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RESEARCH ARTICLE

EXPLORING THE PHARMACOLOGICAL POTENTIAL OF SYNECHOCOCCUS SP. PGDR2 THROUGH PHYTOCHEMICAL AND IN VITRO BIOACTIVITY STUDIES

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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.

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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 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 al., 2024). Among cyanobacteria, the genus *Synechococcus* has

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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 al., 2019).

The anti-inflammatory potential of cyanobacterial metabolites, including those from *Synechococcus* and other marine cyanobacteria, is equally compelling. Cyanobacteria-derived bioactive peptides have been reported to downregulate pro-inflammatory cytokines such as inducible nitric oxide synthase (iNOS), tumor necrosis factor- α (TNF- α), and interleukins, thereby mitigating inflammatory responses in macrophage models (Perera et al., 2023). Anticancer activity is another facet of cyanobacterial bioactivity, with several studies reporting cytotoxic effects against human cancer cell lines. Recent work with fractions from *Synechococcus* sp. has demonstrated notable cytotoxicity against MG-63 osteosarcoma cells, with the profiling for identifying key bioactive molecules such as pheophytin and phorbide derivatives that may underlie these effects (Cavalcante do Amaral et al., 2025). Such anticancer effects are supported by broader evidence that cyanobacterial metabolites can induce apoptosis, inhibit proliferation, and modulate cancer-related signalling pathways *in vitro* (Bouyahaet al., 2024).

Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC-MS) are essential for profiling the complex chemical mixtures produced by cyanobacteria, enabling the identification and quantitation of bioactive constituents. LC-MS analysis facilitates structure-activity relationships and guides the isolation of lead compounds with therapeutic relevance. Integrating bioactivity assays with LC-MS profiling strengthens the methodological framework for evaluating *Synechococcus*-derived compounds, providing deeper insight into their antioxidant, anti-inflammatory, and anticancer actions. Overall, the exploration of *Synechococcus* and related cyanobacterial metabolites represents a promising frontier in natural product research, bridging microbial ecology, analytical chemistry, and biomedical applications. Therefore, this research focused on isolating *Synechococcus* sp. PGDR2 from Retteri Lake and testing the anti-oxidant and anti-cancer properties of its ethanol extract against the MG-63 cell line in a laboratory setting.

Materials and Methods:-

Collection and Isolation:-

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

Morphological Identification:-

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

Molecular Identification using 16S rRNA:-

Extraction and Determination of quality of DNA:-

The Genomic DNA was extracted from the cyanobacterial strain PGDR2. It was extracted using a meticulously prepared extraction buffer for a 500 ml volume, which comprised 20 mM Na₂EDTA (3.7 g) and 100 mM Tris-HCl (6 g). The pH was adjusted to 8, with the subsequent addition of 1.4 M NaCl (40 g) and 2% (w/v) CTAB (10 g). The extraction buffer was preheated to 60 °C, and the sample was mixed well and incubated for 1 h at 60 °C with intermittent shaking at every 10 min. Mixture was cooled down to 37 °C. To 2 ml of this mixture, 1 ml of chloroform: isoamyl alcohol (24:1) was added and gently mixed by inverting the tubes to form an emulsion. It was then centrifuged at 5000xg for 15 min. The clear aqueous phase was transferred to a fresh tube and 150 μ l of 6 M NaCl was added and mixed. To this 1 ml of ice-cold ethanol was added and refrigerated for 1 h at – 20 °C. The pellet was washed several times with ethanol and finally resuspended in 100 μ l of elute buffer. The DNA samples were stored at 4 °C for further analysis (Jagielski et al., 2017). The quality of extracted DNA was checked on 0.8% agarose gels. Agarose powder was dissolved in 1X TAE buffer and boiled until it turned into a clear solution. Once it was cooled to 50 °C, ethidium bromide was added and mixed well. The gel was cast in the gel tray and soaked in

1X TAE buffer in the electrophoresis tank. 3 µl of DNA with 3 µl of gel loading dye was loaded in the wells and run at 70 V for 15 to 20 min. The DNA bands were observed as orange-colored bands in a UV-transilluminator (Genei) (Lee et al., 2012).

Amplification and analysis of DNA by PCR:-

The Polymerase Chain Reaction (PCR) was conducted in a 25 µl volume using the GeneAmp PCR System 9700. The reaction mix included 1X PCR buffer, 0.2 mM of each dNTP, 1 µl of DNA template, 0.2 µl of PhireHotstart II DNA polymerase, and 10 pM each of the 18S F and 18S R primers. The thermal cycling protocol involved an initial denaturation at 95°C for 30 s, followed by 35 cycles of 30 s each at 95°C (denaturation), 56°C (annealing), and 72°C (extension), with a final 10 min extension at 72°C. The resulting PCR products were then verified by electrophoresis on a 1.2% agarose gel prepared with 0.5X TBE buffer and 0.5 µg/ml ethidium bromide, using a 2-log DNA ladder as a molecular standard and visualizing the bands with a UV transilluminator (McInerney et al., 2014). For subsequent steps, the PCR product was purified by mixing 5 µl of the product with 2 µl of ExoSAP-IT (containing Exonuclease I and Shrimp Alkaline Phosphatase). This mixture was incubated at 37°C for 30 min to digest leftover primers and dNTPs, followed by enzyme inactivation at 80°C for 15 min.

Sequencing of purified DNA using BigDye Terminator v3.1:-

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

Phylogenetic analysis using BLAST and phylogenetic tree construction:-

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

Extraction of Synechococcus sp. PGDR2:-

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

Qualitative analysis of Phytochemicals:-

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

In vitro antioxidant activity:-

DPPH assay:-

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

The effectiveness of the sample was quantified as the percentage of DPPH inhibition:

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

ABTS assay:-

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

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

Where, A₀ is the control; A₁ is the test

Protein Denaturation Assay:-

The Protein denaturation assay of *Synechococcus* sp. PGDR2 was carried out with minor modification of Priya et al., 2011. Different concentrations (50, 100, 200, 400, 800 and 1600 µg/mL) of sample, the Diclofenac sodium (reference standard) and control were made up to 4 mL of phosphate buffer solution (0.2 M, pH 7.4). 1 mL of 1mM albumin solution in phosphate buffer was added and incubated at 37 °C in incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 °C in water bath for 15 min. After cooling, the turbidity was measured at 660 nm. The percentage inhibition of denaturation was calculated by using following formula,

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

Anti- cancer activity against MG63 cell line:-**Cell lines and culture medium:-**

The Human Osteosarcoma cell line (MG63) was obtained from the National Centre for Cell Science in Pune, India. These cells were maintained as a stock culture in MEM medium enriched with 10% heat-inactivated Fetal Bovine Serum (FBS) and antibiotics (penicillin at 100 IU/mL and streptomycin at 100 µg/mL). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ until they reached confluency (Soddeet al., 2015).

MTT assay:-

For the MTT assay, a stock of test samples was prepared through a serial two-fold dilution, spanning a concentration range of 6.25 to 100 µg. First, the cells were prepared: the monolayer culture was trypsinized, and the cell density was adjusted to 1.0×10⁵ cells/mL in the appropriate 10% FBS medium. 100 µL of this suspension, resulting in 1×10⁴ cells per well, was then dispensed into each well of a 96-well microtiter plate. After a 24 h incubation period, allowing a partial monolayer to form, the supernatant was removed. The cells were washed once with fresh medium before 100 µL of the test sample (at various concentrations) was added. The plate was incubated for another 24 h at 37°C in 5% CO₂. Following the second incubation, the test solutions were discarded and 20 µL of the MTT solution (2 mg/mL in PBS) was added to each well. The plate was incubated for 4 h under the same conditions (37°C, 5% CO₂). Finally, the supernatant was removed, and 100 µL of DMSO was added to dissolve the formed formazan crystals. Absorbance was measured at 570 nm using a microplate reader, with Doxorubicin serving as the reference standard. Cell viability was calculated using the formula:

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

Results:-**Morphological Identification:-**

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

Figures:-

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

Molecular Identification:-

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

Qualitative analysis:-

The qualitative phytochemical screening of *Synechococcus* sp. PGDR2 (Table 1) revealed the presence of tannins and proteins, while alkaloids, flavonoids, saponins, phenols, cardiac glycosides, steroids, terpenoids, and quinones were absent. The detection of tannins suggests the presence of polyphenolic compounds, which are known for their antioxidant and anti-inflammatory properties. The presence of proteins may indicate bioactive peptides or phycobiliproteins, which are commonly reported in cyanobacteria and are associated with radical scavenging and cytoprotective effects. However, the absence of major secondary metabolites such as flavonoids and terpenoids may explain the comparatively low antioxidant and anticancer activities observed in subsequent assays.

In vitro Anti-oxidant activity:-**DPPH Assay:-**

As shown in Table 2, ascorbic acid exhibited a strong, concentration-dependent increase in DPPH radical scavenging activity, reaching 93.9% inhibition at 320 μg/mL with an IC₅₀ value of 19.97 μg/mL. In contrast, *Synechococcus* sp. extract demonstrated very low inhibition percentages across all tested concentrations (0.8–3.2%), with an IC₅₀ value greater than 320 μg/mL. This indicates negligible DPPH radical scavenging potential under the tested conditions. The poor activity suggests that the extract may lack sufficient hydrogen-donating antioxidants or that the active compounds are present in low concentrations. Since DPPH primarily measures electron or hydrogen atom transfer ability, the weak response indicates limited free radical neutralization capacity in this system.

ABTS Assay:-

In the ABTS assay (Table 3), ascorbic acid again showed strong antioxidant activity with an IC₅₀ value of 20.46 μg/mL and 95.7% inhibition at 320 μg/mL. *Synechococcus* sp. extract showed slightly better activity compared to the DPPH assay but remained weak overall, with inhibition increasing from 1.7% to 25.79% across concentrations and an IC₅₀ value >320 μg/mL. The higher inhibition in ABTS compared to DPPH suggests that the extract may

contain compounds more reactive toward ABTS•⁺ radicals than DPPH radicals. However, the overall antioxidant capacity remains low; indicating limited therapeutic relevance as a primary antioxidant source.

Anti-cancer activity against MG-63 cell line:-

The cytotoxic activity against MG-63 osteosarcoma cells (Table 4) showed a marked difference between the standard drug and the extract. Doxorubicin demonstrated strong cytotoxicity with decreasing cell viability from 54.54% to 15.86% across increasing concentrations and an IC₅₀ value of 9.52 µg/mL, confirming assay reliability. In contrast, *Synechococcus* sp. extract showed minimal cytotoxicity, with cell viability remaining high (78.9–99.08%) even at 100 µg/mL and an IC₅₀ value greater than 100 µg/mL. These findings indicate that the extract does not exert significant antiproliferative effects on MG-63 cells under the tested conditions. The absence of strong cytotoxic secondary metabolites may explain this limited activity. The extract appears relatively non-toxic to osteosarcoma cells, suggesting either low anticancer potential or the need for further purification to isolate active fractions.

Anti-inflammatory Activity (Protein Denaturation Assay):-

The anti-inflammatory activity assessed via protein denaturation assay (Table 5) showed a concentration-dependent inhibition for both the standard and the extract. Diclofenac sodium exhibited strong inhibition (51.2–93.3%) with an IC₅₀ value of 29.43 µg/mL. *Synechococcus* sp. extract demonstrated moderate inhibition, increasing from 7.18% at 50 µg/mL to 81.8% at 1600 µg/mL, with an IC₅₀ value of 235.11 µg/mL. Although less potent than diclofenac, the extract displayed appreciable anti-inflammatory activity at higher concentrations. The observed effect may be attributed to tannins and proteinaceous compounds that stabilize proteins and inhibit denaturation, a mechanism linked to anti-inflammatory potential.

Tables:-

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	+

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

Sample/ (µg/mL)	Conc.	5	10	20	40	80	160	320	IC ₅₀
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

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

Sample/ (µg/mL)	Conc.	5	10	20	40	80	160	320	IC ₅₀
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

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

Sample/ (µg/mL)	Conc.	6.25	12.5	25	50	100	IC ₅₀
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

Table 5. Viability percentage of Protein Denaturation assay

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

Discussion:-

The current study aimed to evaluate the bioactive potential of *Synechococcus* sp. PGDR2, focusing on antioxidant capacity, anticancer activity against MG-63 cells, and anti-inflammatory potential. This investigation marks the first reported use of biotechnology on the *Synechococcus* sp. PGDR2 cyanobacterial strain isolated from Retteri Lake. Prior research on the lake focused on its environmental characteristics, including its physico-chemical parameters (Thangamalathi and Anuradha, 2018). Qualitative phytochemical screening indicated the presence of tannins and proteins, with an absence of flavonoids, alkaloids, and other major secondary metabolite classes. Tannins and proteinaceous compounds are often implicated in radical scavenging and modulation of inflammatory responses, albeit typically less potent than flavonoids or phenolic acids found in other cyanobacterial extracts (Bouyahyaet al., 2024; Singh et al., 2024). In this study, the antioxidant activity of *Synechococcus* sp. PGDR2 extract was low, with DPPH and ABTS assays yielding IC₅₀ values >320 $\mu\text{g/mL}$. By contrast, ascorbic acid showed strong radical scavenging with IC₅₀ values of 19.97 $\mu\text{g/mL}$ (DPPH) and 20.46 $\mu\text{g/mL}$ (ABTS). These high IC₅₀ values suggest that the crude extract lacks sufficient concentrations of potent antioxidant compounds like phycobiliproteins, carotenoids, or phenolic acids that typically exhibit low IC₅₀ ranges (<100 $\mu\text{g/mL}$) in other cyanobacterial studies (Rodrigues et al., 2024; Singh et al., 2024). Published research on *Synechococcus* sp. R42DM demonstrated significant antioxidant activity when phycocyanin was purified, emphasizing that extraction method and purity significantly influence radical scavenging outcomes (Sonaniet al., 2017).

In cyanobacterial antioxidant studies broadly, fractions with enriched pigments such as phycocyanin often exhibit low IC₅₀ values close to those of standards (e.g., 0.57 mg/mL for C-phycocyanin extracts; ~570 $\mu\text{g/mL}$ as reported for *Geitlerinema* sp.), suggesting that biomolecule purity and composition are crucial determinants of activity (Hajiyevaet al., 2025). Comparative studies on other cyanobacteria showed that some strains with richer phenolic profiles or specific metabolites can have IC₅₀ values below 100 $\mu\text{g/mL}$ in radical scavenging assays (*Oscillatoria* sp. ethyl acetate extract, DPPH IC₅₀ \approx 70 $\mu\text{g/mL}$; Sigamaniet al., 2025), indicating moderate to strong activity relative to our crude extract. MTT cytotoxicity testing against MG-63 osteosarcoma cells revealed that the *Synechococcus* sp. extract exhibited minimal growth inhibition across the tested concentration range, with an IC₅₀ >100 $\mu\text{g/mL}$. In contrast, the standard chemotherapeutic agent doxorubicin recorded an IC₅₀ of 9.52 $\mu\text{g/mL}$. These results confirm that *Synechococcus* sp. PGDR2, in its crude form, is not strongly cytotoxic toward MG-63 cells. Previous work on phycocompounds from cyanobacteria reported that certain metabolites such as bartolosides exhibited anticancer activity against osteosarcoma and other cancer cells with IC₅₀ values in the low micromolar range (~22 μM against MG-63 cells), reflecting stronger activity than observed for crude extracts (Sabat et al., 2025).

Similarly, some synergistic cyanobacterial peptides and small molecules demonstrated potent cytotoxicity against diverse human cancer cell lines, highlighting the potential for isolated or fractionated compounds rather than crude biomass (Bouyahyaet al., 2024). The protein denaturation assay revealed that *Synechococcus* sp. PGDR2 exhibited moderate anti-inflammatory activity with an IC₅₀ of 235.11 $\mu\text{g/mL}$, compared to diclofenac sodium's IC₅₀ of 29.43 $\mu\text{g/mL}$. Although the extract is less potent than the nonsteroidal anti-inflammatory drug, the measurable inhibition suggests some anti-denaturation capability. This correlates with reports that cyanobacterial peptides and lipid fractions can suppress inflammatory cytokine expression and modulate inflammatory pathways in macrophage models (Perera et al., 2023). Other cyanobacterial extracts enriched in anti-inflammatory metabolites have been shown to reduce markers such as iNOS, TNF- α , and COX-2 in vitro, indicating that specialized compounds rather than crude mixtures may be necessary to achieve significant inhibitory effects (Singhet al., 2024). Overall, the high IC₅₀ values for antioxidant and anticancer assays in *Synechococcus* sp. PGDR2 reflect limited potency of the crude extract compared to purified compounds or fractions from related cyanobacterial species. This highlights the need for advanced extraction techniques such as solvent partitioning, fractionation, or targeted LC-MS guided isolation to enrich specific bioactive compounds like phycocyanins, peptides, or unique secondary metabolites (Sabat et al., 2025; Martin et al., 2008).

Conclusion:-

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

Conflict of Interest:-

The authors report no conflicts of interest in this work.

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