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Green Synthesis and Characterization of Silver Nanoparticles using Neem (*Azadirachta indica*) Leaf extract

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ABSTRACT: The development of eco-friendly and reliable processes for manufacturing silver nanoparticles (AgNPs) represents a significant milestone in the realm of nanotechnology. Green synthesis is preferable to traditional chemical synthesis because it is less expensive, produces less pollution and improves environmental and human health safety. Hence, the synthesis of silver nanoparticles using neem (*Azadirachta indica*) leaf extract as a reducing and capping agent was carried out in the present study. The synthesis of silver nanoparticles was achieved by treating aqueous silver nitrate solution with neem leaf extract under controlled conditions. The reduction of silver ions to silver nanoparticles was confirmed by observing a change in color from pale yellow to brownish-black, indicating the formation of AgNPs. The synthesized nanoparticles were characterized using various techniques such as UV-visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The UV-Vis absorption spectrum revealed a distinct absorption band centered at 460 nm, indicative of the characteristic surface plasmon resonance of the silver nanoparticles. Furthermore, the SEM image provided evidence of their mild agglomerated spherical shape. XRD confirms the crystalline nature of the synthesized nanoparticles with sharp peaks at various degrees at 38.2^0, 32.3^{0,} and 46.2^0. The stability of the material was confirmed with Zeta potential at the value of -4.02 mV. The characterization results demonstrate the successful formation of silver nanoparticles with desired properties. The synthesized AgNPs hold great potential for various applications such as catalysis, antimicrobial agents, and sensor development.

Keyword: Green synthesis, Neem leaf extract, Silver nanoparticles, Scanning Electron Microscopy, UV-Visible spectrophotometer, Zeta potential.

INTRODUCTION

A particle with a dimension between one to hundred nanometers is referred as a nanoparticle. With its remarkable antimicrobial properties, silver has emerged as a leading contender among nanoparticles, demonstrating immense promise as an exceptionally effective agent in combatting microorganisms. Reduced toxicity, cost effectiveness, and an abundance of decreasing raw materials are all benefits of greener synthesis of AgNPs. Moreover, the exceptional antibacterial (Banerjee *et al.*, 2014; Velusamy *et al.*, 2015) and antifungal (Kim *et al*., 2012; Jafari *et al.*, 2015) properties of silver nanoparticles (AgNPs) have captured significant attention. In addition to these remarkable attributes, AgNPs have garnered widespread recognition for their impressive optical and chemical capabilities. Furthermore, their biocompatibility, low toxicity, and eco-friendly nature further contribute to the growing fascination surrounding these nanoparticles. This uniqueness leads to green synthesis as a promising process for generating nanoparticles. Green synthesis techniques include producing nanoparticles using various plant parts like stem, root, leaf, and microorganisms including bacteria, fungi, and yeast. In contrast to conventional chemical and physical synthesis methods, this approach offers notable advantages in terms of both cost-effectiveness and environmental sustainability (Nangare and Patil 2020). In recent years, the biosynthesis method utilizing plant extracts from *Pelargonium graveolens*, *Medicago sativa*, *Azadirachta indica*, Lemongrass, *Aloe vera*, and *Cinnamomum Camphor* has garnered considerable interest as a viable alternative to conventional methods (Verma and Mehta 2016). In the current study we made attempt on the green synthesis of AgNPs using *Azadirachta indica* leaf extract.

The leaf extract of *Azadirachta indica* plays a pivotal role as a stabilizing agent in the synthesis of various nanoparticles, such as gold, zinc oxide, and silver, among others. The presence of phytochemicals like terpenoids and flavanones in the extract acts as both reducing and capping agents, facilitating the stabilization of the nanoparticles. Notably, when the leaf extract is introduced to a silver salt solution, it triggers the transformation of the silver salt into silver nanoparticles (AgNPs) (Verma and Mehta 2016). As a result, leaf extract simultaneously acts as a reducing agent and a capping agent without the need of chemicals (Lalitha *et al*., 2013). The green synthesis of AgNPs is achieved by treating aqueous silver nitrate solution with neem leaf extract under controlled conditions. The reduction of silver ions to silver nanoparticles can be confirmed by observing a change in color from pale yellow to brownish-black, indicating the formation of AgNPs (Ghazali *et al*., 2022). Hassanin *et al*. (2021) synthesized Ag, Co_3O_4 nanoparticles and different weight ratio of Ag/Co3O4 nanocomposites by using the neem leaf extract as reducing and stabilizing agent for the nanoparticles and the obtained samples were identified by using Fourier-transform infrared (FT-IR) and UV-visible spectrophotometry, Transmission Electron Microscopy (TEM), Scanning electron microscopy (SEM), X-ray energy dispersive spectroscopy (EDAX), and X-ray diffraction (XRD). Zanjage and Khan (2021) conducted a study on the synthesis of silver nanoparticles using the aqueous extract of neem leaves in the presence of aqueous precursor silver nitrate salt ($AgNO_3$) at a concentration of 1 mM. The *Azadirachta indica* (neem) leaf extracts were used to create the silver nanoparticles and characterized the nano particles using X-ray diffractometer (XRD), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) spectroscopy methods according to Saravanan (2021).

In this study, silver nanoparticles (AgNPs) were synthesized using a green approach with neem leaf extract as the key ingredient. The synthesized AgNPs were subsequently subjected to characterization using various techniques, including UV-Visible spectroscopy to analyze their optical properties, Scanning Electron Microscope (SEM) to examine their morphology, X-Ray Diffraction analysis (XRD) to investigate their crystal structure, and Zeta Potential measurement to determine their surface charge properties.

MATERIALS AND METHODS

The experiment was conducted during July 2022 - June 2023 at the Horticulture laboratory, Karunya Institute of Technology and Sciences, Coimbatore. For the green synthesis of nanoparticles, neem leaves were collected from the campus of Karunya Institute of Technology and Sciences, Coimbatore and it was authenticated by the Botanical Survey of India, Coimbatore (TNAU campus). The chemicals were purchased from Hi-Media Laboratories Pvt. Ltd.

A. Synthesis of silver nanoparticles

Preparation of leaf extract. For the preparation of the neem leaf extract, 20 grams of neem leaves were carefully cut into small pieces. The chopped leaves were then subjected to boiling in 100 ml of distilled water at 80°C for a duration of 30 minutes, resulting in the extraction of bioactive compounds from the leaves. To obtain a clear extract, the mixture was filtered using Whatman filter paper, with the filtrate collected in a conical flask. To ensure its preservation, the flask containing the neem leaf extract was stored in a refrigerator at 4°C, ready for subsequent utilization in further experiments.

Green Synthesis of Silver Nanoparticles. To initiate the synthesis of silver nanoparticles (AgNPs), an aqueous solution of silver nitrate ($AgNO_3$) was prepared with a concentration of 1mM. Neem leaf extract, known for its reducing properties, was combined with the $AgNO_3$ solution in a ratio of 1:9 and introduced into a conical flask. The flask containing the mixture was then subjected to heat in a water bath set at 80°C. As the reaction progressed, a noticeable color transformation occurred, shifting from yellow to brown, serving as a visual confirmation of the successful synthesis of silver nanoparticles.

To eliminate any remaining insoluble materials in the solution, the deposited mixture underwent purification through centrifugation at a speed of 3000 rpm for a duration of 10 minutes. This process effectively separated the undesired substances, allowing for the extraction of the supernatant. To convert the supernatant into a powdered form, a lyophilizer (Model: LY 04) was employed, utilizing a freeze-drying technique. By subjecting the supernatant to this process, the moisture content was removed, resulting in the obtainment of silver nanoparticles in a powdered form.

B. Characterization of silver nanoparticles

UV-Visible Spectroscopy. The progress of Ag+ ion reduction was continuously monitored throughout the reaction by measuring the UV-Vis spectrum of the reaction medium. The absorbance readings were recorded within the wavelength range of 200-800 nm, employing a UV-Visible spectrophotometer (Hitachi U-2910 spectrophotometer, Japan). This analytical technique allowed for the observation and quantification of any changes in absorbance, providing valuable insights into the reduction process of Ag+ ions during the synthesis of silver nanoparticles (Renugadevi and Ashwini 2012).

Scanning Electron Microscopy. Scanning Electron Microscopy (JSM-6390) plays a crucial role in providing valuable insights into the sample under investigation. This technique allows for the examination of various aspects, including the external morphology, chemical composition, crystalline structure and material orientation. SEM operates by utilizing a focused electron beam that systematically scans the surface of the sample, detecting secondary or backscattered electron signals. These signals are then processed to generate highly detailed and high-resolution images, enabling a comprehensive characterization of the sample's features and properties.

X-ray Diffraction analysis. To elucidate the crystalline nature of the nanoparticles, X-ray diffraction (XRD) analysis was conducted using the LabX XRD-6100 instrument. The study involved the use of the dried powdered sample for analysis. The nanoparticles were examined using CuKα radiation, with voltage and current settings of 40 kV and 40 mA, respectively. The diffracted intensities were recorded over a range of 2θ angles spanning from 20° to 80°. This XRD analysis facilitated the characterization of the nanoparticles' crystalline structure, providing valuable information about their lattice arrangement and crystallographic properties. (Ibrahim *et al.,* 2015).

Zeta potential. The determination of the nanoparticle's stability, zeta potential was carried out using dynamic light scattering (Zeta Sizer Nano ZS 90). Zeta potential, a measure of the surface charge of particles in suspension, plays a crucial role in their stability and interactions. When particles possess a large negative or positive zeta potential, they tend to repel each other, preventing flocculation or aggregation. Conversely, particles with low zeta potential values lack the repulsive forces necessary to prevent their coming together and flocculating. The zeta potential value serves as a valuable parameter for understanding and predicting the interactions among suspended particles. Moreover, this concept has been employed to study cell adhesion, as surface charge properties are closely related to such adhesion phenomena (Singh *et al.,* 2014).

RESULT AND DISCUSSION

A. Synthesis of silver nanoparticles

Upon the introduction of neem leaf extract into the $AgNO_3$ solution, a distinct and visually apparent color transformation occurred from yellow to dark brown. This noticeable change in color serves as a clear indication of the formation of silver nanoparticles. The phenomenon of color alteration arises due to the reduction of silver ions present in the solution, facilitated by the inherent activity of terpenoids found in the neem leaf extract. These terpenoids actively participate in the reduction process, leading to the generation and stabilization of silver nanoparticles, ultimately manifesting in the observed color change.

B. Characterization of green synthesized nanoparticles

UV-Visible Spectroscopy. UV-Visible spectroscopy was employed as a crucial tool to determine and analyze the absorption characteristics of the synthesized nanoparticles. Fig. 1 displayed the absorption spectrum, revealing significant findings. It was observed that the synthesized nanoparticles exhibited a prominent broadening of the absorption band at 460 nm, which corresponds to the absorption of silver nanoparticles. This outcome, as indicated by the UV-Vis absorption spectrum, confirms the successful formation of silver nanoparticles within the visible range, specifically around 420 nm. This observation can be attributed to the proximity of the conduction and valence bands, facilitating the unrestricted movement of electrons. Consequently, the phenomenon of surface plasmon resonance (SPR) arises, resulting in the distinct absorption band observed in the spectrum (Lakshmi *et al.,* 2015).

Scanning Electron Microscopy. To investigate the surface morphology of the synthesized nanoparticles, Scanning Electron Microscopy (SEM) analysis was performed.

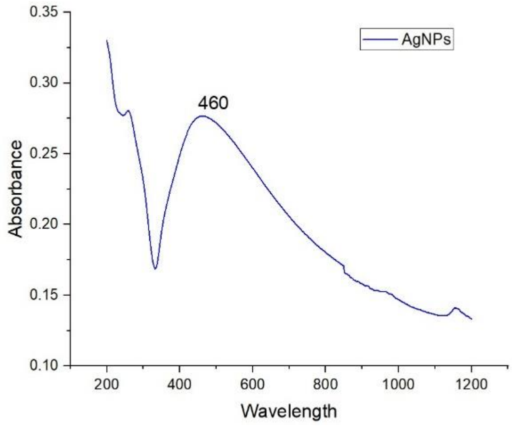

Fig. 1. UV visible spectroscopy of synthesized AgNPs.

Fig. 2 illustrates the results of this analysis, providing valuable insights into the physical characteristics of the synthesized silver material. The SEM image clearly demonstrates that the silver nanoparticles exhibit spherical structures with mild agglomeration. This observation further corroborates the findings of the analysis, confirming the successful synthesis of the nanoparticles with the desired morphological features. In a study conducted by Amooaghaie *et al*. (2015), it was found that the majority of the AgNPs synthesized through green methods exhibited a spherical shape, with some particles showing angular characteristics. Furthermore, it was observed that the nanoparticles tended to agglomerate, forming small aggregates. These findings align with the typical behavior and characteristics of AgNPs synthesized using green approaches, highlighting the consistency of the results across different studies.

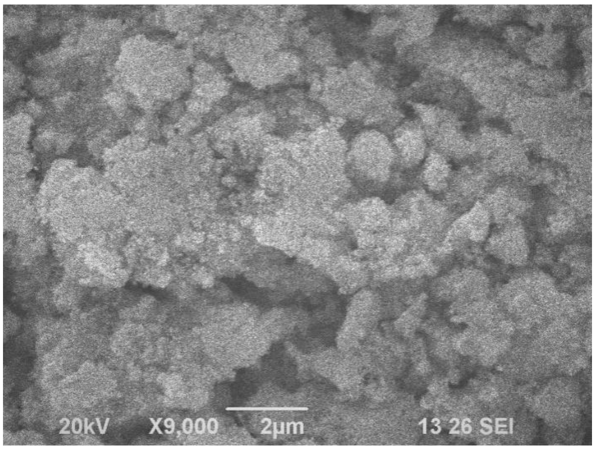

Fig. 2. SEM image of synthesized AgNPs.

X-ray Diffraction analysis. To investigate the crystalline nature of the synthesized silver particles, an X-Ray Diffraction (XRD) analysis was conducted. Fig. 3 displays the XRD pattern obtained, revealing important insights. The synthesized silver particles exhibited sharp peaks at 38.20°, 32.30°, and 46.20°, which were indexed and matched with the Joint Committee on Powder Diffraction Standards (JCDS) card number no: 04-0783. These results indicate that the synthesized silver particles possess a highly crystalline structure. Similar findings were reported by Kumar and Yadav (2009), who also identified crystalline peaks at 32.28°, 46.28°, 54.83°, 67.47°, and 76.69°.

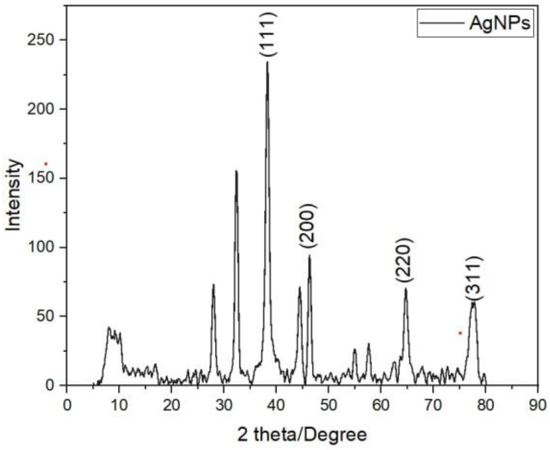

Fig. 3. XRD image of synthesized AgNPs.

The presence of these peaks is attributed to the phytochemical compounds present in the leaf extracts. Moreover, the stronger planes in the XRD pattern further emphasize the presence of silver as a major constituent in the biosynthesis process, consolidating the evidence of successful silver nanoparticle synthesis.

Zeta potential. Zeta potential analysis was employed to determine the surface charge and stability of the synthesized material. Fig. 5 presents the results of this analysis, revealing valuable information. The Zeta potential value of the synthesized material was found to be -4.02 mV. This value indicates that the surface charge of the synthesized material is close to neutral, suggesting a balanced distribution of positive and negative charges on its surface. In a study conducted by Singh *et al*. (2014), it was concluded that spherical nanoparticles exhibited a potential of -5.11 mV, while hexagonal nanoparticles displayed a potential of -15.3 mV. This finding indicates that a change in nanoparticle shape from isotropic (spherical) to anisotropic (hexagonal) led to an increase in the stability of the nanoparticles. The difference in zeta potential values suggests that the anisotropic nanoparticles possessed a higher level of stability compared to their spherical counterparts. This observation highlights the influence of nanoparticle shape on their surface charge and stability, emphasizing the importance of considering shape-dependent properties in the design and application of nanoparticles.

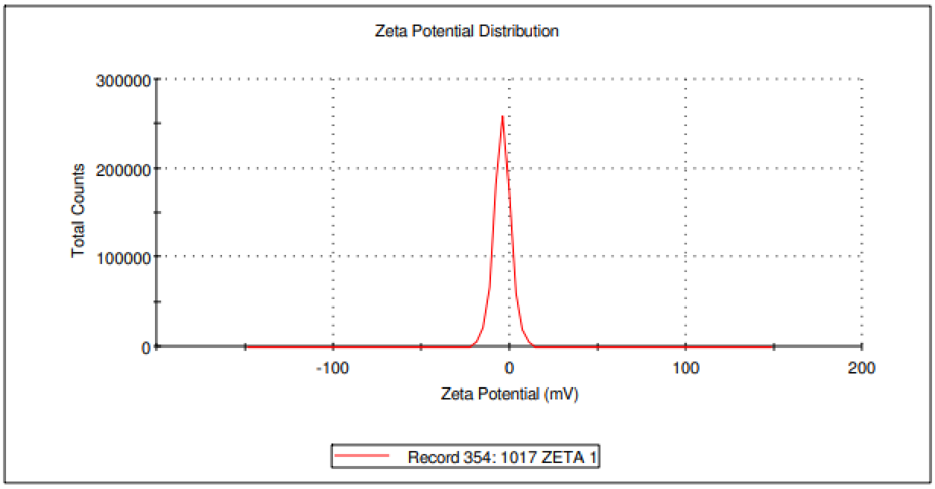

Fig. 4. Zeta potential image of synthesized AgNPs.

CONCLUSION

Silver nanoparticles were successfully synthesized using a neem leaf extract in the presence of $AgNO_3$ and subsequently characterized using various analytical techniques. UV-Visible spectroscopy revealed a distinct color change of the solution from yellow to brown, providing confirmation of the successful synthesis of silver nanoparticles. The UV-Visible spectrum exhibited a significant peak widening at 460 nm, further supporting the presence of synthesized nanoparticles. Scanning Electron Microscope (SEM) analysis unveiled the morphology of the nanoparticles, demonstrating a mild agglomeration and a spherical shape. X-Ray Diffraction (XRD) analysis confirmed the crystalline nature of the synthesized nanoparticles. Lastly, Zeta Potential analysis revealed the stability of the material, with a measured value of -4.02 mV, indicating a near-neutral surface charge. Collectively, these characterizations validate the successful synthesis of silver nanoparticles using neem leaf extract and provide valuable insights into their optical, morphological, crystalline, and surface charge properties.

FUTURE SCOPE

Green nanotechnology is an emerging technology that holds great promise in creating innovative, reliable, and sustainable solutions across various domains. One notable application of green nanotechnology lies in the synthesis of nanoparticles using environmentally friendly methods. These green synthesized nanoparticles exhibit immense potential for evaluating their antimicrobial efficacy, making them particularly relevant in the biomedical field. The utilization of these nanoparticles opens doors to explore their effectiveness in combating microbial infections and developing novel approaches for biomedical applications. The intersection of green nanotechnology and the biomedical field offers exciting opportunities for advancing sustainable and impactful solutions that benefit society at large.

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