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REVIEWER'S REPORT

Manuscript No.: IJAR-56194
Title: Ã‰tude Phytochimique et ActivitÃ© HÃ©molytique de la Pulpe de Balanites aegyptiaca (L.) Del

Recommendation:
Accept as it is .....
Accept after minor revision.....
Accept after major revision .....YES.....
Do not accept (*Reasons below*) .....

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

Reviewer Name: Prof. Dr. Dillip Kumar Mohapatra

Detailed Reviewer's Report

"Étude Phytochimique et Activité Hémolytique de la Pulpe de Balanites aegyptiaca (L.) Del."

Overall Assessment

This manuscript investigates the preliminary phytochemical profile and in vitro hemolytic activity of pulp extracts of *Balanites aegyptiaca* collected from Ségou, Mali. The topic is relevant to ethnopharmacology and medicinal plant research, particularly in the African context. However, the study remains largely descriptive and preliminary, with several methodological and structural weaknesses that limit its scientific impact.

STRENGTHS

1. Ethnopharmacological Relevance

The plant is widely used in traditional African medicine.

Focus on pulp (nutritional part) is interesting since most studies target seeds or bark.

Provides regional data (Ségou, Mali), which adds geographical specificity.

2. Combination of Phytochemical and Biological Evaluation

Integrates:

Qualitative phytochemical screening

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TLC profiling

Hemolytic toxicity testing

Attempts to correlate saponins presence with hemolytic effect.

3. Dual Hemolysis Method

Use of:

Tube macroscopic method

Spectrophotometric quantification

Inclusion of positive and negative controls (PBS and distilled water).

4. Ethical Transparency

Clear conflict of interest and funding statements.
Data availability declared.

WEAKNESSES

A. Major Methodological Weaknesses

1. Lack of Quantitative Phytochemical Analysis

Only qualitative screening (+ / –).

No quantification of:

Total phenolics

Total flavonoids

Total saponins

No HPLC, LC-MS, GC-MS confirmation.

TLC Rf values provided but without reference standards.

This significantly limits scientific robustness.

2. Poor Statistical Treatment

Hemolysis test repeated only twice.

Student t-test mentioned but:

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No p-values reported
No confidence intervals
No statistical table

Sample size insufficient for meaningful inference.

3. Inconsistency in Hemolysis Results

First method: hemolysis at 7 mg/mL (aqueous)

Second method: 46% hemolysis at 2 mg/mL (hydroalcoholic

This suggests:

Either experimental inconsistency

Or major difference in extract composition

Or lack of methodological standardization

The discussion does not adequately justify this discrepancy.

4. Hemolysis Model Limitations

Only bovine erythrocytes used.

No:

Human RBC comparison

Cytotoxicity on mammalian cell lines

LD50 estimation

Dose–response curve modeling

Conclusion "low toxic potential" is therefore premature.

B. Scientific Writing Weaknesses

1. Numerous Formatting Problems

Line numbers embedded

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Typographical errors

Repeated text

Inconsistent units

Rf written as fractions then decimals

Table formatting inconsistent

2. Reference Issues

Several references appear questionable or outdated:

1940 citation (Creac'h)

Some journal details may not correspond to real indexed publications

Possible incorrect citation formatting

Needs verification against databases like:

PubMed

Scopus

3. Over interpretation

Statement:

"Elle peut être une source très importante dans les domaines thérapeutiques…"

This is scientifically exaggerated because:

Only preliminary screening performed.

No isolation of active compounds.

No mechanistic study.

KEY POINTS FOR REVISION

Major Revisions Required:

Add quantitative phytochemical assays:

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Total phenolic content (Folin–Ciocalteu)

Total flavonoid content

Total saponin estimation

Improve statistical analysis:

Increase replicates (minimum n=3–5)

Provide p-values

Add proper graphs with error bars

Clarify discrepancy between two hemolysis methods.

Standardize Rf reporting.

Improve English and French language editing.

Verify and correct references.

Temper conclusions — avoid therapeutic claims without pharmacological validation.

SIGNIFICANCE OF THE STUDY

Scientific Contribution:

The study provides:

Preliminary phytochemical data

Initial toxicity screening

However:

Does not advance beyond known literature

Confirms previously reported presence of saponins and tannins in *Balanites aegyptiaca*

Lacks novel compound identification

Regional Value:

Valuable for:

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Local medicinal plant documentation in Mali

Academic training research

FINAL RECOMMENDATION

Major Revision Required

The manuscript is **not acceptable in its current form**, but could become publishable after substantial revision.

MAJOR REVISION JUSTIFICATION

TITLE

Original:
Étude Phytochimique et Activité Hémolytique de la Pulpe de Balanites aegyptiaca (L.) Del.

Issues:

Title is too broad.

Does not specify "preliminary" nature.

Does not mention extraction solvents.

No indication of in vitro model.

Required Revision:

Revise to:

"Preliminary Phytochemical Screening and In Vitro Hemolytic Activity of Aqueous and Hydroalcoholic Pulp Extracts of *Balanites aegyptiaca* (L.) Del."

ABSTRACT

Line-by-line Concerns:

1. Background Statement

Problem:

Overgeneralized ethnomedicinal claims.

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No references cited in abstract.

Revision:

Add quantitative context.

Avoid therapeutic overstatement.

2. Methods Section

Problem:

No extraction yield reported.

No concentration range justification.

Number of replicates not mentioned.

Revision:

Add extraction yield (% w/w).

Specify n value.

Mention statistical software.

3. Results Section

Problem:

Hemolysis percentages unclear.

No standard deviation.

No p-values.

Discrepancy between two hemolysis methods not clarified.

Revision:

Include mean ± SD.

Clarify statistical significance.

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Explain difference between macroscopic and spectrophotometric results.

4. Conclusion

Problem:

States "low toxicity" without dose–response modeling.

Suggests therapeutic potential prematurely.

Revision:
Replace with:

"The results indicate moderate hemolytic activity possibly associated with saponins. Further quantitative phytochemical and cytotoxic studies are required."

INTRODUCTION

Paragraph 1 – General Medicinal Plant Context

Problem:

Too generic.

No specific global statistics.

Weak scientific framing.

Revision:

Add global data on medicinal plant usage.

Cite indexed databases such as World Health Organization.

Paragraph 2 – Description of *Balanites aegyptiaca*

Problem:

Lacks updated references.

Does not clearly state research gap.

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Previous hemolytic studies not critically reviewed.

Revision:

Clearly state what is unknown:

Has pulp hemolytic activity been quantified before?

Is there regional chemical variation?

Add a gap statement:

"To date, limited data exist on the hemolytic safety profile of pulp extracts collected in Mali."

Paragraph 3 – Study Objective

Problem:

Objective too descriptive.

Revision:
State clear hypothesis:

"This study aimed to evaluate the qualitative phytochemical composition and assess in vitro hemolytic potential of pulp extracts."

MATERIALS AND METHODS

1. Plant Material

Problem:

No herbarium voucher number mentioned.

No authentication authority cited.

Major Revision Required:

Provide voucher specimen number.

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State botanical authentication institution.

2. Extraction Procedure

Problems:

No solvent ratio justification.

No extraction yield reported.

Temperature control unclear.

No reproducibility detail.

Major Revision Required:

Add yield percentage.

Specify extraction duration precisely.

Provide solvent-to-solid ratio.

Mention filtration method.

3. Phytochemical Screening

Problem:

Only qualitative (+/–).

No quantification.

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No reference standards.

Major Revision Required:
Add:

Total phenolic content (Folin–Ciocalteu)

Total flavonoid content

Total saponin estimation

Replicates and calibration curves

Without this, scientific value remains limited.

4. Thin Layer Chromatography (TLC)

Problems:

Rf values inconsistently written.

No standard compounds used.

No visualization wavelength specified.

No densitometry.

Major Revision:

Standardize Rf format (two decimals).

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Include reference standards.

Specify UV wavelength.

Provide TLC plate image if possible.

5. Hemolysis Assay

Major Scientific Concerns:

Only bovine erythrocytes used.

Blood source ethical approval unclear.

Only two repetitions performed.

No dose-response curve plotted.

No EC50 calculated.

No positive control drug (e.g., saponin standard).

Major Revision Required:

Increase replicates (minimum n=3–5).

Provide full dose-response graph.

Calculate % hemolysis relative to distilled water.

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Perform statistical comparison (ANOVA preferable).

Include EC50 estimation.

6. Statistical Analysis

Problem:

Student t-test mentioned but:

No software specified.

No p-values shown.

No confidence intervals.

-

Major Revision:

Specify software (SPSS, GraphPad, etc.).

Report exact p-values.

Include error bars in graphs.

RESULTS

Phytochemical Screening Table

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Problem:

Only qualitative.

No intensity grading.

No replicates reported.

Revision:

Present quantitative results.

Add mean ± SD.

TLC Results

Problem:

Rf values not compared to literature.

No chromatogram figure included.

Revision:

Compare with published Rf ranges.

Include chromatographic image.

Hemolysis Results

Critical Issue:

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Inconsistency between two methods:

7 mg/mL threshold (macroscopic)

46% hemolysis at 2 mg/mL (spectrophotometric)

Major Revision:

Re-run experiment.

Standardize concentration range.

Explain extraction difference.

DISCUSSION

1. Overinterpretation

Problem:

Claims therapeutic value prematurely.

Calls extract "safe" without comprehensive toxicity study.

Revision:

Remove therapeutic extrapolation.

State limitations clearly.

2. Comparison with Literature

Problem:

Very limited critical comparison.

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Old references (e.g., 1940 citation).

Revision:

Include recent studies.

Discuss phytochemical variation by geography.

3. Mechanism of Hemolysis

Problem:

Mentions saponins but no mechanistic explanation.

Revision:

Explain membrane cholesterol interaction.

Cite modern toxicological references.

CONCLUSION

Problem:

Too strong.

Suggests pharmacological application.

Major Revision:
Replace with:

"The study provides preliminary data suggesting moderate hemolytic activity possibly linked to saponins. Comprehensive quantitative and in vivo toxicological studies are necessary before therapeutic consideration."

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Major Issues:

Formatting inconsistent.

Possible non-indexed journals.

Old references.

Required Actions:

Verify indexing in:

PubMed

Scopus

Update to last 10 years where possible.

Follow journal style strictly.

LANGUAGE AND FORMATTING

Major Issues:

Embedded line numbers.

Typographical errors.

Inconsistent decimal separators.

Units formatting inconsistent (mg/ml vs mg/mL).

Requires:

Professional language editing.

Standard SI unit formatting.

Table reformatting.

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SUMMARY OF MAJOR REVISION REQUIREMENTS

Category	Severity
Quantitative phytochemistry	Critical
Statistical analysis	Critical
Hemolysis inconsistency	Critical
Overinterpretation	Major
Reference updating	Major
Language editing	Major

FINAL JUSTIFICATION

The manuscript requires **substantial methodological strengthening, statistical rigor, and moderation of conclusions** before being suitable for publication.

Editorial Recommendation:

Major Revision