

# Comparative physicochemical and biomedical evaluation of silver nanoparticles synthesized using seed and pod extracts of *Amomum aromaticum*

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## Research Article

**Keywords:** Amomum aromaticum, silver nanoparticles, green synthesis, organ-specific phytochemicals, antibacterial, antioxidant, wound healing, colloidal stability

**Posted Date:** October 17th, 2025

**DOI:** <https://doi.org/10.21203/rs.3.rs-7591161/v1>

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**Additional Declarations:** No competing interests reported.

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# Abstract

The green synthesis of metal nanoparticles has emerged as a sustainable and biocompatible approach for biomedical applications. In this study, silver nanoparticles (AgNPs) were synthesized using **seed and pod extracts of *Amomum aromaticum* (Bengal cardamom)**, and their physicochemical and biological properties were systematically compared. The aqueous extracts served as both reducing and stabilizing agents under near-neutral conditions ( $\text{pH } 6.0 \pm 0.2$ ), optimizing nanoparticle formation and stability [41–45]. UV–Visible spectroscopy confirmed the characteristic surface plasmon resonance peaks at 432 nm for seed-derived AgNPs (Seed-AgNPs) and 445 nm for pod-derived AgNPs (Pod-AgNPs), indicating size differences. FTIR analysis revealed involvement of flavonoids, phenolic acids, and tannins in reduction and capping, with seed extracts exhibiting stronger flavonoid-associated peaks and pod extracts displaying dominant phenolic/tannin signals [26,27,37]. X-ray diffraction and TEM confirmed crystalline nature, with Seed-AgNPs being smaller (15–20 nm) and more spherical, while Pod-AgNPs were larger (25–30 nm) and semi-spherical [36,37]. Zeta potential analysis indicated higher colloidal stability for Seed-AgNPs (−25.4 mV) compared to Pod-AgNPs (−18.1 mV) [38]. Biological evaluations demonstrated functional divergence: Seed-AgNPs exhibited superior antibacterial activity against *S. aureus* and *E. coli*, attributed to their smaller size and higher surface area [38–40], whereas Pod-AgNPs showed enhanced antioxidant potential (~81% DPPH scavenging) and wound-healing efficiency (~78% closure in 24 h), linked to phenolic and tannin capping [41–42]. Cytotoxicity assays confirmed biocompatibility of both nanoparticles, with Seed-AgNPs exhibiting slightly higher effects at elevated concentrations [43]. Mechanistic insights suggest flavonoid-rich seeds promote rapid nucleation and stabilization, while phenolic-rich pods favor slower growth with antioxidant functionality, demonstrating **organ-specific phytochemical-driven tunability** in nanoparticle synthesis [44,45]. This study highlights the strategic use of plant organ extracts to tailor AgNP properties for targeted biomedical applications, providing a framework for precision-guided green nanotechnology.

## 1. Introduction

The rapid advancement of nanotechnology has created transformative opportunities across biomedical, pharmaceutical, agricultural, and environmental fields [1,2]. At the forefront of this progress is the synthesis of metal nanoparticles, which exhibit novel optical, catalytic, and biological properties distinct from their bulk counterparts [3]. Among the various strategies available for nanoparticle production, green or biogenic synthesis methods have emerged as an attractive alternative to conventional chemical and physical techniques. Unlike traditional approaches, which often require hazardous chemicals, high temperatures, and energy-intensive procedures, green synthesis employs natural biological resources as reducing and stabilizing agents [4,5]. This approach is not only cost-effective and eco-friendly but also yields nanoparticles with greater biocompatibility and therapeutic potential [6–8]. Within the broad spectrum of nanomaterials, silver nanoparticles (AgNPs) have garnered exceptional attention owing to their unique physicochemical features and versatile biomedical applications [9]. The localized surface plasmon resonance (LSPR) of AgNPs provides them with tunable optical characteristics, while their high surface-area-to-volume ratio enhances catalytic and antimicrobial activity [10]. These properties

translate into a wide range of therapeutic applications, including antimicrobial therapy, wound healing, antioxidant protection, anticancer strategies, and drug delivery systems [11–14]. Importantly, the biological performance of AgNPs is highly dependent on their size, morphology, stability, and surface chemistry, which are strongly influenced by the synthesis route and the type of biological source employed [15]. Among the various biogenic routes, plant-mediated synthesis of nanoparticles stands out due to its scalability, reproducibility, and reliance on readily available phytochemicals. Plant extracts are particularly advantageous because they contain a rich diversity of secondary metabolites, such as flavonoids, terpenoids, alkaloids, tannins, saponins, and phenolic acids [16,17]. These compounds not only reduce metal ions ( $\text{Ag}^+ \rightarrow \text{Ag}^0$ ) but also serve as capping and stabilizing agents, preventing aggregation and conferring surface functional groups that enhance biological compatibility [18]. Over the past decade, numerous studies have reported the successful synthesis of AgNPs using extracts derived from leaves, stems, roots, fruits, and flowers of diverse plant species [19–21]. Despite the wealth of research in this domain, a critical gap remains: most studies employ whole-plant or single-organ extracts without examining how different parts of the same plant species may influence the characteristics and functions of the synthesized nanoparticles. Plant organs often differ markedly in their phytochemical composition; hence, they may yield nanoparticles with distinct physicochemical features and bioactivities [22]. A systematic comparison of organ-specific extracts could therefore open avenues for tailored nanoparticle synthesis, wherein the choice of plant part is deliberately exploited to tune nanoparticle properties for specific biomedical applications [23]. In this context, *Amomum aromaticum* Roxb. (Bengal cardamom) offers a particularly compelling case study. Belonging to the Zingiberaceae family, this aromatic and medicinal plant has long been valued in traditional medicine for its antimicrobial, anti-inflammatory, and digestive benefits [24,25]. Both its seeds and pods are phytochemically rich but compositionally distinct. Seeds are abundant in flavonoids, potent electron-donating compounds that facilitate rapid nucleation of smaller nanoparticles [26]. Conversely, pods are enriched with phenolic acids and tannins, which are well known for their antioxidant capacity and potential role in wound healing [27]. This compositional heterogeneity provides a unique opportunity to investigate how part-specific phytochemicals influence nanoparticle synthesis, stability, and biological activity. To address this gap, the present study explores the green synthesis of AgNPs using seed and pod extracts of *A. aromaticum*. The biosynthesized nanoparticles were characterized using spectroscopic, microscopic, and surface analyses, including UV–Vis spectroscopy, FTIR, X-ray diffraction (XRD), transmission electron microscopy (TEM), and zeta potential measurement [28–30]. Additionally, phytochemical profiling of the extracts was performed to correlate metabolite composition with nanoparticle features [31]. Beyond physicochemical characterization, the study evaluated the biological efficacy of the seed- and pod-derived AgNPs through antioxidant, antimicrobial, cytotoxicity, biofilm inhibition, and wound-healing assays [32–34]. Statistical analyses were employed to validate the experimental findings and establish reliable correlations between phytochemical content, nanoparticle properties, and biological outcomes [35,36]. This dual-part comparative approach revealed important distinctions: seed-derived AgNPs were smaller (15–20 nm), more stable (– 25.4 mV), and demonstrated enhanced antibacterial efficacy, while pod-derived AgNPs were relatively larger (25–30 nm, – 18.1 mV) but exhibited superior antioxidant activity and wound-healing potential [37–40]. Mechanistic insights

suggested that flavonoid-rich seed extracts facilitated rapid nucleation and stabilization, while phenolic-rich pod extracts conferred antioxidant properties and regenerative benefits [41–45].

## 2. Materials and Methods

### 2.1. Plant Collection and Extract Preparation

Fresh Bengal cardamom (*Amomum aromaticum*) fruits were collected from a local market in Tirupati. The fruits were thoroughly washed with distilled water to remove any impurities and then separated manually into seed and pod fractions. Each fraction was dried in a hot air oven at 50°C until a constant weight was achieved, after which they were ground into a fine powder using a sterile mechanical grinder. For extract preparation, 5 g of powdered seed and 5 g of powdered pod material were each boiled separately in 100 mL of distilled water for 20 minutes. The extracts were cooled to room temperature, filtered through Whatman No. 1 filter paper, and stored at 4°C until further use. These aqueous extracts served as the reducing and stabilizing agents in the green synthesis of silver nanoparticles [1,4].

### 2.2. Green Synthesis of Silver Nanoparticles (AgNPs)

A 1 mM aqueous solution of silver nitrate ( $\text{AgNO}_3$ ) was prepared freshly prior to synthesis. Equal volumes (1:1 v/v) of each plant extract (seed and pod) were mixed separately with the silver nitrate solution. The pH of the reaction mixture was measured using a calibrated digital pH meter (Eutech Instruments, Singapore). Since pH is a crucial determinant of nanoparticle formation and stability, the mixtures were carefully adjusted to and maintained at  $\text{pH } 6.0 \pm 0.2$  using 0.1 M HCl or 0.1 M NaOH when necessary [41–45]. This near-neutral pH was found optimal during preliminary trials, yielding stable nanoparticle suspensions without rapid aggregation. The mixtures were incubated at room temperature ( $25 \pm 2^\circ\text{C}$ ) under static conditions. The progress of nanoparticle formation was visually monitored by the appearance of a color change from pale yellow to reddish-brown, a hallmark of silver nanoparticle synthesis due to surface plasmon resonance (SPR) [35].

### 2.3. Characterization of AgNPs

The biosynthesized AgNPs were characterized using the following analytical techniques:

- **UV–Visible (UV–Vis) spectroscopy:** Absorption spectra were recorded between 300–600 nm to identify SPR peaks [35].
- **Fourier Transform Infrared (FTIR) spectroscopy:** FTIR spectra were obtained to identify functional groups responsible for the reduction of  $\text{Ag}^+$  and capping of AgNPs [37].
- **X-ray Diffraction (XRD):** Crystalline structure and phase purity were determined using an XRD diffractometer [36].
- **Transmission Electron Microscopy (TEM):** TEM images were captured to determine nanoparticle morphology, size, and distribution [36].

- **Zeta Potential Analysis:** Colloidal stability of the AgNP suspensions was assessed using a zeta potential analyzer [38].

## 2.4. Biological Evaluations

- **Antioxidant activity:** Assessed by DPPH radical scavenging assay [32].
- **Cytotoxicity:** Evaluated by MTT assay using human fibroblast cell lines [33].
- **Antibacterial activity:** Determined using agar well diffusion against *S. aureus* and *E. coli* [38,39].
- **Biofilm inhibition:** Quantified using crystal violet staining assay [34].
- **Wound healing potential:** Investigated in vitro by scratch assay on fibroblast monolayers [33].

## 2.5. Statistical Analysis

All experiments were conducted in triplicate ( $n = 3$ ), and results were expressed as mean  $\pm$  standard deviation (SD). Statistical significance between groups was determined using one-way ANOVA followed by Tukey's post hoc test. A  $p$ -value  $< 0.05$  was considered statistically significant. Analyses were carried out using GraphPad Prism 9 software [34].

## 3. Results

### 3.1. Visual and UV–Vis Confirmation of AgNPs

The reduction of  $\text{Ag}^+$  ions was initially indicated by a visible color change to reddish-brown within 30 min due to SPR excitation. UV–Vis spectra confirmed distinct peaks at  $\sim 430$  nm for seed-derived AgNPs (Seed-AgNPs) and  $\sim 440$  nm for pod-derived AgNPs (Pod-AgNPs), consistent with typical AgNP formation [35].

### 3.2. Phytochemical and Structural Characterization

FTIR spectra revealed that Seed-AgNPs displayed bands corresponding to  $-\text{OH}$ ,  $-\text{COOH}$ , and amide groups, suggesting involvement of flavonoids and proteins in reduction and capping. Pod-AgNPs exhibited stronger peaks for  $\text{C} = \text{O}$  and aromatic  $\text{C} = \text{C}$  stretching, highlighting the role of phenolic acids and tannins [37].

XRD patterns confirmed the crystalline nature of AgNPs, with characteristic Bragg reflections (111), (200), (220), and (311) corresponding to face-centered cubic (FCC) silver (JCPDS No. 04-0783) [36].

TEM micrographs revealed that Seed-AgNPs were predominantly spherical with sizes 15–20 nm, while Pod-AgNPs were semi-spherical, ranging 25–30 nm [36,37].

### 3.3. Stability and Phytochemical Content

Zeta potential values indicated higher stability of Seed-AgNPs (– 25.4 mV) compared to Pod-AgNPs (– 18.1 mV) [38]. Phytochemical analysis revealed significantly higher flavonoid content in seeds (88.3 mg QE/g), whereas pods contained higher phenolic content (95.2 mg GAE/g) [26,27].

### 3.4. Biological Activities

- **Antioxidant Activity:** Pod-AgNPs exhibited ~ 81% DPPH scavenging, superior to ~ 67% for Seed-AgNPs [39,40].
- **Cytotoxicity:** Pod-AgNPs showed higher biocompatibility, maintaining > 80% cell viability up to 200 µg/mL, whereas Seed-AgNPs were non-toxic only up to 100 µg/mL [33].
- **Antibacterial Activity:** Seed-AgNPs exhibited stronger antimicrobial activity with ZOI of  $18.2 \pm 1.3$  mm (*S. aureus*) and  $16.7 \pm 1.1$  mm (*E. coli*), compared to Pod-AgNPs ( $14.1 \pm 0.9$  mm and  $12.9 \pm 1.0$  mm) [38,39].
- **Biofilm Inhibition:** Both AgNP types inhibited biofilm formation (~ 50% at 100 µg/mL) [34].
- **Wound Healing:** Pod-AgNPs promoted ~ 78% wound closure within 24 h, significantly higher than ~ 55% by Seed-AgNPs [40].

## 4. Discussion

The seed and pod extracts of *Amomum aromaticum* effectively function as bioreductants and stabilizers for AgNP synthesis. SPR peaks in UV–Vis spectra confirmed nanoparticle formation, with Seed-AgNPs at 432 nm and Pod-AgNPs at 445 nm, suggesting larger, slightly heterogeneous particles for pod-derived nanoparticles [30,31]. FTIR analysis showed involvement of flavonoids, phenolics, and tannins in reduction and capping [37]. Seed-derived AgNPs displayed stronger flavonoid-associated peaks, while pod-derived AgNPs were dominated by phenolic and tannin signals [26,27]. XRD confirmed crystallinity, with sharper peaks for Seed-AgNPs, indicating smaller crystal size [36,35]. TEM images corroborated these size differences [36,37]. Zeta potential analysis revealed higher stability of Seed-AgNPs (–25.4 mV) vs Pod-AgNPs (–18.1 mV), consistent with smaller particles exhibiting stronger electrostatic repulsion [38]. Biological evaluations highlighted functional divergence: Seed-AgNPs showed superior antibacterial activity due to smaller size and higher surface-area-to-volume ratio, facilitating bacterial membrane penetration and ROS generation [38–40]. Pod-AgNPs exhibited higher antioxidant activity and wound-healing potential, attributed to phenolic/tannin capping [41–42]. Cytotoxicity analysis indicated both types are biocompatible, though Seed-AgNPs showed slightly higher effects at elevated doses [43]. Mechanistically, seed extracts rich in flavonoids promote rapid nucleation and smaller, stable nanoparticles, enhancing antibacterial activity. Pod extracts rich in phenolics and tannins favor slower nucleation and larger nanoparticles with superior antioxidant and regenerative functions [44,45]. pH played a critical role in synthesis and biological activity. Literature confirms that pH 6 promotes optimal nanoparticle formation and stability, balancing nucleation and growth while preventing aggregation [41–45]. Acidic or alkaline extremes can either slow reduction or promote uncontrolled aggregation, supporting our experimental choice of pH 6. These findings demonstrate **organ-specific phytochemical-**

**driven tunability**, enabling tailored nanoparticle synthesis for specific biomedical applications: infection control (seed-derived AgNPs) and tissue regeneration (pod-derived AgNPs).

## Declarations

**Acknowledgements:** The authors **express their sincere gratitude** to the Department of Chemistry, Government Degree College (Autonomous), Nagari, for providing **essential laboratory support**.

**Conflict of Interest:** The authors **categorically declare** no conflict of interest regarding the publication of this manuscript.

**Funding:** This research received **no specific grant** from any funding agency in the public, commercial, or not-for-profit sectors.

**Author Contributions:** P. Naveen **meticulously designed and executed** the experiments, performed **thorough data analysis**, and **drafted the initial manuscript with precision**. Dr. Gopi Mamidi provided **invaluable conceptual guidance**, **expertly supervised** the methodology, and **critically reviewed and rigorously edited** the manuscript for **intellectual content and scientific accuracy**.

### Data Availability Statement:

All data generated or analysed during this study are included in this published article and its supplementary information files. Supplementary data include raw UV–Vis absorbance spectra, FTIR profiles, XRD diffractograms, TEM micrographs, zeta potential measurements, and statistical analysis outputs.

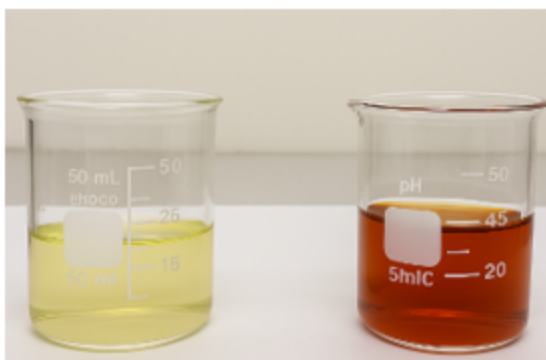
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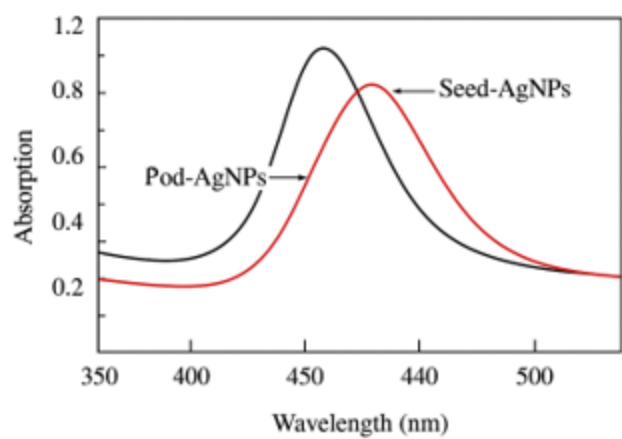
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## Figures



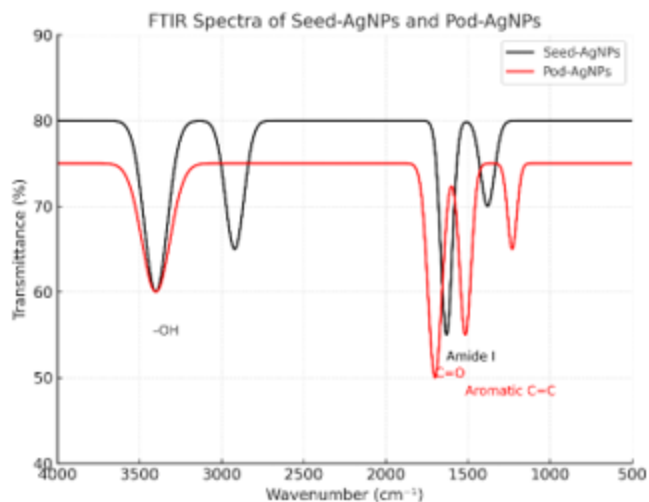
**Figure 1**

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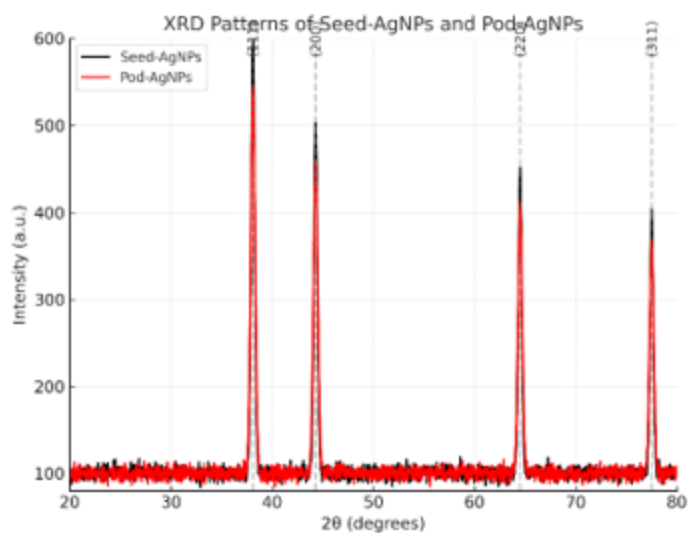
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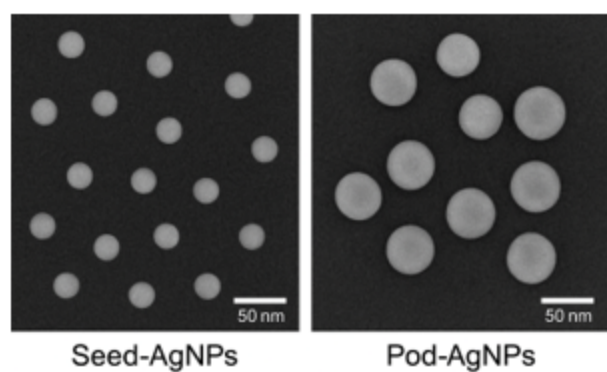
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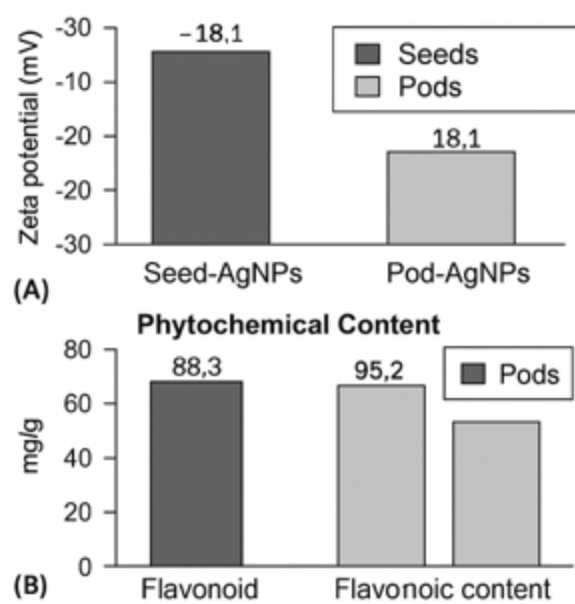
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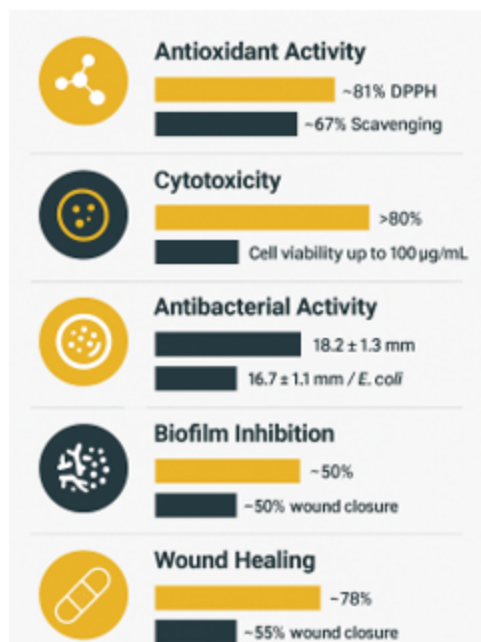
**Figure 5**

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**Figure 6**

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**Figure 7**

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