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REVIEWER'S REPORT

Manuscript No.: IJAR-59542

Title: IN VITRO ?-GLUCOSIDASE INHIBITORY ACTIVITY OF FOUR MEDICINAL PLANTS FROM BOUAFLÃ‰, CÃ"TE DÂ'IVOIRE: INFLUENCE OF PLANT SPECIES AND EXTRACTION PROCEDURE.

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's ID: JPR-094

Detailed Reviewer's Report

Reviewer's Report on the Manuscript

Title: In Vitro α-Glucosidase Inhibitory Activity of Four Medicinal Plants from Bouaflé, Côte d'Ivoire: Influence of Plant Species and Extraction Procedure

1. Strengths of the manuscript

1. Relevant research topic: The study investigates plant-derived α-glucosidase inhibitors, a scientifically relevant area in the search for potential approaches to managing postprandial hyperglycaemia.
2. Comparative experimental design: Four medicinal plant species were evaluated using three extraction procedures and four concentrations, allowing comparison of species-dependent and extraction-dependent activity.
3. Identification of promising extracts: Flacourtia flavescens demonstrated the highest reported inhibition (63.72 ± 0.78%) at 500 µg/mL with ethyl acetate maceration.
4. Quantitative assessment: The manuscript reports percentage inhibition, IC_{50} estimates, and statistical comparisons.

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5. Acknowledgement of limitations: The authors appropriately recognise that the in vitro findings do not establish in vivo antidiabetic efficacy and identify the need for further mechanistic investigation.

2. Weaknesses of the manuscript

- Limited novelty: The manuscript reports that previous studies have investigated α-glucosidase inhibition, including work on Sapium ellipticum. The authors should establish more clearly what is genuinely new about the present comparison.
- Insufficient experimental detail: The number of independent biological and technical replicates is unclear. The authors should report the sample size, replicate structure, and the meaning of the reported error values.
- Absence of a positive control: A standard inhibitor, such as acarbose, was not included. Consequently, the relative potency of the extracts cannot be adequately benchmarked.
- Limitations of the enzyme model: The enzyme source was digestive juice from Archachatina ventricosa, rather than purified mammalian intestinal α-glucosidase. This limits direct clinical relevance.
- Potential assay interference: The manuscript does not adequately establish whether extract colour, reducing substances, or other constituents interfere with the DNS-based spectrophotometric assay.
- Statistical reporting requires improvement: The authors should provide complete ANOVA details, including degrees of freedom, the experimental unit, and clarification of the statistical comparisons. The IC_{50} estimation procedure also requires better justification.
- Outdated plant collection: The plant material was collected in June 2008. The authors should explain the relevance and limitations of these historical samples and provide voucher specimen information or other evidence supporting botanical identification.
- Limited phytochemical evidence: No direct phytochemical profiling or isolation of active constituents was performed in the present study. The proposed relationship between phytochemical composition and inhibitory activity therefore remains speculative.
- Presentation and referencing: Scientific names, spacing, reference formatting, and the consistency of citations require careful editorial revision.

3. Key points requiring attention

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- Clarify the research gap and the specific contribution beyond previously published studies.
- Provide complete information on replication, controls, and statistical methodology.
- Include a suitable positive control in further experiments, preferably acarbose.
- Investigate possible interference in the DNS assay and validate the findings using an appropriate alternative method.
- Justify the use of the snail-derived enzyme and, where feasible, confirm activity using a mammalian enzyme system.

- Improve the reliability and transparency of IC_{50} estimates.
- Provide botanical authentication and voucher specimen details.
- Ensure that all conclusions remain proportional to the evidence and do not imply demonstrated therapeutic efficacy.

4. Scientific significance

The manuscript has moderate preliminary scientific significance. Its main contribution is the comparative evaluation of four medicinal plants under three extraction conditions using a common assay framework.

The results identify Flacourtia flavescens as a candidate for further investigation, while the findings for Bersama abyssinica and Sapium ellipticum also merit follow-up. The very low activity of Pouteria alnifolia under the tested conditions is a useful comparative finding.

However, the study currently provides preliminary enzyme-inhibition evidence rather than proof of antidiabetic efficacy, clinical usefulness, or a validated mechanism of action.

5. Recommendation to the editor

Major Revision

The manuscript addresses a relevant topic and presents potentially useful comparative findings. However, significant improvements in methodological transparency, statistical reporting, assay validation, and the demonstration of novelty are required before the work can be considered suitable for publication.

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I recommend major revision, followed by reassessment of the revised manuscript. If the principal methodological concerns cannot be resolved through clarification or additional validation, rejection should be considered.

6. Confidential comments to the editor

The manuscript may offer a useful preliminary contribution to medicinal-plant screening, particularly through its comparative assessment of extraction procedures. Nevertheless, the absence of a positive control, incomplete information about replication, potential DNS assay interference, and the use of a non-mammalian enzyme source limit the strength of the evidence. I recommend major revision rather than acceptance in its present form. The novelty claim should also be assessed against the existing literature before a final editorial decision.

This is an independent, manuscript-based reviewer assessment. It is not a plagiarism-screening report or a verification of the underlying experimental data.

Justification for Major Revision — Line-by-Line Reviewer Comments

1. Specific reviewer comments and reasons

Comment 1 — Abstract and novelty

Manuscript location: Abstract, lines 6–18; Introduction, lines 36–56

Reviewer comment: The authors should clearly distinguish the novelty of the present study from previous research on plant-derived α-glucosidase inhibitors. The Introduction itself cites previous α-glucosidase research involving Sapium ellipticum and phytochemical work on Flacourtia flavescens.

Reason for major revision: The comparative assessment of four plants and three extraction procedures may offer useful information, but the manuscript does not yet establish sufficiently clearly what new scientific knowledge it contributes beyond the existing literature.

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Required action: Expand the literature comparison, identify what has not previously been investigated, and explain how this study addresses that gap. A novelty statement alone is insufficient without supporting references.

Comment 2 — Enzyme source and biological relevance

Manuscript location: Abstract, lines 9–10; Methods; Discussion, lines 190–197

Reviewer comment: The assay uses α-glucosidase obtained from the digestive juice of Archachatina ventricosa. The authors should provide a stronger justification for using this enzyme source as a model for human intestinal α-glucosidase inhibition.

Reason for major revision: Enzyme characteristics and substrate specificity may differ between a non-mammalian digestive enzyme and mammalian intestinal enzymes. Consequently, the observed inhibition cannot automatically be interpreted as evidence of inhibition of the human enzyme.

Required action: Explain enzyme preparation, source, activity standardisation, and limitations. Validation using a mammalian intestinal α-glucosidase system would strengthen the conclusions; if that experiment is not feasible, the claims must remain explicitly preliminary and model-specific.

Comment 3 — Absence of a positive control

Manuscript location: Methods and Discussion, lines 190–197

Reviewer comment: The authors should include a recognised α-glucosidase inhibitor, such as acarbose, as a positive control, or explain why a suitable reference inhibitor was not used.

Reason for major revision: Without a reference inhibitor, readers cannot benchmark assay performance or interpret the relative potency of the crude extracts against a standard under the same experimental conditions.

Required action: Ideally, perform the assay with a suitable positive control. If no additional experiments can be conducted, clearly acknowledge this limitation and avoid implying that the extracts are comparable to established antidiabetic medicines.

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Comment 4 — DNS assay interference

Manuscript location: Methods; Discussion, lines 192–195

Reviewer comment: The authors should clarify whether extract-only blanks and appropriate reaction controls were used to assess possible interference with the sucrose/DNS colorimetric assay.

Reason for major revision: Crude plant extracts may have intrinsic colour, reducing substances, or other constituents that affect absorbance at 540 nm. Such interference could influence the calculated percentage inhibition independently of enzyme inhibition.

Required action: Describe blank correction and control procedures in sufficient detail. If interference was not assessed, this should be stated as a methodological limitation, and the authors should avoid treating the measured response as definitively enzyme-specific without validation.

Comment 5 — Replicates and reproducibility

Manuscript location: Methods and Results; Abstract, lines 11–15

Reviewer comment: The experimental replication strategy requires clarification. The authors report results as mean ± variation, but the number and type of replicates must be clearly identified.

Reason for major revision: Readers need to know whether the reported variation represents standard deviation, standard error, technical replicates, or independent experiments. Technical repeat measurements alone do not establish independent reproducibility.

Required action: State the number of independent experiments and technical replicates, identify the error measure used, and explain how the final means and variability were calculated. Do not infer or add replicate numbers unless they reflect the actual experiments.

Comment 6 — Statistical analysis

Manuscript location: Abstract, lines 14–16; Statistical analysis and Results

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Reviewer comment: The statistical reporting requires greater detail. The abstract reports \(F = 4.5701\) and \(P < 0.001\), but the statistical model, degrees of freedom, assumptions, and post-hoc comparisons should be reported clearly.

Reason for major revision: A significant overall test does not by itself identify which extracts differ or establish that plant species and extraction procedure independently explain the observed effects. The analysis must also account appropriately for concentration and the experimental design.

Required action: Specify the statistical test, factors included, sample size, assumptions, post-hoc procedure, and exact or appropriately reported \(P\)-values. Reanalyse the data if the current statistical model does not match the design.

Comment 7 — IC_{50} estimates

Manuscript location: Abstract, lines 13–15; Results and Methods

Reviewer comment: The procedure used to calculate IC_{50} values should be described, including the model or interpolation method, data points used, and uncertainty of the estimates.

Reason for major revision: The reported IC_{50} values for F. flavescens are approximately 273–278 µg/mL, whereas the maximum tested concentration is 500 µg/mL. The manuscript should demonstrate how these estimates were derived from the observed concentration–response data and how reliable they are.

Required action: Provide the concentration–response fitting method, fitted parameters where applicable, and confidence intervals or another suitable uncertainty estimate. Ensure the reported values are supported by the actual experimental data.

Comment 8 — Plant authentication and collection details

Manuscript location: Plant material and extraction methods

Reviewer comment: The botanical materials require more complete documentation, including the collection date, plant parts, authentication procedure, voucher specimen information, and storage conditions.

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Reason for major revision: Plant identity, plant organ, collection conditions, and storage may influence chemical composition and biological activity. The manuscript's older collection and phytochemical screening records make it particularly important to establish exactly which materials were tested and how they were authenticated.

Required action: Supply the available collection and authentication records, including voucher numbers and herbarium details if they exist. If voucher specimens were not deposited, disclose this rather than inventing documentation.

Comment 9 — Extraction procedure and reproducibility

Manuscript location: Extraction and sample preparation Methods

Reviewer comment: Provide sufficiently detailed extraction protocols for decoction, aqueous maceration, and ethyl acetate maceration.

Reason for major revision: Differences in extraction conditions can change extract yield and composition. Without clearly reported plant-to-solvent ratios, extraction time and temperature, filtration, concentration, drying, and storage, other researchers cannot reproduce the comparison reliably.

Required action: Report the actual conditions used and, where available, the extraction yield for each preparation. Explain how stock solutions and final assay concentrations were prepared.

Comment 10 — Phytochemical evidence

Manuscript location: Introduction, lines 43–52; Discussion

Reviewer comment: The relationship between the observed inhibitory activity and the phytochemical composition of the extracts requires more cautious interpretation.

Reason for major revision: The manuscript refers to previous phytochemical screening, including work from 2008, but does not directly establish which compounds are present in the tested extracts or responsible for their activity. The suggestion that heat may degrade active constituents is also a possible explanation rather than a demonstrated mechanism.

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Required action: Distinguish earlier phytochemical findings from measurements made in the present study. Either provide current chemical characterisation or explicitly identify it as future work. Present explanations involving specific compounds or heat degradation as hypotheses unless directly tested.

Comment 11 — Interpretation of results and clinical claims

Manuscript location: Discussion, lines 169–197; Conclusion

Reviewer comment: The Discussion and Conclusion should maintain a clear distinction between in vitro enzyme inhibition and antidiabetic efficacy.

Reason for major revision: The findings show activity in a laboratory enzyme assay; they do not establish bioavailability, safety, efficacy in animals or humans, or therapeutic benefit. Crude extracts may also inhibit enzymes through nonspecific interactions.

Required action: Describe F. flavescens as the most active extract under the tested conditions, not as a proven antidiabetic treatment. Retain the limitations concerning the enzyme model, assay interference, toxicity, and lack of in vivo validation.

Comment 12 — References, nomenclature, and presentation

Manuscript location: Throughout; References, lines 215 onward

Reviewer comment: The manuscript requires a careful editorial and bibliographic revision.

Reason for major revision: Inconsistent spacing and formatting of scientific names and author names, as well as inconsistent headings and reference presentation, reduce readability and make it harder to verify the cited literature. Bibliographic accuracy is also essential for evaluating the novelty claim.

Required action: Standardise scientific names in italics, correct formatting and typographical errors, check every in-text citation against the reference list, verify bibliographic details and DOI links, and follow the target journal's style.

2. Which problems actually justify major revision?

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Not every issue above has equal weight. The most important are:

Priority	Issue	Why it matters
Critical	Assay controls and possible DNS interference	Could affect the validity of the inhibition results
Critical	Replication and statistical analysis	Determines whether the reported differences are reliable
High	IC_{50} calculation and uncertainty	Affects the reliability of a key quantitative result
High	Enzyme model and positive control	Limits biological interpretation and assay benchmarking
High	Novelty and comparison with prior studies	Determines whether the work adds enough new knowledge
Moderate	Plant authentication and extraction details	Affects reproducibility and confidence in the materials
Moderate	References and presentation	Important, but usually correctable without new experiments

3. Why not minor revision?

Minor revision would be appropriate if the experiments and analyses were adequately documented and the remaining issues were mostly wording, formatting, or small clarifications.

Here, the manuscript itself acknowledges that it did not include a reference inhibitor and did not specifically evaluate possible DNS interference. It also needs clearer reporting of replication, statistical analysis, and IC_{50} estimation. These matters affect confidence in the central findings, not merely the presentation.

Major revision is therefore justified because the authors must demonstrate that the reported results are methodologically interpretable and statistically supported. Some issues may be resolved by providing missing methodological information or reanalysing existing data; others may require additional experiments. If essential assay-validity problems cannot be addressed, rejection may ultimately be more appropriate.

4. Final recommendation to the editor

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Recommendation: Major Revision

The manuscript investigates a relevant topic and presents a useful comparative screening of four medicinal plants using three extraction procedures. However, important concerns remain regarding assay validation, the absence of a positive reference inhibitor, possible interference in the DNS-based colorimetric assay, incomplete clarification of experimental replication and statistical procedures, and the derivation of IC_{50} values. The use of a non-mammalian enzyme source also limits the biological interpretation of the findings. In addition, the novelty claim requires stronger positioning against previously published studies.

I recommend major revision rather than minor revision because these concerns affect the reliability, reproducibility, and interpretation of the principal findings. The authors should address all methodological and statistical questions transparently, provide supporting information where available, and revise conclusions to reflect the preliminary in vitro nature of the evidence. Where essential validation is not available, the limitations should be explicitly acknowledged. The revised manuscript should be reconsidered only after these issues have been adequately addressed.