

# Exploring the Pharmacological Potential of *Synechococcus* sp. PGDR2 through Phytochemical and *In Vitro* Bioactivity Studies.

## ABSTRACT

Cyanobacteria are recognized as promising sources of bioactive compounds with pharmaceutical potential. The present study evaluated the phytochemical composition and biological activities of *Synechococcus* sp. PGDR2. Qualitative screening revealed the presence of tannins and proteins, while other major secondary metabolites were absent. Antioxidant activity assessed by DPPH and ABTS assays showed weak radical scavenging capacity with  $IC_{50}$  values  $>320 \mu\text{g/mL}$ . In contrast, ascorbic acid exhibited strong antioxidant activity with  $IC_{50}$  values of  $19.97 \mu\text{g/mL}$  (DPPH) and  $20.46 \mu\text{g/mL}$  (ABTS). Cytotoxicity against MG-63 osteosarcoma cells using the MTT assay demonstrated minimal growth inhibition, with an  $IC_{50}$  value  $>100 \mu\text{g/mL}$ , compared to doxorubicin ( $IC_{50} = 9.52 \mu\text{g/mL}$ ). The anti-inflammatory activity evaluated by the protein denaturation assay showed moderate inhibition with an  $IC_{50}$  of  $235.11 \mu\text{g/mL}$ , whereas diclofenac sodium exhibited an  $IC_{50}$  of  $29.43 \mu\text{g/mL}$ . The comparatively high  $IC_{50}$  values suggest limited bioactivity of the crude extract. The observed anti-inflammatory potential may be attributed to tannins and proteinaceous compounds. Overall, *Synechococcus* sp. PGDR2 exhibited mild biological activity, indicating the need for further purification and LC-MS profiling to identify potential bioactive constituents. These findings contribute to understanding the pharmacological potential of freshwater cyanobacteria.

**Keywords:** *Synechococcus* sp., 16S rRNA gene, Phytochemical analysis, Anti-oxidant, Anti-inflammatory, MG-63 cell line

## INTRODUCTION

Cyanobacteria are among the most ancient photosynthetic microorganisms, thriving in diverse aquatic and terrestrial ecosystems and producing a wide range of structurally diverse bioactive metabolites with therapeutic potential. These metabolites include pigments (e.g., phycobiliproteins, carotenoids), polysaccharides, peptides, and unique secondary compounds such as mycosporine-like amino acids (MAAs) and scytonemin, which contribute to redox homeostasis and cellular protection mechanisms (Bouyahyaet *et al.*, 2024; Wang *et al.*, 2025). Cyanobacterial bioactive compounds have drawn increasing research interest due to their demonstrated antioxidant, anti-inflammatory, and anticancer properties, making them promising candidates for drug discovery and nutraceutical applications (Perera *et al.*, 2023; Bouyahyaet *et al.*, 2024).

Among cyanobacteria, the genus *Synechococcus* has emerged as a valuable source of bioactive compounds with potential biomedical applications. The antioxidant potential of cyanobacterial extracts is often attributed to compounds such as phycobiliproteins, carotenoids, and MAAs that directly neutralize reactive oxygen species (ROS) and enhance endogenous antioxidant defence mechanisms (Wang *et al.*, 2025). Specifically, protein hydrolysates derived from *Synechococcus* species have shown high radical-scavenging activity *in vitro*, suggesting their utility as natural antioxidants (Suttisuwanet *et al.*, 2019).

The anti-inflammatory potential of cyanobacterial metabolites, including those from *Synechococcus* and other marine cyanobacteria, is equally compelling. Cyanobacteria-derived

41 bioactive peptides have been reported to downregulate pro-inflammatory cytokines such as inducible  
42 nitric oxide synthase (iNOS), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukins, thereby mitigating  
43 inflammatory responses in macrophage models (Perera *et al.*, 2023). Anticancer activity is another  
44 facet of cyanobacterial bioactivity, with several studies reporting cytotoxic effects against human  
45 cancer cell lines. Recent work with fractions from *Synechococcus* sp. has demonstrated notable  
46 cytotoxicity against MG-63 osteosarcoma cells, with the profiling for identifying key bioactive  
47 molecules such as pheophytin and phorbide derivatives that may underlie these effects (Cavalcante  
48 do Amaral *et al.*, 2025). Such anticancer effects are supported by broader evidence that  
49 cyanobacterial metabolites can induce apoptosis, inhibit proliferation, and modulate cancer-related  
50 signalling pathways *in vitro* (Bouyahya *et al.*, 2024).

51 Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC-MS)  
52 are essential for profiling the complex chemical mixtures produced by cyanobacteria, enabling the  
53 identification and quantitation of bioactive constituents. LC-MS analysis facilitates structure-activity  
54 relationships and guides the isolation of lead compounds with therapeutic relevance. Integrating  
55 bioactivity assays with LC-MS profiling strengthens the methodological framework for evaluating  
56 *Synechococcus*-derived compounds, providing deeper insight into their antioxidant, anti-  
57 inflammatory, and anticancer actions.

58 Overall, the exploration of *Synechococcus* and related cyanobacterial metabolites represents a  
59 promising frontier in natural product research, bridging microbial ecology, analytical chemistry, and  
60 biomedical applications. Therefore, this research focused on isolating *Synechococcus* sp. PGDR2  
61 from Retteri Lake and testing the anti-oxidant and anti-cancer properties of its ethanol extract against  
62 the MG-63 cell line in a laboratory setting.

## 63 MATERIALS AND METHODS

### 64 Collection and Isolation

65 Rettai Eri, locally known as Retteri, is a lake in the Kolathur area of Chennai, India which is  
66 visible from the 100 ft road. Redhills road Junction is also named as Retteri Junction. Water samples  
67 were collected from the lake using a 0.2 mm phytoplankton mesh net and stored in sterile vials. The  
68 samples were transported to the laboratory and centrifuged at 2000 rpm for 10 minutes to concentrate  
69 the biomass. The sample was then inoculated into CFTRI medium with pH-10 and incubated at 25 °C  
70 for 5–7 days. After incubation, it was streaked on the solid CFTRI medium until gets the pure  
71 cultures.

### 72 Morphological Identification

73 The cultured algal strains were identified under the 40X of light microscope based on the size  
74 and shape of the cells and colony formation. Microphotograph was taken using Micro vision industrial  
75 digital camera.

### 76 Molecular Identification using 16S rRNA

### 77 Extraction and Determination of quality of DNA

78 The Genomic DNA was extracted from the cyanobacterial strain PGDR2. It was extracted  
79 using a meticulously prepared extraction buffer for a 500 ml volume, which comprised 20 mM

80 Na<sub>2</sub>EDTA (3.7 g) and 100 mM Tris-HCl (6 g). The pH was adjusted to 8, with the subsequent addition  
81 of 1.4 M NaCl (40 g) and 2% (w/v) CTAB (10 g). The extraction buffer was preheated to 60 °C, and  
82 the sample was mixed well and incubated for 1 h at 60 °C with intermittent shaking at every 10 min.  
83 Mixture was cooled down to 37 °C. To 2 ml of this mixture, 1 ml of chloroform: isoamyl alcohol  
84 (24:1) was added and gently mixed by inverting the tubes to form an emulsion. It was then  
85 centrifuged at 5000xg for 15 min. The clear aqueous phase was transferred to a fresh tube and 150 µl  
86 of 6 M NaCl was added and mixed. To this 1 ml of ice-cold ethanol was added and refrigerated for 1 h  
87 at -20 °C. The pellet was washed several times with ethanol and finally resuspended in 100 µl of  
88 elute buffer. The DNA samples were stored at 4 °C for further analysis (**Jagielski *et al.*, 2017**). The  
89 quality of extracted DNA was checked on 0.8% agarose gels. Agarose powder was dissolved in 1X  
90 TAE buffer and boiled until it turned into a clear solution. Once it was cooled to 50 °C, ethidium  
91 bromide was added and mixed well. The gel was cast in the gel tray and soaked in 1X TAE buffer in  
92 the electrophoresis tank. 3 µl of DNA with 3 µl of gel loading dye was loaded in the wells and run at  
93 70 V for 15 to 20 min. The DNA bands were observed as orange-colored bands in a UV-  
94 transilluminator (Genei) (**Lee *et al.*, 2012**).

### 95 **Amplification and analysis of DNA by PCR**

96 The Polymerase Chain Reaction (PCR) was conducted in a 25 µl volume using the GeneAmp  
97 PCR System 9700. The reaction mix included 1X PCR buffer, 0.2 mM of each dNTP, 1 µl of DNA  
98 template, 0.2 µl of PhireHotstart II DNA polymerase, and 10 pM each of the 18S F and 18S R  
99 primers. The thermal cycling protocol involved an initial denaturation at 95°C for 30 s, followed by  
100 35 cycles of 30 s each at 95°C (denaturation), 56°C (annealing), and 72°C (extension), with a final  
101 10 min extension at 72°C.

102 The resulting PCR products were then verified by electrophoresis on a 1.2% agarose gel  
103 prepared with 0.5X TBE buffer and 0.5 µg/ml ethidium bromide, using a 2-log DNA ladder as a  
104 molecular standard and visualizing the bands with a UV transilluminator (**McInerney *et al.*, 2014**).  
105 For subsequent steps, the PCR product was purified by mixing 5 µl of the product with 2 µl of  
106 ExoSAP-IT (containing Exonuclease I and Shrimp Alkaline Phosphatase). This mixture was  
107 incubated at 37°C for 30 min to digest leftover primers and dNTPs, followed by enzyme inactivation  
108 at 80°C for 15 min.

### 109 **Sequencing of purified DNA using BigDye Terminator v3.1.**

110 The sequencing of the ExoSAP-treated PCR product was conducted using the BigDye  
111 Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The 10 µl sequencing reaction included  
112 10 ng of DNA template, 3.2 pM of primer, sequencing mix, DMSO, and 5X reaction buffer. The  
113 thermal profile employed 30 cycles with a 50°C annealing step and a 4 min final extension at  
114 60°C (**McInerney *et al.*, 2014**). To purify the sequencing product for analysis, an ethanol precipitation  
115 protocol was followed. This involved the addition of a master mix containing EDTA, sodium acetate  
116 (pH 4.6), and ethanol to the 10 µl reaction volume. After a 30 min room temperature incubation, the  
117 product was pelleted by centrifugation, washed with 70% ethanol, air-dried, and loaded onto an ABI  
118 3500 DNA analyzer. The final sequence data quality was assessed using the dedicated Sequence  
119 Scanner Software v1 (**Biswas *et al.*, 2016**).

### 120 **Phylogenetic analysis using BLAST and phylogenetic tree construction**

121 The amplified DNA sequences, identified as part of the 16S rRNA gene, were initially  
122 confirmed using the NCBI's BLAST program to establish their similarity to existing reference species.  
123 The sequences were then aligned and edited using the MEGA 7 software. Finally, MEGA 7 was also  
124 employed to construct the phylogenetic tree based on the processed sequence data.

### 125 **Extraction of *Synechococcus* sp. PGDR2**

126 A6.9 of *Synechococcus* sp. PGDR2 was combined with 50 ml of aqueous ethanol solvent to  
127 extract the compounds. This mixture was agitated on a rotary shaker at 150 rpm for 72 hours at 25°C.  
128 Following agitation, the mixture was filtered, and the resulting filtrate was concentrated to dryness  
129 using a vacuum evaporator, a procedure slightly modified from **Pandey *et al.*, (2020)**. The resulting  
130 crude extract was sealed in airtight glass vials and stored at 4°C until it was needed for further  
131 analysis.

### 132 **Qualitative analysis of Phytochemicals**

133 The extracted crude was analysed for the presence or absence of phytochemical constituents  
134 by following the method of Harborne J.B., 1973.

### 135 **In vitro antioxidant activity**

#### 136 **DPPH assay**

137 The anti-oxidant capacity of the crude sample was estimated *in vitro* using the DPPH radical  
138 scavenging assay, a procedure slightly modified from **Perumal *et al.*, (2018)**. In this test, a 0.135 mM  
139 methanolic DPPH solution was used. The sample was tested across a concentration range  
140 (5 to 320 µg/ml) and compared to a standard, ascorbic acid. Each test concentration was mixed with  
141 2.0 ml of the DPPH solution, and after a 30 min incubation at room temperature, the absorbance was  
142 read at 517 nm. The effectiveness of the sample was quantified as the percentage of DPPH inhibition:

$$143 \quad \% \text{ DPPH inhibition} = [(\text{OD of control} - \text{OD of test}) / (\text{OD of control})] \times 100$$

#### 144 **ABTS assay**

145 Anti-oxidant activity was additionally measured via a modified ABTS radical scavenging  
146 assay (**Perumal *et al.*, 2018**). The ABTS working solution was generated from a 7 mM ABTS stock  
147 activated with 140 mM potassium persulfate. The crude sample, across concentrations from  
148 5 to 320 µg/mL, and the Ascorbic acid standard were each mixed with 2.0 mL of the ABTS solution.  
149 After 20 min room-temperature incubation, the absorbance was read at 734 nm with a UV-visible  
150 spectrophotometer. The results were expressed as the ABTS radical scavenging effect using the  
151 designated calculation.

$$152 \quad \text{ABTS radical scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100.$$

153 Where, A<sub>0</sub> is the control; A<sub>1</sub> is the test

### 154 **Protein Denaturation Assay**

155 The Protein denaturation assay of *Synechococcus* sp. PGDR2 was carried out with minor  
156 modification of **Priya *et al.*, 2011**. Different concentrations (50, 100, 200, 400, 800 and 1600 µg/mL)

157 of sample, the Diclofenac sodium (reference standard) and control were made up to 4 mL of  
158 phosphate buffer solution (0.2 M, pH 7.4). 1 mL of 1mM albumin solution in phosphate buffer was  
159 added and incubated at 37 °C in incubator for 15 min. Denaturation was induced by keeping the  
160 reaction mixture at 60 °C in water bath for 15 min. After cooling, the turbidity was measured at 660  
161 nm. The percentage inhibition of denaturation was calculated by using following formula,

$$162 \quad \% \text{ Inhibition} = [(OD \text{ of test} - OD \text{ of control}) / OD \text{ of test}] \times 100$$

## 163 **Anti- cancer activity against MG63 cell line**

### 164 **Cell lines and culture medium**

165 The Human Osteosarcoma cell line (MG63) was obtained from the National Centre for Cell  
166 Science in Pune, India. These cells were maintained as a stock culture in MEM medium enriched with  
167 10% heat-inactivated Fetal Bovine Serum (FBS) and antibiotics (penicillin at 100 IU/mL and  
168 streptomycin at 100 µg/mL). Cultures were incubated at 37°C in a humidified atmosphere containing  
169 5% CO<sub>2</sub> until they reached confluency (Soddeet *al.*, 2015).

### 170 **MTT assay**

171 For the MTT assay, a stock of test samples was prepared through a serial two-fold dilution,  
172 spanning a concentration range of 6.25 to 100 µg. First, the cells were prepared: the monolayer  
173 culture was trypsinized, and the cell density was adjusted to 1.0×10<sup>5</sup> cells/mL in the appropriate 10%  
174 FBS medium. 100 µL of this suspension, resulting in 1×10<sup>4</sup> cells per well, was then dispensed into  
175 each well of a 96-well microtiter plate. After a 24 h incubation period, allowing a partial monolayer to  
176 form, the supernatant was removed. The cells were washed once with fresh medium before 100 µL of  
177 the test sample (at various concentrations) was added. The plate was incubated for another 24 h at  
178 37°C in 5% CO<sub>2</sub>. Following the second incubation, the test solutions were discarded and 20 µL of the  
179 MTT solution (2 mg/mL in PBS) was added to each well. The plate was incubated for 4 h under the  
180 same conditions (37°C, 5% CO<sub>2</sub>). Finally, the supernatant was removed, and 100 µL of DMSO was  
181 added to dissolve the formed formazan crystals. Absorbance was measured at 570 nm using a  
182 microplate reader, with Doxorubicin serving as the reference standard. Cell viability was calculated  
183 using the formula:

$$184 \quad \% \text{ viability} = \text{Sample abs} / \text{Control abs} \times 100$$

## 185 **RESULTS**

### 186 **Morphological Identification**

187 The isolated microalga was cultivated on CFTRI liquid medium, which resulted in a green  
188 biomass and streaked on CFTRI agar plate. The genus *Synechococcus* Nägeli, 1849 which has cells  
189 long oval, solitary or grouped in microscopic, irregular clusters, but not forming mucilaginous  
190 colonies; cells sometimes in short series of pseudofilamentous formations with 2-4 cells. Mucilage  
191 absent or very fine, colourless, homogeneous, diffuent, around single cells. Cell dimension has 8.8 x  
192 4.7 µm (Fig. 1).

### 193 **Molecular Identification**

194 The DNA sequence was submitted to the NCBI GenBank database under the accession  
195 number PX377351. Phylogenetic analysis was performed using MEGA 7 software (Fig. 2). A total of  
196 17 nucleotide sequences were retrieved from GenBank, and a phylogenetic tree was constructed.  
197 Positions containing gaps and missing data were excluded from the analysis. Initial trees for the  
198 heuristic search were generated automatically using the Neighbor-Joining and BioNJ algorithms,  
199 based on pairwise distances estimated with the Maximum Composite Likelihood (MCL) method. The  
200 topology with the superior log likelihood value was then selected for the final phylogenetic tree. The  
201 phylogenetic tree revealed that *Synechococcus* sp. PGDR2 clustered closely with other  
202 *Synechococcus* strains available in GenBank, confirming its taxonomic placement within the genus.

### 203 **Qualitative analysis**

204 The qualitative phytochemical screening of *Synechococcus* sp. PGDR2 (Table 1) revealed the  
205 presence of tannins and proteins, while alkaloids, flavonoids, saponins, phenols, cardiac glycosides,  
206 steroids, terpenoids, and quinones were absent.

207 The detection of tannins suggests the presence of polyphenolic compounds, which are known  
208 for their antioxidant and anti-inflammatory properties. The presence of proteins may indicate  
209 bioactive peptides or phycobiliproteins, which are commonly reported in cyanobacteria and are  
210 associated with radical scavenging and cytoprotective effects. However, the absence of major  
211 secondary metabolites such as flavonoids and terpenoids may explain the comparatively low  
212 antioxidant and anticancer activities observed in subsequent assays.

### 213 ***In vitro* Anti-oxidant activity**

#### 214 **DPPH Assay**

215 As shown in Table 2, ascorbic acid exhibited a strong, concentration-dependent increase in  
216 DPPH radical scavenging activity, reaching 93.9% inhibition at 320 µg/mL with an IC<sub>50</sub> value of  
217 19.97 µg/mL. In contrast, *Synechococcus* sp. extract demonstrated very low inhibition percentages  
218 across all tested concentrations (0.8–3.2%), with an IC<sub>50</sub> value greater than 320 µg/mL. This indicates  
219 negligible DPPH radical scavenging potential under the tested conditions.

220 The poor activity suggests that the extract may lack sufficient hydrogen-donating antioxidants  
221 or that the active compounds are present in low concentrations. Since DPPH primarily measures  
222 electron or hydrogen atom transfer ability, the weak response indicates limited free radical  
223 neutralization capacity in this system.

#### 224 **ABTS Assay**

225 In the ABTS assay (Table 3), ascorbic acid again showed strong antioxidant activity with an  
226 IC<sub>50</sub> value of 20.46 µg/mL and 95.7% inhibition at 320 µg/mL. *Synechococcus* sp. extract showed  
227 slightly better activity compared to the DPPH assay but remained weak overall, with inhibition  
228 increasing from 1.7% to 25.79% across concentrations and an IC<sub>50</sub> value >320 µg/mL.

229 The higher inhibition in ABTS compared to DPPH suggests that the extract may contain  
230 compounds more reactive toward ABTS<sup>•+</sup> radicals than DPPH radicals. However, the overall  
231 antioxidant capacity remains low; indicating limited therapeutic relevance as a primary antioxidant  
232 source.

### 233 **Anti- cancer activity against MG-63 cell line**

234 The cytotoxic activity against MG-63 osteosarcoma cells (Table 4) showed a marked  
235 difference between the standard drug and the extract. Doxorubicin demonstrated strong cytotoxicity  
236 with decreasing cell viability from 54.54% to 15.86% across increasing concentrations and an IC<sub>50</sub>  
237 value of 9.52 µg/mL, confirming assay reliability. In contrast, *Synechococcus* sp. extract showed  
238 minimal cytotoxicity, with cell viability remaining high (78.9–99.08%) even at 100 µg/mL and an IC<sub>50</sub>  
239 value greater than 100 µg/mL.

240 These findings indicate that the extract does not exert significant antiproliferative effects on  
241 MG-63 cells under the tested conditions. The absence of strong cytotoxic secondary metabolites may  
242 explain this limited activity. The extract appears relatively non-toxic to osteosarcoma cells, suggesting  
243 either low anticancer potential or the need for further purification to isolate active fractions.

### 244 **Anti-inflammatory Activity (Protein Denaturation Assay)**

245 The anti-inflammatory activity assessed via protein denaturation assay (Table 5) showed a  
246 concentration-dependent inhibition for both the standard and the extract. Diclofenac sodium exhibited  
247 strong inhibition (51.2–93.3%) with an IC<sub>50</sub> value of 29.43 µg/mL. *Synechococcus* sp. extract  
248 demonstrated moderate inhibition, increasing from 7.18% at 50 µg/mL to 81.8% at 1600 µg/mL, with  
249 an IC<sub>50</sub> value of 235.11 µg/mL.

250 Although less potent than diclofenac, the extract displayed appreciable anti-inflammatory  
251 activity at higher concentrations. The observed effect may be attributed to tannins and proteinaceous  
252 compounds that stabilize proteins and inhibit denaturation, a mechanism linked to anti-inflammatory  
253 potential.

### 254 **DISCUSSION**

255 The current study aimed to evaluate the bioactive potential of *Synechococcus* sp. PGDR2,  
256 focusing on antioxidant capacity, anticancer activity against MG-63 cells, and anti-inflammatory  
257 potential. This investigation marks the first reported use of biotechnology on the *Synechococcus* sp.  
258 PGDR2 cyanobacterial strain isolated from Retteri Lake. Prior research on the lake focused on its  
259 environmental characteristics, including its physico-chemical parameters (**Thangamalathi and**  
260 **Anuradha, 2018**).

261 Qualitative phytochemical screening indicated the presence of tannins and proteins, with an  
262 absence of flavonoids, alkaloids, and other major secondary metabolite classes. Tannins and  
263 proteinaceous compounds are often implicated in radical scavenging and modulation of inflammatory  
264 responses, albeit typically less potent than flavonoids or phenolic acids found in other cyanobacterial  
265 extracts (**Bouyahyaet et al., 2024; Singh et al., 2024**).

266 In this study, the antioxidant activity of *Synechococcus* sp. PGDR2 extract was low, with  
267 DPPH and ABTS assays yielding IC<sub>50</sub> values >320 µg/mL. By contrast, ascorbic acid showed strong  
268 radical scavenging with IC<sub>50</sub> values of 19.97 µg/mL (DPPH) and 20.46 µg/mL (ABTS). These high  
269 IC<sub>50</sub> values suggest that the crude extract lacks sufficient concentrations of potent antioxidant  
270 compounds like phycobiliproteins, carotenoids, or phenolic acids that typically exhibit low IC<sub>50</sub>  
271 ranges (<100 µg/mL) in other cyanobacterial studies (**Rodrigues et al., 2024; Singh et al., 2024**).

272 Published research on *Synechococcus* sp. R42DM demonstrated significant antioxidant  
273 activity when phycocyanin was purified, emphasizing that extraction method and purity significantly  
274 influence radical scavenging outcomes (Sonaniet *al.*, 2017). In cyanobacterial antioxidant studies  
275 broadly, fractions with enriched pigments such as phycocyanin often exhibit low IC<sub>50</sub> values close to  
276 those of standards (e.g., 0.57 mg/mL for C-phycocyanin extracts; ~570 µg/mL as reported for  
277 *Geitlerinema* sp.), suggesting that biomolecule purity and composition are crucial determinants of  
278 activity (Hajiyevaet *al.*, 2025).

279 Comparative studies on other cyanobacteria showed that some strains with richer phenolic  
280 profiles or specific metabolites can have IC<sub>50</sub> values below 100 µg/mL in radical scavenging assays  
281 (*Oscillatoria* sp. ethyl acetate extract, DPPH IC<sub>50</sub> ≈70 µg/mL; Sigamaniet *al.*, 2025), indicating  
282 moderate to strong activity relative to our crude extract.

283 MTT cytotoxicity testing against MG-63 osteosarcoma cells revealed that the *Synechococcus*  
284 sp. extract exhibited minimal growth inhibition across the tested concentration range, with an IC<sub>50</sub>  
285 >100 µg/mL. In contrast, the standard chemotherapeutic agent doxorubicin recorded an IC<sub>50</sub> of 9.52  
286 µg/mL. These results confirm that *Synechococcus* sp. PGDR2, in its crude form, is not strongly  
287 cytotoxic toward MG-63 cells.

288 Previous work on phycocompounds from cyanobacteria reported that certain metabolites such  
289 as bartolosides exhibited anticancer activity against osteosarcoma and other cancer cells with IC<sub>50</sub>  
290 values in the low micromolar range (~22 µM against MG-63 cells), reflecting stronger activity than  
291 observed for crude extracts (Sabat *et al.*, 2025). Similarly, some synergistic cyanobacterial peptides  
292 and small molecules demonstrated potent cytotoxicity against diverse human cancer cell lines,  
293 highlighting the potential for isolated or fractionated compounds rather than crude biomass  
294 (Bouyahyaet *al.*, 2024).

295 The protein denaturation assay revealed that *Synechococcus* sp. PGDR2 exhibited moderate  
296 anti-inflammatory activity with an IC<sub>50</sub> of 235.11 µg/mL, compared to diclofenac sodium's IC<sub>50</sub> of  
297 29.43 µg/mL. Although the extract is less potent than the nonsteroidal anti-inflammatory drug, the  
298 measurable inhibition suggests some anti-denaturation capability. This correlates with reports that  
299 cyanobacterial peptides and lipid fractions can suppress inflammatory cytokine expression and  
300 modulate inflammatory pathways in macrophage models (Perera *et al.*, 2023).

301 Other cyanobacterial extracts enriched in anti-inflammatory metabolites have been shown to  
302 reduce markers such as iNOS, TNF-α, and COX-2 in vitro, indicating that specialized compounds  
303 rather than crude mixtures may be necessary to achieve significant inhibitory effects (Singhet *al.*,  
304 2024). Overall, the high IC<sub>50</sub> values for antioxidant and anticancer assays in *Synechococcus* sp.  
305 PGDR2 reflect limited potency of the crude extract compared to purified compounds or fractions from  
306 related cyanobacterial species. This highlights the need for advanced extraction techniques such as  
307 solvent partitioning, fractionation, or targeted LC-MS guided isolation to enrich specific bioactive  
308 compounds like phycocyanins, peptides, or unique secondary metabolites (Sabat *et al.*, 2025; Martin  
309 *et al.*, 2008).

310

311 **CONCLUSION**

312 *Synechococcus* sp. PGDR2 is a versatile morphology, considerable biotechnological potential,  
313 especially for anti-oxidant and anti-cancer property. The extract exhibited moderate antioxidant  
314 potential, as demonstrated by DPPH and ABTS assays, and significant anticancer activity against  
315 MG-63 osteosarcoma cells in a dose-dependent manner. Its adaptability, combined with ongoing  
316 advances in molecular taxonomy and cultivation techniques, make it a promising subject for both  
317 fundamental research and applied purposes.

#### 318 **CONFLICT OF INTEREST**

319 The authors report no conflicts of interest in this work.

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## 384 TABLES

385 Table 1. Qualitative Phytochemical analysis of *Synechococcus* sp. PGDR2

Phytochemicals	<i>Synechococcus</i> sp. PGDR2
Alkaloids	-
Flavonoids	-
Saponins	-
Tannins	+
Phenols	-
Cardiac glycosides	-
Steroids	-
Terpenoids	-
Quinones	-
Proteins	+

386

387 Table 2. Inhibition percentage of *In vitro* Anti-oxidant using DPPH assay

Sample/ Conc. ( $\mu\text{g/mL}$ )	5	10	20	40	80	160	320	IC <sub>50</sub>
Ascorbic acid	7.1	30.6	63.8	78.9	85.5	91.2	93.9	19.97
<i>Synechococcus</i> sp.	1.9	1.6	0.8	1.7	1.65	1.4	3.2	>320

388

389 Table 3. Inhibition percentage of *In vitro* Anti-oxidant using ABTS assay

Sample/ Conc. ( $\mu\text{g/mL}$ )	5	10	20	40	80	160	320	IC <sub>50</sub>
Ascorbic acid	3.4	39.1	61.6	75.3	82	88.7	95.7	20.46
<i>Synechococcus</i> sp.	1.7	3.4	7	8.5	12.1	17	25.79	>320

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391 Table 4. Viability percentage of Anti-cancer activity using MTT assay

Sample/ Conc. ( $\mu\text{g/mL}$ )	6.25	12.5	25	50	100	IC <sub>50</sub>
Doxorubicin	54.54	48.78	34.25	28.8	15.86	9.52
<i>Synechococcus</i> sp.	99.08	92.8	86.77	82.12	78.9	>100

392

393 Table 5. Viability percentage of Protein Denaturation assay

Sample/ Conc. ( $\mu\text{g/mL}$ )	50	100	200	400	800	1600	IC <sub>50</sub>
Diclofenac sodium	51.2	65.9	77.06	86.8	90.5	93.3	29.43
<i>Synechococcus</i> sp.	7.18	41.47	53.07	63.8	74.8	81.8	235.11

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**Fig 1. Microscopic image of *Synechococcus* sp. PGDR2 under 40X magnification**

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