

REVIEWER'S REPORT

Manuscript No.: IJAR- 58907

Title: *Antimicrobial potential of shea butter production waste: In vitro antifungal activity of shea hull and press cake methanolic extracts.*

Recommendation:

Accept as it is

Accept after Minor revision.....

Accept after Major revision

Do not accept (*Reasons below*)

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

Reviewer Name: **ANAPANA GOPAL**

Reviewer's Comment for Publication.

General Comments

The manuscript entitled “**Antimicrobial potential of shea butter production waste: In vitro antifungal activity of shea hull and press cake methanolic extracts**” addresses a relevant topic involving the valorization of agro-industrial waste and the search for plant-derived antifungal compounds. The study evaluates methanolic extracts of shea hulls and press cakes against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus fumigatus*, using inhibition-zone measurements and minimum inhibitory concentration (MIC) determination, together with qualitative phytochemical screening.

The manuscript has a potentially useful contribution because shea-processing residues are abundant and may contain bioactive compounds. The experimental results indicate antifungal activity of both extracts. However, the manuscript requires **major revision** before it can be considered for publication. The principal concerns relate to experimental methodology, controls, MIC determination, statistical analysis, interpretation of phytochemical results, and several inconsistencies in terminology and presentation.

Content and Originality

The topic is relevant and has practical importance. The study combines two potentially valuable aspects: **antifungal activity** and **valorization of shea-processing residues**.

The authors report inhibition zones of **13–17 mm** and MIC values of **1.56 mg/mL for *A. flavus* and *A. fumigatus* and 6.25 mg/mL for *C. albicans***.

The manuscript also identifies polyphenols, flavonoids, tannins, saponins, steroids, and triterpenes in both extracts.

However, the claim of novelty needs to be reconsidered. The Introduction states that the biological activity of shea cakes and hulls “**has not been studied to date,**” while the Discussion itself cites Kouakou & Megnanou (2021) as having already demonstrated antifungal activity of methanolic extracts of shea shells and seed cakes.

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Therefore, the authors should clearly explain **what is genuinely new in the present study**, particularly since the cited 2021 study appears closely related.

The novelty may instead lie in the inclusion of *A. flavus* and *A. fumigatus*, but this should be explicitly demonstrated through comparison with the previous study.

Technical Quality

1. Extract preparation requires clarification

The authors state that **100 g of powdered hulls/press cakes were extracted with 1 L of 70% methanol for 48 h**, followed by filtration and evaporation at 40°C.

The method should provide additional information regarding:

- number of independent extraction batches;
- particle size of the powdered material;
- extraction temperature;
- whether extraction was performed once or repeatedly;
- percentage extraction yield;
- final concentration calculation;
- storage duration before testing.

Reporting extraction yield would be particularly valuable for evaluating the practical potential of these wastes as sources of bioactive compounds.

2. Autoclaving the extracts needs justification

The extracts were prepared at 50 mg/mL and **autoclaved at 121°C for 15 minutes** before antifungal testing.

This is a significant methodological point. Heat treatment at 121°C could potentially alter, degrade, or transform thermolabile phytochemicals. The authors should justify why autoclaving was necessary and discuss whether this treatment could influence antifungal activity.

A suitable sterile-filtration approach may be more appropriate for heat-sensitive extracts, depending on extract composition and solubility.

3. Appropriate positive controls are missing

The antimicrobial assay uses **blank discs as negative controls**, but there is no clear indication that a standard antifungal drug was included as a positive control.

A recognized antifungal control would allow the activity of the plant extracts to be placed into an appropriate comparative context.

The authors should include or explain the absence of a standard such as an appropriate azole or other validated antifungal agent.

4. The microbial inoculum preparation needs improvement

The manuscript describes preparation of a 10^{-3} dilution and spreading **10 µL** on Sabouraud agar for determining microbial load.

However, antimicrobial susceptibility testing generally requires a **standardized inoculum concentration**. The manuscript does not clearly report the final inoculum in CFU/mL or an equivalent standardized measure.

The authors should provide:

- initial microbial concentration;
- final inoculum concentration used for the assay;
- method used for standardization;
- number of viable cells/spores used per plate.

This is particularly important for reproducibility.

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5. The MIC methodology is problematic/unclear

The manuscript states that MIC was determined using agar dilution with concentrations from **50 to 0.78 mg/mL**, but subsequently states that MIC was determined **“to the naked eye”** and corresponds to the lowest concentration for which **“no halo is visible around the impregnated disc.”**

This description combines different methodological concepts.

Agar dilution MIC is normally based on **visible growth/no-growth on the agar containing the test compound**, whereas an inhibition halo around a disc corresponds to a diffusion-based assay.

The authors need to clearly state whether MIC was determined by:

- agar dilution;
- disc diffusion using serial extract concentrations; or
- another method.

The MIC method should be rewritten according to the actual experimental procedure.

6. The reported MIC values should be interpreted cautiously

Both extracts produced exactly the same MIC values for each microorganism:

- *C. albicans*: 6.25 mg/mL;
- *A. flavus*: 1.56 mg/mL;
- *A. fumigatus*: 1.56 mg/mL.

This could be a genuine result, but the manuscript should provide the number of independent replicates and clarify whether the MIC determination was repeated independently.

7. Replication and statistical analysis are insufficiently described

The manuscript states that analyses were carried out **in triplicate** and that data were expressed as mean $\pm$ SD. It then reports one-way ANOVA followed by LSD post-hoc testing.

However, Table II reports single inhibition-zone values rather than **mean $\pm$ SD**, despite the Methods stating that experiments were performed in triplicate.

This should be corrected.

The authors should report:

- n for each treatment;
- mean $\pm$ SD;
- ANOVA F-values;
- p-values;
- post-hoc comparisons where appropriate.

If only two extracts are being compared for each organism, the statistical approach should also be reconsidered and clearly justified.

8. Negative and solvent controls

Because the extracts were prepared using **70% methanol**, the authors should demonstrate that residual solvent does not contribute to the observed activity.

The negative control should ideally correspond to the solvent/vehicle used in preparing the test solution, not simply an untreated blank disc.

9. Phytochemical screening is qualitative only

The phytochemical screening demonstrates the presence of several classes of compounds in both extracts.

However, the manuscript subsequently discusses phenolic compounds as likely responsible for antifungal activity.

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Because the screening is qualitative, the study cannot establish which compound class is responsible for the activity.

The Discussion should therefore use cautious language such as **“may contribute”** rather than implying a demonstrated causal relationship.

If feasible, quantitative total phenolic/flavonoid content and chromatographic characterization would substantially strengthen the study.

10. The study should distinguish antifungal activity from therapeutic potential

The Conclusion appropriately describes the extracts as potential sources of bioactive substances. However, the Introduction and Discussion occasionally move toward implications for treatment of infections.

The present experiment is strictly **in vitro** and does not establish:

- toxicity;
- selectivity toward fungal cells;
- pharmacological activity in vivo;
- bioavailability;
- therapeutic efficacy;
- safety.

The manuscript should clearly maintain this distinction.

Language and Presentation

The manuscript requires **substantial English-language editing**.

Examples include:

- “Therefore, ‘agar diffusion’ method were carried out” → grammatically incorrect.
- “Candida sp and Aspergillus are occasional but often dangerous parasites” requires scientific and grammatical revision.
- “they are known to be cause several infectuions” contains grammatical and spelling errors.
- “Antimicrobial test” and “antimicrobial assay” should be used consistently.
- “Analysis test were carried out in triplicate” should be corrected.
- “Statistical was performed” is grammatically incorrect.
- “futher studies” should be “further studies.”
- “oilcake” and “press cake” should be standardized throughout the manuscript.

The scientific nomenclature should also be carefully standardized. For example, ***Candida albicans***, ***Aspergillus flavus***, and ***Aspergillus fumigatus*** should consistently appear in italicized scientific format.

There is also an inconsistency between **“shea shells,” “shea hulls,” “hulls,” “cakes,” “seed cakes,” and “oilcakes.”** The authors should define these materials clearly and then use one terminology consistently.

Structure and Organization

The manuscript follows a generally appropriate structure:

Introduction → Materials and Methods → Results and Discussion → Conclusion → References.

However, the organization can be improved.

Introduction

The Introduction provides useful background on fungal infections and plant-derived antifungal compounds, but it is somewhat broad. It should move more quickly toward:

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1. shea-processing waste;
2. known phytochemical composition;
3. previous evidence of antimicrobial activity;
4. specific knowledge gap;
5. objective/hypothesis of the current study.

The statement that the activity of shea hulls and cakes had not previously been studied needs correction because the manuscript itself cites earlier research on these materials.

Materials and Methods

This section should be strengthened by adding:

- number of independent extracts;
- extraction yield;
- inoculum standardization;
- positive control;
- solvent control;
- exact assay replication;
- precise MIC methodology;
- statistical model.

Results

The Results and Discussion are combined, which is acceptable for some journals, but the presentation would be clearer if the experimental findings were first presented objectively and then interpreted separately.

Tables should report the statistical variation associated with the inhibition-zone measurements.

Discussion

The Discussion provides reasonable interpretation and comparison with previous work. However, it should more clearly distinguish **observed results from proposed mechanisms**.

Conclusion

The Conclusion is generally consistent with the reported findings and appropriately notes that additional isolation, toxicity, and in vivo studies are required before therapeutic applications can be considered.

This cautious statement should be retained and strengthened.

References and Citations

The manuscript contains relevant references covering antifungal resistance, plant-derived antifungal compounds, phytochemistry, and previous studies on shea residues.

However, several points require attention.

1. **The novelty claim should be reconciled with the Kouakou & Megnanou (2021) study**, which appears directly relevant to the present work.
2. The authors should verify that all methodological references actually correspond to the methods used. In particular, the manuscript cites NCCLS (1997) for disc diffusion, while the cited document is specifically a reference method for **broth dilution antifungal susceptibility testing of yeasts**. The methodological citation should therefore be carefully checked.

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3. Reference formatting needs standardization, including:
 - journal names;
 - volume/issue;
 - page numbers;
 - DOI format;
 - capitalization;
 - punctuation.
4. "Sofowra (1993)" should be checked for the correct spelling of the author's name and complete bibliographic information.
5. The references should be checked carefully for consistency between in-text citations and the bibliography.
6. More direct literature on the **antifungal activity and phytochemical composition of *Vitellaria paradoxa* residues** should be incorporated to establish the precise contribution of this manuscript.

Overall Recommendation

The manuscript addresses an interesting and potentially valuable topic, particularly the **valorization of shea-processing residues as possible sources of antifungal compounds**. The reported inhibition zones and MIC values indicate that both shea hull and press-cake methanolic extracts have measurable in vitro activity against the three tested microorganisms.

The study also provides preliminary phytochemical evidence that both extracts contain several classes of secondary metabolites that could potentially contribute to the observed activity.

However, the current manuscript requires substantial revision before its conclusions can be considered sufficiently robust. The most important issues are the **unclear MIC methodology, lack of appropriate positive/solvent controls, insufficient description of inoculum standardization, incomplete statistical presentation, limited characterization of the extracts, and an overstatement of novelty**.

The authors should also carefully revise the manuscript's English, terminology, tables, methodological citations, and reference formatting.

Final Decision:

Major Revision

The manuscript has **potentially publishable preliminary findings**, but significant methodological and presentation improvements are required. In particular, the authors should clarify and strengthen the antifungal assay and MIC methodology, provide appropriate controls and replication details, improve statistical reporting, reconcile the novelty claim with previous literature, and moderate conclusions regarding therapeutic applications.