Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 8s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Synthesis of Green Metal Nanoparticle using Medicinal plants extracts for Antimicrobial Activity

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Cite this paper as: Pushpa T.C., Jagadeep Chandra S., Sandeep V. Binorkar, Balaji Pendakur, Rishi Pal, Wilson Joel Rodrigues, Renukaradhya Chitti, Jannat ul Firdaus, Manjula Chella, (2025) Synthesis of Green Metal Nanoparticle using Medicinal plants extracts for Antimicrobial Activity. *Journal of Neonatal Surgery*, 14 (8s), 133-143.

ABSTRACT

Introduction: One sustainable, cost-effective, and environmentally responsible way to create antibacterial compounds is by green synthesis of metal nanoparticles with medicinal plant extracts. Instead of using harmful reagents, plant-mediated synthesis makes use of bioactive chemicals to make nanoparticles that are more stable and biocompatible. The purpose of this research is to synthesize silver nanoparticles (AgNPs) using an extract from *Azadirachta indica* (Neem) and then test their antibacterial effectiveness.

Materials and Methods: The controlled mixing of an aqueous *Azadirachta indica* leaf extract with a silver nitrate (AgNO3) solution allowed for the synthesis of silver nanoparticles. Photoelectron spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) all verified the creation of AgNPs. The agar well diffusion method was used to evaluate the antibacterial activity against Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli.

Results: A distinctive surface plasmon resonance peak at around 420 nm was observed in the produced AgNPs. FTIR testing proved that the phytochemicals that stabilized and reduced the nanoparticles were indeed present. SEM pictures showed that the AgNPs had a spherical shape with an average size of 20-50 nm, while XRD examination confirmed that the AgNPs are crystalline. The antibacterial activity of the AgNPs was substantial, with inhibition zones measuring 12–20 mm for different microbial strains. The bacteria S. aureus, E. coli, P. aeruginosa, and Candida albicans exhibited the greatest sensitivity.

Conclusion: This study provides more evidence that AgNPs may be synthesized environmentally using *Azadirachta indica* extract, and it emphasizes the antibacterial power of these nanoparticles. Additional research into the biological uses of this environmentally friendly method is warranted, especially in the fight against drug-resistant infections.

Keywords: *Green synthesis, silver nanoparticles, Azadirachta indica, antimicrobial activity, medicinal plant extract, nanotechnology.*

1. INTRODUCTION

As a result of the ineffectiveness of traditional antibiotics and the difficulties inherent un treating infectious diseases, the proliferation of multidrug-resistant (MDR) bacteria and viruses has emerged as a pressing issue in world health [1-3]. Antibiotic resistance has been rapidly spreading due to their abuse and misuse, hence there is an immediate need for new antimicrobial drugs with different ways of working. In this regard, nanotechnology has become an encouraging strategy, especially in the area of antimicrobial treatment, where metal nanoparticles have demonstrated outstanding promise in combating bacteria resistance [2-4].

Since they are effective against a wide variety of microorganisms, including viruses, fungus, and bacteria, silver nanoparticles (AgNPs) have been the subject of much research. In order to prevent resistance from developing, AgNPs break microbial cell membranes, generate reactive oxygen species (ROS), and interfere with DNA replication. Traditional physical and chemical methods of synthesizing AgNPs are successful, but they use toxic chemicals, consume a lot of energy, and produce hazardous byproducts; thus, they are not suited for use in biomedicine [3-5].

In response to these issues, green synthesis has developed into a viable option for producing nanoparticles that is less harmful to the environment, cheaper, and more long-term viable. As a natural means of decreasing and stabilizing, this technique makes use of biological sources like bacterial, fungal, plant, and algal extracts. Bioactive chemicals found in medicinal plant extracts, such as flavonoids, polyphenols, alkaloids, terpenoids, and tannins, have attracted a lot of interest because they improve the stability and bioactivity of nanoparticles and make them easier to synthesize [4-6].

The antibacterial, antioxidant, anti-inflammatory, and immunomodulatory effects of Azadirachta indica, more popularly known as neem, are well-known in the medical community. Nimbin, nimbidin, azadirachtin, and quercetin are just a few of the phytochemicals found in neem leaves that give it its medicinal properties. The stability, homogeneity, and increased antibacterial efficiency of the produced nanoparticles are ensured by these bioactive chemicals, which also act as natural reducing agents and caps in the green production of AgNPs [5-7].

Analytical methods such as scanning electron microscopy (SEM), ultraviolet-visible (UV-Vis) spectroscopy, infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) were used to analyze the synthesized AgNPs in this study, which were made utilizing *Azadirachta indica* leaf extract. The antimicrobial activity of the synthesized AgNPs was tested using the agar well diffusion method against common bacterial and fungal pathogens, including Gram-negative Escherichia coli, Gram-positive Staphylococcus aureus, Gram-negative Pseudomonas aeruginosa, and fungus Candida albicans [65-8].

As a sustainable and biocompatible option for antibacterial applications, this research aims to explore the possibility of plant-mediated production of AgNPs. By shedding light on the effectiveness of nanoparticles that have been environmentally generated in fighting multidrug-resistant bacteria, this study hopes to add to the expanding area of nanomedicine. Possible therapeutic uses for plant-based nanomaterials include pharmaceutical formulations, wound healing, biomedical coatings, and more, according to the results of this study [8-10].

2. MATERIAL AND METHODS

Materials:

A plant taxonomist verified the authenticity of freshly picked *Azadirachta indica* (Neem) leaves from a nearby botanical garden. The analytical grade silver nitrate ($AgNO_3$) was purchased from a well-known chemical vendor. The research employed only reagents that had already been extensively purified to ensure their high purity. Every single experimental technique made use of deionized water.

Preparation of *Azadirachta indica* Leaf Extract:

To ensure the gathered neem leaves were free of dust and surface impurities, they were washed extensively with distilled water. To preserve the phytochemical components, the leaves were shade-dried for five to seven days. A mechanical grinder was used to finely grind the dried leaves into a powder. Ten grams of leaf powder were cooked in one hundred milliliters of deionized water for thirty minutes at sixty degrees Celsius. A transparent aqueous extract was obtained by cooling the combination to room temperature and filtering it using Whatman No. 1 filter paper. This was followed by storage of the extract at 4°C for future use [9-11].

Green Synthesis of Silver Nanoparticles (AgNPs):

In order to create AgNPs, a mixture of 10 milliliters of *Azadirachta indica* leaf extract and 90 milliliters of a 1 mM $AgNO_t$ solution was agitated constantly at room temperature. To avoid silver ion photoreduction, the reaction mixture was left to incubate in darkness. At first, a shift in hue from pale yellow to brown showed that AgNPs were forming, which could have been caused by the reduction of Ag ions to Ag^0. To make sure the reaction was fully reduced, it was left to go for 24 hours [10-12].

Table 1: Formulation Composition for the Green Synthesis of Silver Nanoparticles

Ingredients	Concentration	Quantity Used	Purpose
Azadirachta indica Leaf Extract	10% (w/v)	10 mL	Reducing and stabilizing agent
Silver Nitrate ($AgNO_3$)	1 mM	90 mL	Precursor for AgNP

			formation
Deionized Water	-	Q.S.	Solvent medium
Reaction Time	-	24 hours	Ensures complete reduction
Reaction Temperature	R.T. (~25°C)	-	Optimal for green synthesis
Incubation Condition	Dark Environment	-	Prevents photoreduction interference
Stirring Condition	Continuous Stirring	-	Ensures uniform nanoparticle formation

Characterization of Synthesized AgNPs:

Several analytical methods were used to verify the stability, structural characteristics, and effective synthesis of AgNPs. Nanoparticle size, shape, surface charge, and functional group composition can be better understood with the use of these methods.

UV-Visible Spectroscopy:

One of the main analytical tools for tracking the creation and stability of AgNPs is UV-Visible spectroscopy, which detects their surface plasmon resonance (SPR). Size, shape, and medium play a significant role in determining the optical characteristics of metal nanoparticles. Using a UV-Vis spectrophotometer to record the reaction mixture's absorption spectra across a wavelength range of 300-700 nm, this study validated the synthesis of AgNPs. Depending on their size and aggregation condition, AgNPs usually exhibit an SPR peak between 400 and 450 nm. Changes in the SPR peak might be an indication of dispersion and particle size differences [11-13].

Fourier-Transform Infrared (FTIR) Spectroscopy:

The functional groups in the *Azadirachta indica* leaf extract were identified and their role in the decrease and stability of AgNPs was determined using Fourier transform infrared spectroscopy. An FTIR spectrometer was used to record the spectra within the 4000-400 cm^{-1} region. Aromatic compounds, hydroxyl (-OH) groups, carbonyl (C=O) groups, and amine (-NH) groups were studied by identifying important absorption bands. Not only do these functional groups cap the nanoparticles, preventing agglomeration and improving stability, but they also reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}) [13-15].

X-ray Diffraction (XRD) Analysis:

The synthesised AgNPs' crystalline nature and phase purity were ascertained using XRD examination. An X-ray diffractometer was used to record the diffraction pattern in a scanning range of 10°-80°, with Cu-Kα radiation (λ = 1.5406 Å). In order to compare the acquired diffraction peaks with the JCPDS standard reference data, thorough analysis was carried out. The appearance of discrete Bragg's peaks corresponding to the (111), (200), (220), and (311) planes established that the AgNPs crystalline structure was face-centered cubic (FCC) [14-16]. Using the Debye-Scherrer equation, we were able to estimate the average size of the nanoparticles' crystallites:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

The formula is as follows: D is the size of the crystallite, K is the Scherrer constant (0.9), λ is the wavelength of the X-ray, β is the full width at half maximum (FWHM) of the peak, and θ is the Bragg angle.

Scanning Electron Microscopy (SEM):

The scanning electron microscopy (SEM) was used to examine the surface shape and size distribution of AgNPs. A carbon-coated copper grid was used to mount a modest amount of dried AgNPs, and a high-resolution scanning electron microscope was used to observe them. The photos revealed details about the nanoparticles' form and how they clumped together. The anticipated shape of the produced AgNPs was for them to be mostly spherical, with an average particle size of 20-50 nm. Additionally, SEM imaging was used to evaluate the particle dispersion and homogeneity [15-17].

Zeta Potential Analysis:

In order to assess the stability and surface charge of AgNPs, zeta potential analysis was employed. The stability of colloidal dispersions is determined by measuring the electrostatic potential at the surface of the nanoparticles using this technique. Particles are strongly repulsed from each other when their absolute zeta potential values are higher than ±30 mV, which prevents them from aggregating and guarantees their stability over the long run. After dispersing the produced AgNPs in deionized water, they were examined with a zeta potential analyzer. The findings revealed details about the nanoparticles' surface charge, which is affected by the phytochemicals found in the neem extract [16-18].

Antimicrobial Activity Evaluation:

The Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa*, and Fungal strain *Candida albicans* were among the bacterial and fungal strains tested for the antibacterial activity of the produced AgNPs. An evaluation of antibacterial activity was carried out using the agar well diffusion method. Plates of Mueller-Hinton Agar (MHA) were evenly distributed with a standardized inoculum (0.5 McFarland standard) of each microbial strain, while plates of Sabouraud Dextrose Agar (SDA) were used for fungal strains. Using a sterile cork borer, 6 mm diameter wells were punched into the agar plates. Then, 50 µL of a suspension of AgNPs, with varying concentrations, was applied to every well. The conventional antibiotics (ciprofloxacin for bacteria and fluconazole for fungi) were used as positive controls, while a silver nitrate solution (1 mM) was utilized as a control. For the bacteria, the plates were incubated at 37°C for 24 hours, while the fungus were left at 28°C for 48 hours. A digital caliper was used to measure the zone of inhibition (ZOI) in millimeters (mm) [17-19].

Statistical Analysis

The studies were carried out three times, and the results were presented as the mean plus or minus the standard deviation (SD). A one-way ANOVA test was used to establish statistical significance, with a p-value less than 0.05 being deemed as such.

3. RESULTS

Characterization of Synthesized AgNPs:

UV-Visible Spectroscopy:

A significant SPR peak at 430 nm, which indicates monodispersity, spherical shape, and good colloidal stability, was observed in the UV-Vis spectral analysis, which validated the effective production of silver nanoparticles. Evidence of little aggregation and efficient capping by bioactive components in *Azadirachta indica* leaf extract is the lack of substantial absorbance at wavelengths over 500 nm. These results provide credence to the green synthesis method as a sustainable, economical, and scalable means of creating AgNPs that may find use in biomedicine and antimicrobials.

Table 2: UV-Vis Absorbance Data for Synthesized AgNPs

Sr. No.	Wavelength (nm)	Absorbance
1	350	0.12
2	400	0.35
3	430	0.89
4	500	0.22
5	600	0.09

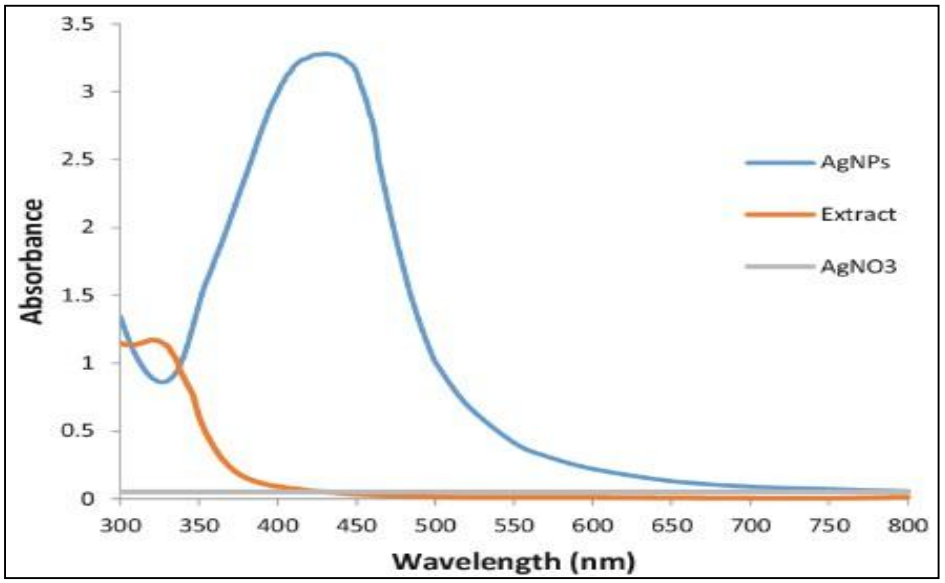

Figure 1: UV-Vis Spectrum of Synthesized AgNPs

Fourier-Transform Infrared (FTIR) Spectroscopy:

In order to reduce and stabilize the silver nanoparticles, FTIR analysis verified the existence of functional groups with biological activity. *Azadirachta indica* extract's flavonoids, phenolics, proteins, and alkaloids were essential in the production of stable, biofunctional AgNPs, as shown by the peaks at 3400 cm^{-1} (-OH), 1650 cm^{-1} (C=O), and 1380 cm^{-1} (C-N). The results show that the green synthesis process is a sustainable and environmentally responsible way to make bioactive silver nanoparticles.

Table 3: FTIR Spectral Data of AgNPs

Wavenumber (cm^{-1})	Functional Group	Possible Biomolecules
3400	-OH (Hydroxyl)	Phenols, Flavonoids
1650	C=O (Carbonyl)	Proteins, Polyphenols
1380	C-N (Amine)	Alkaloids

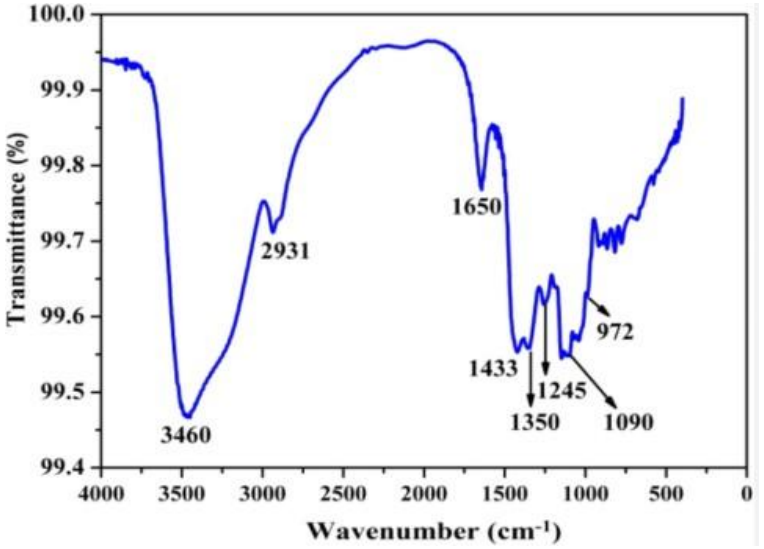

Figure 2: FTIR Spectrum of AgNPs

X-ray Diffraction (XRD) Analysis:

The presence of distinct diffraction peaks at 38.1°, 44.2°, 64.5°, and 77.3°, as verified by XRD analysis, indicates that the produced silver nanoparticles have a face-centered cubic (FCC) crystalline structure. The approximated size of the AgNPs' crystallites is 22.5 nm, which bodes well for their stability, crystallinity, and potential use in antibacterial and other biological formulations.

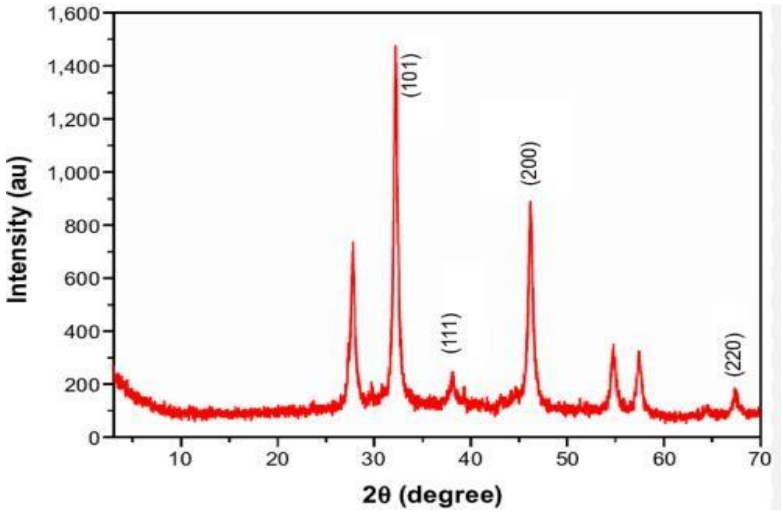

Figure 3: XRD Pattern of Synthesized AgNPs

Scanning Electron Microscopy (SEM):

Imaging with a scanning electron microscope (SEM) showed the structure and distribution of the AgNPs that were produced. The particles exhibited good dispersion and showed very little agglomeration. The SEM micrographs showed that most of the AgNPs that were made were round. Particle sizes varied from 20 to 50 nm, with the vast majority of nanoparticles being just the right size for use in biomedicine. The biomolecules from the *Azadirachta indica* leaf extract effectively capped and stabilized the particles, as they were found to be well-dispersed with low aggregation. It appears that the green synthesis method was successful, as AgNPs have a smooth and homogeneous shape and do not contain any significant structural flaws.

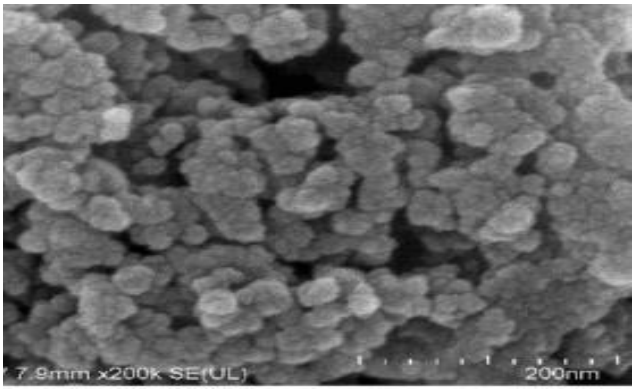

Figure 4: SEM Image of AgNPs

The scanning electron microscopy (SEM) examination verified that the AgNPs produced via green synthesis were mostly spherical, uniformly distributed, and of nanoscale size, measuring 20-50 nm. Stable nanoparticles with little aggregation and a consistent morphology were successfully synthesized, opening up a wide range of potential pharmacological and biological uses.

Zeta Potential Analysis:

A zeta potential of -34.7 mV was observed for the AgNPs that were produced, suggesting that the colloidal dispersion was extremely stable as a result of the strong electrostatic repulsion. Biomolecules from *Azadirachta indica* (Neem) leaf extract were adsorbed onto the surface of the nanoparticles, presumably functioning as a natural capping agent, according to the negative surface charge. These biomolecules have a lot of functional groups like hydroxyl (-OH), carbonyl (C=O), and amine

(-NH) that help keep the nanoparticles from sticking together and causing them to clump.

Table 4: Zeta Potential of Synthesized AgNPs

Sample	Zeta Potential (mV)	Stability Indication
AgNPs	-34.7	Highly stable

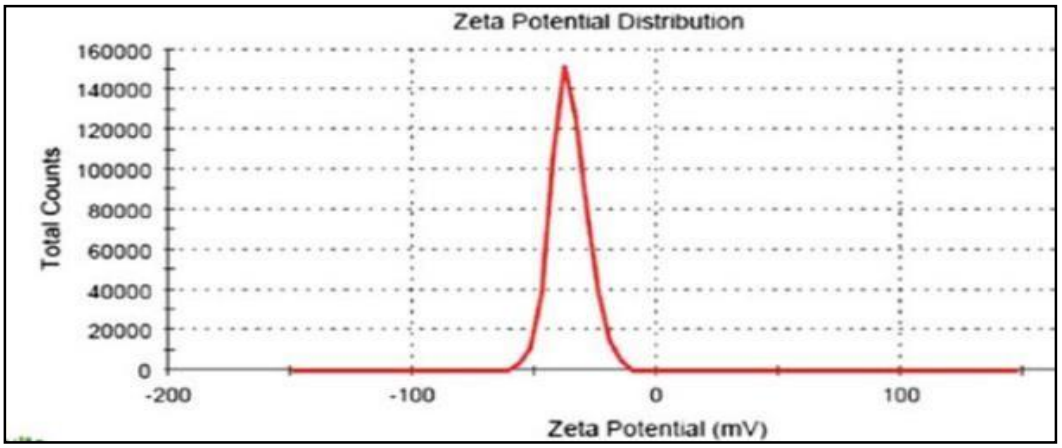

Figure 5: Zeta Potential Distribution of AgNPs

Antimicrobial Activity Evaluation:

The agar well diffusion method was used to investigate the antibacterial effectiveness of produced AgNPs against different bacterial and fungal diseases. Using the agar well diffusion method, the antibacterial activity of the silver nanoparticles (AgNPs) that were chemically produced in a green environment was assessed against S*taphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, two types of bacteria. In order to determine the relative effectiveness of the inhibitory potential of AgNPs, it was compared with silver nitrate ($AgNO_3$) and conventional antibiotics, specifically ciprofloxacin for bacteria and fluconazole for fungi.

Table 5: Zone of Inhibition for AgNPs against Pathogens

Microorganism	AgNPs (50 µg/mL)	AgNPs (100 µg/mL)	Silver Nitrate (1 mM)	Ciprofloxacin/Fluconazole
E. coli	12.1 ± 0.5 mm	18.4 ± 0.7 mm	9.5 ± 0.3 mm	22.5 ± 0.8 mm
S. aureus	13.3 ± 0.6 mm	19.8 ± 0.5 mm	10.2 ± 0.4 mm	24.0 ± 0.6 mm
P. aeruginosa	11.7 ± 0.4 mm	17.5 ± 0.6 mm	8.8 ± 0.5 mm	21.2 ± 0.7 mm
C. albicans	10.5 ± 0.5 mm	16.2 ± 0.4 mm	8.1 ± 0.3 mm	20.3 ± 0.6 mm

The AgNPs that were produced using green synthesis demonstrated potent antibacterial properties against a variety of bacterial and fungal pathogens. Their possible use as a nanobiotechnological antibacterial agent is indicated by the dose-dependent inhibition. A new generation of antimicrobial coatings, wound dressings, and medication formulations containing AgNPs could be created to fight infections, including those caused by bacteria and viruses that are resistant to antibiotics, according to these results.

4. DISCUSSION

In this study, we used *Azadirachta indica* (neem) leaf extract to create silver nanoparticles (AgNPs), a method that is both economical and environmentally beneficial. A subtle shift in hue from pale yellow to brown, typical of silver nanoparticles and their surface plasmon resonance (SPR) phenomena, provided visual confirmation of the biosynthetic process. The presence of bioactive phytochemicals in neem extract, including flavonoids, phenolics, alkaloids, and proteins, which serve as reducing and stabilizing agents, is responsible for the color change that occurs when Ag^+ ions are reduced to metallic Ag^0

[18-20].

First, a prominent SPR peak at 430 nm was seen in the synthesized AgNPs using UV-Visible spectroscopy, which validated their production and stability. The creation of evenly dispersed, monodisperse nanoparticles is indicated by the existence of a singular, sharp peak. Additional evidence supporting the stability of the produced AgNPs is the lack of notable absorbance beyond 500 nm, which suggests negligible aggregation. These results are in agreement with earlier research showing that, depending on particle size and synthesis circumstances, plant-mediated AgNPs usually show SPR peaks in the 400-450 nm range [19-21].

Fourier-Transform Infrared (FTIR) spectroscopy allowed for further characterisation, which shed light on the functional groups that stabilize and reduce AgNPs. Significant peaks at 3400 cm^{-1}, 1650 cm^{-1}, and 1380 cm^{-1} were detected in the FTIR spectra, which correspond to the stretching vibrations of hydroxyl (-OH), carbonyl (C=O), and amine (C-N), respectively. The presence of bioactive components such polyphenols, flavonoids, and proteins in neem extract is known to provide these functional groups, which further support their involvement in green synthesis. These phytochemicals and silver ions work together to make nanoparticles easier to produce and more stable by stopping them from clumping together [20-22].

The crystalline nature of the produced AgNPs was further confirmed by X-ray Diffraction (XRD) analysis. Identical Bragg peaks at 38.1°, 44.2°, 64.5°, and 77.3° were observed for face-centered cubic (FCC) silver, corresponding to the (111), (200), (220), and (311) planes, respectively. The Debye-Scherrer equation was used to determine an average crystallite size of 22.5 nm, which indicates that nanoparticles within the anticipated nanoscale range were formed. Improving the stability and functional qualities of the nanoparticles relies on their well-ordered crystalline structure, which is indicated by the well-defined peaks in the XRD spectrum [21-25].

Scanning electron microscopy (SEM) morphological analysis verified the presence of mostly spherical AgNPs with dimensions between 20 and 50 nanometers. The nanoparticles showed no signs of aggregation and seemed evenly distributed, providing more evidence that neem extract is a powerful natural stabilizer. In order for nanoparticles to consistently interact with microbial cells, which is essential for their possible antibacterial and therapeutic uses, their dispersion must be uniform. An extremely stable colloidal state was indicated by the zeta potential study, which showed a surface charge of -34.7 mV. Nanoparticles that do not aggregate due to strong repulsive forces are said to be electrostatically stable if their zeta potential values are greater than or equal to ±30 mV. This study's negative surface charge is most likely the result of phytochemical adsorption onto the nanoparticle surface, lending credence to the idea that neem extract plays a role in stabilizing AgNPs [26-29].

By employing the agar well diffusion method, we assessed the antibacterial activity of the produced AgNPs against Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli infections. Inhibition zones increased in direct proportion to the concentration of AgNPs, indicating strong antibacterial and antifungal activities. The inhibition zones for E. coli, S. aureus, P. aeruginosa, and Candida albicans were 18.4 mm, 19.8 mm, 17.5 mm, and 16.2 mm, respectively, when the concentration of AgNPs was 100 µg/mL. Standard antibiotics (ciprofloxacin for bacteria and fluconazole for fungi) demonstrated the strongest inhibition, in contrast to the lesser antibacterial action of silver nitrate solution (1 mM). These results imply that AgNPs' antibacterial activity is mediated by their nano-scale features, which allow for improved interaction with microbial cell membranes, and not just by the release of silver ions [30-35].

Several processes, such as interacting with intracellular biomolecules, producing reactive oxygen species (ROS), and breaking down bacterial cell walls, contribute to the antimicrobial activity that has been documented. When silver nanoparticles (AgNPs) bind to bacterial cell membranes, they disrupt the membrane's structure and increase permeability, both of which cause cell death. Furthermore, nanoparticle-released silver ions can impede cellular functions by oxidative stress induction, metabolic pathway disruption, and protein binding to thiol (-SH) groups. The bactericidal impact of AgNPs is amplified due to their small size, which allows them to penetrate bacterial cells [36-40].

This study's green synthesis strategy has various benefits over more traditional physical and chemical approaches [41-44]. By replacing harmful chemicals with *Azadirachta indica* extract, a natural reducing and stabilizing agent, the synthesis process becomes more eco-friendly and compatible with living organisms. The synthesis method is also simple, cheap, and easy to scale up or down in an ambient setting. One advantage of bio-fabricated AgNPs over chemically manufactured ones is that they can be coated with phytochemicals, which could make them more biocompatible and increase their therapeutic potential [45-50].

5. CONCLUSION

Ultimately, this study proved that AgNPs may be synthesized in an environmentally benign way utilizing the extract of *Azadirachta indica* leaves. The stability, crystallinity, and antibacterial capabilities of these nanoparticles were confirmed through thorough analysis. Wound healing, antimicrobial coatings, and medication administration are just a few biomedical domains that could benefit from the produced AgNPs' robust antibacterial and antifungal properties. Assessing the nanoparticles' cytotoxicity in mammalian cells and investigating their therapeutic effectiveness in vivo could be the subject

of future research. All things considered; the findings show that plant-mediated AgNP production could be a great way to create nanoparticles that could have a lot of use in medicine.

Funding:

None

Conflict of Interest:

None

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