Plant Extract as Green Synthesis Agent for Silver Nanoparticle Synthesis and Evaluation of Their Antibacterial Activity

Rajesh K. M.[1*], Bhagyalakshmy M.[2], Lakshmi K. P.[3], Malavika K. M.[4], Nandhana M. P.[5], Laxmi Priya S.[6]
[1,2,3,4,5,6] Post Graduate and Research Department of Chemistry, Sree Narayana College, Nattika, Thrissur- 680566, Kerala, India.

Abstract- Silver nanoparticles (AgNPs) have gained significant attention due to their eco-friendly synthesis and remarkable antibacterial properties. In this study, AgNPs were synthesized with a silver nitrate solution and bio-reducing agents extracted from neem (**Azadirachta indica*****) leaves through a simple and cost-effective water bath treatment method. The synthesized nanoparticles were characterized using UV-Visible spectroscopy and X-ray diffraction (XRD) analysis. The antibacterial activity of the AgNPs was evaluated against* Staphylococcus aureus *using standard antimicrobial assays. The results demonstrated significant antibacterial efficiency. This eco-friendly approach provides a sustainable and green alternative for nanoparticle synthesis, reducing the hazardous environmental impact associated with conventional chemical methods.***

Keywords: Anti bacterial study, Plant extract, Silver Nano Particles

1. Introduction:

Silver nanoparticles (Ag-NPs) are versatile nano materials exhibiting widespread applications in consumer products like textiles, cosmetics, bandages, cleaning agents and contraceptives [1]. They also plays significant role in life sciences and biomedical fields. Ag-NPs are found to exhibit diverse functional properties such as catalytic activity, electrical conductivity and antimicrobial effects including antibacterial, antifungal, antiviral, anti-inflammatory, antigenic, antioxidant, and anticancer abilities [2]. Their superior antimicrobial efficacy, even at low concentrations, makes them more effective than conventional antimicrobial agents. This effectiveness is primarily due to their specific interactions with pathogens, including the disruption of mitochondrial function, induction of reactive oxygen species (ROS) production, suppression of ATP synthesis, and ultimately, DNA damage, leading to microbial cell death.

Various techniques are employed to synthesize Ag-NPs with controlled size and shape, including physical, chemical, and biological methods. Physical synthesis ensures uniform size distribution and high purity, while chemical synthesis, the most commonly used method, involves the reduction of silver ions to silver atoms, often using reducing, capping, and stabilizing agents to prevent the unwanted agglomeration of colloids. However, the environmental and biological risks associated with chemical synthesis have driven by interest in green synthesis methods, which utilize clean, non-toxic, and sustainable approaches. It also provides advantage of being cost effective, less time consuming and environment friendly. There is no need to use high temperature, high pressure or toxic chemicals [3]. Recently, biosynthetic methods employing different plants extract have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nano materials [4, 5, 6]. Green nanotechnology, employing naturally occurring reagents such as sugars, microorganisms, fungi, enzymes, and plant extracts, has gained significant attention due to its environmental friendliness and potential biomedical applications [7-11]. Despite of the advantages of physical and chemical synthesis methods, their high cost and environmental toxicity highlight the potential advantage of green synthesis.

Plants are a promising source for the green synthesis of nanoparticles, offering non-toxic, naturally occurring capping agents and eliminating the need for maintaining microbial cultures. Several plant extracts, including Azadirachta indica (neem), Zingiber officinale (ginger), Capsicum frutescens (cayenne pepper), Allium sativum (garlic), marigold flowers, Ziziphora tenuior, and Ocimum tenuiflorum (holy basil), have been successfully utilized in the biosynthesis of Ag-NPs. Among these, Azadirachta indica, a medicinal plant from the Meliaceae family, is known for its antimicrobial properties due to its phytochemical constituents. Neem has various phytochemicals identified to be carbohydrates, alkaloids, steroids, phenols, saponins and flavonoids. The advantage of using Neem leaves for bioreduction of metal ions is that it is easily available throughout the year. Studies have suggested that biomolecules like protein, phenols, flavonoids and some phytochemicals has ability to reduce the metallic ions to the nanosize and also play an important role in the capping of the nanoparticles for its stability [12, 13]. These compounds act as reducing agents, facilitating the synthesis of Ag-NPs. Ag-NPs synthesized using Azadirachta indica leaf extract have demonstrated antimicrobial activity, been incorporated into wash-durable antimicrobial fabrics, and contributed to the degradation of textile effluents.

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that commonly colonizes human skin and mucous membranes. It is a facultative anaerobe, meaning it can survive with or without oxygen. This bacterium is known for causing a range of infections, from minor skin infections to life-threatening conditions such as pneumonia, endocarditis, and sepsis.

The present study involves the synthesis of silver nano particles from silver nitrate solution using plant extract of Azadirachta indica as natural bio reducing agent using slightly modified methods reported [14] and tried to establish its antibacterial activity on Staphylococcus aureus. However, the antibacterial and antioxidant potential of Ag-NPs synthesized using Azadirachta indica leaf extract remains unexplored.

2. Experimental:

2.1. Preparation:

2.1.1. Preparation of Neem Leaf Extract:

Fresh neem leaves (figure 1) were collected from the local area, thoroughly washed with tap and deionized water, and air-dried. A total of 20 g of finely chopped leaves was treated in 75 mL of deionized water at 60°C for 15–20 minutes. After cooling, the extract was vacuum-filtered using Whatman No. 42 filter paper and stored at 4°C for future use.

Figure 1. Neem leaves

2.1.2. Green Synthesis of Silver Nanoparticles:

To synthesize silver nanoparticles (Ag-NPs), a 1 mM silver nitrate ($AgNO_3$) solution (purchased from Nice Chemicals) was prepared. Subsequently, 10 mL of neem (*Azadirachta indica*) leaf extract was added to 60 mL of the $AgNO_3$ solution. The mixture was stirred at room temperature for 10 minutes and then heated in a water bath at below 40°C for 5 minutes. A small portion of the solution was taken for UV-Visible spectroscopy analysis, while the remaining portion was dried in an oven at 50–60°C for further use.

2.2. Characterisation:

The UV-Visible absorbance measurement of the sample was recorded at room temperature between 200 – 800 nm using JASCO (V-660) model UV-Visible spectrophotometer. A solvent of water was used as a reference. A little of extracted sample was mixed with the reference solvent to make the solution. The solution was properly diluted with the reference solvent and its absorption was taken. The X-ray diffraction pattern was recorded using Bruker AXS D8 advance X ray Diffractometer with Ni filtered Cu Kα radiation source. The intensities were obtained in the 2θ ranges from 10 to 70^0.

2.3Antimicrobial activity by well diffusion method:

The silver nanoparticles (Ag-NPs) synthesized using *Azadirachta indica* plant extract were evaluated for their antimicrobial activity against *Staphylococcus aureus* using the well diffusion method. Pure cultures of the organism were subcultured in Mueller–Hinton broth at 35^0C on a rotary shaker at 200 rpm. Mueller–Hinton agar (MHA) plates were prepared, and *Staphylococcus aureus* was evenly spread on the surface. Three wells (7 mm in diameter) were punched into the agar, and the nanoparticle sample was pipetted into the wells. Clindamycin was used as a positive control. The plates were then incubated for 24 hours, and the zone of inhibition was measured to assess antimicrobial activity.

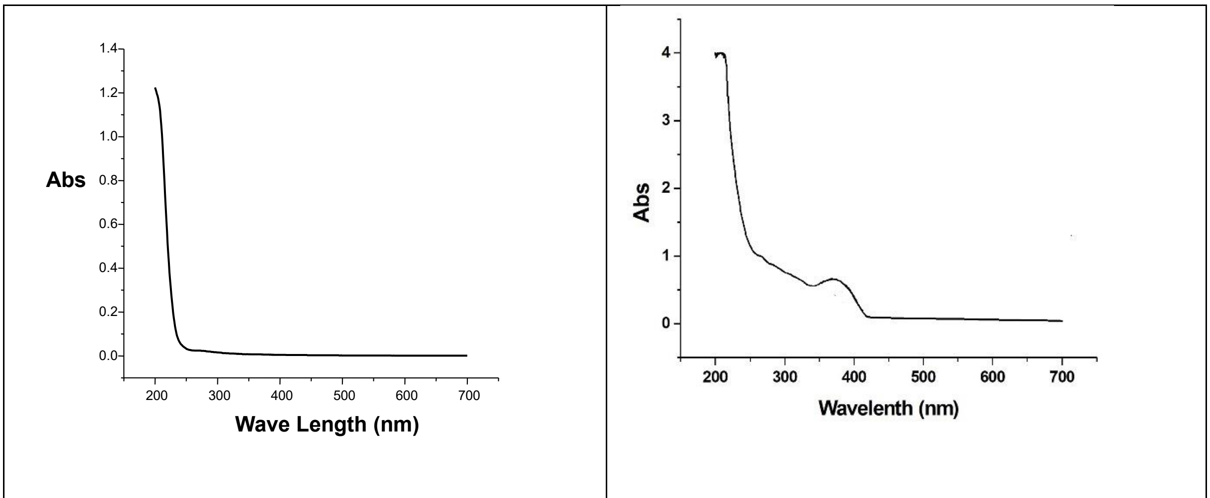
3. Result and Discussion:

The formation of silver nanoparticles (Ag-NPs) was visually indicated by a colour change in the solution, as shown in Figure 2. While pure silver nitrate is colourless, neem extract exhibits a greenish-yellow hue. Upon treatment of the silver nitrate solution with neem extract, the solution turned brown, confirming the reduction of Ag^+ ions and the formation of Ag-NPs. This transformation was observed as the colour changed from yellow to dark brown. Notably, the colour change occurred within just 5 minutes when the solution was heated in a water bath below 40°C. To the best of our knowledge, this is one of the shortest reported times for the green synthesis of silver nanoparticles using neem extract.

Figure 2. Visual observation of silver nanoparticle (Ag-NP) formation: Azadirachta indica extract; (Left) silver nitrate solution (middle) and (C) silver nanoparticles. (Right)

Figure 3(a-b) illustrates the UV-Visible spectra of pure silver nitrate and the synthesized silver nanoparticles (Ag-NPs). The UV-Vis spectrum of the silver nitrate solution does not exhibit any distinct absorption peak. However, the neem extract-treated silver nitrate solution shows a characteristic peak around 375 nm, confirming the formation of Ag-NPs. This peak corresponds to the Surface Plasmon Resonance (SPR) phenomenon, which occurs when specific wavelengths of light induce collective oscillations of conduction electrons in the metal nanoparticles. The strong interaction between metal nanoparticles and incident light gives rise to the surface plasmon absorption band (SPAB) [14, 17].

The observed peak at 375 nm aligns well with previous reports and is a distinct indicator of Ag-NP formation, confirming their nanoscale nature. The peak was broad and of low intensity, suggesting that the synthesized nanoparticles were relatively small in size and exhibited a wide size distribution. The SPR band is highly influenced by the shape and size of the nanoparticles, further validating their successful synthesis through the neem extract-mediated reduction process.



(a) (b)

Figure 3. UV- Visible spectrum of (a) silver nitrate (b) silver nitrate nano particle

In our study, the plasmon resonance band of the biosynthesized Ag-NPs was observed at 375 nm, whereas other reports have typically shown bands in the range of 400 to 445 nm. This shift may be attributed to the lower concentration of neem leaf extract in our sample. Similar effects have been reported, where an increase in plant extract concentration resulted in a higher absorption intensity. This suggests that the concentration of biomolecules in the extract plays a crucial role in influencing the optical properties and size distribution of the synthesized silver nanoparticles [18].

Neem extract functioned as both a reducing and stabilizing agent in the synthesis of silver nanoparticles (Ag-NPs). The bioactive compounds present in neem extract, including phenolics, tannins, saponins, flavonoids, terpenoids, fatty acids, and alkaloids, facilitated the reduction of Ag^+ to Ag^0, leading to the formation of nanoparticles (Present studies does not concentrated on the separation and isolation of these photchemicals compounds). Additionally, these biomolecules played a crucial role in stabilizing the Ag-NPs, preventing aggregation, and ensuring their uniform dispersion. This dual functionality of neem extract highlights its effectiveness as a natural and eco-friendly agent for nanoparticle synthesis [2].

Figure 4 shows the X-ray diffraction pattern of AgNPs obtained. The XRD image shows three peaks at 2θ values of 38.4, 44.4, 64.5 and 77.2 respectively. JCPDS data reveals that, the above peaks can be assigned to (111), (200) and (220) and (311) reflections of face centered cubic silver nanoparticles [1]. The X-ray diffraction studies undoubtedly conclude that the silver nanoparticles formed in this method are crystalline in nature. The sharp and narrow peaks indicates the nano structure of the particle.

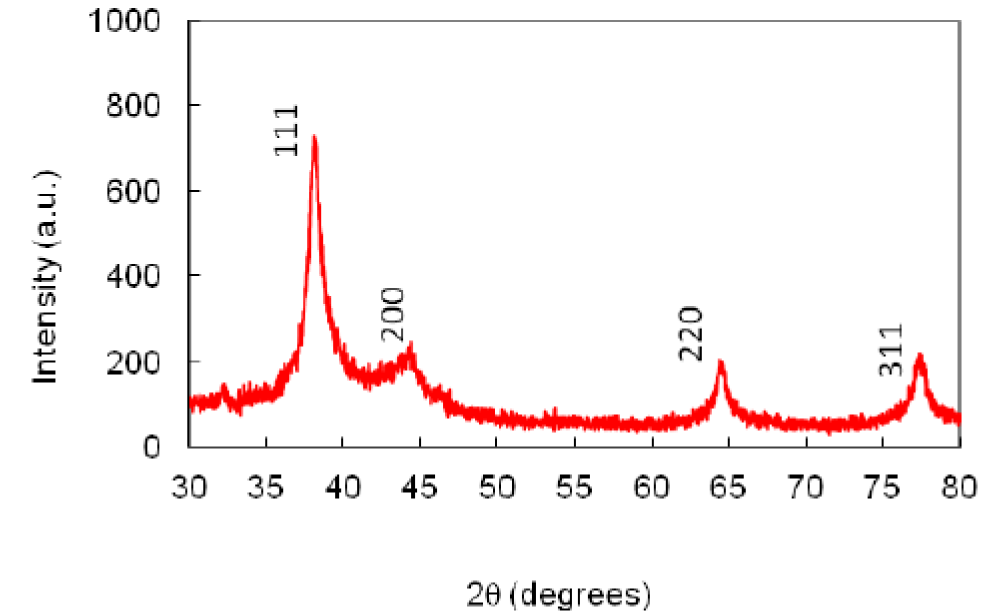


Figure 4. XDR Spectrum of silver nano particles obtained using neem extract

The antimicrobial activity of silver nanoparticles (Ag-NPs) synthesized using the natural plant extract of *Azadirachta indica* was evaluated against the pathogenic organism *Staphylococcus aureus* using the well diffusion method, as shown in Figure 5. The effectiveness of the Ag-NPs was determined by measuring the diameter of the inhibition zones (mm) around each well containing the nanoparticle solution. The results are summarized in Table 1, demonstrating the potential of biosynthesized Ag-NPs as an effective antimicrobial agent.

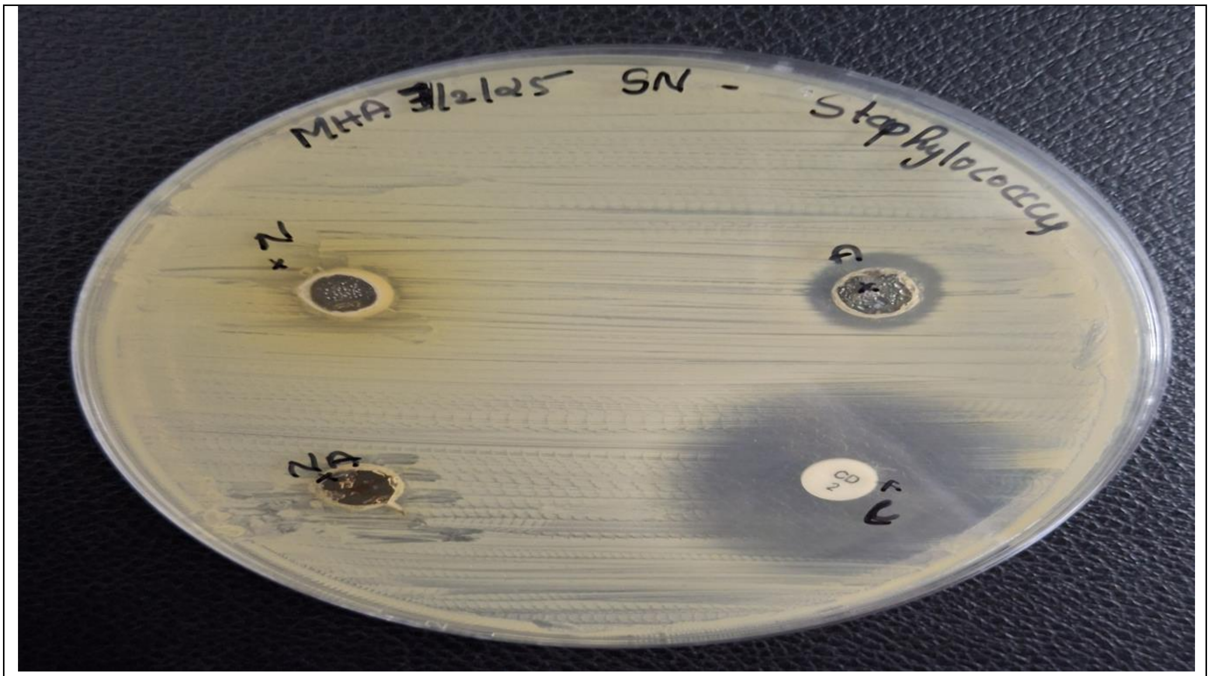


Figure 5. Antibacterial study of Staphylococcus aureus – C- Control, N- Silver nitrate, NA- Plant extract of Azadirachta indica and A-Plant extract + Silver nitrate

ORGANISM	SAMPLE SOURCE	INHIBITION ZONE DIAMETER(mm)
Staphylococcus aureus	Neem Extract	1
	Silver Nitrate	0

	Silver nitrate + Neem extract	11

Table 1. Zone of inhibition of different samples against Staphylococcus aureus.

As observed in Figure 5 and Table 1, the silver nanoparticles (Ag-NPs) synthesized using neem extract exhibited significant antibacterial activity. Several factors influence the antibacterial effectiveness of Ag-NPs, with surface area being a crucial determinant. An increased surface area enhances the interaction between the nanoparticles and microbial cells, improving their antibacterial efficacy. During the reduction of silver nitrate into Ag-NPs by the plant extract, the surface area of the nanoparticles increases, facilitating better contact with microorganisms. The nanoparticles primarily target the respiratory chain and disrupt cell division, ultimately leading to bacterial cell death.

4. Conclusion:

We successfully synthesized silver nanoparticles (Ag-NPs) using neem (*Azadirachta indica*) extract as a green reducing agent and silver nitrate ($AgNO_3$) as the precursor. The formation of Ag-NPs was confirmed through a visual color change, UV-Visible spectroscopy, and XRD analysis. The biosynthesized Ag-NPs demonstrated strong antibacterial activity against *Staphylococcus aureus*.

This study provides evidence that plant extract-stabilized nanoparticles could serve as promising candidates for future biomedical and pharmaceutical applications. The synthesis process offers a cost-effective and eco-friendly alternative to conventional methods, with the advantage of low reaction temperatures and short synthesis times, making it suitable for large-scale industrial production. Given the widespread availability of neem leaves, the active nano-compound derived from them can be developed into an effective antibacterial agent that is safe, affordable, and free from the adverse effects associated with synthetic alternatives.

5. Acknowledgment:

We are deeply grateful for the financial support provided by the DBT-STAR program of Sree Narayana College, Nattika. We also extend our sincere thanks to Veta Genomics, Thrissur, as well as the Departments of Botany and Zoology at Sree Narayana College for their valuable contributions to the antibacterial studies and data interpretation.

6. References:

1. Pandian, H., et al. (2023). Azadirachta indica leaf extract mediated silver nanoparticles impregnated nano composite film (AgNP/MCC/starch/whey protein) for food packaging applications. Environmental Research, 216 (2), 114641.
2. Mudenda, S., Banda, M., Mohamed, S., & Chabalenge, B. (2023). Phytochemical composition and antibacterial activities of azadirachta indica (Neem): significance of traditional medicine in combating infectious diseases and antimicrobial resistance. Journal of Pharmacognosy and Phytochemistry, 12(5), 256-263.
3. Sangeetha V., & Ponnuswami, V. (2014). Synthesis and Characterization of Silver Nanoparticles using Moringa (Moringa oleifera LAM.) Pod Extract. Biosciences, 7(8), 675-679.
4. Peter, L., Sivagnanam, S., & Jayanthi A. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society Volume 19 (3), 311-317.
5. Shankar, S.S., Ahmad, A., & Sastry,.M. (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnology. Progress. 19 1627-1631
6. Chandran S.P., el al., (2006).Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology. Progress. 22, 577-583.
7. Mukherjee, P., et al. (2001). Bioreduction of $AuCl_4$ ions by the fungus, Verticillium sp. and surface trapping of the gold nanoparticles formed. Angewandte Chemie International Edition, 40(19), 3585-3588.
8. Ahmad, A., et al. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids and Surfaces B: Biointerfaces, 28, 313-318.

9. Vigneshwaran, N., et al. (2006). A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohydrate research, 341(12), 2012-2018.
10. Shankar, S. S., et al. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. Journal of colloid and interface science, 275(2), 496-502.
11. Edwards-Jones, V., (2006). Antimicrobial and barrier effects of silver against methicillin-resistant Staphylococcus aureus. Journal of Wound Care, 15, 285-290.
12. Bindhani, B. K., and Panigrahi, A. K., (2014). Green Synthesis of Gold Nanoparticles Using Neem (Azadirachta indica L). Leaf Extract and Its Biomedical Applications. IJBR, 15(3), 457-464.
13. Ahmed, S., et al. (2015). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences 9(1) 173, 1-7.
14. Anitha Kumari, S., Anita. K. Patlolla & Madhusudhanachary, P. (2022). Biosynthesis of Silver Nanoparticles Using Azadirachta indica and Their Antioxidant and Anticancer Effects in Cell Lines Micromachines, 13(9), 1416.
15. Otunola, G.A. et al. (2017). Characterization, antibacterial and antioxidant properties of silver nanoparticles synthesized from aqueous extracts of Allium sativum, Zingiber officinale, and Capsicum frutescens. Pharmacognosy. Magazine, 13, S201–S208.
16. Adebayo-Tayo, B.; Salaam, A. & Ajibade, A. (2019). Green synthesis of silver nanoparticle using Oscillatoria sp. extract, its antibacterial, antibiofilm potential and cytotoxicity activity. Heliyon, 5(10), e02502.
17. Dharamvir Singh A. et al. (2014). Synthesis and Characterization of Sol–Gel Prepared Silver Nanoparticles. International Journal of Nanoscience. 13(1) 1450004
18. Ahmed, S., Ahmad, M.S. & Swami, B.L (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and. Applied . Sciences. 9(1), 1–7