



## Effect of temperature and time of reaction on green synthesis of silver nanoparticles using *Malvaviscus arboreus*, their antimicrobial activities

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### Abstract

Mother nature has long served as a rich source of medicinal compounds and therapeutic substances. One such plant is *Malvaviscus arboreus* (commonly known as sleeping hibiscus), an evergreen shrub native to India. Green synthesis has emerged as an environmentally friendly alternative for producing nanoparticles, utilizing biological entities such as bacteria, algae, fungi, and plants. This approach offers a sustainable route for nanoparticle fabrication. In this context, higher temperatures were observed to accelerate the nucleation and formation of silver nanoparticles (AgNPs), resulting in an increase in the intensity of the absorbance peak. This suggests a higher yield of nanoparticles at elevated temperatures. The consistent wavelength of the absorbance peak (around 440-450 nm) indicates that the AgNPs synthesized under varying conditions maintained a relatively similar size. However, a slight enhancement in absorbance at higher temperatures could be attributed to the presence of smaller nanoparticles, as smaller particles exhibit stronger surface plasmon resonance (SPR). Furthermore, the study demonstrated a direct correlation between reaction time and AgNP synthesis. Longer reaction times resulted in increased nanoparticle production, as evidenced by higher absorbance values. In summary, both temperature and reaction time significantly influenced the quantity and potentially the size distribution of the synthesized AgNPs, with higher values leading to more efficient nanoparticle formation.

**Keywords:** Green synthesis, Silver nanoparticles, *Malvaviscus arboreus*, Antimicrobial activities

### Introduction

Nanotechnology is a science that brings together different fields like biology, chemistry, physics, medicine, and engineering to create and use tiny materials and devices. This idea was first imagined by Richard P. Feynman in 1959 in his lecture "There's plenty of room at the bottom," where he introduced the concept of working on extremely small scales (Feynman, 1960) [4]. Among these advancements, silver nanoparticles (AgNPs) have gained significant attention because they are useful in many areas of modern life, such as healthcare, air and water purification, food production, cosmetics, clothing, and household products (Marambio-Jones and Hoek 2010) [10].

Silver nanoparticles are special because they have unique optical, electrical, and thermal properties. These properties make them valuable for uses like medical treatments, sensors, imaging technologies, and diagnostic tools. Traditionally, metallic nanoparticles like AgNPs are made using physical or chemical methods (Kruiset *et al.*, 2000; Sondiet *et al.*, 2003) [8, 14]. However, these methods often have downsides, including high production costs, harmful by-products, and environmental pollution. As concerns about global warming and environmental damage grow, the need for eco-friendly alternatives has become more important.

Green synthesis has emerged as a promising alternative for producing nanoparticles. This approach uses natural materials like bacteria, algae, fungi, and plants to create nanoparticles (Mittal *et al.*, 2013) [11]. Among these, plants are preferred because they offer a simple, fast, cost-effective, and eco-friendly way to produce nanoparticles in a single step (Huang *et al.*, 2007) [5]. The main process behind plant-based nanoparticle synthesis involves phytochemicals—natural compounds like flavonoids, terpenoids, carboxylic acids, and others. These compounds

help reduce metal salts into nanoparticles and act as enzymes or catalysts during the process (Jagajjanani and Paria, 2013) [6].

Nature has long been a source of medicine and therapeutic substances. One plant, *Malvaviscus arboreus* (commonly known as sleeping hibiscus), is an evergreen shrub found throughout India. It has been widely used in Ayurvedic medicine for its health benefits. *Malvaviscus arboreus* contain many active compounds, including flavonoids, anthocyanins, and organic acids, which have various medicinal properties. These include promoting hair growth, supporting heart and brain health, controlling blood sugar levels, and acting as a cooling agent (Shanmugasundaramet *et al.*, 1997; Adhirajanet *et al.*, 2003; Kora, 2023) [1, 7].

Despite its many uses, there hasn't been much research on using *Malvaviscus arboreus* petals to produce silver nanoparticles. In this study, a simple and eco-friendly method was used to create AgNPs from hibiscus petals using water as a solvent. The resulting nanoparticles were tested for their antimicrobial properties and potential applications in medicine. This approach highlights a sustainable way to produce valuable materials while reducing environmental harm.

### 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<sub>3</sub>), with a purity of 99.5%, was sourced from Sigma-Aldrich, India. Fresh specimens of *Malvaviscus arboreus* 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

Fresh and healthy leaves were first washed thoroughly with tap water and then with distilled water to remove dirt and impurities (Fig. 1A). The cleaned leaves were cut into small pieces and left to air-dry at room temperature until all moisture was removed. Once dried, the leaves were ground ((Fig. 1B) using a mortar and pestle.



**Fig 1:** (A) Fresh leaves of *Malvaviscus arboreus* (B) Air dried and grounded leaves

To prepare the extract, 20 grams of the powdered leaves were mixed with 100 mL of water and boiled for 30 minutes. The mixture was then filtered using Whatman No. 1 filter paper to remove solid residues, resulting in a clear liquid extract. This extract was stored in sterilized 250 mL Erlenmeyer flasks and refrigerated at 4°C for use in future experiments. Sterile conditions were maintained throughout the process to avoid contamination and ensure reliable results.

### Synthesis of Ag NPs

For synthesizing silver nanoparticles (AgNPs), 10 mL of this leaf extract was mixed with 50 mL of 10mM silver nitrate ( $\text{AgNO}_3$ ) solution. The mixture was incubated in a water bath, and the synthesis process was monitored by observing a change in the solution's color.

To understand how time and temperature affected the nanoparticle synthesis, the mixture was incubated for up to 60 minutes at varying temperatures (20°C, 30°C, 40°C, 50°C and 60°C). The reaction was periodically analyzed at different intervals (5, 15, 30, 45, and 60 minutes) using a UV-visible spectrophotometer to scan wavelengths between 400–600 nm. A dark reddish-brown color indicated successful synthesis of AgNPs.

The resulting solution was centrifuged at 10,000 rpm for 45 minutes, repeating the process three times with washing in deionized water to purify the nanoparticles. Finally, the purified nanoparticles were lyophilized (freeze-dried) and stored at 4°C for further analysis and characterization.

### Characterization of Silver Nanoparticles (AgNPs)

The synthesis of AgNPs was confirmed using UV-visible spectroscopy by scanning the sample over a wavelength range of 200–700 nm. This analysis verified the characteristic absorption peak of AgNPs, indicating their formation.

FTIR analysis of the dried AgNPs was conducted using the potassium bromide (KBr) pellet method, with a sample-to-

KBr ratio of 1:100. The spectra were acquired using 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  $\text{cm}^{-1}$  to detect functional groups responsible for the synthesis and stabilization of AgNPs. This analysis provided detailed information about the chemical interactions between the nanoparticles and the plant extract.

The size, shape, and structural characteristics of the nanoparticles were analyzed using a Phillips TECHNAI FE 12 TEM instrument. TEM provided high-resolution images at the nanoscale, allowing precise determination of the nanoparticles' morphology and dimensions, confirming their successful synthesis.

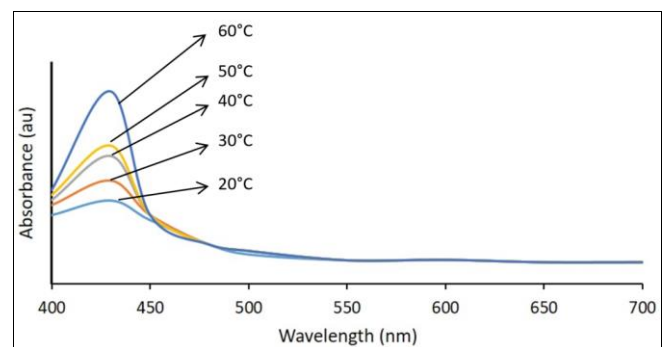
### Estimation of antibacterial activity

Bacterial culture (*E. coli* with an OD around 0.1) is uniformly spread on the surface of the agar plate using a sterile swab to create a bacterial lawn. The antibiotic activity was estimated with well diffusion method. 50  $\mu\text{L}$  of different concentrations of AgNP solution (5-25  $\mu\text{g}/\text{ml}$  named as S1 to S4 respectively) was poured in the wells. Along with the samples a negative control (C) is also considered and poured with sterile distilled water.

### Results and Discussion

UV-Vis spectroscopy is an essential method for verifying the formation and evaluating the stability of metal nanoparticles in aqueous solutions. When silver nitrate is mixed with the leaf extract, the solution undergoes a significant color change from a pale yellowish-green to brown, which indicates the synthesis of silver nanoparticles (AgNPs). This color transformation arises from a phenomenon known as surface plasmon resonance (SPR), where free conduction electrons collectively oscillate in response to an electromagnetic field.

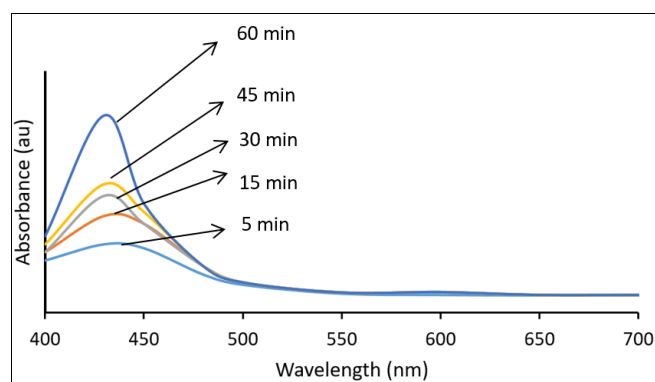
At lower temperature the peaked are broad representing a diversity in shape and size of nanoparticle while with in an increase in reaction temperature the peak became narrow and high. According to Mie theory, non-spherical nanoparticles, such as rods or prisms, typically display multiple SPR bands. However, the presence of a single peak in this study confirms the formation of spherical nanoparticles (Lahiri, 2016). The reaction temperature found to affect significantly influence nanoparticle formation, as reflected in the UV-Vis spectra (Fig 2).



**Fig 2:** UV-Vis absorption spectrum of reaction mixture at different incubated at different temperature

As far the time of reaction is concerned, initially the peaks are found broadened, may be due to rapid nucleation and variation in shapes and distribution. But as the time

increases the peak is found to be narrowed down and high representing quality (spherical shape) and quantity of nanoparticles (Fig. 3).



**Fig 3:** UV-Vis absorption spectrum of reaction mixture as measured at different time intervals

A comparison of FTIR spectra of the leaves extract of *Malvaviscus arboreus* with the FTIR spectra of synthesized AgNPs was carried out to analyse the shifts and changes in peak positions, which indicate the functional groups involved in the synthesis and stabilization (capping) of silver nanoparticles (AgNPs).

The shifts and functional groups observed in the analysis of the leaves extract and silver nanoparticles (AgNPs) provide valuable insights into the chemical interactions and changes during nanoparticle synthesis. In the case of the hydroxyl groups (O–H), the leaves extract exhibits a broad peak at  $3480\text{ cm}^{-1}$ , which indicates the presence of free and bonded hydroxyl groups in polyphenols, flavonoids, and other phytochemicals. This broad band suggests that these compounds are rich in hydroxyl groups, which could play a role in the reduction of metal ions during nanoparticle formation. When silver nanoparticles are synthesized, the peak shifts to  $3435\text{ cm}^{-1}$ , indicating that the hydroxyl groups are involved in reducing  $\text{Ag}^+$  ions and also in capping the AgNPs, stabilizing them by interacting with the nanoparticle surface. Moving to the carbonyl groups (C=O), the leaves extract shows a peak at  $1608\text{ cm}^{-1}$ , which corresponds to the stretching vibration of carbonyl groups, typically found in compounds like flavonoids and ketones. This suggests that these compounds in the extract are important in the chemistry of the plant material. However, in the presence of AgNPs, the peak shifts to  $1585\text{ cm}^{-1}$ , which indicates that the carbonyl group may be donating electrons to stabilize the nanoparticles. The shift reflects the interaction between the carbonyl group and the silver ions during the reduction process.

A significant characteristic of AgNPs is the metal–oxygen bonds (Ag–O), where a peak at  $619\text{ cm}^{-1}$  is observed. This peak is distinctive of metal–oxygen interactions and confirms the reduction of  $\text{Ag}^+$  ions to form AgNPs. The presence of this peak further suggests that oxygen-containing biomolecules from the plant extract are involved in both reducing silver ions and capping the nanoparticles, ensuring their stability in the solution. Finally, the presence of  $\text{CO}_2$  absorption is noted in the AgNPs spectra with a peak at  $2413\text{ cm}^{-1}$ . This peak is attributed to  $\text{CO}_2$  adsorbed on the nanoparticle surface or possibly interacting with the environment. The absorption of  $\text{CO}_2$  could indicate that the nanoparticles have a high surface area, which is typical for

nanoparticles, and that they may have the ability to interact with gases in the surrounding atmosphere. Hence, the observed shifts and functional groups suggest that the plant extract contains a variety of functional groups, including hydroxyl, carbonyl, and aromatic groups, which participate actively in the reduction and stabilization of AgNPs. The interactions of these groups with silver ions contribute to the formation of well-stabilized nanoparticles, with several chemical functional groups playing roles in capping and stabilizing the AgNPs during the synthesis process. The shifts in O–H, C=O, and N–H peaks confirm the role of hydroxyl, carbonyl, and amine groups in reducing  $\text{Ag}^+$  ions and stabilizing AgNPs. The appearance of new peaks (e.g.,  $2915$  and  $619\text{ cm}^{-1}$ ) in the AgNP spectra indicates the formation of Ag–O bonds and additional capping by alkyl or other compounds. Decrease in intensity of some peaks (e.g., N–H bending) suggests specific molecules preferentially bind to the AgNP surface.

### Antibacterial activity

The antibacterial activity assay of silver nanoparticles (AgNPs) shows significant inhibitory effects against *E. coli* culture used in the study. AgNPs produced noticeable clear zones around the wells, indicating strong antibacterial activity (Fig. 7).

The size of the inhibition zone varied between 7 mm (S1), 10 mm (S2), 13 mm (S3) to 15 mm (S4). The S1 sample with smaller size nanoparticles exhibited higher antibacterial activity. Zone of inhibition (in mm) of *Escherichia coli* in response to increasing concentrations (5–25  $\mu\text{g/mL}$ ) of AgNPs (Table 1).

**Table 1:** Zone of inhibitions of *E. coli* against synthesized AgNPs

Sample	Concentration of AgNPs ( $\mu\text{g/mL}$ )	Zone of inhibition (in mm)
Control (C)	---	---
S1	5	$7 \pm 0.1$
S2	10	$10 \pm 0.1$
S3	15	$13 \pm 0.2$
S4	25	$15 \pm 0.2$

### Conclusion

Higher temperatures promote faster nucleation and formation of AgNPs, leading to an increase in the absorbance peak intensity. This suggests that more nanoparticles are synthesized at elevated temperatures. The consistent wavelength ( $\sim 440\text{--}450\text{ nm}$ ) indicates that the AgNPs maintain a relatively similar size, though smaller particles might slightly enhance absorbance due to stronger SPR. The results from the study demonstrate that longer reaction times lead to an increase in the synthesis of AgNPs, as reflected by higher absorbance. However, the peak wavelength ( $\sim 440\text{--}450\text{ nm}$ ) remains consistent, suggesting that while the number of nanoparticles increases, their size remains relatively unchanged over time. In summary, temperature and time of reaction is found to influence the quantity and possibly the size distribution of AgNPs, directly with higher temperatures and reaction time leading to higher absorbance due to more efficient nanoparticle synthesis.

### Conflict of interest

The authors declare that they have no conflict of interest.

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