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REVIEWER'S REPORT

Manuscript No.: IJAR-56315

Title: In Vitro Ovicidal Activity of *Tabernaemontana pandacaqui* (Pandakaki) Leaf Ethanolic Extract Against *Ascaris lumbricoides* in Varying Concentrations

Recommendation:
Accept after major revision .....

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

Detailed Reviewer's Report

The manuscript investigates the *in vitro* ovicidal activity of **Tabernaemontana pandacaqui** leaf ethanolic extract against *Ascaris lumbricoides* ova. The topic is relevant and locally important, and the concentration- and time-dependent trends are internally consistent.

However, after cross-checking the manuscript, **substantial methodological, analytical, and reporting limitations remain**, which prevent acceptance in the journal in its current form.

Major Comments

1. Experimental design and biological endpoint

- The primary endpoint is subjective morphological grading (0–3) of ova.
- There is no functional viability assessment (e.g., embryonation failure, larval development arrest, hatching inhibition).
- Morphological damage alone does not demonstrate ovicidal lethality or loss of infectivity, which is a major limitation for parasitology journals.
- This concern is not addressed in the other reviewer's report and remains a core scientific weakness.

This is a decisive limitation and must be acknowledged or addressed experimentally.

2. Sample size, replication, and unit-of-analysis

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- The methods state: "Six eggs will be included for each trial, whereas one egg will be used for one concentration or control", which is internally inconsistent.
- Results report N = 27 or N = 36, but it is unclear:
    - whether these represent independent biological replicates, repeated observations of the same egg or
    - pooled observations across trials.
- Eggs appear to be treated as independent observations, raising a strong concern of pseudoreplication.

This is a major issue. It must be clarified and statistically corrected.

3. Statistical analysis

- Morphological scores are ordinal, yet analyzed using parametric tests (ANOVA, independent t-tests) without justification.
- No assessment of normality or variance homogeneity is reported.
- Reporting $p = 0.000$ is incorrect and must be replaced with $p < 0.001$.
- The interpretation that "not significantly different from mebendazole" implies equivalence is statistically incorrect.

4. Positive control (mebendazole) preparation

- The manuscript states use of mebendazole (500 mg) as a positive control.
- It is not explained how this solid-dose drug was solubilized, what solvent was used, or whether it is appropriate for in vitro ovicidal testing.
- Dose comparability between extract (% v/v) and mebendazole (mg) is not scientifically justified.
- This concern stands fully after cross-checking.

5. Overinterpretation of results

Claims such as:

- "credible natural alternative,"
- "comparable to mebendazole,"
- "recommended for helminth control"
- are not supported by the presented data.

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- The p-value of 0.060 does not demonstrate equivalence.
- No equivalence or non-inferiority testing was conducted.

Conclusions must be substantially toned down.

Minor but Important Issues

Language quality is below journal standard, with frequent grammatical and typographical errors.
Methods are inconsistently written in future tense, despite being a completed study.
Ethics and waste disposal sections are excessively detailed for a results paper.
Excessive low-resolution microscopy images reduce clarity; representative composite figures are needed.
References show formatting inconsistencies and partial mismatches with in-text citations.

Final Recommendation: Major Revision Required

The manuscript has potential, but to meet the journal standards the authors must:

- Clarify and correct replication, sample size, and unit-of-analysis.
- Revise statistical methods appropriate for ordinal data.
- Remove or substantially temper claims of equivalence to mebendazole.
- Improve methodological transparency, especially positive control preparation.
- Rewrite the Discussion to critically interpret findings in light of limitations.
- Perform professional language editing.
- Condense figures and non-essential sections.

Without these revisions, the study does not yet reach the methodological rigor and interpretive restraint expected of the journal.