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Investigation into the Stability Enhancement and Anticancer Efficacy of Phycocyanin Extracted from Spirulina Platensis

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Abstract: Phycocyanin, a pigment–protein complex obtained from *Spirulina platensis*, is well recognized for its remarkable antioxidant and anticancer potential. Despite these promising properties, its limited structural stability restricts its practical use in pharmaceutical and food formulations. In this work, the biological activity and stability of phycocyanin were investigated through a combination of spectroscopic characterization, antioxidant evaluation, encapsulation trials, and molecular docking studies. Ultraviolet–visible spectroscopy was employed to assess pigment purity, while Fourier-transform infrared spectroscopy verified the presence of characteristic chromophore peaks around 1019 cm^{-1}, confirming structural integrity. The total antioxidant capacity assay revealed a strong radical scavenging ability, and the *in vitro* cytotoxicity test on K562 leukemia cells demonstrated substantial growth inhibition with a half-maximal inhibitory concentration (IC_{50}) of approximately 54.4 µg. Molecular docking analysis indicated specific interactions between phycocyanin and the G-protein-coupled receptor GPRC5D, a target associated with bone marrow malignancies, thereby suggesting its potential as a safe, naturally derived therapeutic agent. To overcome instability under environmental stress, edible oil-based encapsulation using homogenization was applied, resulting in improved pH and thermal resistance. The encapsulated form-maintained stability within pH 4.8–7.5 and tolerated temperatures up to 60 °C. Overall, this study highlights phycocyanin as a promising bioactive molecule for anticancer and pharmaceutical applications, warranting further *in vivo* and clinical investigations.

Keywords: *Phycocyanin, Spirulina Platensis, Antioxidant Activity, Encapsulation, Anticancer Effect, Molecular Docking, GPRC5D.*

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I. INTRODUCTION

Phycocyanin (PC) is a pigment–protein complex consisting of a phycobiliprotein linked with the chromophore phycocyanobilin [14]. Extracted primarily from *Spirulina platensis*, PC is widely recognized for its strong antioxidant and anticancer potential [5, 8, 18]. Structurally, it comprises α and β subunits, with phycocyanobilin acting as the principal chromophore responsible for its vivid blue color and biological activity [5, 8, 18]. Functionally, PC neutralizes reactive oxygen species (ROS) and mitigates oxidative stress, a major factor contributing to cancer progression [8]. Several studies have demonstrated its ability to induce apoptosis through mitochondrial mechanisms and inhibit PI3K/AKT signaling pathways, thereby suppressing tumor cell proliferation [11,16]. Despite these promising properties, the compound's practical use remains restricted due to its poor stability under extreme temperature, pH fluctuations, and oxidative environments [3]. To address this limitation, encapsulation using edible oils has been investigated as an effective approach to improve PC's stability, enhance bioavailability, and ensure consistent therapeutic efficacy [13].

Cancers originating within the bone marrow present major therapeutic challenges due to their tendency for late detection, recurrence, and resistance to conventional chemotherapeutic approaches. Among them, multiple myeloma is a malignant proliferation of plasma cells that disrupts normal hematopoiesis, resulting in immune suppression and extensive bone deterioration. Because of its strong chemoresistance, developing alternative treatment strategies has become an area of critical research interest. The G-protein-coupled receptor GPRC5D has been reported to be highly overexpressed in multiple myeloma cells and is currently being explored as a promising therapeutic target [20]. The receptor's molecular structure and functional relevance are illustrated in Fig. 2. Considering these limitations of existing therapies, naturally derived bioactive molecules such as phycocyanin offer potential as multi-

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targeted therapeutic agents capable of modulating several cancer-related pathways simultaneously.

This study investigates the anticancer potential of phycocyanin (PC) through *in vitro* cytotoxicity testing against K562 leukemia cells, combined with molecular docking analysis to explore its binding interactions with the GPRC5D receptor, which is associated with multiple myeloma. By integrating both biochemical experiments and computational modeling approaches (Fig. 1), the research aims to establish PC as a promising natural therapeutic candidate for bone marrow–related cancers.

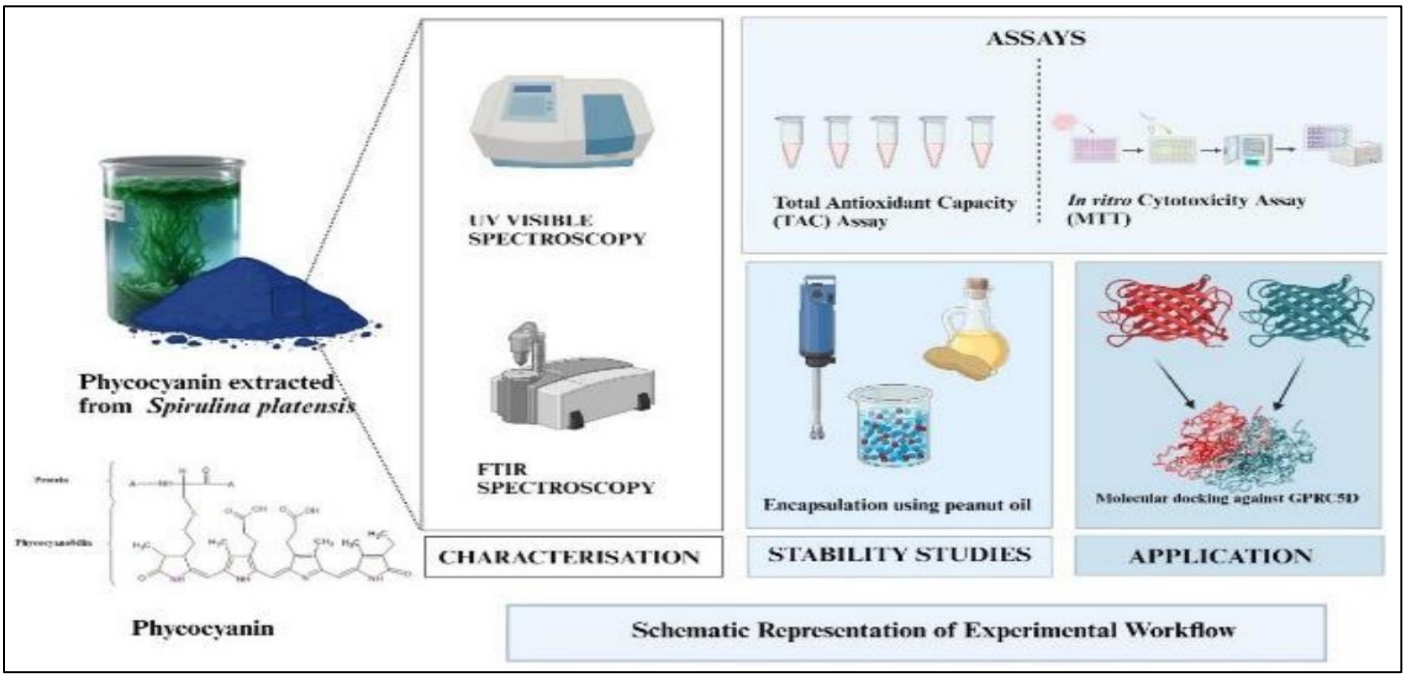

Fig 1 Graphical Abstract

II. MATERIAL AND METHODS

The materials employed in this study included wood-pressed edible peanut oil and phycocyanin powder, both procured from Urban Platter (Mumbai, India). The K562 human leukemia cell line was obtained from the National Centre for Cell Science (NCCS), Pune. Cell culture experiments were performed using Roswell Park Memorial Institute (RPMI) medium supplemented with fetal bovine serum (FBS). The chemicals utilized comprised MTT solution (1 mg/mL), dimethyl sulfoxide (DMSO), and Tween 80 (used as an emulsifier). Additional analytical-grade reagents included disodium hydrogen phosphate, ammonium molybdate, sulfuric acid, ascorbic acid, acetic acid, sodium acetate, and monobasic sodium phosphate. Molecular docking studies were conducted using the HADDOCK 2.4 server to evaluate the binding interactions between phycocyanin and the target protein.

➢ *Characterization of Phycocyanin*

A stock solution of phycocyanin (10 mg/mL) was initially prepared in deionized water and subsequently diluted to a working concentration of 1 mg/mL. All experiments were conducted in triplicate to ensure data reliability. The absorbance of the samples was recorded in the wavelength range of 500–700 nm using a Shimadzu UV–visible spectrophotometer. The purity index of phycocyanin was determined by calculating the ratio of absorbance at 620 nm (A_{620}, corresponding to phycocyanin) to that at 280 nm (A_{280}, representing total protein content). Fourier-transform infrared (FTIR) spectroscopy was employed to analyze the functional groups and structural features of the sample. The spectra were obtained using an attenuated total reflectance (ATR) FTIR setup with a resolution of 4 cm^{-1} over a scanning range of 400–4000 cm^{-1}.

➢ *Antioxidant Activity*

For the antioxidant assay, phycocyanin (PC) stock solution at a concentration of 10 mg/mL was used to prepare working samples in varying volumes ranging from 10 to 50 µL, each diluted to a total volume of 100 µL with distilled water. To each sample, 1 mL of reagent solution was added, consisting of 28 mM disodium hydrogen phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid prepared in distilled water. The reaction mixtures were incubated at 95 °C for 90 minutes, followed by cooling to room temperature. The absorbance of each sample was then measured spectrophotometrically at 675 nm. Ascorbic acid (1 mg/mL) was used as the standard antioxidant reference for comparison [17].

➢ *Anticancer Activity Assessment*

The cytotoxic potential of phycocyanin (PC) was evaluated using the MTT assay on K562 human leukemia cells. Briefly, cells cultured in RPMI medium were supplemented with phosphate-buffered saline (PBS, pH 7.2), MTT solution (1 mg/mL), and 100% dimethyl sulfoxide (DMSO). The K562 cell line, obtained from the National Centre for Cell Science (NCCS), Pune, was incubated for 3–4 hours at 37 °C in a humidified atmosphere containing 5% CO_2.

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After incubation, the absorbance of the resulting formazan crystals was measured spectrophotometrically at 570 nm. The percentage of cell viability was calculated, and a linear regression plot of concentration (µg) versus cytotoxicity (%) was used to determine the half-maximal inhibitory concentration (IC_{50}) value.

➢ *Molecular Docking Studies*

Molecular docking analysis was carried out to investigate the binding affinity of phycocyanin (PC) with the selected target protein associated with bone marrow cancer. The structural model of phycocyanin (Chain B; PDB ID: 1HA7) was retrieved from the RCSB Protein Data Bank, while the target receptor GPRC5D (Accession: AF-Q9NZDI-F1-V4) was obtained from the AlphaFold protein structure database. Prior to docking, both protein structures were refined by removing water molecules (HOH) and heteroatoms to eliminate unnecessary conformational noise. The refined structures were then uploaded to the HADDOCK 2.4 web server, where active binding sites were predicted using the CastP and FTSite servers. Docking simulations were performed using the default HADDOCK parameters. The resulting protein–ligand interactions were analyzed using the Protein–Ligand Interaction Profiler (PLIP) and visualized with PyMOL and UCSF Chimera software for detailed interpretation of molecular interactions.

➢ *Encapsulation of Phycocyanin*

Phycocyanin (PC) encapsulation was performed using the homogenization technique with edible peanut oil as the carrier medium. The emulsion was formulated by mixing 32.5 mL of edible peanut oil, 1.5 mL of phycocyanin solution, and 0.5 mL of Tween 80, which served as an emulsifying agent. The total volume was adjusted to 50 mL with deionized water. The mixture was then subjected to homogenization at 13,000 rpm for 5 minutes using a high-speed homogenizer to ensure uniform dispersion and droplet formation. A control emulsion, prepared under identical conditions but without phycocyanin, was used for comparison [2].

➢ *Stability Studies*

The stability of both encapsulated and unencapsulated forms of phycocyanin (PC) was evaluated under varying pH and temperature conditions. For pH stability assessment, equal volumes of the emulsified sample and buffer solutions with pH values of 4.8, 6.8, and 7.5 were prepared and incubated at 55 °C for 30 and 60 minutes. Following incubation, the mixtures were centrifuged at 9,500 rpm for 10 minutes, and the absorbance of the supernatant representing the amount of unencapsulated PC was measured spectrophotometrically at 620 nm [3].

Similarly, temperature stability was examined by incubating equal volumes of the emulsified sample and buffer solutions (pH 4.8 and 5.9) at two different temperatures, 37 °C and 60 °C, for 30 minutes. The samples were then centrifuged at 9,500 rpm for 10 minutes, and the absorbance of the supernatant was again recorded at 620 nm to determine the proportion of released PC [3].

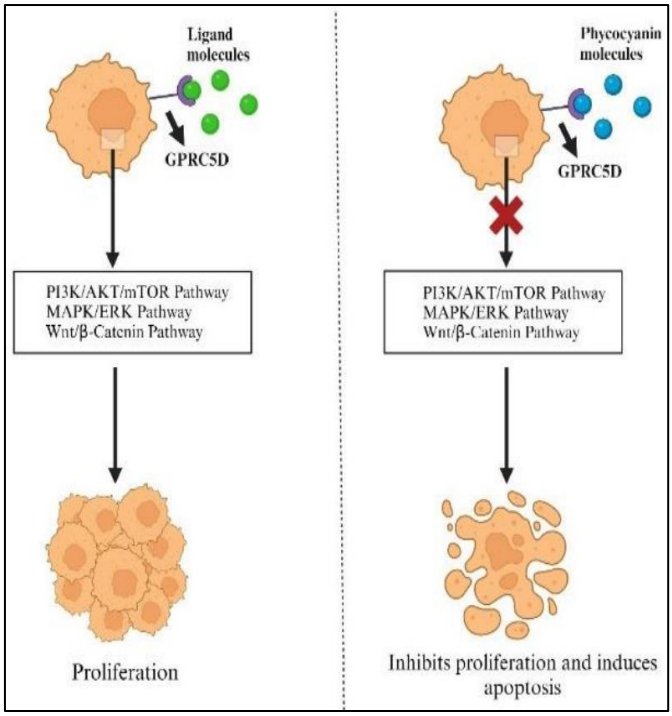

Fig 2 Phycocyanin's Role against GPRC5D.

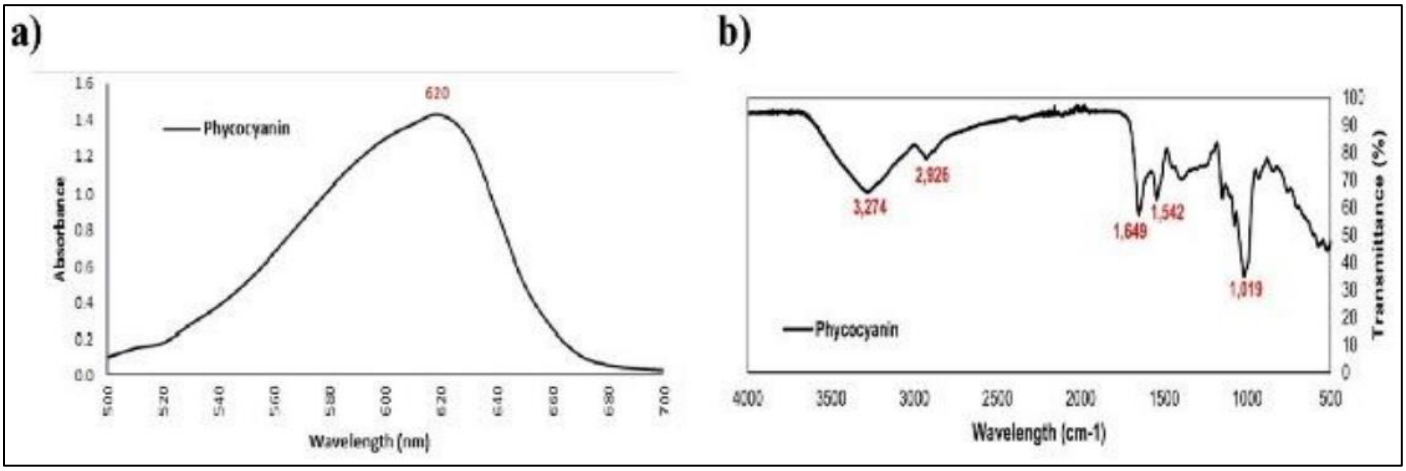

Fig 3 Characterization Studies – a) Absorption Spectrum Obtained from UV – Vis Spectroscopy and b) FTIR Spectrum of Purified PC.

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III. RESULTS

➢ *Characterization of PC*

The absorption profile of phycocyanin (PC) was recorded using UV–visible spectroscopy in the wavelength range of 500–700 nm. Based on the mean values from triplicate measurements, a prominent absorption peak was observed at 620 nm, as illustrated in Fig. 3a [9]. The calculated purity ratio (A_{620}/A_{280}) of PC was 2.78, indicating partial purity, which suggests its suitability for potential applications in food and cosmetic formulations [4]. The FTIR spectrum further verified the presence of characteristic functional groups associated with phycocyanobilin at 1019 cm^{-1}. Additional absorption peaks were detected at 1649 cm^{-1} (Amide I), 1542 cm^{-1} (Amide II), and 3274 cm^{-1} (O–H stretching), corresponding to protein and hydroxyl functional groups. These spectral features, depicted in Fig. 3b, align well with previously reported literature values for purified phycocyanin [12].

➢ *Antioxidant Potential of PC*

The total antioxidant capacity (TAC) of phycocyanin (PC) was evaluated based on the formation of a green phosphate–molybdenum complex, which results from the reduction of molybdenum (VI) to molybdenum (V). The antioxidant response of PC displayed a clear concentration-dependent enhancement, as indicated by increasing absorbance values measured at 695 nm. When compared to the standard ascorbic acid reference, each corresponding PC concentration demonstrated a proportional rise in antioxidant activity, confirming its strong radical scavenging ability. These observations are presented in Fig. 4, further validating the potent antioxidant nature of phycocyanin [17].

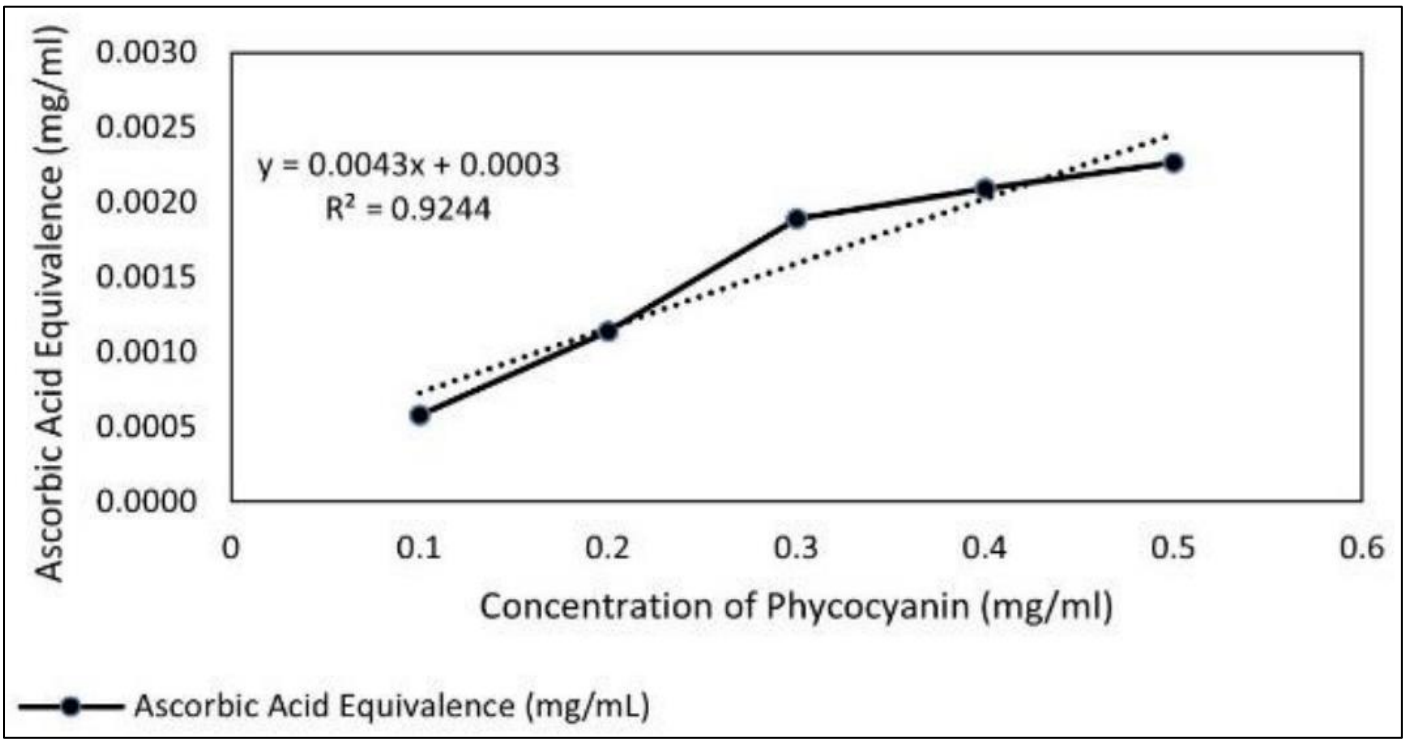

Fig 4 Concentration of PC (mg/ml) vs Ascorbic Acid Equivalence (mg/ml).

➢ *Anticancer Activity*

The MTT assay measures the metabolic activity of cells through the reduction of yellow tetrazolium salt (MTT). The cytotoxic effects of/The MTT assay was utilized to assess the cytotoxic potential of phycocyanin (PC) by quantifying cellular metabolic activity through the reduction of the yellow tetrazolium salt (MTT) to insoluble formazan crystals. The cytotoxic response of K562 human leukemia cells exposed to varying PC concentrations (10–100 µg/mL) is depicted in Fig. 5. The half-maximal inhibitory concentration (IC_{50}) was calculated to be 54.4 µg/mL using the linear regression equation $y = 0.764x + 8.471$, generated through data analysis in Microsoft Excel. After 24 hours of treatment, PC demonstrated a marked dose-dependent cytotoxic effect, with 78% inhibition observed at the highest concentration (100 µg/mL) compared to the untreated control (Table 1) [11].

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Fig 5 Anticancer Activity with IC_{50} against K562 Cells.

Table 1 MTT Assay with IC_{50} for Sample at Different Concentrations

Concentration (µg)	Phycocyanin			
	Cytotoxicity (%)	*Cell Viability (%)*	*Cytotoxic Reactivity*	*With IC_{50} Value*
0	0	100	None	54.4 µg
10	17	83	Slight	
20	24	76	Mild	
30	33	67	Mild	
40	41	59	Mild	
50	50	50	Mild	
60	62	38	Moderate	
70	66	34	Moderate	
80	70	30	Moderate	
90	73	27	Severe	
100	78	22	Severe	

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➢ *Molecular Docking Application*

The HADDOCK 2.4 web tool was used for protein-protein docking [6,7]. Molecular docking studies of PC were done against GPRC5D which is highly expressed in multiple myeloma and identified Cluster 2 as the most stable binding conformations when compared to other clusters through plots and values provided in the summary report of the web server. The HADDOCK score of -25.8 shows the binding efficiency and a Z-score of -2.1 is a statistical measure of a number of standard deviations. A more negative Z-score shows better docking results. The van der Waals interaction energy of –55.3 indicates favourable spatial complementarity between PC and the GPRC5D binding pocket. The negative electrostatic energy of –114.2 suggests strong ionic and polar interactions, reinforcing the binding stability. A desolvation energy of –37.4 reflects the effective displacement of water molecules at the interaction interface, thereby contributing to complex formation. While the high restraints violation energy of 897.9 may point to potential deviations in defined active site parameters or conformational flexibility of residues, it does not negate the overall docking quality. Additionally, the substantial buried surface area of 2489.9 Å² supports the existence of a well-embedded and stable protein-ligand complex. Detailed HADDOCK docking parameters are summarized in Table 2. The residues of GPRC5D (LEU 256, PRO 260, VAL 259, ARG 267) are not yet confirmed to be a part of the orthosteric binding site but their consistent interactions with CYB 184 (Phycocyanobilin) residues of PC as shown in Fig 6, suggests a potential for allosteric modulation which may still influence the protein's functional conformation. The interactions were visualized using the PLIP tool in PyMOL, further supporting the therapeutic potential of PC. The results suggest that PC has a good binding affinity to GPRC5D, indicating its potential for targeted cancer therapy.

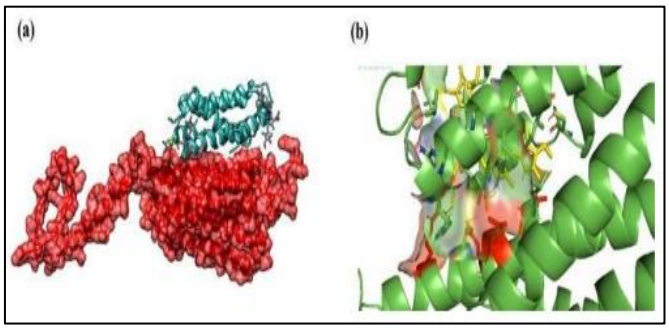

Fig 6 Protein-Protein Docking of PC against GPRC5D Protein – a) shows Docked Complex Structure with Chain A – GPRC5D Protein (Red) and Chain B – Phycocyanin (Cyan Blue) and b) Shows Small Molecule (Phycocyanobilin – CYB 184) – [Yellow] Binding to GPRC5D Protein Residues (LEU 256, PRO 260, VAL 259, and ARG 267) – [Red]

Table 2 Docking Summary from HADDOCK Web Server

Parameter	Value
Best Cluster	2
HADDOCK Score	−25.8 ± 19.2
Cluster Size	9
RMSD	16.7 ± 0.3
Van der Waals	−55.3 ± 11.8
Electrostatic	−114.2 ± 25.6
Desolvation	−37.4 ± 3.4
Buried Surface	2489.9 ± 360.9
Z-Score	−2.1

➢ *Encapsulation Stability*

Encapsulation stability (%) refers to the proportion of phycocyanin (PC) successfully retained within the oil–water emulsion. To examine the thermal protective effect of edible oil, PC was encapsulated in peanut oil and its stability was compared to that of the unencapsulated form. The results indicated that encapsulated PC retained its stability for a longer duration at 55 °C compared to free PC, as presented in Table 3. Optimal pH stability was observed at 4.8, 6.8, and 7.5, where encapsulated samples demonstrated enhanced resistance to degradation relative to unencapsulated ones. As shown in Table 4, encapsulation effectively preserved the structural integrity of PC over extended incubation periods. Furthermore, temperature-dependent degradation studies conducted at 37 °C and 60 °C for 30 and 60 minutes revealed that encapsulated PC exhibited significantly greater thermal stability than its unencapsulated counterpart, consistent with findings reported in earlier literature [3].

Table 3 Encapsulated Stability (%) of PC at 55°C

Time (min)	pH 4.8 Free	pH 4.8 Encapsulated	pH 6.8 Free	pH 6.8 Encapsulated	pH 7.5 Free	pH 7.5 Encapsulated
0	100	100	100	100	100	100
30	79.6	92.5	83.3	88.3	65.8	84.6
60	78.4	85.0	77.0	78.2	63.0	81.1

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Table 4 Effect of Temperature on PC Stability

Temperature (°C)	pH 4.8 Free	pH 4.8 Encapsulated	pH 5.9 Free	pH 5.9 Encapsulated
37	97.7	99.7	98.7	99.5
60	75.6	99.3	65.6	98.9

IV. DISCUSSION

Spectral characterization of phycocyanin (PC) using UV–visible spectroscopy revealed a distinct absorption maximum at 620 nm, consistent with previously reported values for standard phycocyanin, thereby confirming its purity [9]. The calculated purity ratio (A_{620}/A_{280}) was 2.78, indicating partial purity suitable for food and cosmetic-grade applications [4]. FTIR analysis further validated the structural integrity of PC by identifying characteristic peaks corresponding to the chromophoric group phycocyanobilin at 1019 cm^{-1}, along with other functional groups such as Amide I (1649 cm^{-1}), Amide II (1542 cm^{-1}), and hydroxyl (O–H) stretching at 3274 cm^{-1}, aligning with standard spectral profiles reported in earlier studies [12]. The presence of phycocyanobilin is particularly significant as it contributes to the compound's antioxidant activity and provides insight into its molecular architecture. The total antioxidant capacity (TAC) assay demonstrated an increasing formation of the green phosphate–molybdenum complex with higher PC concentrations, showing a positive correlation with ascorbic acid equivalents, thus confirming its strong antioxidant potential [17]. Similarly, results from the MTT assay indicated concentration-dependent cytotoxicity against K562 human leukemia cells, with an IC_{50} value of 54.4 µg/mL, signifying notable anticancer activity compared with standard references [11].Given the sensitivity of PC to environmental conditions such as pH and temperature, encapsulation using edible peanut oil via the homogenization technique proved effective in enhancing its stability. The encapsulated PC maintained its integrity at 55 °C for up to 60 minutes and exhibited optimal pH stability between 4.8 and 7.5. It also retained thermal stability up to 60 °C, suggesting improved bioavailability and sustained functionality compared to the unencapsulated form.

To further explore its therapeutic potential, molecular docking studies were performed with GPRC5D-a receptor protein overexpressed in multiple myeloma and involved in critical signaling pathways. The docking results revealed strong binding interactions, underscoring the potential of phycocyanin as a natural anticancer candidate. However, further *in vivo* and clinical investigations are recommended to elucidate its detailed molecular mechanisms and validate its pharmacological significance.

V. CONCLUSION

This study highlights the enhanced stability and bioactivity of phycocyanin (PC) extracted from *Spirulina platensis*, emphasizing the effectiveness of edible oil–based encapsulation in preserving its structural and functional integrity. The results demonstrate that PC exhibits strong antioxidant and anticancer properties, as confirmed through total antioxidant capacity (TAC) analysis and cytotoxicity evaluation on K562 leukemia cells. Molecular docking studies further revealed a significant interaction between PC and the GPRC5D receptor, which is overexpressed in multiple myeloma, suggesting its potential as a therapeutic target. Collectively, these findings establish PC as a promising natural bioactive compound with considerable potential in cancer therapy and oxidative stress management. Moreover, the encapsulation approach proved to be an efficient strategy for improving PC's stability and bioavailability, laying the groundwork for future *in vivo* research and clinical translation in pharmaceutical applications.

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