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Antifungal Activities of *Moringa Oleifera* Ethanolic Leaf Extract against *Aspergillus Niger*

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Abstract

***Aspergillus niger* is a filamentous fungus commonly found in soil, decaying organic matter, and indoor environments. While it is widely used in various industrial applications, it is also an opportunistic pathogen capable of causing severe infections, particularly in immunocompromised individuals. The increasing resistance of fungi to conventional antifungal treatments has underscored the need for alternative remedies.*Moringa oleifera* is known for its medicinal properties and has demonstrated antimicrobial effects against a range of pathogens. In this study, fresh *Moringa oleifera* leaves were collected, authenticated, and processed for ethanolic extraction at the University of the Philippines–Los Baños (UPLB) Chemistry Institute. The extract was subsequently tested against *A. niger* at BIOTECH-UPLB, using varying concentrations (25%, 50%, 75%, and 100%). The fungal isolate, *Aspergillus niger* (accession number BIOTECH 3225), was obtained from the Philippine National Collection of Microorganisms (PNCM), a recognized repository for microbial strains in the Philippines. Itraconazole was used as the positive control. A t-test was conducted to compare the antifungal effects of the 100% *Moringa oleifera* ethanolic leaf extract and Itraconazole against *A. niger*.The results showed that the *Moringa* extract produced no inhibition zones (0 mm), while Itraconazole demonstrated significant inhibition (13.86 mm and 12.38 mm). The t-test revealed a statistically significant difference (p = 0.000), indicating that Itraconazole is significantly more effective than the *Moringa* extract in inhibiting *A. niger* growth. These findings suggest that the ethanolic leaf extract of *Moringa oleifera* lacks antifungal activity against *A. niger* under the tested conditions. However, further research is warranted—exploring different plant parts, extraction methods, or fungal targets—to fully assess the broader antimicrobial potential of *M. oleifera*.**

Keywords: Aspergillus niger, Moringa oleifera, ethanolic leaf extract, Itraconazole, antifungal effects, antimicrobial

1. Introduction

Fungal infections caused by *Aspergillus niger*, a filamentous fungus from the *Aspergillus* genus, present a significant health challenge, particularly for immunocompromised individuals. This opportunistic pathogen is capable of causing invasive aspergillosis, a life-threatening condition that primarily affects those undergoing chemotherapy, organ transplantation, or living with HIV/AIDS. Denning (2024) highlights the gravity of this disease, noting that it accounts for approximately 1.8

million deaths annually, with a mortality rate reaching 85.2%, especially among patients with chronic obstructive pulmonary disease, lung cancer, and hematologic malignancy.

The clinical diversity of *Aspergillus* species is unmatched, with infections typically originating from environmental sources rather than direct human-to-human transmission. However, emerging evidence suggests that some species may have the potential for aerosol-based transmission (Rudramurthy et al., 2019). The prevalence of specific Aspergillus species also varies by geography. For example, *A. flavus* is more common in tropical countries like India, Pakistan, Sudan, and Saudi Arabia, where it is the predominant cause of aspergillosis. These geographical differences emphasize the need to understand the unique biological and environmental factors influencing fungal distribution and disease patterns

In the Philippines, fungal infections remain a significant but under-recognized public health issue. Despite the country's tropical climate and rising population of immunocompromised individuals such as those with tuberculosis, cancer, HIV/AIDS, and autoimmune diseases there is still no national surveillance system for fungal diseases. Estimates using data from global and national health sources reveal that aspergillosis and candidiasis are among the most common fungal infections in the country. Chronic pulmonary aspergillosis (CPA) alone is believed to affect over 77,000 Filipinos, primarily those with a history of tuberculosis. In addition, allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) are estimated to impact over 280,000 individuals combined. These figures highlight the pressing need for improved fungal disease monitoring and targeted interventions in the Philippines (Batac & Denning, 2017). This situation is further complicated by the alarming rise in resistance to commonly used antifungal medications.

Effective antifungal agents play a vital role in improving healthcare outcomes for patients with fungal infections. Timely and appropriate treatment can prevent the progression of superficial infections to invasive ones and enhance the quality of life for patients living with chronic fungal conditions (Alegbeleye, 2018).

Recent advancements in healthcare have brought about significant shifts in the epidemiology of invasive fungal infections, with growing concerns about antifungal resistance. The increasing prevalence of resistance, coupled with the appearance of less common fungal species, complicates treatment strategies, as effective therapies for these resistant strains are not yet well-established. (Logan et al., 2022).

As Lockhart et al. (2023) emphasize, the emergence of antifungal-resistant strains poses a critical public health threat and highlights the urgent need for novel therapeutic strategies. Rani et al. (2018) also support the call for alternative treatment options, noting that current antifungals are increasingly losing their effectiveness due to resistance.

In a study by Idris, Agustien, and Mansyurdin (2025), their research showed that the combination of these plant extracts demonstrated significant antifungal activity, particularly when used together. This suggests that these medicinal plant extracts, especially in combination, possess strong antifungal properties and could serve as a natural alternative to chemical fungicides, which can have harmful environmental and health impacts.

Moringa oleifera is a plant known for its wide range of medicinal properties, attributed to its phytochemical constituents such as alkaloids, flavonoids, glycosides, and tannins. Numerous studies have demonstrated its antifungal effects against organisms like *Candida* species and *Saccharomyces cerevisiae*, indicating its potential as a natural therapeutic agent (Pareek et al., 2023).

As resistance to conventional antifungal drugs continues to rise among *Aspergillus* species, exploring alternative treatments such as *Moringa oleifera* offers a promising solution. While Moringa's antifungal properties have been tested against species like *A. flavus, A. fumigatus, Candida* species and *Saccharomyces cerevisiae*, research specifically targeting *A. niger* remains scarce. Given the unique challenges that *A. niger*presents, particularly in immunocompromised individuals, it is crucial to investigate the efficacy of Moringa against this pathogen. Addressing this gap in research could pave the way for more sustainable and effective treatments for infections caused by this persistent and difficult-to-treat fungus.

The study titled "Antifungal Activities of *Moringa oleifera* Ethanolic Leaf Extract against *Aspergillus niger*" evaluated the antifungal properties of *Moringa oleifera* against this specific strain. Furthermore, it aimed to determine the optimal concentration of the extract using the disk diffusion method. This research represents an important contribution to the development of plant-based antifungal strategies, supporting the global shift toward sustainable and natural healthcare solutions.

2. Objective of the Study

The general objective of this study is to determine the antifungal activities of the ethanolic leaf extract of *Moringa oleifera* against *Aspergillus niger*. Specifically, the study aims to:measure the zone of inhibition of Itraconazole (positive control) against *Aspergillus niger*. determine the zone of inhibition of different concentrations of ethanolic leaf extract of *Moringa oleifera* against *Aspergillus niger*, specifically at 25%, 50%, 75%, 100%. identify the most effective concentration of ethanolic leaf extract of *Moringa oleifera* against *Aspergillus niger*.determine if there is a significant difference between the zone of inhibition exhibited by the most effective concentration of *Moringa oleifera* ethanolic leaf extract and Itraconazole (positive control) against *Aspergillus niger*.

3. Materials and Methods

The research method used in this study was experimental research design. According to Sereyrath Em (2024), in an experimental study, the cause of an effect is examined by exposing one or more variables to experimental treatments or conditions.In this study, the researcher collected *Moringa oleifera* (malunggay) leaves and sent them to the University of the Philippines-Los Baños for extraction. After the extract was successfully obtained, it was brought to the National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB), where professional analysts conducted the experiments. The necessary equipment was available at the facility to ensure accurate and reliable results.The primary sources of data were the results that came from the experimentation using *Moringa oleifera* leaves that were collected from a subdivision in City of Biñan, Laguna and were extracted by the Chemistry Institute of UPLB against the fungal culture of *Aspergillus niger* which was obtained from Philippine National Collection of Microorganism (PNCM) BIOTECH- UPLB.The researcher collected Moringa oleifera leaves from a subdivision in the City of Biñan, Laguna. The samples were authenticated by a

Registered Forester from the College of Forestry and Natural Resources, University of the Philippines, Los Baños, Laguna, in February 2025. The sample consisted of leaves and branches.

All materials, including glassware, filters, and instruments, were thoroughly cleaned, sterilized, and calibrated to ensure consistency and accuracy throughout the study. The glassware, such as beakers, flasks, and amber bottles, were properly washed and disinfected to prevent contamination. The rotary evaporator and spectrophotometer were also prepared for use by the University of the Philippines, Los Baños staff, ensuring all equipment met the necessary standards for reliable results.

*Plant's Ethanolic Leaf Extract Preparation.*The extraction process began with the collection of 2000 grams of fresh Moringa oleifera (malunggay) leaves, which were carefully selected to ensure they were free from damage or contaminants. The leaves were then air-dried at room temperature to remove surface moisture. After air drying, they were further oven-dried at a controlled temperature to ensure complete removal of moisture while preserving their beneficial compounds. Once fully dried, the leaves were ground into a fine powder using a blender or milling machine. This step increases the surface area of the plant material, improving the efficiency of the extraction process.The powdered leaves were then processed at the University of the Philippines-Los Baños (UPLB) Chemistry Institute using 95% ethanol as the solvent. The leaves were soaked in ethanol at a 1:4 (w/v) ratio (one-part leaf powder to four parts ethanol) and left for 48 hours with continuous shaking. This method, known as maceration, allowed the ethanol to dissolve bioactive compounds such as flavonoids, alkaloids, and tannins. After 48 hours, the mixture was filtered to remove solid residues. First, cheesecloth was used to separate large particles, followed by a second filtration using Whatman No. 1 filter paper to ensure a pure extract. The filtered extract was then concentrated using a rotary evaporator set at 40°C and 0.09 MPa to remove excess ethanol. This process ensured that only the desired plant compounds remained while preventing heat damage to sensitive components. The concentrated extract was transferred into a sterile amber bottle to protect it from light exposure, which could degrade its active compounds. It was then stored in a refrigerator to maintain its stability until further testing.

Fungal Culture. The *Aspergillus niger* fungal culture used in the study was obtained from the Philippine National Collection of Microorganisms (PNCM), BIOTECH-UPLB, with accession number BIOTECH 3225. The isolate was identified, authenticated, and maintained under standard conditions. It was grown on Potato Dextrose Agar (PDA) and incubated at 25–30°C for 3 to 5 days to ensure optimal growth and sporulation for experimental use. Proper storage, handling, and growth of the fungal culture were carried out in accordance with established standards.

Disk Diffusion Assay. The collected extract and fungal culture were brought to the Antibiotics Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB) for antifungal susceptibility testing. The *Aspergillus niger* fungal culture was inoculated into Sabouraud's dextrose broth (SDB) at 37°C for six (6) hours. This step ensured that the fungus grew optimally and was prepared for standardization.Following incubation, the fungal suspension was standardized using a spectrophotometer. The turbidity was adjusted with Nutrient Broth (NB) and SDB to achieve an absorbance reading between 0.08 and 0.13 at 625 nm, which corresponds to a 0.5 McFarland standard. This standardization was performed in accordance with the guidelines set by the Clinical and Laboratory Standards Institute for filamentous fungi.After standardization, the fungal inoculum was evenly spread

across the surface of Sabouraud Dextrose Agar (SDA) plates. Sterile 6 mm filter paper disks were prepared and impregnated with 25 µL of the plant extract at varying concentrations (25%, 50%, 75%, and 100%). The disks were then carefully placed onto the surface of the inoculated plates. Itraconazole was used as the positive control. All tests were carried out with multiple repetitions. The inoculated plates were incubated at 35°C for 48 hours, following CLSI recommendations for optimal fungal growth and accurate susceptibility results. After incubation, zones of inhibition were measured using a transparent ruler. A zone of clearance exceeding 6 mm in diameter was interpreted as indicative of antifungal activity. The procedure was based on standard practices followed at the Antibiotics Laboratory of BIOTECH, University of the Philippines Los Baños (BIOTECH-UPLB).

The researcher collected *Moringa oleifera* leaves, which were authenticated by a Registered Forester from the College of Forestry and Natural Resources, UPLB. The collected leaves underwent the extraction process conducted by the Chemistry Institute of the University of the Philippines Los Baños, and they were then brought to the Antibiotics Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB) to undergo antifungal susceptibility testing against *Aspergillus niger*.The procedures, materials, as well as their proper handling and storage, followed in accordance with the standard. Both the procedures in the study and the validation of results were conducted by professional analysts. The test was done in multiple repetitions.

T-test was conducted to evaluate the significant difference between the zones of inhibition produced by *Moringa oleifera* ethanolic leaf extract at varying concentrations (25%, 50%, 75%, 100%) and those produced by Itraconazole (positive control) against *Aspergillus niger*.

After completing the tests, all used samples and materials were sterilized using an autoclave at 121°C for 15 minutes under 15 psi pressure to fully eliminate the fungi and avoid contamination from other microbes. Afterwards, the sterilized items were placed in leak-proof containers and sealed in a yellow bag marked “infectious” to ensure safe and proper disposal in accordance with CDC protocols.

4. Results and Discussion

Zone of Inhibition of Itraconazole (Positive Control) against *Aspergillus niger*

Table 1. Zone of Inhibition of Itraconazole (Positive Control) against *Aspergillus niger*

Antibiotic		**Trial**	**Zone of Inhibition (mm) Replicates**					**Average Zone of Inhibition (mm)**
			1	**2**	**3**	**4**	**5**	
Itraconazole		**1**	14.8	13.9	11.1	15.9	13.6	**13.86**
		2	13.9	11.4	12.2	12.5	11.9	**12.38**

Table 1 illustrates the antifungal efficacy of itraconazole, a triazole-class antifungal, against *Aspergillus niger* as assessed through the agar well diffusion method. The average inhibition zones recorded across two independent trials—13.86 mm and 12.38 mm—reflect the consistent fungistatic activity of itraconazole against this filamentous fungus. These results are consistent with those reported

by Poulsen et al. (2021), who found that itraconazole demonstrated notable in vitro activity against *A. niger*, with a minimum inhibitory concentration (MIC) exceeding 16 mg/L.These results are consistent with those reported by Poulsen et al. (2021), who found that itraconazole demonstrated notable in vitro activity against *A. niger*, with a minimum inhibitory concentration (MIC) exceeding 16 mg/L.

In a separate study conducted by Shakya (Hada) et al. (2021), the antifungal susceptibility of various environmental *Aspergillus* species, including *A. niger*, was tested using the NCCLS M38-A guidelines. Itraconazole was among the agents evaluated and showed considerable effectiveness, with most isolates exhibiting MIC values as low as 0.125 µg/mL. Notably, no resistance was detected among the environmental strains tested in their study.

However, contrasting results were reported by Moin et al. (2020), who examined the susceptibility profiles of clinically significant *Aspergillus* isolates in Pakistan. Their findings revealed variable resistance patterns, with some *A. niger* isolates showing resistance not only to itraconazole but also to other triazoles like voriconazole. Their proteomic analysis revealed that exposure to itraconazole disrupted multiple metabolic pathways, including the tricarboxylic acid (TCA) and glyoxylate cycles, and notably reduced peroxidase enzyme activity—further highlighting the drug's impact at the cellular level.

These findings underscore a growing concern regarding emerging azole resistance in *A. niger*, which appears to vary based on geographical and clinical context. Despite this, itraconazole remains a widely utilized antifungal, valued for its broad-spectrum activity and good oral bioavailability. It is particularly useful in treating chronic, endemic, and invasive mycoses. However, due to its pharmacokinetic variability and potential interactions with other drugs, therapeutic drug monitoring (TDM) is often recommended to optimize its efficacy and safety (Taylor & Francis, 2013).

Zone of Inhibition of Different Concentrations of Ethanolic Leaf Extract of *Moringa oleifera* against *Aspergillus niger*

Table 2. Zone of Inhibition of Different Concentrations of Ethanolic Leaf Extract of *Moringa oleifera* against *Aspergillus niger*

Concentration of *Moringa oleifera* ethanolic extract	**Trial 1 and 2 Zone of Inhibition (mm) Replicates**					**Average Zone of Inhibition (mm)**
	1	**2**	**3**	**4**	**5**	
25%	NIL	NIL	NIL	NIL	NIL	NIL
50%	NIL	NIL	NIL	NIL	NIL	NIL
75%	NIL	NIL	NIL	NIL	NIL	NIL
100%	NIL	NIL	NIL	NIL	NIL	NIL
Legend: NIL stands for "no growth of microorganisms"						

Table 2 shows that no antifungal activity was observed in any of the concentrations of Moringa oleifera ethanolic leaf extract tested in all trials. No zones of inhibition were recorded at any of the tested

concentrations (25%, 50%, 75%, and 100%), indicating a complete lack of antifungal activity under the experimental conditions. Despite the lack of antifungal effect against *A. niger* observed in this study, these results did not preclude the effectiveness of *Moringa oleifera* against other fungal cultures. As shown in the study of Ahmad et al. (2021), it examined the antifungal activity of *Moringa oleifera* leaf and seed extracts against Botrytis cinerea, the pathogen responsible for gray mold disease in tomatoes, and found both extracts to demonstrate significant antifungal properties.

Another study by Isitua et al. (2016) evaluated the antifungal activity of *Moringa oleifera* leaf extracts against *Candida albicans*, a common cause of fungal infections in humans. The study found that both the methanolic and ethanolic leaf extracts of *Moringa oleifera* demonstrated significant antifungal activity against *Candida albicans*, with the methanol extract showing a higher zone of inhibition compared to ethanol.

Based on these findings, one possible inference is that *Moringa oleifera* may exhibit species-specific antifungal activity and could be ineffective against *Aspergillus niger.* Additionally, the ethanol extraction method used may not have effectively isolated the antifungal compounds, or the concentration of bioactive compounds in the extract may not have been potent enough to inhibit *A. niger*. It is also possible that the phytochemicals degrade during extraction or storage, leading to a loss of activity These findings suggest that further investigation using different extraction methods, solvents, or fungal targets may be necessary to fully explore the antifungal potential of *Moringa oleifera*.

Most Effective Concentration of Ethanolic Leaf Extract of *Moringa oleifera* Against *Aspergillus niger*

Table 3. Most Effective Concentration of Ethanolic Leaf Extract of *Moringa oleifera* Against *Aspergillus niger*

Concentration of *Moringa oleifera* ethanolic extract	**Trial 1 and 2 Zone of Inhibition (mm) Replicates**					**Average Zone of Inhibition (mm)**
	1	**2**	**3**	**4**	**5**	
100%	NIL	NIL	NIL	NIL	NIL	NIL

Table 3 shows that the 100% concentration of *Moringa oleifera* ethanolic leaf extract exhibited no antifungal activity against *Aspergillus niger*, as evidenced by a mean zone of inhibition of 0.00 mm. This indicates that, at its highest tested concentration, the ethanolic extract was ineffective against this particular fungal strain.This finding aligns with the study by Suraka et al. (2021), who examined the antifungal potential of methanolic and ethanolic extracts of *M. oleifera* leaves against *Aspergillus flavus* and *Rhizopus stolonifer*. Their results demonstrated that while both extracts had antifungal effects, the methanolic extract was significantly more potent, with minimum inhibitory concentrations (MICs) of 75 mg/mL for *A. flavus* and 100 mg/mL for *R. stolonifer*. This suggests that the type of solvent used in extraction plays a critical role in determining antifungal efficacy.

Moreover, Malhotra and Mandal (2018) confirmed the presence of bioactive phytochemicals—including tannins, flavonoids, glycosides, terpenoids, and phenols—in both aqueous and ethanolic extracts of *M. oleifera* leaves. While the ethanolic extract exhibited strong antibacterial activity against

both *Staphylococcus aureus* and *Escherichia coli*, its antifungal potential, at least against *A. niger*, appears limited.

Although *Moringa oleifera* is rich in phytochemicals with known antimicrobial properties, its antifungal efficacy—particularly in ethanolic form—may be constrained by factors such as the extraction method, target organism, and concentration. Methanolic extracts seem to be more effective against fungi, highlighting the importance of optimizing extraction techniques to fully harness the plant's antifungal potential. Moreso, despite the lack of antifungal effect against *A. niger* observed in this study, these results do not preclude the effectiveness of *Moringa oleifera* against other fungal cultures.

Comparison of the Most Effective Concentration of *Moringa oleifera* Ethanolic Leaf Extract and Itraconazole (Positive Control) Against *Aspergillus niger*

Table 4: Comparison of the Most Effective Concentration of *Moringa oleifera* Ethanolic Leaf

Trial	Sample/Concentration	Mean	Sd	Inferential statistics	p-value	Decision and Interpretation
1	**100% *Moringa oleifera* ethanolic leaf extract**	.000	.000	t=-17.371	.000*	H_0 rejected, Significant
	Itraconazole (positive control)	13.86	1.78			
2	**100% *Moringa oleifera* ethanolic leaf extract**	.000	.000	t=-29.393	.000*	H_0 rejected, Significant

Table 4 presents the comparative analysis between the highest concentration (100%) of *Moringa oleifera* ethanolic leaf extract and itraconazole (positive control) in inhibiting the growth of *Aspergillus niger*. The results showed that 100% *Moringa oleifera* extract produced no inhibition zones, with a mean of 0 mm across both trials. In contrast, itraconazole exhibited significant antifungal activity, with mean inhibition zones of 13.86 mm and 12.38 mm. An independent sample t-test was conducted, resulting in t-values of -17.371 and -29.393, both with p-values of 0.000. Since the p-values were less than the 0.05 significance level, this indicates a statistically significant difference between the two treatments.

Therefore, the null hypothesis was rejected, confirming that itraconazole was significantly more effective than the 100% concentration of *Moringa oleifera* ethanolic leaf extract in inhibiting *A. niger* under the experimental conditions. This highlights the extract's limited antifungal bioactivity against *A. niger*. The lack of inhibition by the *Moringa* extract suggests that its bioactive compounds may not be effective against *A. niger* at the tested concentration or under the experimental conditions. It could also indicate that ethanol extraction may not have isolated the necessary active compounds.

A previous study by Salah Omar Abdulali Habberrih et al. (2021) demonstrated that *Moringa oleifera* seed extracts were effective in inhibiting the growth of *Rhodotorulamucilaginosa* when used at higher concentrations. This suggests that *Moringa oleifera* had the potential to combat fungal growth, though the current study indicates that its effectiveness may be limited under specific experimental conditions. In contrast, itraconazole's strong antifungal activity can be attributed to its well-defined mechanism targeting ergosterol biosynthesis. This result suggests that further studies with different concentrations, solvents, or fungal species are needed to better explore the potential of *Moringa oleifera* as an antifungal agent.

5. Conclusion and Recommendations

Based on the salient findings of the study, the following conclusions were drawn. Itraconazole, the positive control, demonstrated consistent and significant antifungal activity across two trials, with average zones of inhibition of 13.86 mm and 12.38 mm, confirming its effectiveness in inhibiting *Aspergillus niger* growth. In contrast, Moringa oleifera ethanolic leaf extract, regardless of concentration (25%, 50%, 75%, or 100%), showed no measurable antifungal activity, with all trials yielding inhibition zones of 0 mm, suggesting that the extract does not contain antifungal compounds against *A. niger* under the conditions tested. Even at the highest concentration of 100%, the extract failed to exhibit any antifungal activity against *Aspergillus niger*, indicating that it lacks the necessary properties to inhibit the growth of this fungal strain under the experimental conditions. The comparison between the 100% concentration of Moringa oleifera extract (0 mm inhibition zone) and Itraconazole (mean zone of inhibition = 13.86 mm) clearly highlighted the difference in effectiveness. Itraconazole was significantly more effective than the Moringa oleifera extract, which showed no antifungal activity. Therefore, the null hypothesis was rejected, confirming that Itraconazole is significantly more effective than Moringa oleifera ethanolic leaf extract in inhibiting *Aspergillus niger*.

In accordance with the results and conclusions of the study, the researcher humbly suggests the following recommendations for possible applications and furtherance of the study. Future researchers could delve into and explore the potential antifungal effects of other parts of the *Moringa oleifera* plant. Additionally, exploring alternative polar solvents could provide a broader understanding of *Moringa oleifera*'s antifungal capabilities and reveal more effective extraction techniques for its active compounds. The usage of the Soxhlet method or other extraction methods available could enhance the recovery of phytochemical compounds in *Moringa oleifera* that are responsible for its antifungal effects. Lastly, future studies could investigate the potential antifungal effects of other parts of the *Moringa oleifera* plant, perhaps even exploring synergistic effects when combined with silver nanoparticles, to enhance its therapeutic potential against fungal infections.

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