RESEARCH PAPER

Green Synthesis of Silver Nanoparticles Using Azadirachta indica Extract and Their In-Vitro Evaluation

Ruchita Shrivastava[1], Pratibha Kurkute[2], Chhavi Mangla[3], D Nagaraju[4], Tulika Mishra[5*]

[1] Visiting Lecturer, Department of Botany, Govt. Homescience PG Lead College, Narmadapuram (MP), India.
[2] Assistant Professor, Sahakar Maharshi Bhausaheb Santuji Thorat Arts, Commerce and Science College, Sangamner, India.
[3] Assistant Professor, Department of Botany, Dayanand College, Hisar, Haryana - 125001, India.
[4] Associate Professor of Botany, Government City College (A) Nayapul, Hyderabad, Telangana, India.
[5*] Assistant Professor, Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, India.
Email: tulika.mishra.2000@gmail.com

ABSTRACT
The research examines environmentally friendly methods for producing silver nanoparticles through the use of Azadirachta indica leaf extract while evaluating their potential laboratory applications. The plant-based method for drug development provides both an economical solution and an environmentally sustainable approach to drug production. It uses phytochemicals found in plants to stabilize and reduce compounds through the action of flavonoids, terpenoids, and phenolic compounds. The nanoparticles known as AgNPs form when a specific color transformation occurs and this concept scientists validate through multiple testing techniques which include UV–Visible spectroscopy and FTIR and SEM and XRD. The synthesized nanoparticles should display a spherical structure with a solid body and a surface plasmon resonance peak that occurs at 420 nm. The results of the in-vitro test demonstrate that the nanoparticles possess powerful antibacterial properties along with strong antioxidant abilities. Membrane destruction leads to the production of reactive oxygen species within the system. The research demonstrates that AgNPs produced through green synthesis method exhibit potential as durable nanomaterials suitable for use in biological and pharmaceutical environments.

Keywords: Azadirachta Indica, AgNPs, UV–Visible Spectroscopy, FTIR, SEM, XRD, Synthesized Nanoparticles, Pharmaceutical Environments etc.

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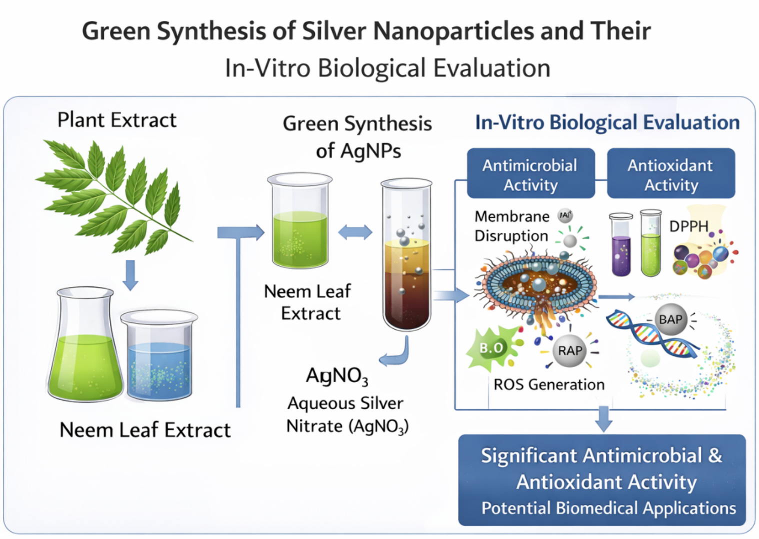

Figure 1:Graphical Abstract, Source: Author Generated

1. Introduction

Nanotechnology has advanced rapidly, significantly transforming contemporary medical and pharmaceutical domains through the creation of metal nanoparticles with distinctive physical and chemical characteristics [1]. Researchers have investigated silver nanoparticles (AgNPs) as one of the most scrutinized nanomaterials due to their potent antibacterial, antioxidant, and anti-inflammatory properties [2]. These nanoparticles exhibit remarkable therapeutic efficacy against various diseases owing to their elevated surface area-to-volume ratio and their capacity to interact with microbial membranes. The current international figures demonstrate that antimicrobial resistance (AMR) leads to approximately 1.27 million deaths each year which creates an urgent need for new antimicrobial solutions like nanomaterials.

The conventional techniques for producing AgNPs through chemical reduction and physical methods need to use dangerous substances while consuming large amounts of power and creating hazardous materials for the environment [3]. The existing restrictions have driven scientists to develop new production methods which use environmentally friendly approaches that follow the principles of green chemistry [4]. The synthesis process which uses plants as the main source for producing natural biomolecules to create reducing and stabilizing agents creates a cost-effective and environmentally friendly production method. The tree species Azadirachta indica has gained major popularity because people recognize its established medicinal properties and its wide variety of therapeutic plant compounds [5].

The leaves of Azadirachta indica contain multiple bioactive compounds which include flavonoids and terpenoids and phenolics and alkaloids that play essential roles in the creation of nanoparticles [6]. The chemicals function as dual components which help to convert silver ions (Ag^+) into metallic silver (Ag^0) while they also act as capping agents which improve the stability of nanoparticle formation [7].

Phytochemical	Functional Role in AgNP Synthesis	Biological Significance
Flavonoids	Electron donation (reduction)	Antioxidant
Terpenoids	Stabilization of nanoparticles	Anti-inflammatory
Phenolics	Redox activity	Free radical scavenging
Alkaloids	Surface capping	Antimicrobial

Table 1: Key Phytochemicals in Azadirachta indica and Their Functional Roles, Source: Author Generated

When nanoparticles are made, surface plasmon there always resonance causes a noticeable change in color. The green synthesis then process is good for the environment and can be used on a large scale in industry situation.

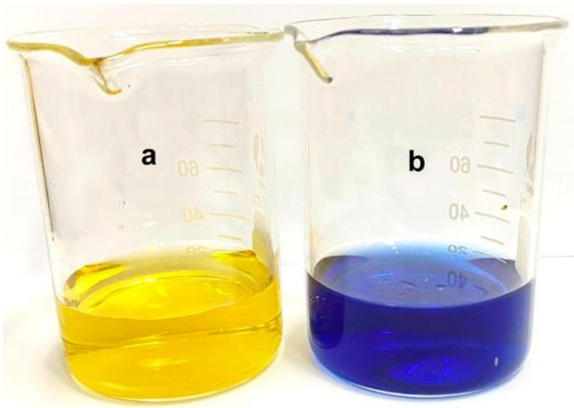

Figure 2: Preparations for Nano plates, Source: https://doi.org/10.3390/colloids9030031

AgNPs created through plant extracts demonstrate better biological activity than their chemically made counterparts [8]. The mechanism of their action begins with the destruction of microbial cell membranes which leads to the production of reactive oxygen species (ROS) while they disrupt essential internal elements such as DNA and proteins.

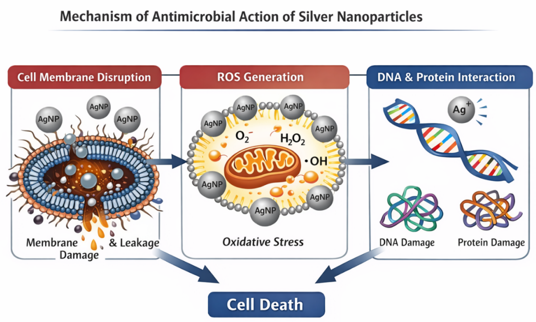

Figure 3: Mechanism of Antimicrobial Action of Silver Nanoparticles, Source: Author Generated

The study investigates the green synthesis of silver nanoparticles through Azadirachta indica leaf extract which transforms into their potential biological activities through in vitro testing [9. 10]. The research aims to elucidate the mechanisms underlying nanoparticle creation and its functional implications in addressing microbial resistance, therefore aiding in the advancement of sustainable nanotechnology-based treatment approaches.

2. Materials and Methods

2.1 Plant Material and Extract Preparation

Researchers selected fresh Azadirachta indica leaves because these leaves contain high phytochemical levels and people use them for medicinal purposes. The collected leaves undergo a two-phase cleaning procedure which starts with washing in distilled water to eliminate dust and surface contaminants and continues with shade-drying at ambient temperature to protect thermolabile bioactive compounds. The dried leaves are then pulverised into a fine powder using a sterilised mechanical grinder [7].

The researchers heated 10 grams of powdered material in 100 millilitres of distilled water for 15 to 20 minutes to extract phytoconstituents. The solution is subjected to cooling and filtration through Whatman No. 1 filter paper to obtain a clear aqueous extract. The extract is stored at 4°C which keeps its phytochemical content stable for 24 to 48 hours.

2.2 Green Synthesis of Silver Nanoparticles

Researchers create silver nanoparticles through green synthesis which requires them to combine plant extract with 1 mM silver nitrate aqueous solution. The continuous agitation process maintains a ratio of 1:10 which combines extract with $AgNO_3$ solution at ambient temperatures and temperatures that reach up to 60 degrees Celsius. The reaction mixture shows a palpable color shift from pale yellow to dark brown because AgNPs were produced through surface plasmon resonance. The extract contains phytochemicals which act as reducing agents to convert Ag^+ ions into metallic silver (Ag^0) while they stabilize nanoparticles by performing capping processes [11]. The factors that influence reactions through pH temperature and reaction duration create definite impact on how nanoparticle size and shape and production amount. The researchers isolated the produced nanoparticles through centrifugation process which they followed by washing to remove all biomolecules that did not attach.

2.3 Characterization Techniques

Scientists use two types of telescopes to study silver nanoparticles. The lens device lets them see the shape of the particles, their internal structure, and the process of making them. UV–Visible spectroscopy serves as a fundamental scientific instrument which scientists employ to investigate various methods of nanoparticle production. The unique surface plasmon resonance (SPR) peak appeared at 420 nanometres which falls within the wavelength range of 400 to 450 nanometres. The results showed that AgNPs had been produced.

Fourier Transform Infrared Spectroscopy (FTIR) enables scientists to identify functional groups which participate in both stability and reduction reactions.

The peaks which correspond to hydroxyl (–OH) and carbonyl (C=O) and amine (–NH) groups show displacement because the chemical compounds interact with silver ions. Scanning Electron Microscopy (SEM) provides information about the particle shape and size distribution of nanoparticles which scientists expect to have spherical shapes and even size distribution. X-ray Diffraction (XRD) research verifies the crystalline structure of AgNPs which show distinct Bragg reflection peaks that correspond with face-centered cubic (FCC) silver configurations [11, 12].

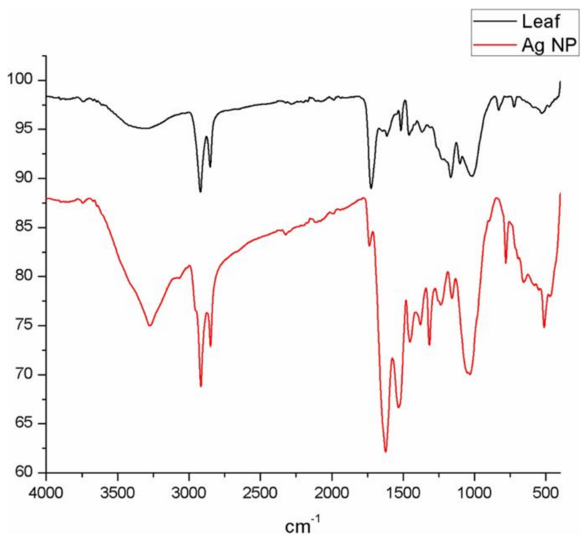

Figure 4:Green synthesis of silver nanoparticle, Source: https://www.nature.com/articles/s41598-024-69120-0?

2.4 In-Vitro Evaluation

Scientists conducted in vitro biological tests on green-synthesized AgNPs to evaluate their efficacy in eliminating microbes while preserving cellular integrity. The disc diffusion method assesses the effectiveness of various substances against standard Gram-positive and Gram-negative bacterial strains. Researchers placed AgNP-coated discs, devoid of microorganisms, onto agar plates containing pre-existing bacterial cultures. After the incubation period here, the researchers here took measurements of the inhibition zones for thi\s matter.

Laboratory tests here for DPPH radical scavenging and FRAP (Ferric Reducing Antioxidant Power) measure to know how well antioxidant enzymes work in this nature. The tests in this manner can see how well AgNPs can fight free radicals and lower reactive stress at the same time here. AgNPs have better than more biological activity because their smaller size makes them utmost active on the surface, which eventually helps them interact better with Azadirachta indica phytochemicals herein. These situations make them useful in both medical and industrial settings.

3. Results

The process of making silver nanoparticles from Azadirachta indica leaf extract works because both visual and chemical tests show that it works. The first change that can be seen in the reaction mixture is when its color changes from pale yellow to dark brown. This happened because the nanoparticles developed a surface plasmon resonance (SPR) phenomenon. The first visual change shows that AgNPs have started to form in the solution [13]. The UV–Visible spectroscopy method should show a strong absorption peak that covers a wide range with its peak value at 420 nm, which is the standard absorption pattern for silver nanoparticles. The peak location and intensity give information about the size distribution and strength of the nanoparticles. Uniform particle formation produces a more intense peak than other methods of particle creation [14].

Parameter	Expected Observation	Interpretation
Absorption Peak	~420 nm	Confirms AgNP formation
Peak Width	Moderate	Indicates polydispersity
Peak Intensity	High	Stable nanoparticle formation

Table 2: Expected UV–Visible Spectral Characteristics of AgNPs, Source: Author Generated

The FTIR spectral analysis will reveal significant alterations in absorption bands associated with functional groups such as amine (–NH), carbonyl (C=O), and hydroxyl (–OH). The study indicates that antioxidants function as stabilizing agents that prevent silver ions from oxidizing while maintaining the stability of nanoparticles through direct interactions with caps. The SEM analysis is expected to display spherical nanoparticles exhibiting a uniform size distribution within the 10 to 50 nanometer range [15]. The absence of significant agglomeration supports the finding that plant-based biomolecules effectively maintain system stability. The XRD analysis would show clear diffraction peaks that match the FCC structure of metallic silver. This would prove that the nanoparticles are crystalline structures.

The in vitro antimicrobial test should demonstrate that AgNPs exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The disc diffusion assays would show bigger inhibition zones than those of plant extract alone which means that the nanoparticles would make the plant extract more effective [16].

The DPPH and FRAP antioxidant tests should show that they can pick up a lot of free radicals. This is due to the phytochemicals and silver nanoparticles that are attached to the surface.

Assay	Expected Outcome	Significance
Antimicrobial (Disc Diffusion)	Large inhibition zones	Broad-spectrum activity
DPPH Assay	High % scavenging	Strong antioxidant capacity
FRAP Assay	Increased reducing power	Electron-donating ability

Table 3: Conceptual Summary of In-Vitro Biological Activity, Source: Author Generated

Overall, the required results support the idea that green synthesis-based structure with Azadirachta indica makes stable, bioactive based silver nanoparticles that have a lot of promise for use in medicine.

4. Discussion

The sustainable production process for silver nanoparticles (AgNPs) through Azadirachta indica extract demonstrates an environmentally friendly method which delivers high performance results [8,9]. The process complies with green chemistry regulations. The biosynthesis process occurs when plant chemicals transform into their final forms. The substances function as agents which both reduce and maintain stability. The proteins transfer electrons to silver ions (Ag^{+}) which leads to the formation of metallic silver (Ag^{0}). The proteins create a protective layer around the nanoparticles which prevents them from forming clumps [10]. This technology has two functions, so it doesn't need chemical stabilizers from outside sources.

AgNPs display an intricate yet potent mechanism which effectively eradicates microbial organisms [16]. The nanoparticles penetrate into microbial cell walls which results in increased permeability and structural damage to the cells. The AgNPs generate reactive oxygen species (ROS) which induce oxidative stress in bacterial cells while they damage proteins and lipids and nucleic acids. The silver ion release causes the poison to become more dangerous because it disrupts enzyme function and DNA replication [11]. The combination of these factors results in cell death. The nanoscale dimensions of AgNPs increase their surface reactivity while enabling deeper penetration into microbial cells which enhances their ability to kill microbes.

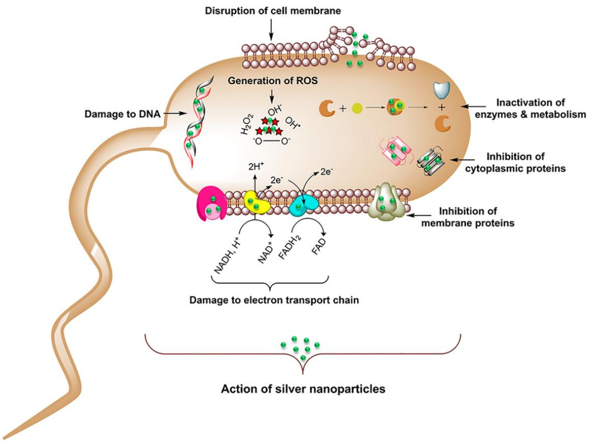

Figure 5: Silver nanoparticles produced from Cedecea sp. exhibit antibiofilm activity and remarkable stability, Source: https://www.nature.com/articles/s41598-021-92006-4?

Plant-mediated synthesis presents numerous benefits when compared to chemical and physical synthesis methods. The traditional methods require substantial energy consumption and they utilize hazardous reducing agents such as sodium borohydride which creates safety and environmental problems [18]. The use of Azadirachta indica extract for green synthesis produces environmentally friendly results which naturally decompose and generate less waste materials. The literature shows that plant-derived AgNPs produce the same or better biological activity because their surface phytochemicals work together. The research work of Ahmed et al. and Iravani has shown that plant-mediated nanoparticle production works effectively. Neem extract shows effectiveness because it contains bioactive compounds. The research shows that Azadirachta indica AgNPs have stronger antibacterial properties against dangerous bacteria which includes Staphylococcus aureus and Escherichia coli based on previous scientific studies [12]. The benefits come with requirements that must be addressed. The phytochemical makeup changes depending on where you are, what time of year it is, and what the weather is like. The method makes different types of nanoparticles with different sizes and frequencies. To keep the nanoparticles' size and performance during large-scale production, the extraction and synthesis processes must be standardized. Green-synthesized AgNPs exhibit excellent potential for biomedical applications which include drug delivery systems and wound healing and antimicrobial coatings and diagnostic tools. Their safe use and ability to work with living organisms make them ideal tools for translational research. Future studies should concentrate on in-vivo validation and toxicity profiling and clinical application to achieve maxim.

5. Conclusion

The research demonstrates how to create sustainable silver nanoparticles through the application of Azadirachta indica leaf extract which demonstrates their potential for biological research applications [2]. The plant-based method employs natural plant compounds to function as both reducing agents and stabilizing agents whereas it provides an environmentally sustainable approach to nanoparticle production which remains economically viable throughout its entire operational lifespan. The synthesized silver nanoparticles exhibit potent antimicrobial properties alongside their capability to safeguard against oxidative damage. Their diminutive size, coupled with their pronounced surface activity and ability to interact with plant-derived biological materials, elucidates this phenomenon.

The theoretical results correspond with existing literature which demonstrates that neem-based green synthesis produces stable nanoparticles with biological activity [17]. The method requires resolution of two major problems which include plant composition changes and the necessity to establish standardized procedures which will enable successful large-scale operations. The scientific community must prioritize the testing of these nanoparticles via experimental studies, alongside thorough safety evaluations and assessments of their clinical applicability. The amalgamation of green nanotechnology with biomedical applications offers significant prospects for the development of innovative treatments aimed at oxidative stress-related diseases and infections exhibiting treatment resistance.

Acknowledgement

Conflict of Interest

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