

**Peer Review for Manuscript/INVITRO ANTIFUNGAL ACTIVITY OF SOME ETHANOL
PLANT EXTRACTS AGAINST CANDIDA SPECIES ISOLATED FROM HIGH
VAGINAL SWABS OF WOMEN ATTENDING A HOSPITAL IN ENUGU STATE,
NIGERIA.**

Dear Editor,

I hope this message finds you well. I would like to express my gratitude for the opportunity to serve as a peer reviewer for the manuscript titled "*In Vitro Antifungal Activity of Some Ethanol Plant Extracts Against Candida Species Isolated from High Vaginal Swabs of Women Attending a Hospital in Enugu State, Nigeria.*" It is an honor to contribute to the evaluation of such an important and timely piece of research.

This paper focuses on the evaluation of the antifungal activity of ethanol plant extracts from *Moringa oleifera*, *Vernonia amygdalina*, and *Ocimum gratissimum* against *Candida* species, which are known to cause vaginal candidiasis. Vaginal candidiasis is a common fungal infection among women worldwide, and its treatment often faces challenges due to the increasing resistance of *Candida* species to conventional antifungal drugs like azoles and echinocandins.

The research aims to explore natural alternatives by testing the efficacy of these medicinal plants on *Candida* strains resistant to multiple antifungal drugs. The study specifically targets six isolates of *Candida tropicalis*, *Candida albicans*, *Candida parapsilosis*, and *Candida krusei*, which were obtained from women attending a hospital in Enugu State, Nigeria.

Key Findings:

- *Moringa oleifera* demonstrated the highest antifungal activity, particularly against resistant *Candida albicans* with an inhibition zone diameter of 19 mm at a concentration of 200 mg/ml.
- *Vernonia amygdalina* and *Ocimum gratissimum* also showed significant antifungal effects but were less effective than *Moringa oleifera*.
- *Candida tropicalis* was sensitive to all the plant extracts, while *Candida krusei* was completely resistant to all tested extracts, even at the highest concentrations.
- The study highlights the potential of these plants as sources for antifungal drug development, especially for treating infections caused by drug-resistant *Candida* species.

Focus: The paper emphasizes the therapeutic potential of natural plant-based treatments as alternatives to conventional antifungal drugs, particularly in light of the increasing antifungal resistance observed in clinical settings. The experimental approach used the agar well diffusion method to assess the efficacy of the plant extracts.

Overall, the article aims to contribute to the search for effective antifungal treatments by exploring the antifungal properties of selected medicinal plants against resistant *Candida* species.

In summary, this research underscores the need for further investigation into plant-derived antifungal agents as a means to address the growing issue of drug resistance in *Candida* species.

INTRODUCTION

The focus on the antifungal properties of *Moringa oleifera*, *Vernonia amygdalina*, and *Ocimum gratissimum* is well-justified given their long-standing use in traditional Nigerian medicine. The manuscript effectively highlights the urgent need for alternative treatments due to the increasing resistance of *Candida* species to conventional antifungal drugs like azoles and echinocandins. By targeting medicinal plants, this study contributes to the growing body of research exploring the therapeutic potential of natural products in overcoming drug resistance. The introduction section provides a well-rounded background and successfully establishes the relevance of the research.

Materials and Method

Strengths:

1. **Relevance and Timeliness:**

2. This study addresses a significant and pressing issue—the growing resistance of *Candida* species to conventional antifungal treatments, particularly in developing countries like Nigeria. The use of alternative treatments, such as ethanol plant extracts from *Moringa oleifera*, *Vernonia amygdalina*, and *Ocimum gratissimum*, is timely given the rise of drug-resistant fungal strains and the increased interest in natural, plant-based remedies.

3. **Scientific Rigor:**

The study follows a robust scientific methodology, including the use of standard techniques such as the agar well diffusion method and McFarland standard for inoculum preparation. The serial dilution of plant extracts and measurement of inhibition zones provide clear, quantitative data that allow for a comparative evaluation of the antifungal efficacy of different plant concentrations.

4. **Detailed Plant Identification and Extraction:**

The careful identification of plant species, complete with herbarium numbers, adds credibility to the study. The Soxhlet extraction method used to obtain the ethanol extracts is a well-established technique, ensuring that the active compounds from the plants are effectively isolated.

5. **Clear Data Presentation:**

The materials and methods section are detailed, allowing for reproducibility. The progression from initial collection of plant material to the antifungal susceptibility testing is logically structured, ensuring that readers can easily follow the experimental process.

Areas for Improvement:

6. Lack of Controls and Comparative Data:

The study would benefit from the inclusion of positive controls (i.e., commercially available antifungal agents like fluconazole or amphotericin B) alongside the plant extracts. This would provide a comparative baseline, allowing the reader to gauge the efficacy of the plant extracts relative to conventional antifungal treatments. Without this, it's difficult to assess how these extracts fare in practical, clinical settings.

7. Statistical Analysis:

While the inhibition zones were measured, the study could benefit from a more detailed statistical analysis. For example, including standard deviations, confidence intervals, or ANOVA tests would add depth and reliability to the data interpretation. This would also help quantify the significance of the differences observed between plant extract concentrations and *Candida* strains.

8. Toxicity and Safety Considerations:

One limitation of the study is the lack of any mention of the toxicity of the plant extracts. While in vitro efficacy is demonstrated, understanding whether these extracts are safe for human application, especially in the sensitive vaginal environment, is crucial. Future research should consider in vivo studies or cytotoxicity assays to ensure that these plant extracts are safe for potential therapeutic use.

9. Mechanism of Action:

While the study demonstrates that the plant extracts possess antifungal properties, it lacks any discussion or exploration of the possible mechanisms of action. A review of existing literature on the antifungal compounds in *Moringa oleifera*, *Vernonia amygdalina*, and *Ocimum gratissimum*, and how these may disrupt fungal cells, would provide a deeper scientific understanding of their effectiveness.

10. Future Directions:

Although the conclusion suggests that these plants be investigated for antifungal drug development, the study would benefit from more detailed recommendations for future research. For example, examining the synergistic effects of these plant extracts with existing antifungal drugs could open new avenues for combination therapy, particularly against resistant *Candida* strains.

RESULTS AND DISCUSSION

The **Results and Discussion** section of the article presents key findings regarding the antifungal activity of ethanol extracts from selected medicinal plants against various *Candida* species. Here are the main points:

- **Inhibition Zone Measurements:** The study found that the **Extract of *Moringa oleifera*** exhibited the highest inhibition zone diameter of **19mm** at a concentration of **200mg/ml** against ***Candida albicans* 1**. This was followed closely by ***Vernonia amygdalina*** and ***Ocimum gratissimum***, which showed inhibition zone diameters of **18.67mm** and **18mm** at concentrations of **100mg/ml** and **200mg/ml**, respectively.
- **Sensitivity and Resistance Patterns:** The results indicated that ***Candida tropicalis* 1** was sensitive to all the plant extracts at all tested concentrations. In contrast, ***Candida parapsilosis*** demonstrated resistance to the extract of ***Ocimum gratissimum*** across all concentrations. Notably, ***Candida krusei*** was found to be completely resistant to all plant extracts at the highest concentration of **200mg/ml**.
- **Concentration-Dependent Activity:** The antifungal activity of the extracts from ***Moringa oleifera*** and ***Ocimum gratissimum*** against ***Candida tropicalis* 1** was observed to be concentration-dependent, meaning that higher concentrations resulted in greater inhibition of fungal growth.
- **Therapeutic Potential:** The varying degrees of antifungal activity exhibited by the plant extracts suggest that these plants possess significant therapeutic potential. The study emphasizes the importance of exploring these natural remedies, especially in the context of rising antifungal resistance to commonly used drugs like **Fluconazole**.
- **Recommendations for Future Research:** The authors recommend further investigation into these plant extracts for their potential use in formulating antifungal drugs, particularly against *Candida* species that have developed resistance to conventional treatments.

FINAL COMMENTS ON THE PAPER

- The paper titled "INVITRO ANTIFUNGAL ACTIVITY OF SOME ETHANOL PLANT EXTRACTS AGAINST CANDIDA SPECIES ISOLATED FROM HIGH VAGINAL SWABS OF WOMEN ATTENDING A HOSPITAL IN NENUGU STATE, NIGERIA" presents valuable findings regarding the antifungal properties of selected medicinal plants.
- **Areas for Improvement:**

- **Methodological Clarity:** While the methodology is generally sound, providing more detailed information on the extraction process and the specific concentrations tested would enhance reproducibility. For instance, the rationale behind the chosen concentrations could be elaborated upon to justify their selection.
- **Discussion of Resistance Mechanisms:** The paper could benefit from a more in-depth discussion on the mechanisms of resistance observed in certain *Candida* species, particularly ***Candida krusei*** and ***Candida parapsilosis***. This would provide a better understanding of the implications of the findings and the potential for developing effective treatments.
- **Broader Context:** Including a comparison with existing literature on antifungal activities of these plants against other pathogens could strengthen the discussion and highlight the novelty of the findings.

Based on the provided contexts, there is no specific mention of the graphics or visual representations used in the paper. However, the effectiveness of graphics in scientific papers is crucial for conveying complex data and results clearly.

While the contexts do not provide specific details about the graphics, it is generally advisable for the authors to review and improve any visual elements in the paper to enhance clarity, data presentation, and overall impact.

FINAL Decision

Overall, the paper presents promising results that contribute to the field of antifungal research. With some improvements in methodological clarity and depth of discussion, it would be well-positioned for publication. The authors are encouraged to address the suggested points to enhance the quality and impact of their work.