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Green Synthesis and Physicochemical Characterization of Silver Nanoparticles Using *Semecarpus anacardium* Nut Extract: Optimization of Reaction Parameters and Structural Evaluation

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Abstract

The development of environmentally compatible routes for metallic nanoparticle synthesis has increased interest in plant-mediated nanotechnology. In the present study, silver nanoparticles (AgNPs) were synthesized using an aqueous nut extract of *Semecarpus anacardium* as a biological reducing and stabilizing system. The synthesis process was optimized by examining the extract-to-silver nitrate mixing ratio, reaction pH, and temperature. Fresh *S. anacardium* nuts were shade-dried, powdered, and extracted in Milli-Q water. The extract was subsequently reacted with 1 mM silver nitrate. Among the tested conditions, an extract: AgNO₃ ratio of 1:4, pH 8, and room temperature (approximately 28 °C) produced the most favourable nanoparticle formation. The reduction of silver ions was initially indicated by a change in the reaction mixture from colourless to dark brown. UV-Visible spectroscopic analysis further confirmed nanoparticle formation through a characteristic surface Plasmon resonance band at 425 nm. Optical density increased between 4 and 24 h, indicating progressive nanoparticle formation. Electron microscopic observations showed predominantly spherical and dispersed nanoparticles. TEM analysis indicated particle dimensions of approximately 17.1–24.3 nm, whereas particle-size distribution analysis showed a dominant fraction near 60 nm and an average particle size of approximately 40 nm. Energy-dispersive X-ray analysis showed a characteristic silver-related signal near 3 keV. The results demonstrate that *S. anacardium* nut extract can serve as an effective biological matrix for the synthesis of nanoscale silver under mild reaction conditions.

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Introduction

Silver nanoparticles (AgNPs) have received sustained attention because their nanoscale dimensions confer distinctive optical, physicochemical, catalytic, and biological properties. However, conventional physical and chemical synthesis routes may require elevated energy input or reagents that are undesirable from environmental and biological perspectives. These

concerns have encouraged the development of greener synthesis approaches based on biological materials, particularly plant extracts, which can provide natural molecules capable of reducing metal ions and contributing to nanoparticle stabilization (Akhter *et al.*, 2024; Thomas *et al.*, 2024). Plant-mediated synthesis is particularly attractive because plant extracts contain chemically diverse constituents, including phenolics, flavonoids, terpenoids, proteins, sugars, and other

metabolites. Depending on their composition, these molecules may participate in the conversion of Ag^+ to elemental silver while simultaneously influencing particle nucleation, growth, surface chemistry, and aggregation behaviour (Fahim *et al.*, 2024; Zulfiqar *et al.*, 2024). Consequently, the biological source and reaction conditions are important determinants of the final characteristics of phytosynthesized AgNPs.

The physicochemical properties of green-synthesized nanoparticles are strongly influenced by experimental parameters. Changes in extract concentration, precursor concentration, extract-to-metal ratio, pH, temperature, and reaction time can alter the rate of reduction and the balance between nucleation and particle growth. These factors may subsequently affect nanoparticle size, morphology, dispersion, stability, and optical behaviour (Fares *et al.*, 2024; Shafie *et al.*, 2026). Therefore, optimization of the synthesis conditions is essential before detailed characterization or biological evaluation.

Among the parameters influencing plant-mediated nanoparticle synthesis, pH has a particularly important role because it can modify the ionization state and reactivity of phytochemicals involved in the reduction process. Likewise, temperature can accelerate reaction kinetics but may also influence particle growth and stability. Recent reviews emphasize that optimization of these variables is necessary for improving reproducibility and controlling nanoparticle characteristics (Bhurre *et al.*, 2025; Fahim *et al.*, 2024). The formation of AgNPs can be evaluated using complementary analytical approaches. UV–Visible spectroscopy is widely employed as an initial method because metallic silver nanoparticles exhibit surface plasmon resonance, producing characteristic absorption within the visible region. Electron microscopy provides additional information regarding particle morphology and size, while energy-dispersive X-ray spectroscopy contributes to elemental confirmation (Akhter *et al.*, 2024; Thomas *et al.*, 2024).

Semecarpus anacardium L.f., commonly known as the marking nut, is an important medicinal plant with a chemically rich fruit and nut system. Recent literature documents the presence of several phytochemical classes and highlights the pharmacological importance of its natural constituents (Mishra *et al.*, 2024). Its phytochemical diversity provides a scientific basis for investigating the nut extract as a biological reaction medium for nanoparticle synthesis. Although *S. anacardium* has attracted considerable pharmacological

attention, the optimization of AgNP synthesis using its nut extract provides an opportunity to explore its function as a plant-derived reducing and stabilizing matrix. In the present work, aqueous *S. anacardium* nut extract was employed for the synthesis of silver nanoparticles under mild conditions. The effects of extract-to- AgNO_3 ratio, pH, and temperature were evaluated, followed by optical and morphological characterization of the synthesized nanoparticles. The specific objectives were therefore to synthesize AgNPs using aqueous *S. anacardium* nut extract, optimize the extract-to-silver nitrate ratio, determine the influence of pH and temperature on nanoparticle formation, confirm nanoparticle synthesis using UV–Visible spectroscopy and to examine nanoparticle morphology, particle-size characteristics, and elemental composition using electron microscopic and energy-dispersive approaches.

Materials and Methods

Collection and authentication of plant material

Fresh and healthy nuts of *Semecarpus anacardium* were collected from the Sirumalai Hills, Eastern Ghats, Tamil Nadu, India (10.1942° N, 77.9967° E). The plant material was authenticated at the Herbarium, Department of Botany, Government Arts College, Salem, Tamil Nadu, India.

Recent literature confirms the importance of careful identification and chemical characterization of *S. anacardium* because its fruits and nuts contain multiple biologically active constituents and require appropriate handling during experimental applications (Mishra *et al.*, 2024).

Preparation of aqueous nut extract

Mature nuts were selected, shade-dried, and processed into powder. The powdered material was mixed with 100 mL of Milli-Q water and heated at 50–60 °C for 20 min. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was collected and stored at 4 °C until further use.

The use of aqueous extraction was consistent with the objective of developing a simple biological synthesis system without introducing an additional synthetic reducing agent. Plant extracts can provide naturally occurring compounds that participate in the reduction and surface stabilization of nanoparticles (Mittal *et al.*, 2013; Shereen *et al.*, 2024).

Preparation of silver precursor

A 1 mM aqueous silver nitrate solution was prepared using Milli-Q water and used as the silver-ion precursor for nanoparticle synthesis. The interaction between plant-derived metabolites and Ag^+ ions forms the basis of plant-mediated silver nanoparticle production. The composition of the biological extract and the reaction environment can influence both the reduction rate and the characteristics of the resulting nanoparticles (Irvani, 2011; Zulficar *et al.*, 2024).

Green synthesis of silver nanoparticles

The aqueous nut extract was mixed with 1 mM AgNO_3 solution. Based on the optimization experiments, the reaction mixture was adjusted to pH 8 and prepared at an extract-to-silver nitrate ratio of 1:4 (v/v). The reaction was maintained at room temperature. Changes in colour were monitored at 10-min intervals during the first hour. The reaction progress was subsequently monitored using UV–Visible spectroscopy from 4 to 24 h. The experimental design was based on the principle that plant-mediated synthesis is sensitive to reaction variables. Previous studies have similarly demonstrated that pH, temperature, reaction duration, and relative concentrations of extract and silver precursor can substantially affect AgNP formation (Ahmed *et al.*, 2016; Fahim *et al.*, 2024).

Optimization of extract-to-silver nitrate ratio, pH and temperature

To determine the effect of mixing ratio, 10% nut extract was combined with 1 mM AgNO_3 at ratios of 1:4, 1:3, 1:2, and 1:1 (v/v). The reaction mixtures were compared on the basis of their optical response and progression of nanoparticle formation. The influence of pH was investigated by allowing the 10% nut extract to react with 1 mM AgNO_3 over a pH range of 5–9 while maintaining the 1:4 mixing ratio. The optimized 1:4 extract-to- AgNO_3 system was evaluated at room temperature and at elevated temperatures ranging from 50 to 80 °C.

UV–Visible spectroscopic analysis

The optical properties of the colloidal reaction system were examined using a Shimadzu UV-1800 UV–Visible spectrophotometer. Aliquots of the colloidal solution were monitored at 4-h intervals from 4 to 24 h. Distilled water was used as the blank, and absorbance spectra

were recorded from 300 to 800 nm. UV–Visible spectroscopy remains a widely used preliminary characterization method because the appearance and position of the surface plasmon resonance band provide evidence for the formation and optical behaviour of AgNPs (Akhter *et al.*, 2024; Shafie *et al.*, 2026).

Electron microscopic and elemental characterization

The morphology of the synthesized AgNPs was examined using scanning electron microscopy (SEM). Transmission electron microscopy (TEM) was used to investigate particle shape and size distribution. Energy-dispersive X-ray spectroscopy (EDX/EDAX) was employed for elemental confirmation. The combined use of optical, microscopic, and elemental approaches is consistent with current recommendations for the characterization of plant-mediated AgNPs (Fares *et al.*, 2024; Thomas *et al.*, 2024).

Results and Discussion

Visual evidence of nanoparticle formation

The synthesis of AgNPs was initially indicated by a visible transformation of the reaction mixture. Before the addition of plant extract, the silver nitrate solution was colourless. Following interaction with the *S. anacardium* nut extract, the reaction mixture gradually developed a dark-brown appearance, indicating the reduction of silver ions and formation of colloidal nanoparticles. Colour development is a common preliminary indicator of AgNP formation because metallic nanoparticles interact strongly with visible light through collective electron oscillation at the nanoparticle surface. Similar visual transitions have been described in recent studies of plant-mediated AgNP synthesis (Shereen *et al.*, 2024; Fares *et al.*, 2024). The observed colour change in the present study therefore provided the first experimental indication that compounds present in the nut extract participated in the conversion of Ag^+ ions into nanoscale metallic silver (Figure 1).

Optimization of reaction parameters

The formation of AgNPs was influenced by the extract-to-silver nitrate ratio, pH, and reaction temperature. Among the evaluated mixing ratios, the 1:4 extract-to- AgNO_3 ratio produced the most favourable optical response and was selected as the optimized condition. Similarly, nanoparticle formation improved as the reaction environment changed from acidic toward

alkaline conditions, with pH 8 identified as the optimum value. At higher pH, the optical density increased relative to acidic conditions, indicating more favourable nanoparticle formation under the selected experimental system. Room temperature was identified as the preferred condition when compared with the tested higher temperatures. This finding is particularly relevant from a green-synthesis perspective because efficient nanoparticle formation under ambient conditions reduces the need for additional energy input.

As summarized in Table 1, the optimized system consisted of a 1:4 extract-to-AgNO₃ ratio, pH 8, and room-temperature conditions. These observations are consistent with the broader understanding that pH, temperature, and the relative availability of biological reducing molecules influence the nucleation and growth of phytosynthesized nanoparticles (Akhter *et al.*, 2024; Fahim *et al.*, 2024; Zulfiqar *et al.*, 2024). The present results also demonstrate that more severe reaction conditions are not necessarily required for effective nanoparticle production. Mild alkaline conditions may improve the availability or reactivity of phytochemical constituents involved in silver-ion reduction, whereas room-temperature synthesis provides an energy-efficient route (Figure 2).

UV-Visible confirmation of nanoparticle formation

UV-Visible spectroscopic analysis provided further confirmation of AgNP formation. The synthesized colloidal system displayed a characteristic surface plasmon resonance band at 425 nm. In addition, optical density increased between 4 and 24 h, indicating continued development of the nanoparticle population during the monitored reaction period (Figure 3). The presence of a distinct visible-region absorption band is characteristic of silver nanoparticle formation. The exact position and shape of the SPR band can be influenced by particle size, morphology, dispersion, and the surrounding chemical environment (Thomas *et al.*, 2024; Fares *et al.*, 2024). The observed 425 nm peak therefore provides strong optical evidence for the formation of AgNPs in the *S. anacardium* reaction system. The progressive increase in absorbance over time further supports continued reduction of silver ions and growth of the nanoparticle population.

Morphological and elemental characteristics

SEM examination demonstrated that the synthesized AgNPs were highly dispersed and predominantly

associated with spherical morphology. The nanoparticles appeared distributed across the examined surface, with smaller nanoparticles also observed in association with larger biological material, suggesting the presence of plant-derived surface components. TEM provided more detailed information regarding nanoparticle morphology and size. The particles were predominantly spherical, although some aggregates were also observed.

Importantly, particles within aggregates were not necessarily in direct contact, suggesting that plant-derived molecules may have contributed to separation and surface capping. The TEM observations indicated particle dimensions in the approximate range of 17.1–24.3 nm. Particle-size distribution analysis showed that the major fraction was near 60 nm, while the reported average particle size was approximately 40 nm.

The difference between directly observed TEM dimensions and the average value obtained from the reported size distribution reflects the heterogeneous nature of the synthesized colloidal population. Such differences can occur because microscopic observations describe individual particles, whereas particle-size distributions may reflect broader populations, including aggregates or differently sized fractions (Shafie *et al.*, 2026; Thomas *et al.*, 2024).

The combined observations summarized in Table 2 support successful formation of nanoscale silver particles. Recent literature emphasizes the importance of using multiple analytical methods because no single technique can fully establish nanoparticle identity, morphology, size, surface chemistry, and composition (Akhter *et al.*, 2024; Bhurre *et al.*, 2025; Thomas *et al.*, 2024).

The results demonstrate that *S. anacardium* nut extract can function as a biologically derived reaction medium for AgNP synthesis under relatively mild conditions. The optimized reaction required only a 1:4 extract-to-silver nitrate ratio, pH 8, and room temperature. This is advantageous because it avoids the need for aggressive reducing agents or sustained high-temperature treatment. The phytochemical richness of *S. anacardium* may contribute to this activity. Recent reviews describe the plant as a source of multiple phenolic and other bioactive constituents, supporting its potential to participate in biologically mediated chemical transformations (Mishra *et al.*, 2024). However, the present findings should be interpreted within the scope of the available experimental data.

Table.1 Optimization conditions for *Semecarpus anacardium*-mediated AgNP synthesis.

Parameter	Conditions evaluated	Selected condition	Experimental interpretation
Extract: AgNO ₃ ratio	1:4, 1:3, 1:2, 1:1	1:4	Highest favourable optical response
pH	5–9	8	Increased optical density under mildly alkaline conditions
Temperature	Room temperature; 50–80 °C	Room temperature (~28 °C)	Efficient synthesis without additional heating
AgNO ₃ concentration	1 mM	1 mM	Used for optimized synthesis
Monitoring period	4–24 h	Up to 24 h	Progressive increase in optical density

Table.2 Physicochemical characteristics of synthesized AgNPs.

Characterization feature	Experimental observation	Interpretation
Visual appearance	Colourless to dark brown	Preliminary evidence of AgNP formation
UV–Visible SPR maximum	425 nm	Optical confirmation of colloidal AgNPs
Reaction monitoring	Increasing OD from 4–24 h	Progressive nanoparticle formation
SEM morphology	Predominantly scattered/spherical particles	Morphological evidence of nanoparticle formation
TEM morphology	Mainly spherical particles with some aggregates	Nanoscale morphology
TEM size range	17.1–24.3 nm	Individual nanoscale particles
Particle-size distribution	Majority near 60 nm	Heterogeneous particle population
Average particle size	Approximately 40 nm	Overall reported particle size
EDX/EDAX	Characteristic signal near 3 keV	Elemental evidence supporting silver presence

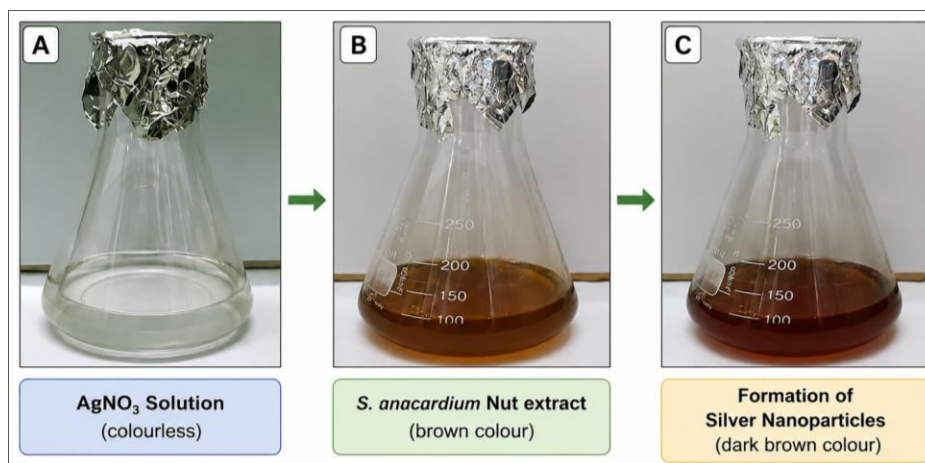
Figure.1 Visual stages in the green synthesis of *Semecarpus anacardium*-mediated silver nanoparticles.

Figure.2 Optimization of reaction parameters for the synthesis of *Semecarpus anacardium* nut-mediated AgNPs. The experimental data identified an extract: (A) AgNO₃ ratio of 1:4, (B) pH 8 and (C) room temperature as the preferred conditions for nanoparticle synthesis.

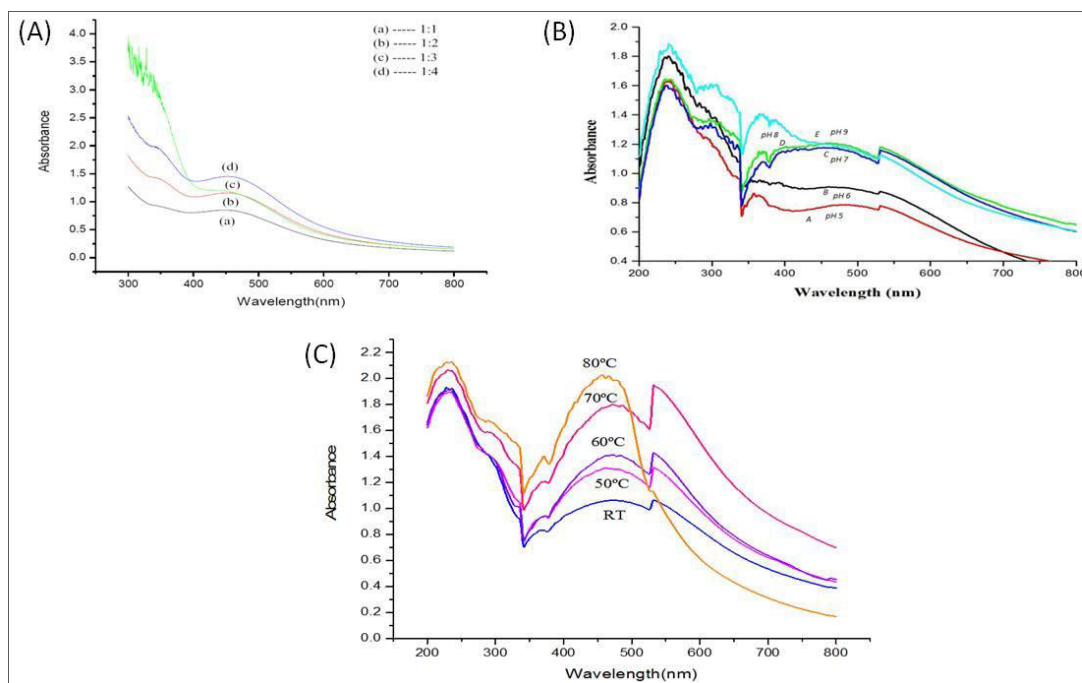
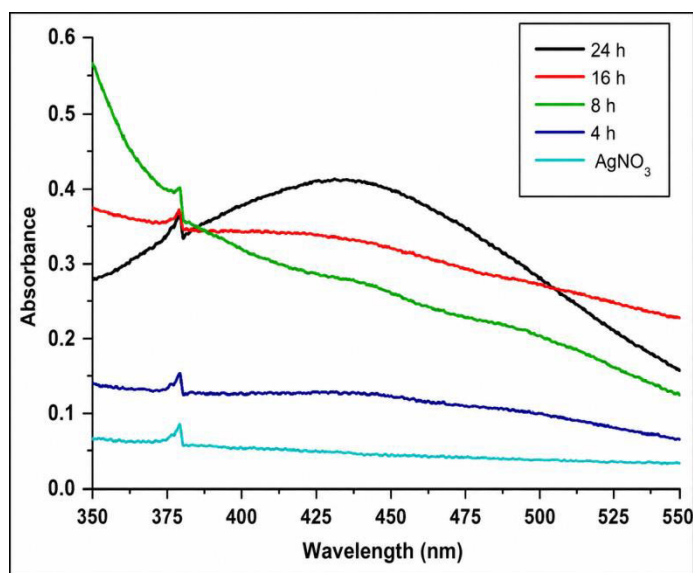


Figure.3 UV–Visible spectroscopic confirmation of silver nanoparticle formation using *Semecarpus anacardium* nut extract. The characteristic surface plasmon resonance band centred at approximately 425 nm confirms the formation of colloidal AgNPs.



While the results establish successful nanoparticle formation through colour change, UV–Visible spectroscopy, electron microscopy, particle-size observations, and elemental analysis, detailed identification of the specific molecules responsible for

reduction and capping would require additional chemical analyses.

Current research also emphasizes the need for improved reproducibility, scale-up strategies, and systematic safety

assessment before broad biomedical or industrial applications of plant-derived AgNPs can be established (Fahim *et al.*, 2024; Zulfiqar *et al.*, 2024). Thus, the present study provides a foundation for further work involving detailed phytochemical characterization of the nut extract, nanoparticle stability assessment, crystallographic analysis, and evaluation of biological activity.

In conclusion, the present investigation established an environmentally compatible approach for synthesizing silver nanoparticles using aqueous *Semecarpus anacardium* nut extract. The synthesis process was influenced by the experimental reaction conditions, with an extract-to-AgNO₃ ratio of 1:4, pH 8, and room temperature identified as the preferred parameters. Nanoparticle formation was initially indicated by the development of a dark-brown colour and subsequently confirmed by a characteristic UV-Visible surface Plasmon resonance band at 425 nm. Electron microscopic observations demonstrated predominantly spherical nanoparticles, with TEM indicating individual particle dimensions of approximately 17.1–24.3 nm. Particle-size distribution analysis showed a heterogeneous population with a reported average size of approximately 40 nm. EDX/EDAX analysis provided elemental evidence supporting the presence of silver. Collectively, these findings demonstrate that *S. anacardium* nut extract can function as a biological matrix for the synthesis of nanoscale silver under mild conditions. Further studies should investigate surface chemistry, crystallinity, long-term colloidal stability, phytochemical capping mechanisms, reproducibility, and potential applications of the synthesized nanoparticles.

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