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REVIEWER'S REPORT

Manuscript No.: IJAR-59684

Title: Phytochemical characterization and in vitro evaluation of antisickling, anticoagulant, and anti-inflammatory activities of bark extracts from Enantia chlorantha and Alstonia boonei,

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

Rating	Excel.	Good	Fair	Poor
Originality		X		
Techn. Quality		X		
Clarity			X	
Significance			x	

Reviewer's ID: JPR-142

Detailed Reviewer's Report

General assessment

The manuscript investigates the phytochemical composition and preliminary pharmacological activities of aqueous bark extracts of *Enantia chlorantha* and *Alstonia boonei*, with particular emphasis on their potential antisickling, antioxidant, anti-inflammatory and anticoagulant properties.

The research addresses an important public health problem, particularly in sub-Saharan Africa, where sickle cell disease represents a substantial healthcare burden. The comparative evaluation of two traditionally used medicinal plants is relevant and may provide a foundation for further pharmacological investigations.

The manuscript includes several experimental approaches, including phytochemical screening, DPPH radical scavenging, phosphomolybdenum antioxidant capacity, protein denaturation, erythrocyte membrane stabilization, antisickling evaluation and whole-blood clotting-time assessment.

Nevertheless, despite its potential scientific value, the manuscript requires substantial revision before it can be considered for publication. Important concerns relate to the characterization of blood donors, experimental reproducibility, numerical inconsistencies, statistical interpretation and overstatement of proposed pharmacological mechanisms.

Major comments

1. Clarification of blood genotype

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The Methods section identifies the blood samples as originating from individuals with the heterozygous AS genotype, whereas the manuscript repeatedly refers to these individuals as patients with sickle cell disease. The authors should clearly distinguish sickle cell trait (HbAS) from sickle cell disease genotypes (HbSS and HbSC). The number of donors, electrophoresis results and independent biological replicates should be reported.

2. Validity of hydroxyurea comparison

The interpretation that the plant extracts outperform hydroxyurea requires reconsideration. Hydroxyurea primarily exerts its clinical antisickling effects through increased fetal hemoglobin production and other systemic mechanisms. An isolated erythrocyte assay cannot adequately reproduce these effects. The authors should explain the limitations of this comparison and avoid suggesting clinical superiority over hydroxyurea.

3. Inconsistencies in numerical results

There are discrepancies between the abstract and Results section concerning IC50 values for protein denaturation and erythrocyte membrane stabilization. Furthermore, the antisickling percentages reported for *E. chlorantha* differ between the Results sections. All experimental values must be cross-checked against the original laboratory records, tables and figures.

4. Anticoagulant activity

The Lee–White method measures whole-blood clotting time, whereas the manuscript refers to plasma coagulation. The authors should correct this terminology and clarify the number and characteristics of blood donors. The proposed mechanisms involving thrombin inhibition and fibrin formation require experimental evidence or should be presented as hypotheses.

5. Statistical methodology

The authors should clearly distinguish technical replicates from independent biological replicates. The number of independent experiments and blood donors should be stated. The IC50 estimation procedures, statistical assumptions, confidence intervals and multiple-comparison methods also require clarification.

6. Phytochemical characterization

The study primarily reports qualitative screening and total phytochemical content. However, individual compounds such as echitamine, alstonine, palmatine and berberine are discussed as possible active constituents without direct analytical identification in the investigated extracts. The authors should distinguish literature-reported constituents from compounds demonstrated in the present investigation.

7. Mechanistic interpretation

The proposed mechanisms involving direct HbS binding, inhibition of polymer nucleation, membrane penetration and thrombin inhibition are not directly demonstrated by the experimental methods. These

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explanations should be appropriately qualified and presented as potential mechanisms requiring further investigation.

8. Anti-inflammatory activity

The BSA denaturation and heat-induced hemolysis assays require more complete methodological descriptions, including BSA concentration, erythrocyte preparation, appropriate extract blanks and independent sample numbers. The results should be interpreted as preliminary in-vitro screening findings rather than established clinical anti-inflammatory activity.

9. Phytochemical and antioxidant reporting

The authors should standardize the units used for total phenolics, flavonoids, tannins, proanthocyanidins and total antioxidant capacity. The use of QE, GAE, APE, PAE, CE, VCE and TE should be checked against the actual calibration standards and methods.

10. Discussion and conclusion

The discussion should be revised to distinguish experimentally observed findings from literature-derived explanations and proposed mechanisms. Claims concerning therapeutic efficacy, prevention of vaso-occlusive crises and development of affordable phytomedicines should be moderated because the study does not include animal or clinical efficacy experiments.

4. Minor comments

1. Remove the duplicated statement concerning the management of sickle cell disease in the Introduction.
2. Correct grammatical errors, spelling inconsistencies and inappropriate scientific terminology throughout the manuscript.
3. Standardize the scientific names and botanical author citations.
4. Ensure that all abbreviations are defined at first occurrence.
5. Improve the consistency of headings and subsection numbering.
6. Standardize the presentation of statistical values, including p-values, IC50 values and standard deviations.
7. Improve the resolution and readability of the figures and microscopic images.
8. Provide appropriate scale bars and magnification information for microscopy images.
9. Revise figure legends to include sample sizes, experimental conditions and statistical comparisons.
10. Verify the bibliographic details and relevance of all references, particularly those supporting toxicity and proposed molecular mechanisms.
11. Correct the terminology used for whole-blood clotting time and plasma coagulation.

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12. Include a clearly defined limitations subsection.
13. Ensure that the abstract accurately reflects the final verified experimental findings.