

Research Article

Green synthesis of silver nanoparticles using leaves of *Lantana camara*: Impact of extract quantity, reaction time on shape, size and antibacterial activity

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Abstract

Types of plant extract, concentration of precursor solution and the incubation time play a significant role in the kinetics of nanoparticle formation during green synthesis. The present study focused on the effect of these factors on the size, uniformity and antibacterial activity of synthesized silver nanoparticles (AgNPs). *Lantana camara* leaf extract was used as a reducing and stabilizing agent for the biosynthesis of AgNPs. The synthesis process involved the careful preparation of leaf extract (10%), followed by the reaction of different volumes 10, 5, 2 and 1ml of extract with 50ml of 1.0mM silver nitrate solution under controlled conditions with reaction time of 10, 30, 45 and 1hr respectively-vis spectrum indicated a characteristic single peak at 423nm, 429nm, 435nm and 443nm for samples following increasing quantity of leaf extract. Transmission electron microscopy (TEM) revealed a decrease in the size of nanoparticles and an increase in incubation time. The size of the particles was 12 nm, 15nm, 17nm and 23nm, respectively, during the incubation time of 10, 30, 45 and 1hr. The antibacterial efficacy of the AgNPs was evaluated against *Escherichia coli* O157:H7, and a significant increase in antibacterial activity was demonstrated with a decrease in size. FTIR revealed the involvement of hydroxyl, carbonyl and amide groups in reducing and stabilizing agents present in leaf extract for the synthesis of nanoparticles. This study authenticates the advantages of using *L. camara* for green synthesis of silver nanoparticles; more prolonged incubation with lower concentrations of precursor solution resulted in smaller nanoparticles with higher antibacterial activity.

Keywords: Antibacterial activity, Extract concentration, Green synthesis, *Lantana camara*, Reaction time, Silver nanoparticles**INTRODUCTION**

Green synthesis of nanoparticles, especially metal nanoparticles, has emerged as a sustainable and eco-friendly alternative to traditional chemical and physical methods. This approach leverages biological entities such as plant extracts, bacteria, fungi, and algae to reduce and stabilise metal ions, leading to the formation of nanoparticles. The method is considered green because it avoids the use of harmful chemicals, employs natural reducing agents, and produces minimal environmental waste (Mittal *et al.*, 2013). Plant-

based green synthesis of nanoparticles has attracted particular attention due to the availability of diverse phytochemicals that serve as natural reducing agents. Plants contain secondary metabolites like flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds, which can efficiently reduce metal ions such as silver (Ag^+), gold (Au^{3+}), and others into their respective nanoparticles (Srikar *et al.*, 2016)

For instance, in the synthesis of silver nanoparticles (AgNPs), the functional groups such as hydroxyl and carbonyl groups present in these metabolites of plant extracts, interact with silver ions (Ag^+), reducing them

to elemental silver (Ag^0) while simultaneously stabilizing the nanoparticles to prevent agglomeration. Various plants, such as *Azadirachta indica* (neem), *Zingiber officinale* (Ginger), *Asparagus racemosus* (Shatavari) and *Ocimum sanctum* (holy basil) etc., have been explored for synthesizing nanoparticles with enhanced biological activities, including antimicrobial, anticancer, antioxidant properties and drug delivery. (Jadoun et al., 2021; Giri et al., 2023; Arshad et al., 2024).

Silver nanoparticles are particularly favoured in green synthesis due to their broad-spectrum antimicrobial properties (Wanget al., 2017; Roy et al., 2017).

The emergence of antibiotic-resistant bacterial strains has become a significant global health concern, necessitating the search for alternative antimicrobial agents (Murray et al., 2022). Nanoparticles, particularly metal-based nanoparticles such as silver (AgNPs), gold (AuNPs), and zinc oxide (ZnO NPs), have gained considerable attention due to their potent antibacterial properties (Zhang et al., 2022). These nanoparticles exhibit bactericidal activity through multiple mechanisms, including cell membrane disruption, reactive oxygen species (ROS) generation, and interference with DNA replication and protein synthesis (Mondal et al., 2024).

Among various nanoparticles, silver nanoparticles (AgNPs) have demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, making them a promising candidate for biomedical applications (Wang et al., 2017).

As research in green nanotechnology progresses, emphasis is placed on the large-scale production of nanoparticles using biogenic methods. These advances aim to reduce the cost of nanoparticle synthesis while maintaining consistency and enhancing the biocompatibility of the nanoparticles. A major advantage of plant-mediated synthesis is its simplicity and scalability through the potential for producing nanoparticles with unique properties that depend on various factors, e.g., the condition of reaction, time and the plant species used etc. (Rana et al., 2020).

Lantana camara is a perennial shrub from the Verbenaceae family, native to Central and South America, but it has spread worldwide and is now classified as an invasive species in many regions. Its colourful flowers and ability to thrive in diverse conditions have contributed to its ornamental popularity. However, it poses ecological challenges by displacing native plant species and being toxic to livestock (Ruwanza, 2020). *Lantana camara* contains a wide variety of bioactive compounds, such as terpenoids, flavonoids, phenolics, alkaloids, and essential oils, which are responsible for its medicinal properties, including antimicrobial, anti-inflammatory, and antioxidant effects (Shah et al., 2020).

Recent research has focused on optimizing the condi-

tions for green synthesis, including plant species selection, extraction methods, pH, temperature, and precursor concentrations (Jadoun et al., 2021). The quantity of plant extract used in green synthesis directly impacts the production of nanoparticles. The plant extract acts as an electron donor, facilitating the transformation of metal ions into nanoparticles. Reducing the amount of plant extract used leads to a decrease in the number of nanoparticles produced (Radulescu et al., 2023). At the same time, an increase in reaction time results in the shift of absorption peak towards a higher wavelength, which indicates the larger size of nanoparticles (Liaquat et al., 2022).

Thus, the present study has been carried out on the green synthesis of silver nanoparticles using leaves extract of *L. camara* and the effect of extract concentration and reaction time on the kinetics of particle synthesis and characters.

MATERIALS AND METHODS

All reagents utilized in this study were of analytical grade and employed as received, with no additional purification steps. Silver nitrate (AgNO_3), with a purity of 99.5%, was sourced from Sigma-Aldrich, India. Fresh specimens of *L. camara* were harvested from the local region of Meerut district, Uttar Pradesh, India. Solutions necessary for the synthesis processes were prepared using deionized water. To ensure contamination-free conditions, all glassware was cleaned using a solution of aqua regia (a 1:3 ratio of nitric acid to hydrochloric acid) and subsequently rinsed thoroughly with deionized water to maintain high standards of cleanliness.

Preparation of the leaf extract

In the present study, *Lantana camara* leaf extract was used as a biological material for synthesis of silver nanoparticles (Fig.1). For the preparation of the extract, fresh and healthy leaves were thoroughly rinsed with tap water and then distilled water to remove dust and other visible impurities. The leaves were cut into small pieces and air-dried at room temperature to eliminate moisture completely. The dried leaves were ground into a fine powder using a mortar and pestle. Approximately 10 grams of this powder were added to 100 mL of water and kept at 60°C for 30 minutes. The resulting extract was filtered through Whatman No. 1 filter paper to remove any particulate matter, yielding a clear solution. This solution was then stored in 250 mL Erlenmeyer flasks and refrigerated at 4°C for future experiments. Throughout the process, sterile conditions were maintained to ensure the accuracy and effectiveness of the results, preventing contamination.

Silver nanoparticle (Ag NPs) synthesis with varying concentrations of extract

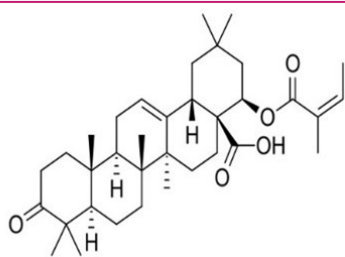


Fig. 1. *Lantana camara* and chemical structure of Lamtidines

To synthesize silver nanoparticles (AgNPs), a 50 mL solution of 1mM AgNO_3 (Banerjee *et al.*, 2014) was prepared in a 100 mL reaction vessel. Subsequently, 10ml (S1), 5ml (S2), 2ml (S3) and 1 ml (S4) of aqueous leaf extract were added to each silver nitrate solution. The pH of solution was 7.0, and the volume of each sample was made up to 60 ml with deionized water. These reaction mixtures were then homogenized and kept for reaction in a dark environment at 37°C to prevent any light-induced degradation of the silver nitrate. The time of reaction given to samples were 15min, 30min, 45min and 1hr for S1, S2, S3 and S4 respectively. In addition, a negative control setup was established without the Silver nitrate to serve as a negative control (C). The successful formation of AgNPs was confirmed by the observation of a distinct brown colour, which is characteristic of silver nanoparticles (Gupta *et al.*, 2024).

UV-visible spectra were recorded at the respective time intervals between wavelengths of 200 to 700 nm (Quevedo *et al.*, 2021) in a spectrophotometer (Labindia Dual Beam UV-VIS 2000 Spectrophotometer, Model Name/Number: UV2000)

Following the respective reaction time, the resulting colloidal solutions underwent centrifugation at 10,000 rpm for 15 minutes to isolate and purify the synthesized AgNPs. The pellets obtained from centrifugation were washed twice with deionized water to remove any unreacted precursors or by-products. Finally, the purified nanoparticles were re-dispersed in deionized water, preparing them for subsequent experiments and analyses. This thorough washing and re-dispersion ensured that the AgNPs were well-characterized and suitable for various applications.

Characterization of Ag NPs

The synthesis of AgNPs was confirmed by UV-visible spectroscopy having a wavelength in the range of 200-700 nm. The FTIR analysis of the dried silver nanoparticles (AgNPs) was performed using the potassium bromide (KBr) pellet method at a 1:100 sample-to-KBr ratio (Smith, 2011). The spectra were acquired with a Jasco FT/IR-6300 spectrometer equipped with a JASCO IRT-7000 Intron Infrared Microscope, operating in transmittance mode. The instrument was set to a resolution of 4 cm^{-1} to ensure accurate detection of

functional groups involved in the synthesis and stabilization of the AgNPs. This setup provided detailed insights into the chemical interactions between the nanoparticles and the plant extract.

The size and structural characteristics of the nanoparticles were determined using transmission electron microscopy (TEM -Phillips TECHNAI FE 12 instrument). This technique provided detailed images at the nanoscale, allowing for precise confirmation of particle morphology and dimensions.

Estimation of antibacterial activity

Escherichia coli O157:H7 (procured from King George's Medical University, Lucknow) was used to analyze the antibacterial activity of the synthesized nanoparticles in this study. Bacterial culture (*E. coli* with an OD around 0.1) was uniformly spread on the surface of the agar plate using a sterile swab to create a bacterial lawn. The antibiotic activity was estimated with a Well diffusion method (Syame *et al.*, 2020). 50 μL of different samples were poured into the wells (S1, S2, S3 and S4). Along with the samples, a negative control (C) was also considered poured with sterile distilled water.

RESULTS AND DISCUSSION

UV-visible spectrophotometer analysis

UV-V spectroscopy is a key technique for confirming the formation and assessing the stability of metal nanoparticles in an aqueous solution. When the leaf extract was added to the silver nitrate solution, a noticeable color shifted from clear yellowish green to brown (Fig.2b), signifying the formation of silver nanoparticles (AgNPs). This color change was due to the collective oscillation of free conduction electrons, a phenomenon called surface plasmon resonance (SPR) caused by the interaction with an electromagnetic field.

In the UV-Vis spectrum of AgNPs, all the samples (S1, S2, S3 and S4) showed a single absorption peak at

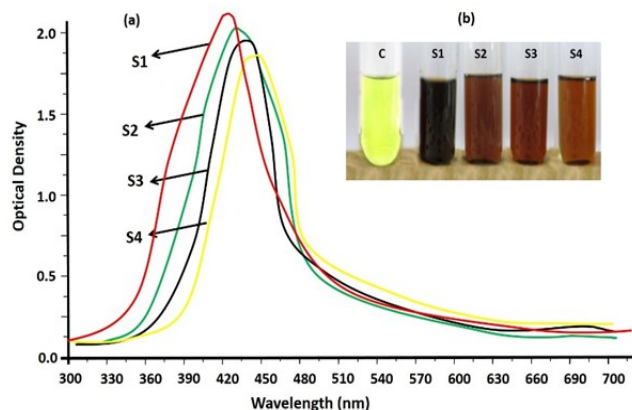


Fig. 2. (a) UV-visible spectrum (b) Colour change confirming the successful formation of AgNPs

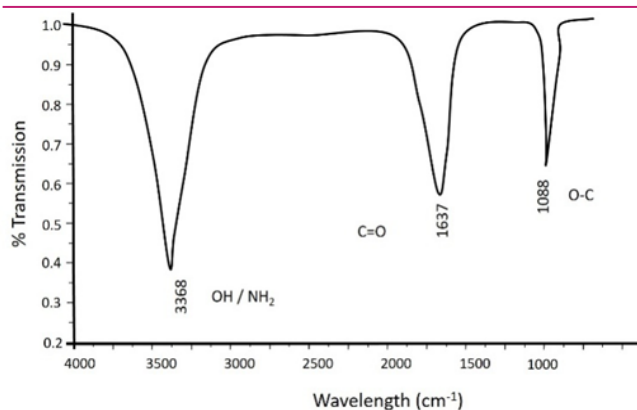


Fig. 3. Fourier Transform Infrared (FTIR) spectrum of synthesized AgNPs

different wavelengths for different samples (Fig.2a).As per Mie theory, non-spherical particles like prisms or rods would show two or more SPR bands, in this case a single peak indicates the formation of spherical nanoparticles (Lahiri, 2016).

The concentration of the leaf extract mixed in the sample and the reaction time affected nanoparticle formation, as indicated by the UV-Vis spectra. In the case of the S1 sample, there was a sharp peak at 423 nm, with greater height, indicating a smaller nanoparticle size and higher quantity. As the reaction time increased and the amount of leaf extract decreased, the peak shifted to 429 nm (S2), 435 nm (S3), and 443 nm (S4), depicting an increase in nanoparticle size with a lower quantity of nanoparticles.

Fourier Transform Infrared Analysis (FTIR)

FTIR spectroscopy plays a crucial role in identifying interactions between silver nanoparticles (AgNPs) and various functional groups in leaf extract.

The FTIR spectrum displayed distinct peaks that indi-

cated the presence of these functional groups at different wave numbers (Fig. 3). A strong peak at 1637 cm^{-1} corresponded to carbonyl groups ($\text{C}=\text{O}$), which participated in the formation of nanoparticles. Additionally, a broader but less intense peak at 1088 cm^{-1} was linked to the O-C stretching vibrations. A prominent band at 3368 cm^{-1} was attributed to amine groups ($-\text{NH}_2$) in aromatic amines and hydroxyl groups ($-\text{OH}$) in alcohols. The amide I and amide II bands, which appeared due to the stretching vibrations of carbonyl and N-H groups within protein amide linkages, demonstrated a high affinity for binding with AgNPs, thus contributing to their stabilization.

From the FTIR analysis, the presence of hydroxyl and carbonyl groups, which are found in carbohydrates, flavonoids, terpenoids, and phenolic compounds, were identified as effective reducing agents, responsible for reducing Ag^+ ions to Ag^0 , leading to the formation of silver nanoparticles. Furthermore, the study suggests that the carbonyl groups in amino acid residues and peptides in the proteins can effectively bind metal ions, potentially forming a protective coating around the nanoparticles. This layer prevents the particles from aggregating, stabilising the solution's nanoparticles.

Analysis of the size of synthesized nanoparticles using Transmission electron microscopy (TEM)

The size of the synthesized silver nanoparticle was determined using TEM, which showed that the particles were spherical in shape with sizes varying from 12, 15, 17 and 23 nm of S1, S2, S3 and S4, respectively (Fig. 4).

Antibacterial activity

The antibacterial activity assay of silver nanoparticles (AgNPs) showed significant inhibitory effects against *E. coli* O157:H7 culture used in the study. AgNPs produced

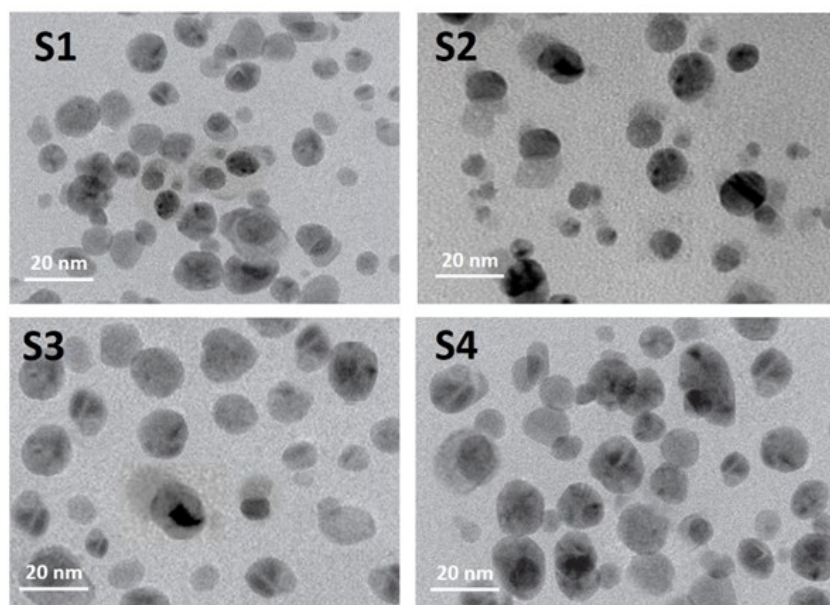


Fig. 4. Transmission electron microscopy (TEM) images of S1, S2, S3 and S4 samples, depicting different sizes of AgNPs

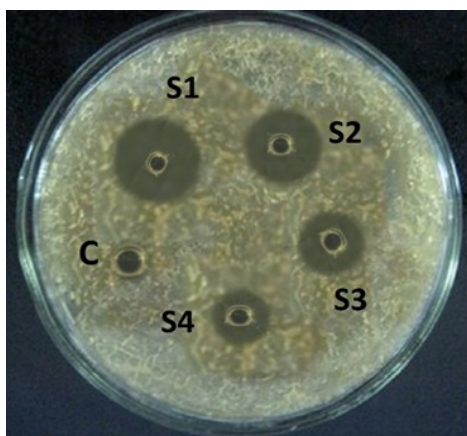
Table 1. Comparison of antibacterial activities of green synthesized NPs *Escherichia coli* O157:H7 in the present study with AgNPs synthesized with other biological material reported in literature

Size of AgNPs	Plants Used for synthesis	Conc. Of NPs	ZOI (in mm)	Reference
2–10	<i>Clerodendrum inerme</i>	250 µg/mL	17	Khan <i>et al.</i> , 2020
8–20	<i>Dioscorea bulbifera</i>	500 µg/mL	15	Ghosh <i>et al.</i> , 2012
8–50	<i>Allium ampeloprasum</i>	300 µg/mL	13	Jalilian <i>et al.</i> , 2020
20	<i>Umbrella</i>	250 µg/mL	16	Elemike <i>et al.</i> , 2020
17.3	<i>Curcuma zanthorrhiza</i>	100 µg/mL	9	Aiswariya & Jose, 2021
12	<i>Lantana camara</i>	100µg/mL	18	Present work

clear zones around the wells, indicating strong antibacterial activity (Fig. 5). The size of the inhibition zone varied between 21mm (S1), 15mm (S2), 13mm (S3) and 11mm (S4). The S1 sample with smaller-size nanoparticles exhibited higher antibacterial activity which is very well justified by the reasons mentioned in the literature, e.g., increased surface area (Donget *et al.*, 2009) enhanced silver ion release and generation of reactive oxygen species (Menichetti *et al.*, 2023).

The antimicrobial efficacy of silver nanoparticles (AgNPs) synthesized in the present study was compared with AgNPs synthesized using various plant extracts, as reported in previous literature (Table1). The results indicated that *Lantana camara*-mediated AgNPs (12 nm) exhibited a superior antibacterial effect against *E. coli* O157:H7, with a zone of inhibition (ZOI) of 18 mm at a 100 µg/mL nanoparticle concentration. This inhibition zone was higher than that observed for *Curcuma zanthorrhiza*-synthesized AgNPs of similar size (17.3 nm), which exhibited a ZOI of only 9 mm at the same concentration (Aiswariya & Jose, 2021).

Among the reported studies, *Clerodendrum inerme*-synthesized AgNPs (2–10 nm) showed a comparable antibacterial effect, with a ZOI of 17 mm at a higher nanoparticle concentration (250 µg/mL) (Khan *et al.*, 2020). However, despite their lower concentration, the AgNPs synthesized in the present study exhibited a slightly greater inhibition zone, suggesting enhanced antibacterial potential was due to their optimized size and bioactive coating.

**Fig. 5.** Nanoparticles of different samples (C, S1 to S4) against *Escherichia coli* showing different zones of inhibitions

Additionally, AgNPs synthesized using *Dioscorea bulbifera* (8–20 nm), *Allium ampeloprasum* (8–50 nm), and *Umbrella* plant (20 nm) exhibited inhibition zones of 15 mm, 13 mm, and 16 mm, respectively, at higher nanoparticle concentrations ranging from 250–500 µg/mL (Ghosh *et al.*, 2012; Jalilian *et al.*, 2020; Elemike *et al.*, 2020). The lower ZOIs observed in these studies suggest that higher nanoparticle concentrations were required to achieve antibacterial effects comparable to those obtained in the present study with a significantly lower concentration (100 µg/mL).

Overall, these findings emphasized the efficiency of *L. camara*-mediated AgNPs in inhibiting *E. coli* O157:H7, even at lower concentrations, which can be attributed to their small size (12 nm), high surface area, and potentially stronger interaction with bacterial membranes ((Menichetti *et al.*, 2023). The results further support the significance of optimizing nanoparticle synthesis conditions to achieve maximum antimicrobial efficacy.

As per the scientific literature (Azad *et al.*, 2023; Velgsova *et al.*, 2024), the amount of time a plant extract reacts with metal ions greatly influences how quickly and evenly silver nanoparticles form. Longer incubation times can change the size and uniformity of the nanoparticles. The plant chemicals help to reduce and stabilize the metal ions, and how they interact during this time affects the final properties of the nanoparticles (Kazemi *et al.*, 2023). In the present study, longer incubation resulted in the increased size of silver nanoparticles. At the same time, plant extract provides electrons that help convert metal ions into nanoparticles; hence, the quantity of plant extract directly affects nanoparticle production. Fewer nanoparticles will be formed if less plant extract is used (Rana *et al.*, 2020). Hence, in the present study, the sample with a higher quantity of extract and lesser reaction time resulted in a higher quantity of small-sized nanoparticles, while as the quantity of extract decreased and reaction time increased, that resulted in the formation of a smaller quantity of large-sized nanoparticles.

Conclusion

This study demonstrated that the quantity of *L. camara* leaf extract and reaction time significantly influenced the shape, size and antibacterial activity of green-

synthesized silver nanoparticles (AgNPs). The bioactive compounds present in the extract played a crucial role in nanoparticle formation and stabilization. The synthesized nanoparticles were spherical in shape. Higher extract concentrations and shorter reaction times (15 min) resulted in smaller AgNPs (12 nm). These small-sized nanoparticles exhibited enhanced antibacterial activity against *E. coli* O157:H7, as evident from the larger inhibition zone, measuring 21 mm. Conversely, lower extract concentrations combined with prolonged reaction times resulted in the synthesis of fewer, larger nanoparticles (15, 17, and 23 nm), corresponding to a decrease in antibacterial efficacy, with inhibition zones measuring 15, 13, and 11 mm, respectively. These findings highlight the crucial role of extract-mediated synthesis parameters in controlling nanoparticle characteristics, reinforcing that smaller-sized AgNPs exhibit stronger antimicrobial effects due to their increased surface area and bioactivity. Optimizing extract quantity and reaction time can enhance AgNP synthesis efficiency, offering valuable insights for future biomedical and antimicrobial applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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