Green Synthesis of Silver Nanoparticles from Rosebay leaf extract for Antifungal Activities

Dr. P. Bhavani[1*], Ms. G. Ruby[2]

[1*]Assistant Professor**,** R.V. Govt.Arts College Chennai, Tamilnadu- 600 048, India.

[2]Assistant Professor**,** PERI Institute of Technology**,** Chennai, Tamilnadu- 600 048, India.

Abstract

Nanotechnology has emerged as a promising approach in antifungal activities, Silver nanoparticles (AgNPs) are among the most studied nanomaterials due to its antifungal properties against a variety of pathogens. The controlled release and targeted delivery of antifungal agents through nanotechnology enhance efficiency while minimizing toxicity, making it a revolutionary tool in combating fungal diseases in both human health and crop protection. This study aimed to assess the antifungal properties of silver nanoparticles (AgNPs) synthesized through a green chemistry approach using the leaf extract of Rosebay. The antifungal activity was evaluated against three pathogenic Candida albicans isolates using the gel diffusion method and by determining the minimum inhibitory concentration (MIC). AgNPs were successfully synthesized utilizing Rosebay leaf extract and silver nitrate, with the plant extract serving as both a reducing and stabilizing agent. The synthesized nanoparticles were characterized through Energy Dispersive X-ray (EDX), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Atomic Force Microscopy (AFM). The crystalline structure of the AgNPs was confirmed using X-ray diffraction analysis.

Keywords: Nanotechnology, Antifungal agent, Energy Dispersive X-ray (EDX), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Atomic Force Microscopy (AFM).

INTRODUCTION

Fungal infections cause significant threats to human health, agriculture, and food security. The rise of antifungal resistance makes necessary the development of alternative treatments. Silver nanoparticles (AgNPs) have gained attention as effective antifungal agents due to their ability to disrupt fungal cells, prevent biofilm formation, and enhance treatment effectiveness. Other nanoparticles, such as zinc oxide (ZnO), copper oxide (CuO), and chitosan-based nanocomposites, exhibit strong antifungal effects by inhibiting biofilm formation and fungal growth [1]. These nanomaterials are increasingly used in medicine, agriculture, and food preservation to prevent fungal infections.

Many conventional physical and chemical methods used to produce nanomaterials often result in toxic byproducts that can be harmful to the environment. As a result, researchers are increasingly turning to safer alternatives, such as biological synthesis and green chemistry. Green chemistry has been widely adopted for the too easy synthesis of silver nanoparticles (AgNPs) due to its simplicity and environmental benefits [2]. The growing emphasis on sustainability has led to a

shift toward eco-friendly nanoparticle synthesis methods, with plant extracts being commonly used as natural reducing agents[3]. Silver nanoparticles are extensively utilized in both industry and medicine due to their antibacterial, antifungal, and antiparasitic properties. They are a crucial component of nanotechnology, as they do not cause biosynthesis alterations in living cells, thereby preventing microbial resistance development [4].

Silver nanoparticles (AgNPs) have emerged as powerful antifungal agents due to their unique physicochemical properties. Their broad-spectrum efficiency against pathogenic fungi, combined with their biocompatibility, makes them valuable in medical, agricultural, and environmental applications [5]. There are two primary approaches to synthesizing nanoparticles: the bottom-up and top-down methods. The top-down approach involves breaking down larger structures into nanoscale materials through mechanical processes. In contrast, the bottom-up approach assembles atoms or molecules to form nanoscale structure. AgNPs can be synthesized through chemical, physical, and biological methods. Among these, green synthesis has gained popularity due to its eco-friendly nature [6-7]. Plant extracts, bacteria, fungi, and algae serve as natural reducing and stabilizing agents, eliminating the need for hazardous chemicals. This study focuses on evaluating the effectiveness of silver nanoparticles synthesized via green chemistry using Rosebay as an antifungal agent.

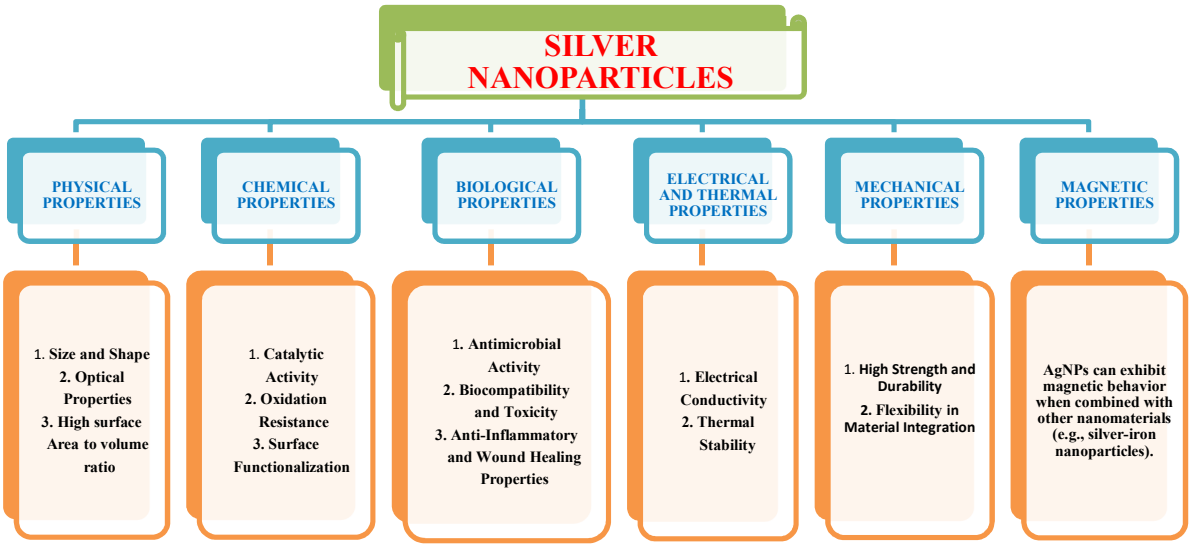

Figure: 1 Properties of Silver Nanoparticles

Figure 2: Application of Silver Nanoparticles

Materials and Methods:

Preparation of Silver Nitrate Solution:

To prepare the solution, 16.98 g of $AgNO_3$ was dissolved in 100 mL of distilled water at room temperature. The mixture was then stirred for 15 minutes to ensure uniformity. To prevent oxidation, the prepared solution was stored in a dark container.

Preparation of Rosebay:

The leaves of Rosebay were thoroughly washed multiple times with water, followed by sterilization using 50% alcohol to eliminate any foreign contaminants such as dust particles. Afterward, they were rinsed more than five times with distilled water and cut into small pieces. To prepare the extract, five grams of finely chopped Rosebay leaves were boiled in 20 mL of sterile distilled water for 45 minutes. The resulting broth solution was filtered using Whatman filter paper. This extraction process was repeated several times, and the final broth solution was stored in a refrigerator at 30°C for further study.

Synthesis of silver Nanoparticles:

For the synthesis of silver nanoparticles (AgNPs), a specific volume of Rosebay extract (1 mL) was added to 9 mL of 0.01M $AgNO_3$ solution. The mixture was then heated at 80°C for 15 minutes, as shown in Figure 3. The solution gradually turned brown, indicating the successful formation of AgNPs.

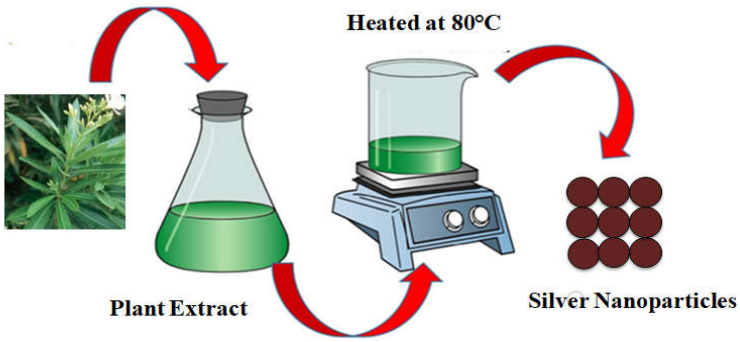

Figure 3: Synthesis of Silve Nanoparticles

Concentration of Prepared Silver Nanoparticles:

The concentration of silver nanoparticles (AgNPs) was determined by the below calculation.

Calculation of the Average Number of Atoms per Nanoparticle
The number of atoms per nanoparticle (N) was calculated using the following equation:

$$\mathbf{N} = \frac{N_A \ MD^3}{6\pi\rho}$$

Where

N = Number of atoms per nanoparticle
π = 3.14
ρ = Density of silver (10.5 g/cm³)
D = Average diameter of nanoparticles (20.56 nm)
M = Atomic mass of silver (107.868 g)
N_A = Avogadro's number (6.023×10^{23})

Assuming that all silver ions were completely converted into AgNPs, the calculated value of N was 22,105,954 atoms per nanoparticle.

Determination of Molar Concentration of AgNPs Solution

The molar concentration of the AgNPs colloidal solution was calculated using the following formula

$$\mathbf{C} = \frac{\mathbf{N_T}}{\mathbf{NV}}$$

Where

C = Molar concentration of Ag NPs colloidal solution
N_T = Total number of silver atoms introduced as $AgNO_3$ (1mM)
N = Number of atoms per nanoparticle (from equation 1)
V = Volume of the reaction colloidal solution (in liters)

Based on these calculations, the molar concentration of the AgNPs solution was determined to be **487.95 µg/mL**.

Instrumentation:

The production of silver nanoparticles was initially indicated by a color change from pale yellow to brown. Their formation was further confirmed using X-ray diffraction (XRD). Additional characterization was performed using a scanning electron microscope (SEM) to analyze the morphology, size, and distribution of the nanoparticles [8-9]. Atomic force microscopy (AFM) was employed to verify the shape and determine the average grain size of the synthesized nanoparticles.

The silver nanoparticles (AgNPs) synthesized from Rosebay were evaluated for their antifungal activity using the well-diffusion method on agar plates and the minimum inhibitory concentration (MIC) method.The inoculum was prepared using 24-hour-old cultures grown on Sabouraud dextrose agar at 25°C. A single colony was suspended in a sterile saline solution (0.85%) to match the 0.5 McFarland standard (0.5–2.5 × 10^5 CFU/mL) [9-10]. The Candida albicans suspension was then evenly spread onto three different plates using sterile cotton swabs.

The antifungal activity of the synthesized AgNPs was evaluated using the broth micro dilution assay. The minimum inhibitory concentration (MIC) of the AgNPs solution was determined following the EUCAST guidelines using a 96-well microdilution plate. The AgNPs solution was serially diluted in YPD broth (2% yeast extract, 3% peptone water, 3% dextrose) in a 1:2 ratio to obtain seven different concentrations. A 10 µL aliquot of the prepared Candida albicans suspension was added to each well[11-15].

Two positive controls were included. One contains Candida albicans with YPD broth and another with Candida albicans and Rosebay leaf extract. Additionally, two negative controls were used: YPD broth with the AgNPs solution and YPD broth with Nystatin solution. For the well-diffusion assay, 55 µL of the serially diluted AgNPs solution was added to seven wells (each 5 mm in diameter) on the agar plates. A standard Nystatin solution was used as a positive control in the eighth well, while a 55 µL $AgNO_3$ solution served as a negative control in the ninth well. After 24 hours of incubation at 25°C, the inhibition zones of different AgNP suspensions were measured to assess their antifungal effectiveness [15-20].

Result and Discussion

X-ray diffraction (XRD) studies

The polycrystalline nature of silver nanoparticles (AgNPs) was confirmed through X-ray diffraction (XRD) analysis. The XRD spectrum displayed four prominent peaks at diffraction angles (2θ) of 40.1°, 44.5°, 64.9°, and 77.2°, corresponding to the (115), (210), (225), and (315) crystallographic planes of silver. These patterns indicate that the AgNPs possess a face-centered cubic (FCC) structure, with an average crystalline size calculated to be 30.01 nm.

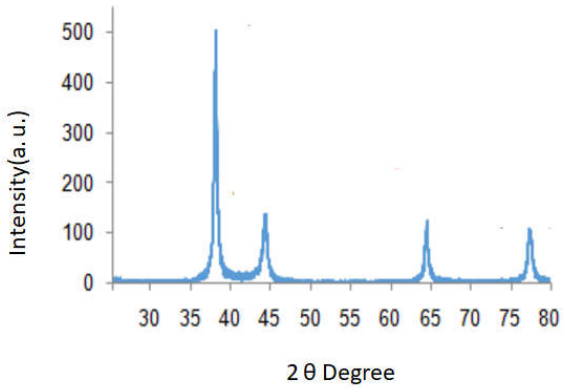

Figure 4: XRD of Silver Nanoparticles synthesized

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis of the synthesized AgNPs, derived from plant extract, revealed their morphological characteristics. The nanoparticles exhibited a relatively uniform and spherical shape, with diameters ranging between 38.1 nm and 80 nm. The presence of larger white areas was attributed to the agglomeration of smaller nanoparticles. The crystalline size of the AgNPs was determined using the Debye-Scherrer equation.

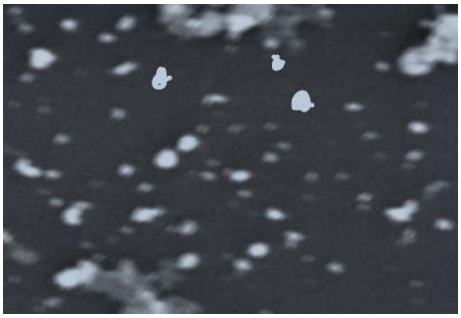

Figure 5: SEM of Silver Nanoparticles synthesized

EDX Spectrum Analysis

The initial composition of the synthesized AgNPs was assessed using Energy Dispersive X-ray (EDX) spectrum analysis. As shown in Figure 6, a distinct signal at approximately 3 keV confirms the formation of AgNPs derived from *Rosebay*. The spectra further reveal the presence of oxygen, in addition to high-purity silver nanoparticles, which constitute 83.54% of the total weight. The detected oxygen peaks may be attributed to the preparation of AgNPs under atmospheric conditions.

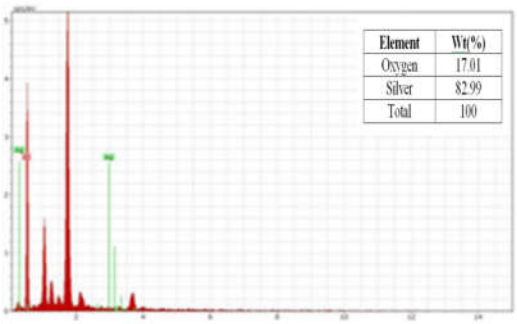

Figure 6: EDX Spectrum Ananlysis of Silver Nanoparticles synthesized

Atomic Force Microscopy (AFM) Analysis

The synthesized AgNPs, which exhibit considerable variation in shape. However, the majority of the nanoparticles appear to be spherical. The average grain size of the synthesized nanoparticles was determined to be approximately 55–59.05 nm.

Antifungal Activity of Silver Nanoparticles (AgNPs)

A preliminary antifungal screening was conducted using the disc diffusion method to evaluate the efficacy of the prepared AgNPs solution against different isolates of Candida albicans. The results demonstrated notable antifungal activity at wells 1, 2, and 3, with AgNP concentrations of 488.02 µg/mL, 244.03 µg/mL, and 122.02 µg/mL, respectively (Figure 7).

The highest inhibition zone was observed at the AgNPs concentration of 488.02 µg/mL, with inhibition decreasing proportionally as the nanoparticle concentration decreased. A statistically significant difference was observed in inhibition zones between different AgNP concentrations and the positive control.

Figure 7: Antifungal Activity of Silver Nanoparticles synthesized

Minimum Inhibitory Concentration (MIC)

MIC analysis revealed that the third titer of the serial dilution, containing AgNPs at a concentration of 122.02 µg/mL, effectively inhibited the studied isolate of Candida albicans.

Fungicidal Properties of Silver Nanoparticles

Silver nanoparticles are well-established antifungal agents. They exhibit a strong affinity for binding to chitin microfibers in the fungal cell wall through hydroxyl-carbon moieties, thereby disrupting the fungal cell's reverse osmosis process, ultimately leading to cell damage.Compared to other metal-based antifungal agents, AgNPs demonstrate superior fungicidal making them a promising alternative for treating fungal infections.

Conclusion

In this study, a green chemistry approach was employed as a rapid, eco-friendly, and efficient method for synthesizing silver nanoparticles using Rosebay leaf extract. The synthesized nanoparticles were predominantly spherical in shape, and the process did not require any chemical reagents. The findings confirm the antifungal properties of AgNPs produced from Rosebay leaf extract through green synthesis. This method offers a simple, cost-effective, safe, and efficient alternative to conventional antifungal agents.

References

1. Abdel-Raouf, N., Al-Enazi, N. M., Ibraheem, I. B. M., Alharbi, R. M., & Alkhulaifi, M. M. (2019). Biosynthesis of silver nanoparticles by using of the marine brown alga Padina pavonia and their characterization. Saudi Journal of Biological Sciences, 26(7), 1207–1215. https://doi.org/10.1016.

2. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 9(1), 1–7. https://doi.org/10.1016.

3. Arokiyaraj, S., Arasu, M. V., Vincent, S., Prakash, N. U., Choi, S. H., & Oh, Y. K. (2014). Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: An in vitro study. International Journal of Nanomedicine, 9, 379–388. https://doi.org/10.2147.

4. Ashokkumar, S., Ravi, S., Kathiravan, V., & Velmurugan, S. (2015). Synthesis of silver nanoparticles using A. indicum leaf extract and their antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134, 34–39. https://doi.org/10.1016.

5. Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., De, S. P., & Misra, A. (2009). Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 339(1–3), 134–139. https://doi.org/10.1016.

6. Bindhu, M. R., & Umadevi, M. (2013). Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 101, 184–190. https://doi.org/10.1016.

7. Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., & Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology Progress, 22(2), 577–583. https://doi.org/10.1021.

8. El-Rafie, H. M., Mohamed, A. A., Shaheen, T. I., Hebeish, A., & El-Rafie, M. H. (2014). Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. Carbohydrate Polymers, 113, 86–93. https://doi.org/10.1016.

9. Fayaz, A. M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P. T., & Venketesan, R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine, 6(1), 103–109. https://doi.org/10.1016.

10. Gardea-Torresdey, J. L., Gomez, E., Peralta-Videa, J. R., Parsons, J. G., Troiani, H., & Jose-Yacaman, M. (2003). Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. Langmuir, 19(4), 1357–1361. https://doi.org/10.1021.

11. Wady A F et al (2014) Effect of a Silver Nanoparticles Solution on Staphylococcus aureus and Candida spp. J. Nanomaterials. Hindawi, 2014, 1–7. doi: 10.1155/2014/545279.

12. Wijesena R N et al (2017) In-situ formation of supramolecular aggregates between chitin nanofibers and silver nanoparticles. Carbohydrate Polymers 173, 295–304. doi: 10.1016.

13. Nam K Y (2011) In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. J. Adv. Prosthodontics 3(1), 20– 24. doi: 10.4047/jap.2011.3.1.20.

14. Samaka H M et al (2018) Genotyping and Antifungal Susceptibility Profile of Candida albicans Isolated from Cancer Patients. J. Chem. Pharmaceut. Sci. 11(3), 236–241.

15. Manjumeena R et al (2014) Biogenic nanosilver incorporated reverse osmosis membrane for antibacterial and antifungal activities against selected pathogenic strains: An enhanced eco-friendly water disinfection approach. J. Environ. Sci. and Hlth. Part A, 49(10), 1125–1133. doi: 10.1080/10934529.2014.897149.

16.Manokari M and Shekhawat M S (2016) Production of Zinc oxide nanoparticles using extracts of Passiflora edulis Sims. f. flavicarpa Deg. 47(2), 267–278.

17. Khanra K, Bhattacharyya N and Bengal W (2016) Biological application of green silver nanoparticle synthesized from leaf extract of Rauvolfi serpentina Benth. Asian Pac. J. Trop. Dis. 1808(July). doi: 10.1016/S2222-1808(16)61085-X.

18. Khatoon N, Mazumder J A and Sardar M (2017) Current Biotechnological Applications of Green Synthesized Silver Nanoparticles. J. Nanosci. 2(1), 1–8. doi: 10.4172/2572-0813.1000107.

19. Kim K J et al (2009) Antifungal activity and mode of action of silver nano-particles on Candida albicans. BioMetals 22(2), 235–242. doi: 10.1007/s10534-008-9159-2.

20. Lara H H et al (2015) Effect of silver nanoparticles on Candida albicans biofilms: An ultrastructural study. J. Nanobiotech. 13(1), 1–12. doi: 10.1186/s12951-015-0147-8.