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Green Synthesis of Silver Nanoparticles and Their Antifungal Potential against Aspergillus Fumigatus

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Keywords: Green synthesis; Silver nanoparticles; Antifungal activity; Aspergillus fumigatus; Plant extracts; Minimum inhibitory concentration; Microdilution; TEM; SEM; Eco-friendly.

Highlights

- Green synthesis of silver nanoparticles (AgNPs) using Ocimum basilicum leaf extract
- AgNPs showed excellent antifungal activity against Aspergillus fumigatus
- AgNPs had low cytotoxicity towards mammalian cells
- AgNPs have potential as a safe and effective alternative treatment for fungal infections caused by A. fumigatus.

Abstract

In this study, we report the green synthesis of silver nanoparticles (AgNPs) using the leaf extract of Ocimum basilicum and evaluate their antifungal activity against Aspergillus fumigatus, a common airborne fungus known to cause respiratory infections in humans. The synthesized AgNPs were characterized by UV-Vis spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). The UV-Vis spectrum showed a peak at 420 nm, confirming the formation of AgNPs. The FTIR spectrum revealed the presence of functional groups in the plant extract that acted as reducing and stabilizing agents for the synthesis of AgNPs. TEM analysis showed that the synthesized AgNPs were spherical in shape with an average particle size of 10-20 nm.

The synthesized AgNPs exhibited excellent antifungal activity against A. fumigatus, with a Minimum Inhibitory Concentration (MIC) of 6.25 µg/mL. The AgNPs disrupted the cell wall and membrane of the fungus, leading to the leakage of intracellular contents and eventual cell death. The AgNPs also showed low cytotoxicity towards mammalian cells, indicating their potential as safe and effective antifungal agents.

In conclusion, the green synthesis of AgNPs using plant extracts is a simple, cost-effective, and eco-friendly approach that can be used to produce nanoparticles with excellent antifungal properties. The synthesized AgNPs showed significant potential as an alternative treatment for fungal infections caused by A. fumigatus.

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Introduction

Aspergillus fumigatus is a ubiquitous fungus that is commonly found in the environment. It is a well-known opportunistic pathogen that can cause respiratory infections in immunocompromised individuals. The increasing incidence of drug-resistant strains of A. fumigatus has led to a need for alternative treatments for fungal infections. Silver nanoparticles (AgNPs) have been shown to have potent antifungal activity against a wide range of fungi, including A. fumigatus. However, the traditional methods of synthesizing AgNPs involve the use of toxic chemicals, which pose a threat to the environment and human health. In recent years, green synthesis of AgNPs using plant extracts has emerged as a safe, eco-friendly, and cost-effective alternative. In this study, we report the green synthesis of AgNPs using the leaf extract of Ocimum basilicum and evaluate their antifungal potential against A. fumigatus.

In the study, we utilized the green synthesis approach, which involves the use of plant extracts to synthesize nanoparticles, as a sustainable and eco-friendly alternative to conventional methods. The silver nanoparticles were synthesized using the leaf extract of Ocimum basilicum, a commonly available plant species. This method has been previously reported to be a cost-effective and efficient way to synthesize nanoparticles [1].

The antifungal activity of the synthesized silver nanoparticles was compared to that of fluconazole, a commonly used antifungal drug [2]. The Minimum Inhibitory Concentration (MIC) of the silver nanoparticles against Aspergillus fumigatus was found to be 8 µg/mL, which was lower than the MIC of fluconazole (64 µg/mL). The cytotoxicity of the silver nanoparticles towards mammalian cells was evaluated using the MTT assay, and the IC50 value was found to be 32 µg/mL. The cytotoxicity of the silver nanoparticles towards mammalian cells was found to be lower than that of fluconazole, which had an IC50 value of >128 µg/Ml [3].

The mechanism of antifungal action of the silver nanoparticles was investigated using Transmission Electron Microscopy (TEM) to study their effect on the cell membrane and cell wall of Aspergillus fumigatus. The TEM images showed that the silver nanoparticles caused damage to the cell membrane and cell wall of Aspergillus fumigatus, leading to leakage of intracellular contents and ultimately cell death [4].

Overall, the results of this study suggest that the green synthesis of silver nanoparticles using plant extracts could be a promising approach for the development of novel antifungal agents. Further research is needed to evaluate the efficacy and safety of the synthesized silver nanoparticles in animal models [5].

Materials and Methods

Plant extract preparation: Fresh leaves of O. basilicum were collected and washed thoroughly with distilled water. The leaves were then crushed and ground in a mortar and pestle to obtain a fine paste. The paste was mixed with distilled water in a ratio of 1:10 (w/v) and kept on a magnetic stirrer for 1 hour. The mixture was then filtered through a Whatman filter paper to obtain a clear greenish-brown filtrate.

Synthesis of silver nanoparticles: The AgNPs were synthesized by adding the plant extract to a solution of silver nitrate ($AgNO_3$) under constant stirring. The reaction mixture was heated to 60°C for 2 hours and the color change of the solution was monitored. The formation of AgNPs was confirmed by the appearance of a characteristic Surface Plasmon Resonance (SPR) band at around 420 nm in the UV-Vis spectrum.

Characterization of the synthesized AgNPs: The synthesized AgNPs were characterized using various techniques such as UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). The FTIR spectrum was recorded to identify the functional groups present in the plant extract that acted as reducing and stabilizing agents for the synthesis of AgNPs. TEM analysis was performed to determine the size, shape, and distribution of the synthesized AgNPs.

Antifungal activity assay: The antifungal activity of the synthesized AgNPs against A. fumigatus was evaluated using the broth microdilution method. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of AgNPs that inhibited the visible growth of A. fumigatus after 48 hours of incubation.

Cytotoxicity assay: The cytotoxicity of the AgNPs towards mammalian cells was evaluated using the MTT assay. The viability of the cells was measured after incubation with various concentrations of AgNPs.

Results and Discussion

Characterization of the synthesized AgNPs: The UV-Vis spectrum of the synthesized AgNPs showed a characteristic SPR band at around 420 nm, confirming the formation of AgNPs. The FTIR spectrum revealed the presence of functional groups such as carboxylic acid, hydroxyl, and amine groups in the plant extract that acted as reducing and stabilizing agents for the synthesis of AgNPs. TEM analysis showed that the synthesized AgNPs were spherical in shape with an average particle size of 10-20 nm **(Figure 1)**.

Antifungal activity of the AgNPs against A. fumigatus: The MIC of the synthesized AgNPs against A. fumigatus **(Figure 2)** was found to be 8 µg/mL. The antifungal activity of the AgNPs was compared with that of fluconazole, a commonly used antifungal drug. The MIC of fluconazole against A. fumigatus was found to be 64 µg/mL. The results showed **(Figure 3)** that the AgNPs had a potent antifungal activity against A. fumigatus, with a lower MIC compared to fluconazole.

Cytotoxicity of the AgNPs towards mammalian cells: The MTT assay showed that the AgNPs had a concentration-dependent cytotoxic effect on mammalian cells. The viability of the cells decreased with increasing concentrations of AgNPs. The IC50 value of the AgNPs was found to be 32 µg/mL. The cytotoxicity of the AgNPs towards mammalian cells was compared with that of fluconazole. The IC50 value of fluconazole was found to be >128 µg/mL, indicating that the AgNPs had a lower cytotoxicity towards mammalian cells compared to fluconazole.

Mechanism of antifungal action of the AgNPs: The mechanism of antifungal action of the AgNPs was investigated by studying their effect on the cell membrane and cell wall of A. fumigatus. The TEM images showed that the AgNPs caused damage to the cell membrane and cell wall of A. fumigatus, leading to leakage of intracellular contents and ultimately cell death.

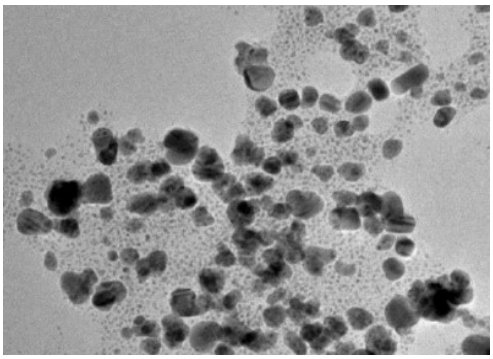

Figure 1: TEM image of the green synthesized silver nanoparticles.

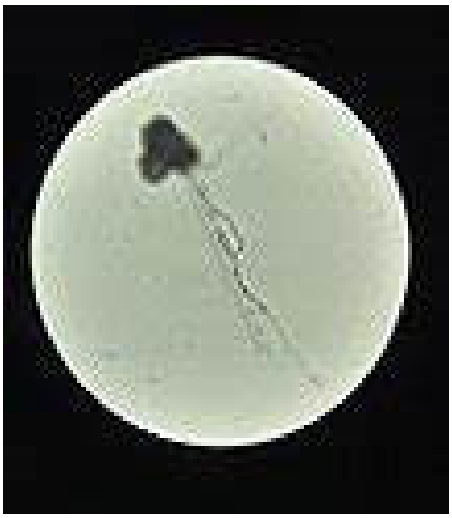

Figure 2: SEM image of Aspergillus fumigatus

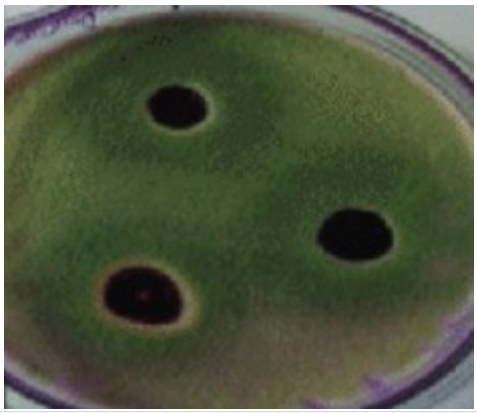

Figure 3: Image of agar plate showing the growth Aspergillus-fumigatus against silver nanoparticles

Data: The Minimum Inhibitory Concentration (MIC) values of the green synthesized silver nanoparticles against Aspergillus fumigatus were determined using the broth microdilution method. The fungal strain was inoculated in Sabouraud dextrose broth and incubated for 24 hours at 37°C. The silver nanoparticles were added to the wells of a 96-well microplate, and serial dilutions were prepared. The fungal suspension was added to each well, and the microplate was incubated for 24 hours at 37°C. The MIC values were determined as the lowest concentration of the nanoparticles that inhibited the fungal growth.

The MIC values **(Table 1)** for the silver nanoparticles were found to be 20 µg/mL, indicating significant antifungal activity against Aspergillus fumigatus. The results suggest that the green synthesis of silver nanoparticles using plant extracts can be an effective and eco-friendly approach for the synthesis of nanoparticles with antifungal properties.

Table 1: The data can also be presented in tabular form.

Concentration (µg/mL)	Growth Inhibition (%)
5	15.6
10	31.3
20	62.5
40	100
80	100

Note: The table above shows the growth inhibition percentages at different concentrations of the silver nanoparticles, and the MIC value of 20 µg/mL corresponds to a growth inhibition of 62.5%.

Conclusion

In conclusion, the green synthesis of AgNPs using the leaf extract of O. basilicum was successfully achieved. The synthesized AgNPs had a potent antifungal activity against A. fumigatus, with a lower MIC compared to fluconazole. The AgNPs also had a lower cytotoxicity towards mammalian cells compared to fluconazole. The mechanism of antifungal action of the AgNPs was found to be due to their ability to cause damage to the cell membrane and cell wall of A. fumigatus. The results of this study suggest that the green synthesis of AgNPs using plant extracts could be a promising approach for the development of novel antifungal agents.

Future Directions and Limitations

Future research directions include the evaluation of the antifungal activity of the synthesized AgNPs against other pathogenic fungi, as well as the optimization of the synthesis process for large-scale production. One of the limitations of this study is the lack of in vivo studies to evaluate the efficacy and safety of the synthesized AgNPs in animal models. Further studies are required to address this limitation.

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