration was 161.5 ± 4.85 mg. L− 1.

3.2. Analyzing extracted C-PC using HPLC

Table 2 presents the results of the HPLC analysis of the standard and test samples. The results of chromatogram graphs from the HPLC analysis of both standards and test samples have been presented in **Figure 2** (supplementary **Figure S1**). The area analysis data of the standard sample is comparable to that of the extracted sample, confirming high purity with an $\frac{A_{620}}{A_{280}}$ the ratio is 1.34. In contrast, the $\frac{A_{620}}{A_{280}}$ the ratio for the C-PC extract after the freezing and thawing step was 4.23.

Table 2. Comparison of chromatogram analyses of the standard and extracted samples

Samples	Test Area	Standard Area
1	83127	83133
2	83130	83129
3	83132	83127
SD of area	2.52	3.05
Area average	83129.67	83129.67
RSD of area	0.003	0.003

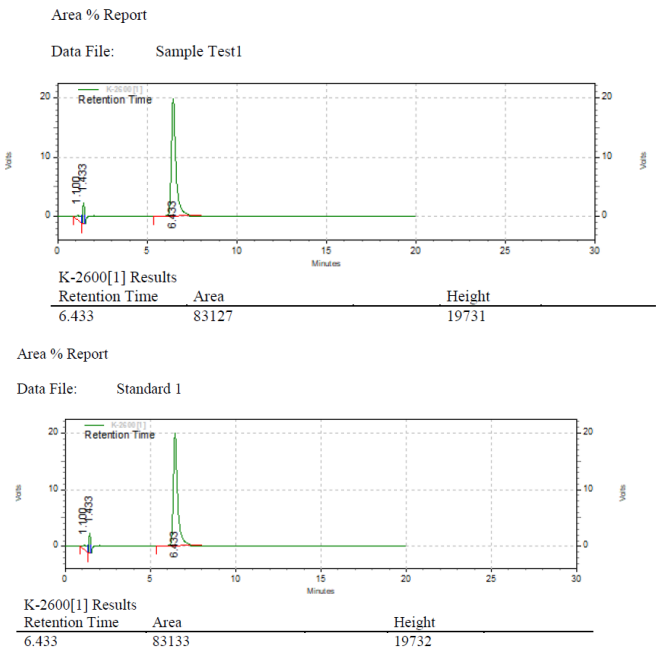

Figure 2. Chromatogram graphs from the HPLC analysis of both standard and test samples.

3.3. Investigation of C-PC on the PBMCs cytotoxicity

The MTT assay was conducted after 48 hours to assess the effect of C-PC on PBMC viability. The viability of PBMCs exposed to C-PC (200 and 1000 µg.ml^{-1}) did not differ significantly from that of control cells not exposed to C-PC. After 48 hours of exposure to C-PC, the viability of the PBMCs was more than 95% for both C-PC concentrations.

3.4. The effect of EC-PC and NEC-PC on gene expressions

Multi-group analysis of gene expression was performed between unstimulated, PHA-stimulated cells in the presence of NEC-PC (200 µg.ml^{-1}), NEC-PC (1000 µg.ml^{-1}), EC-PC (200 µg.ml^{-1}), and EC-PC (1000 µg.ml^{-1}) using Kruskal-Wallis test. Results showed no significant differences in Catalase (P=0.06), SOD1 (P = 0.5), SOD2 (P = 0.3), and IL-17 (P = 0.2) gene expressions among PBMCs treated with different concentrations of C-PC (200 and 1000 µg/ml) and in encapsulated and non-encapsulated forms, **Figure 3**. However, pairwise analysis of IL-17 gene expression showed that stimulation of the cells in the presence of Encapsulated PC (1000 µg.ml^{-1}) decreased the IL-17 gene expression in PBMCs (P = 0.05).

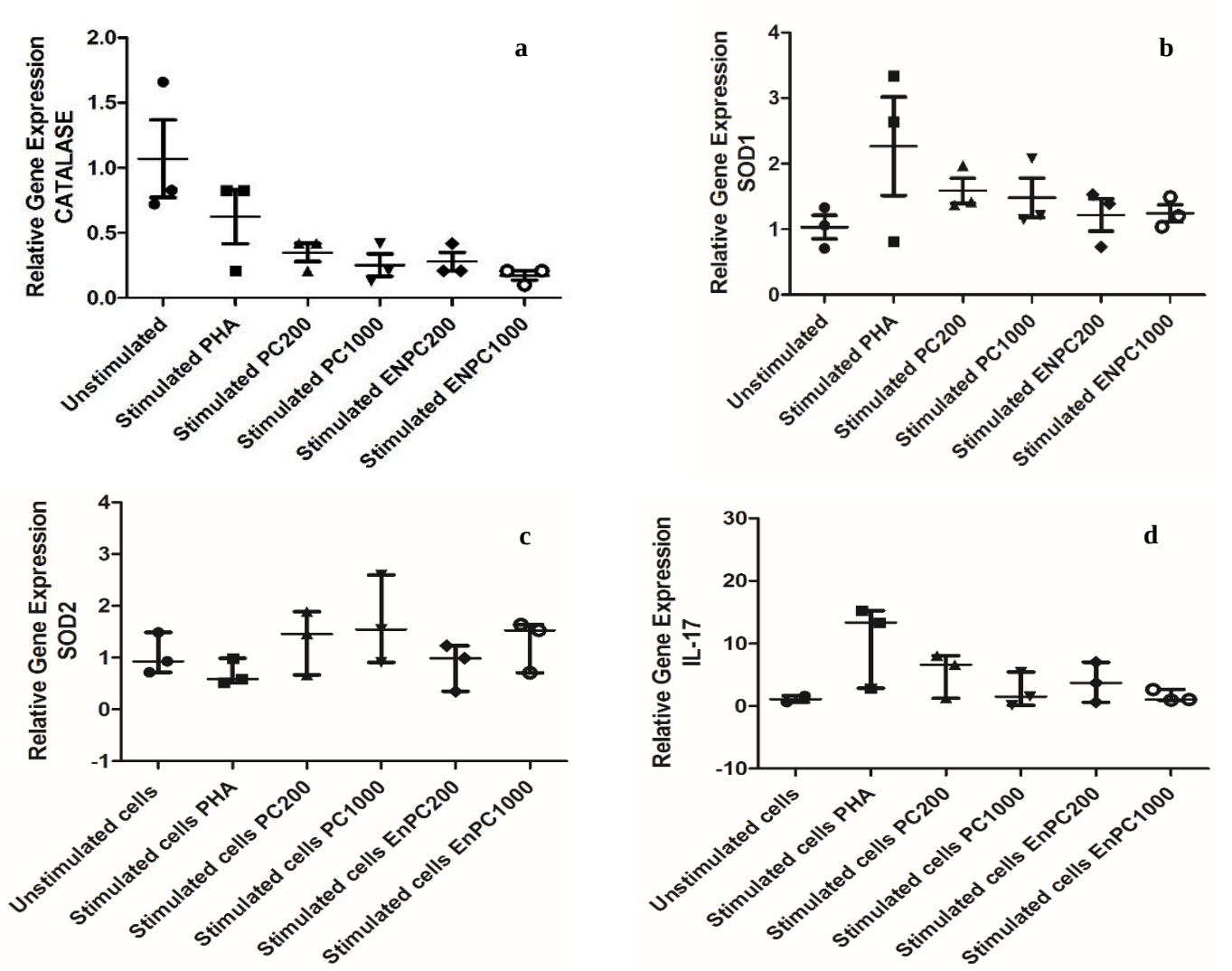

Figure 3. Relative gene expression of a. Catalase, b. SOD1, c. SOD2, and d. IL-17. Gene expression was compared among unstimulated PBMCs, stimulated PBMCs with PHA, and stimulated PBMCs in the pretense of EC-PC (200,1000 µg.ml$^{-1)}$ or NEC-PC (200,1000 µg.ml$^{-1)}$. P value was calculated using Kruskal-Walli's test. PBMCs: Peripheral blood mononuclear cells, PHA: phytohemagglutinin, EC-PC: encapsulated Phycocyanin PC, NEC-PC: non-encapsulated Phycocyanin.

3.5. Dissection

In humans, many diseases are associated with inflammation and oxidative stress [33, 34]. Phycocyanin (C-PC) is known for its anti-oxidant and anti-inflammatory properties [18, 19]. In this study, we assessed the effect of phycocyanin on Catalase, SOD1, SOD2, and Il-17 gene expression in PBMCs from three healthy individuals in the encapsulated and non-encapsulated forms. Our findings revealed that treating PBMCs with C-PC at 200 and 1000 µg.ml-1 concentrations did not result in significant differences in catalase, SOD1, SOD2, and IL-17 gene expression in PMBCs from healthy subjects. These results are consistent with those of Pentón-Rol et al., in which PC was found to upregulate the CD4+CD25high subset in the PBMC from MS patients but not in a healthy individual [35]. Further research is needed to investigate gene expression analysis in patients with inflammatory disorders such as psoriasis and rheumatoid arthritis.

Numerous studies investigated the immunomodulatory effects of C-PC [36]. The results regarding the effect of phycocyanin on the level of anti-oxidant and anti-inflammatory compounds are inconclusive. Results of a study on experimental autoimmune encephalitis (EAE), an animal model of multiple sclerosis (MS), revealed that phycocyanin decreases the oxidative stress markers [35]. The use of C-PC has been shown to have a beneficial effect on allergic patients. When PBMCs were exposed to PC, there was a decrease in the production of IL-4 [37]. Using C-PC resulted in an increase in Catalase and SOD activities in the liver of animals with hypercholesterolemia. However, the gene expression SOD-1 in HepG2 cells did not significantly differ when treated with C-PC [38]. Treating PBMCs from three healthy subjects with 200 µg/ml of C-PC did not significantly differ in the number of Treg cells [35].

Studies have demonstrated that capsulation improved the stability and anti-oxidant activity of phycocyanin [21]. Microencapsulation of phycocyanin involves enclosing phycocyanin particles within a coating or sheath made from various compositions, including Cetyl Alcohol, Polyvinylpyrrolidone K30 (PVP K30), Hydroxypropyl MethylCellulose 6 CPS (HPMC 6CPS), Polyethylene Glycol 4000 (PEG 4000) and Ethanol 75%. This coating serves several purposes: it helps control the release of phycocyanin, protects the compound, enhances its stability, and improves its physical and chemical properties. Using any of these components to microencapsulate phycocyanin effectively enhances its pharmacological properties and regulates its release [39]. The results of studies on phycocyanin encapsulation indicate that encapsulation improves its stability against temperature changes and extends its shelf-life [21, 40]. Our findings showed no significant difference in gene expression in PBMCs in encapsulated and non-encapsulated PC presence. However, pairwise analysis showed that encapsulated PC (1000 µg/ml) decreased IL-17 gene expression. Similar results were found in a study examining the effect of C-PC on IL-17 expression [41].

4. Conclusion

In conclusion, these results contribute to the existing research on the potential therapeutic use of C-PC in treating inflammatory conditions. However, further investigations, including in vivo studies and clinical trials, are needed to validate and extend these findings and to elucidate the specific molecular mechanisms underlying the observed effects of C-PC on anti-oxidant and inflammatory gene expression. Exploring the potential synergistic effects of C-PC with existing anti-inflammatory drugs may offer novel therapeutic strategies. Additionally, further optimization of encapsulation techniques to enhance stability and bioavailability is a promising avenue for future research.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets used and/or analysed during the current study are available from
the corresponding author on reasonable request.

Athors Contributions

FE and MRP: carried out the experiments. BE: performed the data analysis. BE, FE and FPS contributed

to the conception of the work, editing, and revising the manuscript. MRP, FE, BE, SH, MME, and FPS wrote the manuscript. All authors read and approved the final manuscript.

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The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of graphics, tables, or their corresponding captions.

References

1. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nature medicine. 2019;25(12):1822-32.
2. Pant T, Uche N, Juric M, Zielonka J, Bai X. Regulation of immunomodulatory networks by Nrf2-activation in immune cells: Redox control and therapeutic potential in inflammatory diseases. Redox Biology. 2024:103077.
3. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204.
4. Holdsworth SR, Gan P-Y. Cytokines: names and numbers you should care about. Clinical journal of the American Society of Nephrology: CJASN. 2015;10(12):2243.
5. Speer H, D'Cunha NM, Alexopoulos NI, McKune AJ, Naumovski N. Anthocyanins and human health—A focus on oxidative stress, inflammation and disease. Anti-oxidants. 2020;9(5):366.
6. Padureanu R, Albu CV, Mititelu RR, Bacanoiu MV, Docea AO, Calina D, et al. Oxidative stress and inflammation interdependence in multiple sclerosis. Journal of clinical medicine. 2019;8(11):1815.
7. Yildirim M, Inaloz H, Baysal V, Delibas N. The role of oxidants and anti-oxidants in psoriasis. Journal of the European Academy of Dermatology and Venereology. 2003;17(1):34-6.
8. Ighodaro O, Akinloye O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire anti-oxidant defence grid. Alexandria journal of medicine. 2018;54(4):287-93.
9. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerging microbes & infections. 2013;2(1):1-5.
10. Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best practice & research Clinical gastroenterology. 2010;24(2):121-32.
11. Hörl WH. Nonsteroidal anti-inflammatory drugs and the kidney. Pharmaceuticals. 2010;3(7):2291-321.
12. Yatoo M, Gopalakrishnan A, Saxena A, Parray OR, Tufani NA, Chakraborty S, et al. Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review. Recent patents on inflammation & allergy drug discovery. 2018;12(1):39-58.
13. Singh JA, Hossain A, Kotb A, Wells G. Risk of serious infections with immunosuppressive drugs and glucocorticoids for lupus nephritis: a systematic review and network meta-analysis. BMC medicine. 2016;14(1):1-12.
14. Fernández-Rojas B, Hernández-Juárez J, Pedraza-Chaverri J. Phycocyanin a nutraceutical with antioxidant activity. Improving Health and Nutrition through Bioactive Compounds: Elsevier; 2025. p. 67-78.
15. Nuhu AA. Spirulina (Arthrospira): An important source of nutritional and medicinal compounds. Journal of Marine biology. 2013;2013.
16. Moraes CC, Sala L, Cerveira GP, Kalil SJ. C-phycocyanin extraction from Spirulina platensis wet biomass. Brazilian Journal of Chemical Engineering. 2011;28(1):45-9.
17. Sotiroudis TG, Sotiroudis GT. Health aspects of Spirulina (Arthrospira) microalga food supplement. Journal of the Serbian Chemical Society. 2013;78(3):395-405.
18. Thangam R, Suresh V, Princy WA, Rajkumar M, Senthilkumar N, Gunasekaran P, et al. C-Phycocyanin from Oscillatoria tenuis exhibited an anti-oxidant and in vitro antiproliferative activity through induction of apoptosis and G0/G1 cell cycle arrest. Food chemistry. 2013;140(1-2):262-72.
19. Romay C, Gonzalez R, Ledon N, Remirez D, Rimbau V. C-phycocyanin: a biliprotein with anti-oxidant, anti-inflammatory and neuroprotective effects. Current protein and peptide science. 2003;4(3):207-16.

20. Chaiklahan R, Chirasuwan N, Bunnag B. Stability of phycocyanin extracted from Spirulina sp.: influence of temperature, pH and preservatives. Process Biochemistry. 2012;47(4):659-64.
21. Suzery M, Majid D, Setyawan D, Sutanto H, editors. Improvement of stability and anti-oxidant activities by using phycocyanin-chitosan encapsulation technique. IOP Conference Series: Earth and Environmental Science; 2017: IOP Publishing.
22. Pentón-Rol G, Lagumersindez-Denis N, Muzio L, Bergami A, Furlan R, Fernández-Massó JR, et al. Comparative neuroregenerative effects of C-phycocyanin and IFN-beta in a model of multiple sclerosis in mice. Journal of Neuroimmune Pharmacology. 2016;11(1):153-67.
23. Nasser T, Emamshoushtari MM, Helchi S, Saeidi A, Pajoum Shariati F. Mitigating membrane fouling in an internal loop airlift membrane photobioreactor containing Spirulina platensis: effects of riser cross-sectional area and hydrophilic baffles. Preparative Biochemistry & Biotechnology. 2023:1-9.
24. Sohani E, Pajoum Shariati F, Pajoum Shariati SR. Assessment of various colored lights on the growth pattern and secondary metabolites synthesis in Spirulina platensis. Preparative Biochemistry & Biotechnology. 2023;53(4):412-23.
25. Bennett A, Bogorad L. Complementary chromatic adaptation in a filamentous blue-green alga. The Journal of cell biology. 1973;58(2):419-35.
26. Boussiba S, Richmond AE. Isolation and characterization of phycocyanins from the blue-green alga Spirulina platensis. Archives of Microbiology. 1979;120:155-9.
27. Kumar D, Dhar DW, Pabbi S, Kumar N, Walia S. Extraction and purification of C-phycocyanin from Spirulina platensis (CCC540). Indian Journal of Plant Physiology. 2014;19:184-8.
28. Parikh DM. Handbook of pharmaceutical granulation technology. Drugs and the pharmaceutical sciences. 2005;81.
29. Hernandez FYF, Khandual S, López IGR. Cytotoxic effect of Spirulina platensis extracts on human acute leukemia Kasumi-1 and chronic myelogenous leukemia K-562 cell lines. Asian Pacific Journal of Tropical Biomedicine. 2017;7(1):14-9.
30. Esmaeili B, Mansouri P, Doustimotlagh AH, Izad M. Redox imbalance and IL-17 responses in memory CD 4+ T cells from patients with psoriasis. Scandinavian journal of immunology. 2019;89(1):e12730.
31. Esmaeili B, Rezaee SAR, Layegh P, TAVAKKOL AJ, Dye P, GHAYOOR KE, et al. Expression of IL-17 and COX2 gene in peripheral blood leukocytes of vitiligo patients. 2011.
32. Liu W, Saint DA. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Analytical biochemistry. 2002;302 (1):52-9.
33. Wood SK. The role of inflammation and oxidative stress in depression and cardiovascular disease. Cardiovascular Implications of Stress and Depression: Elsevier; 2020. p. 175-209.
34. Rekatsina M, Paladini A, Piroli A, Zis P, Pergolizzi JV, Varrassi G. Pathophysiology and therapeutic perspectives of oxidative stress and neurodegenerative diseases: a narrative review. Advances in therapy. 2020;37(1):113-39.
35. Pentón-Rol G, Martínez-Sánchez G, Cervantes-Llanos M, Lagumersindez-Denis N, Acosta-Medina EF, Falcón-Cama V, et al. C-Phycocyanin ameliorates experimental autoimmune encephalomyelitis and induces regulatory T cells. International immunopharmacology. 2011;11(1):29-38.
36. Ravi M, De SL, Azharuddin S, Paul SF. The beneficial effects of Spirulina focusing on its immunomodulatory and anti-oxidant properties. Nutrition and Dietary Supplements. 2010;2:73-83.
37. ElSheikh SM, Shalaby MA, Hafez RA, Metwally WS, El-Ayoty YM. The immunomodulatory effects of probiotic bacteria on peripheral blood mononuclear cells (PBMCS) of allergic patients. American Journal of Immunology. 2014;10(3):116.
38. Sheu M-J, Hsieh Y-Y, Lai C-H, Chang C-C, Wu C-H. Antihyperlipidemic and anti-oxidant effects of C-phycocyanin in golden syrian hamsters fed with a hypercholesterolemic diet. Journal of traditional and complementary medicine. 2013;3(1):41-7.
39. Pourmand MR, Shariat FP, Ardjmand M. Evaluation of Lipophilic Properties of Thermoplastic Granule in Formulation of Sustained Release Matrix in Solid Dosage Forms. Pharmaceutical Chemistry Journal. 2019;53(7):660-7.
40. Pradeep H, Nayak CA. Enhanced stability of C-phycocyanin colorant by extrusion encapsulation. Journal of Food Science and Technology. 2019;56:4526-34.
41. Haryanti E, Gondo HK. Induction of interleukin-6 trigger an apoptosis through IL-17 and stat3 pathway that alleviated by phycocyanin treatment. Jurnal Ilmiah Kedokteran Wijaya Kusuma. 2018;5 (2):6-10.

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2025: 21 (1): 183- 190
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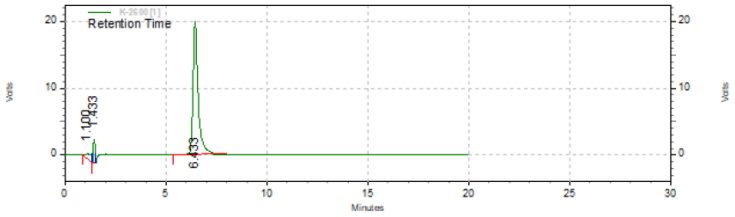
Figure S1: Chromatogram graphs from the HPLC analysis of both standards and test samples.

Area % Report

Data File: Sample Test2

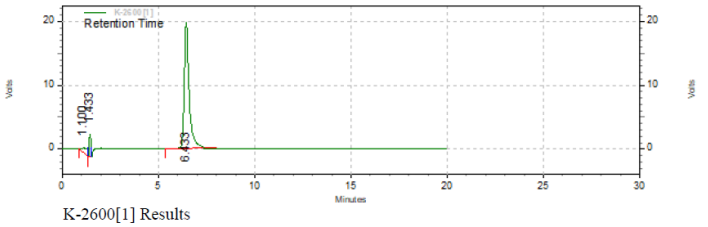


K-2600[1] Results

Retention Time	Area	Height
6.433	83130	19731

Area % Report

Data File: Sample Test3

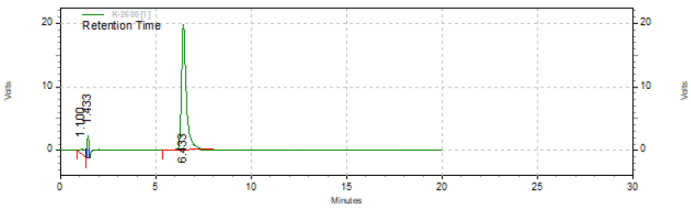


K-2600[1] Results

Retention Time	Area	Height
6.433	83132	19732

Area % Report

Data File: Standard 2

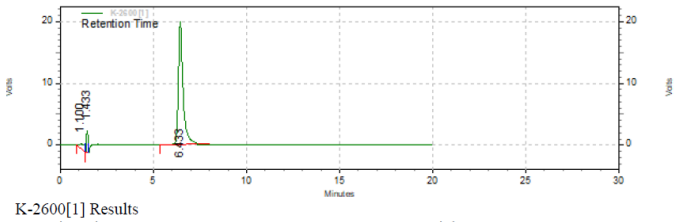


K-2600[1] Results

Retention Time	Area	Height
6.433	83129	19731

Area % Report

Data File: Standard 3



K-2600[1] Results

Retention Time	Area	Height
6.433	83127	19732