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REVIEWER'S REPORT

Manuscript No.: IJAR-56632

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

Recommendation:

Accept as it is .....

Accept after minor revision...YES.....

Accept after major revision .....

Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality		√		
Clarity		√		
Significance			√	

Reviewer's ID: JPR-094

Detailed Reviewer's Report

1. Strengths of the Manuscript

1. **Relevant and Emerging Research Area**

The study focuses on cyanobacterial bioactive compounds, which are gaining increasing attention in pharmaceutical and biotechnological research due to their diverse pharmacological activities.

2. **Isolation of a Natural Microbial Resource**

The isolation of *Synechococcus* sp. PGDR2 from Retteri Lake contributes to microbial biodiversity exploration and highlights freshwater cyanobacteria as potential sources of bioactive metabolites.

3. **Integration of Molecular Identification**

The use of **16S rRNA gene sequencing and phylogenetic analysis** strengthens the taxonomic identification of the cyanobacterial strain.

4. **Multiple Bioactivity Assays**

The study evaluates several biological activities including:

* Antioxidant activity (DPPH and ABTS assays)

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* Anti-inflammatory activity (protein denaturation assay)

* Anticancer activity (MTT assay on MG-63 cell line)

This multi-assay approach provides a broader evaluation of pharmacological potential.

5. **Use of Standard Controls**

The inclusion of appropriate standards (ascorbic acid, diclofenac sodium, and doxorubicin) improves the reliability of comparative bioactivity evaluation.

6. **Clear Presentation of Experimental Methods**

The Materials and Methods section describes the extraction process, molecular identification, and assay procedures in sufficient detail for reproducibility.

2. Weaknesses of the Manuscript

1. **Limited Bioactivity Observed**

The extract exhibited **very weak antioxidant and anticancer activities**, with high IC_{50} values (>320 µg/mL and >100 µg/mL respectively), which limits the pharmacological significance of the findings.

2. **Only Qualitative Phytochemical Analysis**

The study reports only qualitative screening.

Quantitative estimation of phenolics, flavonoids, or proteins is missing, which could better explain the observed biological activities.

3. **Lack of Chemical Characterization**

No analytical techniques such as:

* LC-MS

* GC-MS

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* HPLC

were used to identify specific bioactive compounds.

4. **Limited Mechanistic Insight**

The anticancer activity was evaluated only by the **MTT assay**, without investigating mechanisms such as:

* apoptosis induction

* ROS generation

* cell cycle arrest.

5. **Inconsistency Between Results and Conclusion**

The conclusion claims **moderate antioxidant and significant anticancer activity**, whereas the results show **weak antioxidant activity and minimal cytotoxicity**. This inconsistency needs correction.

6. **Statistical Analysis Not Clearly Presented**

The manuscript lacks details regarding:

* number of experimental replicates

* standard deviations

* statistical significance tests.

7. **Minor Language and Formatting Issues**

Several grammatical errors and formatting inconsistencies are present, including:

* spacing issues in references

* typographical errors (e.g., *Synechocoocus*)

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* inconsistent use of units.

3. Significance of the Study

The study contributes to **natural product research and cyanobacterial biotechnology** by reporting preliminary pharmacological screening of a freshwater cyanobacterial strain (*Synechococcus* sp. PGDR2). Although the observed bioactivities are limited, the work highlights the importance of **further purification and chemical characterization** to identify potential bioactive compounds.

The study may serve as a **baseline investigation** for future work on cyanobacterial metabolites from Indian freshwater ecosystems.

4. Key Points

* Isolation and identification of *Synechococcus* sp. PGDR2 from Retteri Lake.

* Presence of tannins and proteins detected in phytochemical screening.

* Weak antioxidant activity observed in DPPH and ABTS assays.

* Minimal cytotoxic effect against MG-63 osteosarcoma cells.

* Moderate anti-inflammatory activity in the protein denaturation assay.

* Suggestion for future studies involving purification and LC-MS profiling.

5. Recommendation

Decision: Minor Revision

Justification

The manuscript addresses a relevant topic and presents preliminary data on the bioactivity of a cyanobacterial strain. However, several issues need correction before publication, including:

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* Revision of the conclusion to match the results

* Improvement in language and formatting

* Clarification of statistical analysis

* Minor methodological explanations.

The manuscript would benefit from these improvements but does not require major experimental redesign.

Minor Revision Justification (Line-by-Line with Issues)

Title

Line 1–2

Issue: The title is slightly long and could be simplified for clarity.

Justification: Minor editorial improvement suggested; scientific meaning remains unchanged.

ABSTRACT

Line 7

Issue: "Qualitative screening revealed the presence of tannins and proteins" – method for phytochemical screening is not mentioned in the abstract.

Justification: A brief mention of the method would improve clarity.

Line 9

Issue: "IC_{50} values >320 µg/mL" formatting should be consistent throughout the manuscript.

Justification: Minor formatting correction.

Line 13–14

Issue: Anti-inflammatory assay method is mentioned but the mechanism is not briefly explained.

Justification: Minor clarity improvement.

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Line 15–16

Issue: Statement "limited bioactivity" is correct but could be expressed more precisely.

Justification: Minor language revision.

Line 20–21 (Keywords)

Issue: Missing space in "Keywords:Synechococcus".

Justification: Typographical correction.

INTRODUCTION

Line 28

Issue: Citation style inconsistent ("Bouyahyaet al." instead of "Bouyahya et al.").

Justification: Formatting correction.

Line 35

Issue: "defence" spelling should follow journal style (defense/defence).

Justification: Minor language consistency.

Line 45–47

Issue: Sentence structure is long and may affect readability.

Justification: Minor editorial improvement recommended.

Line 51–56

Issue: LC-MS is discussed although LC-MS analysis was not conducted in this study.

Justification: Clarify that LC-MS is suggested for future studies.

MATERIALS AND METHODS

Collection and Isolation

Line 69–71

Issue: Grammar issue in "until gets the pure cultures."

Justification: Should be corrected to "until pure cultures were obtained."

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Morphological Identification

Line 73

Issue: "under the 40X of light microscope" wording is awkward.

Justification: Minor grammatical correction.

Molecular Identification

Line 98

Issue: Primers described as "18S F and 18S R" while the section title states 16S rRNA gene.

Justification: Clarification needed (likely typographical error).

Line 100

Issue: Degree symbol formatting inconsistent (◦C).

Justification: Minor formatting correction.

Extraction

Line 126

Issue: Typographical error "A6.9 gof Synechococcus sp."

Justification: Should be "6.9 g of Synechococcus sp."

Phytochemical Analysis

Line 133

Issue: Typo in "constituents".

Justification: Minor spelling correction.

Antioxidant Assays

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Line 137

Issue: "anti-oxidant" and "antioxidant" used inconsistently.

Justification: Standardize terminology.

Line 143

Issue: Equation formatting inconsistent.

Justification: Minor formatting revision.

Protein Denaturation Assay

Line 162

Issue: Formula may contain an error. Standard formula typically uses (OD control – OD test)/OD control ×100.

Justification: Clarification needed but does not affect experimental results.

MTT Assay

Line 172–174

Issue: Cell density formatting inconsistent.

Justification: Minor formatting correction.

RESULTS

Morphological Identification

Line 188–190

Issue: Taxonomic description slightly lengthy for results section.

Justification: Minor structural improvement.

Molecular Identification

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Line 201–202

Issue: Typographical error "Synechocoocus".

Justification: Should be "Synechococcus".

Qualitative Analysis

Line 204–206

Issue: Reference to Table 1 should be standardized.

Justification: Minor formatting correction.

Antioxidant Activity

Line 218

Issue: "very low inhibition percentages" wording could be more precise.

Justification: Minor language refinement.

Cytotoxic Activity

Line 238

Issue: Sentence structure slightly repetitive.

Justification: Minor editorial revision recommended.

Anti-inflammatory Activity

Line 250–252

Issue: Mechanistic explanation could be slightly expanded.

Justification: Minor clarity improvement.

DISCUSSION

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Line 257

Issue: Claim "first reported use of biotechnology on Synechococcus sp. PGDR2" should be supported with citation if possible.

Justification: Minor clarification.

Line 304–308

Issue: Discussion is appropriate but could be slightly condensed to improve readability.

Justification: Minor structural suggestion.

CONCLUSION

Line 311–316

Major inconsistency (Minor revision category)

Issue: The conclusion states:

> "moderate antioxidant potential"
>
> "significant anticancer activity"

However, **results indicate weak antioxidant and minimal anticancer activity**.

Justification: Conclusion should be revised to match results.

REFERENCES

Line 331–383

Issue: Several references contain spacing errors (e.g., "Bouyahyaet al.").

Justification: Minor formatting corrections.

TABLES

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Table 1–5

Issues:

Table titles formatting inconsistent.

Units not consistently formatted (µg/mL).

Some headings unclear (e.g., "Viability percentage of Protein Denaturation assay").

Justification: Minor formatting improvements required.

FIGURE

Line 400

Issue: Figure caption should include scale bar and brief description.
Justification: Minor improvement.

Final Recommendation

Decision: Minor Revision

Reason

The manuscript is scientifically sound and methodologically adequate, but requires:

grammatical corrections

formatting adjustments

clarification of primer type

correction of conclusion consistency.

No major experimental redesign or additional data is required.

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