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Antifungal Efficacy of Silver Nanoparticle-Potentiated Moringa oleifera Leaf Extract Against Candida albicans: An In Vitro Study

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ABSTRACT

Background: The rise of antifungal resistance in Candida albicans, a significant fungal pathogen, necessitates the exploration of novel therapeutic agents. This study evaluated the antifungal efficacy of Moringa oleifera leaf extract, both alone and enhanced with biogenically synthesized silver nanoparticles (AgNPs), against clinical isolates of Candida albicans. Aim: This study assessed the antifungal efficacy of a Moringa oleifera-silver nanoparticle formulation against Candida albicans by comparing its activity to the plain Moringa extract and the standard drug, Amphotericin B. Materials and Methods: Moringa oleifera leaf extract was prepared and used to synthesize AgNPs. Five clinical isolates of Candida albicans were subjected to various formulations using the agar well diffusion assay to determine antifungal activity. The tested groups included methanolic Moringa extract alone, and the extract combined with 1 mM, 2 mM, 3 mM, and 4 mM concentrations of AgNPs. As a positive control, amphotericin B was employed. After 24 hour, antifungal efficacy was measured by the zones of inhibition (ZOI). Result: Moringa methanol extract showed moderate antifungal activity (mean ZOI = 13.2 mm), which was enhanced by silver nanoparticles in a dose-dependent manner, reaching 17.2 mm at 4 mM, however, the differences among AgNP concentrations were not statistically significant ($p > 0.05$). All Moringa formulations remained less potent than Amphotericin B (24.2 mm, $p < 0.05$) Conclusion: Silver nanoparticles biogenically synthesized using Moringa oleifera leaf extract significantly enhance the extract's inherent antifungal properties against Candida albicans. The synergistic, dose-dependent activity suggests that this combination holds promise as a potential alternative or adjunctive therapeutic agent, meriting further investigation.

Keywords: Antifungal Activity, Candida albicans, Moringa oleifera, Silver Nanoparticles (AgNPs), Synergistic Effect

How to cite this article: Prakash SJ, Jagannatha GV, Benedict CH, Nagaland T, Shanmugam P, Appadurai M. Antifungal efficacy of silver nanoparticle-potentiated Moringa oleifera leaf extract against Candida albicans: an in vitro study. Int J Drug Deliv Technol. 2026;16(5): 1137-1149. DOI: 10.25258/ijddt.16.5.152

INTRODUCTION

Fungi are highly diverse, with approximately 12 million species worldwide—most of which remain uncategorized. They collectively cause around 1.6 million deaths annually.[1,2] Fungal pneumonias and invasive infections in organs like the brain, kidneys, and bloodstream are significant causes of death, especially in immunocompromised people.[3] Fungi are usually harmless residents of the human microbiome, but can lead to contamination if immunity is compromised.[4] *Candida albicans,* the most common species in its genus, causes about 70% of

mucosal and systemic fungal infections worldwide. It is the leading cause of fatal invasive infections, with a mortality rate of about 40% in hospitals.[5]

In October 2022, the WHO[6] Listed 19 fungal pathogens, including *Candida albicans*, as high-priority threats to human health. Despite increasing risks, fungal infections are underfunded, with limited diagnostics, treatments, and research, exacerbating their global impact. The rise of multidrug-resistant pathogens, such as *Candida*, exacerbates the problem, narrows treatment options, and underscores the need for new antifungal drugs. Fungal infections impose significant economic burdens, including healthcare costs and lost productivity, burdening patients and healthcare systems.[7]

Management of fungal infections involves antifungal agents such as polyenes, azoles, echinocandins, and allylamines, each with distinct mechanisms of action.[8] Amphotericin B, a polyene from Streptomyces nodosus, has been a mainstay for severe infections such as *Candida albicans* since the 1950s, especially for critically ill or immunocompromised patients.[9,10] Amphotericin B kills fungi by linking to ergosterol in their cell membranes, creating pores that disrupt cell integrity and cause rapid pathogen death and recovery from candidiasis.[11]

Amphotericin B is an effective antifungal but has severe toxicities like nephrotoxicity and anaemia, requiring careful monitoring. Hence, to combat this, herbal alternatives with fewer side effects are increasingly studied. [12] Exploring medicinal plants offers a promising solution amid rising drug resistance and current limitations.[7,13]

The adaptable Moringa oleifera plant, which belongs to the Moringaceae family, has been grown for centuries all over the world because of its therapeutic and nutritional advantages. Native to India, it thrives in tropical regions.[14,15] Its leaves are rich in nutrients and antioxidants, contributing to its traditional use in treating over 300 diseases, including cancer.[16] It possesses anti-diabetic, anti-inflammatory, antioxidant, anti-apoptotic, and neuroprotective properties, thanks to compounds such as flavonoids, phenols, and vitamins C and E.[14,17]

Oral candidiasis, primarily caused by *Candida albicans*, affects individuals with compromised immune systems, resulting in discomfort and potential complications. Resistance and side effects from traditional antifungals highlight the need for safer options. Plant-based phenolic compounds, with their antifungal and antioxidant properties, can inhibit the biofilm formation, as well as the growth of *Candida*. They may serve as safer, effective alternatives or supplements for treating oral candidiasis. [18]

Moringa methanolic extract (MME) shows promise as an antifungal, effective against *Candida albicans*, and with potential for treating fungal infections.[19] However, the low lipophilicity of MEE limits its skin penetration.[20] This can be addressed through nano-formulation. Nanotechnology enhances herbal remedies by overcoming limitations like poor bioavailability, low solubility, and instability of plant compounds.[21,22] It utilizes the small size, high surface area, and customizable surfaces of nanoparticles for improved biological interactions.[23] Nanoparticles improve medicinal plant efficacy by increasing bioavailability, stability, and cellular uptake, enabling targeted, sustained release, and overcoming drug resistance.[24,25] Common nanoparticles like zinc, copper, bismuth, gold, titanium, and iron are used as plant adjuvants.[16,20,26] They enhance the antioxidant, antimicrobial, and anti-inflammatory effects of plant extracts, thereby amplifying the benefits of herbal medicine. Our study utilized silver nanoparticles (Ag-NPs) due to their broad-spectrum antimicrobial properties to enhance the antifungal effect of *Moringa* leaf extract.

Silver nanoparticles (Ag-NP) enhance skin permeation, control drug release, and have potent antimicrobial activity at low concentrations. [27] They have minimal toxicity to cells, thereby reducing the risk of resistance.[28] Their antifungal action involves damaging fungal cell walls and membranes, increasing permeability, and generating reactive oxygen species (ROS) that damage DNA.[29]

This study aims to develop a safer and more effective antifungal treatment by combining the natural antifungal properties of *Moringa oleifera* leaf extract with silver nanoparticles to enhance activity and reduce side effects. An in vitro assessment against *Candida albicans* was conducted to evaluate this formulation.

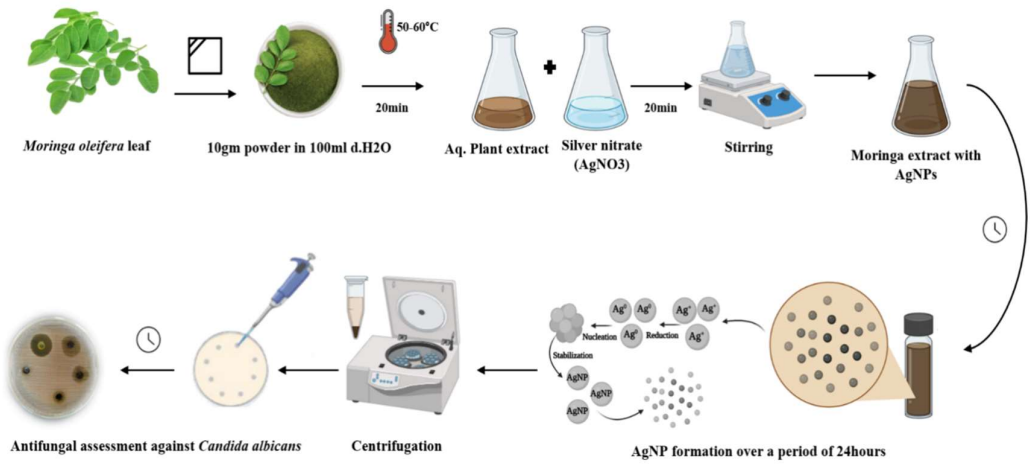
MATERIALS AND METHODS

Preparation of *Moringa oleifera* Leaf Extracts

Fresh *Moringa oleifera* leaves were harvested, washed, allowed to dry in the shade, and then ground into powder form. To prepare the methanolic extract, 10 g of this powder were mixed with 100 ml of methanol and stirred at 45°C for 30 minutes. After filtering the mixture, extract was concentrated by evaporating the solvent. For the aqueous mixture, 10 g of powder was combined with 100 ml of deionized water, exposed to heat at 50-60°C for 20 minutes, cooled, and filtered.

Green Synthesis and Characterization of Silver Nanoparticles (AgNPs)

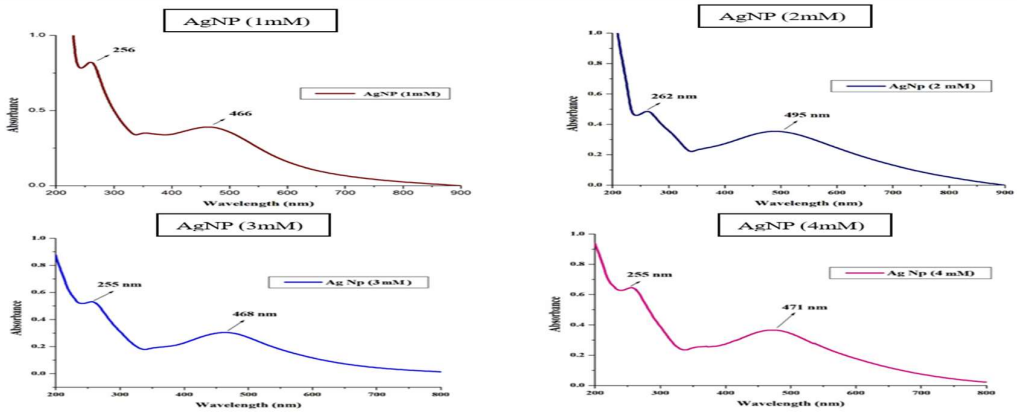
Aqueous solutions of silver nitrate ($AgNO_3$) at concentrations of 1 mM, 2 mM, 3 mM, and 4 mM were formulated. To synthesize AgNPs, 10 ml of *Moringa* extract was blended with each $AgNO_3$ solution and mixed for 20 minutes at normal temperature (Figure 1). The formation of AgNPs was confirmed by a colour change from yellow to light black.



Process of synthesizing AgNPs using moringa leaf extract

The synthesized AgNPs were assessed using several methods. UV-Vis spectroscopy confirmed their formation by showing absorbance peaks between 400 and 500 nm (Figure 2). Dynamic Light Scattering (DLS) measured the nanoparticles' hydrodynamic size and Polydispersity Index (PDI). Zeta Potential (ZP) analysis evaluated the electrostatic charge and stability of the nanoparticle dispersion.

Fungal Strains and Culture



UV-Vis spectroscopy absorption graph of AgNP

This study utilized five clinical isolates of *Candida albicans*. The strains were kept on Sabouraud Dextrose Agar (SDA) and stored at 4°C. For experimental procedures, fungal suspensions were made in peptone water and adjusted to a 0.5 McFarland standard, equating to 1.5×10^8 cells/ml.

Evaluation of Antifungal Activity

The antifungal activity was analyzed using the agar well diffusion method on Mueller-Hinton Agar (MHA) with 2% glucose. A lawn of each *C. albicans* isolate was prepared, and 5 mm wells were drilled into the agar. Each well was filled with 100 µl of the test solutions: methanolic Moringa extract, *Moringa*-AgNP formulations (1 to 4 mM), Amphotericin B served as the positive control, while 90% DMSO (Dimethyl sulfoxide) is used as negative control. Following an overnight incubation period at 37°C, the inhibition zones' diameters were measured in millimetres.

Statistical Analysis

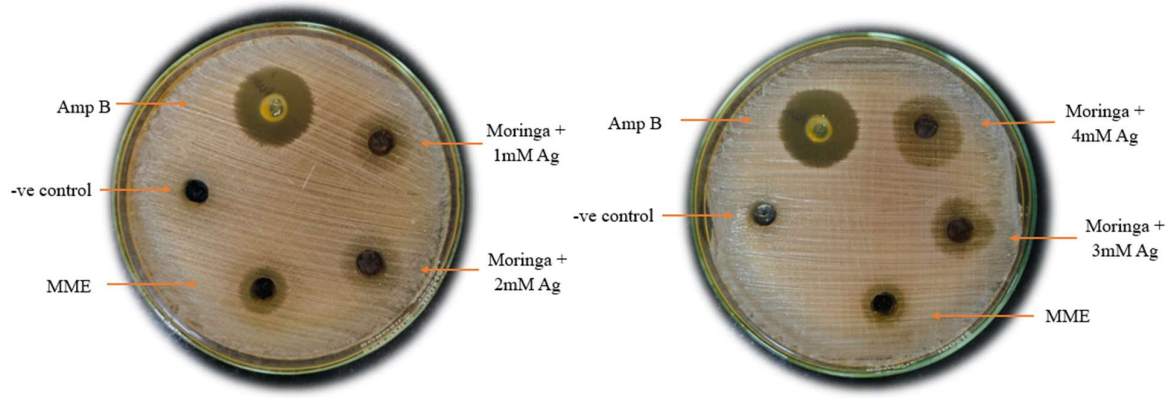
The statistical analyses were performed using IBM SPSS, Version 25.0 (IBM Corp., Armonk, NY, USA). Antifungal efficacy (Zone of Inhibition) was compared between the plain extract of Moringa oleifera and four different concentrations of AgNP potentiated Moringa extract keeping Amphotericin B as positive control, using One-way Analysis of variance (ANOVA). Results were deemed statistically significant if the p-value was below 0.05. All quantitative data are expressed as the mean ± standard deviation (SD).

RESULTS

The antifungal activity of *Moringa oleifera* methanolic extract (MME) and its combination with silver nanoparticles (AgNPs) was assessed against five clinical isolates of *Candida albicans*. MME alone showed moderate antifungal effects, with a mean zone of inhibition (ZOI) of 13.2 mm ± 1.92 mm. An apparent dose-dependent increase was observed when AgNPs were added to the extract, with the mean ZOI rising as the concentration of AgNPs increased: 13.4 ± 1.67 mm at 1 mM, 15.0 ± 0.70 mm at 2

mM, 16.8 ± 0.44 mm at 3 mm, and reaching a maximum of 17.2 ± 1.09 mm at 4 mM (Figure 3). Amphotericin B, used as the positive control, displayed significantly higher antifungal activity than all tested formulations, with a mean ZOI of 24.2 mm ± 2.68 mm ($p < 0.05$). However, differences in activity among the *Moringa* formulations were not statistically significant ($p > 0.05$). These findings are depicted in Table 1 and Figure 4.

- Mean ZOI of Moringa leaf extract and silver nanoparticles' antifungal activity



Zone of inhibition of antifungal activity of the prepared solutions

Antifungal Activity (ZOI) of Moringa Extract and Silver Nanoparticles Against Candida albicans (mean ±SD)

Sample	Moringa methanol extract(MME)	Moringa +1 mM Ag	Moringa + 2 mM Ag	Moringa +3 mM Ag	Moringa +4 mM Ag	Amphotericin B
1	14	12	16	17	19	26
2	12	14	15	17	17	23
3	16	16	15	16	17	28
4	11	13	14	17	17	22
5	13	12	15	17	16	22
Mean	13.2	13.4	15	16.8	17.2	24.2
SD	1.92	1.67	0.70	0.44	1.09	2.68
One-way ANOVA statistics, F= 31.736, P < 0.05						

DISCUSSION

The present study evaluated the antifungal effects of Moringa leaf extract, alone and combined with silver nanoparticles (AgNPs), against *Candida albicans*, a pathogen responsible for a range of infections. *Moringa oleifera* leaves are rich in bioactive compounds[20] like flavonoids, alkaloids, phenolics, saponins, tannins, and isothiocyanates, which contribute to their antifungal properties. The results confirmed that *Moringa* leaf extract possesses innate antifungal activity, supporting previous research findings.[7,14,16,19] These compounds in *moringa* exhibit antifungal effects by damaging fungal cell membranes, disrupting essential enzymes, and interfering with cellular processes.[30] Phenolic compounds affect ion balance, gene transcription, membrane integrity, and ergosterol biosynthesis in *Candida albicans*.[31] Flavonoids such as luteolin, quercetin, and rutin in *Moringa oleifera* also strongly inhibit *C. albicans*.[17] This broad-spectrum activity highlights *Moringa oleifera* as a promising natural antifungal agent.

Silver Nanoparticles (AgNPs) exhibit strong antifungal effects[32,33] by disrupting fungal cell membranes and walls, resulting in the leakage of cellular contents and cell death. They primarily inhibit mycelial growth and spore germination, thereby interfering with the early stages of the fungal life cycle. AgNPs also bind to enzymes, induce the production of reactive oxygen species (ROS), and cause oxidative DNA damage, while disrupting energy metabolism, signalling pathways, and genetic processes.[34]

This study highlights the enhanced antifungal effect of *Moringa* leaf extracts combined with AgNPs. It is essential to note that the nanoparticles are synthesized using a green method, which utilizes plant compounds, such as polyphenols and flavonoids from *M. oleifera*, as natural reducing agents to convert silver ions into nanoparticles.[35,36]

The combination of *Moringa oleifera* leaf extracts with silver nanoparticles (AgNPs) significantly improved antifungal effectiveness, marking an important discovery in this study. This synergistic effect boosted the fungicidal activity at all tested AgNP concentrations (1 mM, 2 mM, 3 mM, and 4 mM $AgNO_3$), with the most substantial effect seen at 4 mM. Our study found the size of AgNPs ranging from 40-75nm, averaging 55-60nm, aligning with previous research using *Moringa oleifera* extract that reported nanoparticle sizes around 57nm. This consistency supports the robustness of our green synthesis method.[37] AgNPs are effective antifungal agents that disrupt cell membranes and enhance the uptake of phenolic compounds from *Moringa* leaf extract, creating a synergistic effect. This combination enhances antifungal efficacy and may lead to safer and more potent antifungal strategies. [32]

The study shows UV-visible spectra between 460-500 nm, aligning with typical AgNP surface plasmon resonance (SPR) bands,[38] usually between 400-500 nm, depending on size and shape. Increased AgNP concentration correlates with higher absorbance, indicating more nanoparticle formation. The 4 mM sample peaks at ~495 nm, suggesting it's optimal for the desired optical properties.[35] Higher precursor concentrations generally produce more nanoparticles and greater optical density, which is vital for controlling synthesis and tailoring optical features.[39,40] Increasing AgNP concentration enhances antifungal activity by disrupting fungal cells and improving the delivery of *Moringa oleifera's* bioactive compounds. However, these combinations are less effective than liposomal Amphotericin B (LAmB).

Amphotericin B (positive control) has a broad antifungal range and is highly effective against pathogens like *Candida albicans*, a common cause of fungal infections. It is the "gold standard" for treating systemic fungal infections, especially in critically ill or immunocompromised patients.[10] First, this drug binds to the fungal cell wall in liposomes. Then, it's released to target the fungal membrane, probably because it has a higher affinity for ergosterol.[11] Although effective, it has high risk of toxicity like nephrotoxicity, infusion reactions, electrolyte imbalances, and anaemia, requiring close monitoring.[41] Amphotericin B is potent against *Candida* but has safety concerns. The *Moringa*-AgNPs are less strong but safer due to their herbal *Moringa* component. In this study, 95% DMSO was used as a negative control and showed no inhibitory effects on five *Candida* isolates, confirming that the solvent did not affect antifungal results.

The rise of drug-resistant *Candida* strains limits treatment options and increases patient mortality risk.[42] This highlights the urgent need for new antifungal agents with different mechanisms. *M. oleifera* leaf extract, known for its antifungal properties and enhancement when combined with AgNPs, offers a promising alternative. Using natural products like *Moringa* leaves with nanotechnology could provide a safer, more sustainable solution for fungal infections, potentially overcoming some issues of traditional antifungals.

Further research is necessary to understand how *Moringa* leaf extract and AgNPs work together. Analysing fungal cell morphology with TEM and SEM, and identifying bioactive compounds using GC-MS and HPLC, could clarify their mode of action. Though in vivo research is crucial to assess safety and effectiveness for possible therapy the current study suggests potential for exploring new antifungal agents.

This study demonstrates that combining Moringa leaf extract with silver nanoparticles (AgNPs) significantly enhances antifungal activity against Candida albicans, underscoring their potential for developing effective, less toxic treatments. This foundational work underpins additional research on mechanisms and in vivo studies to help translate these findings into clinical practice.

Strengths and Limitations

This study highlights several key strengths in that it confirmed the antifungal effects of *Moringa* leaf extract reinforced with AgNPs against *Candida albicans*, showcasing their individual and combined potency. Of significant benefit is the green synthesis technique used for synthesizing the AgNPs, making it both eco-conscious and cost-effective. Furthermore, the well-characterized nanoparticles ensure consistency and reproducibility in our findings. Significantly, this combination offers a potential solution to the issue of drug resistance, which is becoming increasingly problematic.

The study focused solely on Candida albicans and did not investigate the mechanism of interaction between Moringa leaf extract, AgNPs and the fungus. Future research shall include other *Candida* species, particularly drug-resistant strains, and further explore these mechanisms. Despite these limitations, the promising preliminary results highlight the potential of *Moringa* leaf extract potentiated by AgNPs as antifungal agents, providing a solid foundation for future studies.

CONCLUSION

The present study indicated that *Moringa oleifera* leaf extract reinforced with silver nanoparticles enhances the antifungal activity against *Candida albicans* in a dose-dependent manner. Combining natural substances with nanotechnology presents a promising new approach to antifungal treatment. With the rising prevalence of drug resistance, this formulation could be valuable for further research and in vivo studies aimed at developing effective clinical therapies.

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Acknowledgments – We express our gratitude to the Faculty Of Allied Health Sciences, Chettinad Academy of Research and Education, for their methodological guidance and technical support, as well as access to their laboratory facilities.