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Biogenic Selenium Nanoparticles from *Coleus amboinicus* and *Solanum trilobatum* Extracts: Synthesis, Characterization and Comparative Biomedical Applications

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Abstract

Green synthesis of selenium nanoparticles (SeNPs) using plants offers ecofriendly means to produce biocompatible and bioactive nanomaterials for medicinal purposes. *Coleus amboinicus* and *Solanum trilobatum* species have significant phytochemical properties, but their efficacy for the synthesis of SeNPs has not been compared. In the current study, aqueous extracts of these plants were used to reduce sodium selenite to produce SeNPs. Several characterization techniques, including UV–Vis spectroscopy, FTIR, XRD and SEM, were used for characterization of the synthesized nanoparticles. DPPH assay was used to determine antioxidant activity of the SeNPs and MIC and MBC assays were performed to assess antibacterial activity of the SeNPs against clinical Staphylococcus aureus strains. Excellent crystalline and spherical nanoparticles with average sizes ranging from 70 to 100 nm were obtained from both extracts. The FTIR technique indicated that various functional groups of the plant extracts contributed to nanoparticle stabilization. At a concentration of 100 µg/mL, C-SeNPs displayed greater DPPH scavenging capability, around 81%, in comparison to S-SeNPs, which showed around 77%. On the other hand, S-SeNPs exhibited a higher antibacterial activity against S. aureus strains due to larger inhibition zones as well as better MIC and MBC results than C-SeNPs. This is evidence of the significant role that phytochemicals play in the biological activity of SeNPs. Thus, while C-SeNPs were able to achieve better antioxidant performance, S-SeNPs showed stronger antibacterial properties.

Keywords: Antioxidant, *Coleus amboinicus*, Green Synthesis, Selenium Nanoparticles, *Solanum trilobatum*.

Introduction

Antimicrobial resistance (AMR) represents a significant and emerging global health threat, with pathogens such as *Staphylococcus aureus* increasingly developing resistance to commonly used antibiotics. The continuous emergence and spread of antimicrobial-resistant microorganisms have created major challenges in the effective management of infectious diseases and increased the need for alternative therapeutic strategies. Without effective new interventions, the World Health Organization anticipates that antimicrobial resistance could contribute to up to 10 million deaths annually by 2050 (1, 2). Therefore, there is an urgent need to identify and develop new therapeutic agents that provide effective antimicrobial activity while minimizing toxicity and other limitations associated with conventional treatments. Nanotechnology offers promising opportunities in this context, particularly selenium nanoparticles (SeNPs), which possess antimicrobial and antioxidant activities. Selenium is an essential trace mineral required for the development and function of selenoproteins and plays important roles in physiological processes, including redox regulation and immune system function (3, 4). Studies have shown that SeNPs exhibit greater biological activity than bulk selenium, together with lower toxicity and improved bioavailability. Their unique physicochemical properties support antibacterial activity through mechanisms such as bacterial membrane disruption, induction of oxidative stress, interaction with DNA and proteins and inhibition of biofilm formation (5, 6). SeNPs can also scavenge free radicals and enhance endogenous antioxidant activity, thereby protecting cells from

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oxidative injury associated with excessive free radicals and infection-related oxidative stress (7, 8). Nanoparticles can be produced using various methods that considerably influence their physicochemical and biological properties. Conventional physical and chemical approaches may involve complex procedures, high energy consumption, toxic reducing agents and chemical waste. Consequently, plant extracts have emerged as attractive alternatives for nanoparticle synthesis. Green synthesis using plants is advantageous because phytochemical constituents can act as reducing and stabilizing agents during nanoparticle production. This approach is cost-effective, environmentally friendly and can facilitate the production of biocompatible nanoparticles (7, 8). Plant extracts may also provide an additional benefit because their bioactive constituents can become associated with nanoparticles and contribute to their biological properties. However, the influence of plant source and phytochemical composition on the physico-chemical and biological characteristics of synthesized nanoparticles needs to be considered. Among medicinal plants, *Coleus amboinicus* (Indian borage) and *Solanum trilobatum* (climbing brinjal, "thuthuvalai") possess well-documented traditional and therapeutic properties. *C. amboinicus* contains bioactive constituents such as rosmarinic acid, flavonoids and essential oils rich in carvacrol, which have been associated with antioxidant and antibacterial activities (9, 10). These phytochemicals may contribute to the reduction and stabilization of SeNPs during synthesis and influence their biological characteristics. Similarly, *S. trilobatum* is an important medicinal plant traditionally used in Siddha and Ayurvedic medicine and widely utilized in the management of respiratory diseases and infections. It contains phytochemicals including alkaloids such as solasodine, flavonoids and saponins, which possess antimicrobial, anti-inflammatory and antioxidant activities (11, 12). The distinct phytochemical profiles of these plants provide a basis for their potential application in the green synthesis of SeNPs. Plant-derived compounds may participate in selenium precursor reduction and remain associated with the nanoparticle surface as capping or stabilizing molecules. These interactions may influence nanoparticle size, shape, surface characteristics, stabilization and ultimately biological activity.

Although previous studies have demonstrated the potential of plant extracts for nanoparticle synthesis and highlighted the antioxidant and antimicrobial properties of plant-derived phyto-chemicals and SeNPs, several limitations remain. Much of the existing research has focused on nanoparticles synthesized using individual plant extracts, with relatively limited comparative assessment of different medicinal plant sources under similar synthesis conditions. Consequently, it remains difficult to determine how differences in phytochemical composition influence the physico-chemical characteristics and biological perfor-mance of resulting SeNPs. In particular, the relationship between phytochemical-mediated nanoparticle stabilization and antibacterial efficacy, including minimum inhibitory concen-tration (MIC) and minimum bactericidal concentration (MBC), has not been sufficiently explored. Furthermore, SeNPs produced using different plant extracts may not show the same patterns of antioxidant and antibacterial activities. Therefore, these properties should be evaluated comparatively rather than assuming that enhanced antioxidant activity will necessarily result in enhanced antibacterial activity.

Therefore, the present study was designed to comparatively investigate SeNPs synthesized using aqueous extracts of *C. amboinicus* and *S. trilobatum*. The study aimed to synthesize and characterize the resulting SeNPs using UV–Visible spectroscopy, Fourier- transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). It further aimed to evaluate and compare their antioxidant activities and assess their antibacterial activities against clinical isolates of S. aureus using MIC and MBC assays. By comparing the physicochemical characteristics and biological activities of SeNPs synthesized from two distinct medicinal plant extracts under comparable conditions, this study seeks to provide information on the influence of plant-derived phytochemicals on nanoparticle properties and bioactivity. The novelty of the present study lies in the comparative evaluation of *C. amboinicus*- and *S. trilobatum*-mediated SeNPs, linking their physicochemical characteristics with antioxidant and antibacterial performance. This approach provides insight into the potential

contribution of different plant phytochemical compositions to the functional properties of SeNPs and highlights the potential of green nanotechnology for developing sustainable and biologically active nanomaterials for biomedical applications.

Methodology

Collection and Preparation of Plant Materials

Leaves of *Coleus amboinicus* and *Solanum trilobatum* were collected from the Herbal Garden, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Poonamallee High Road, Velappanchavadi, Thiruverkadu, Tamil Nadu, India – 600077 (13.055775° N, 80.124602° E). The collected plant materials were authenticated before use. The leaves were thoroughly washed with water to remove surface debris, air-dried under shade and finely ground into powder. The powdered samples were stored in tightly sealed, airtight containers under appropriate conditions until further use, as previously reported (10, 13, 14).

Preparation of Aqueous Extracts

Water extracts of *C. amboinicus* and *S. trilobatum* were made by an appropriate amount of plant powder in distilled water. The solution was boiled, then filtered and concentrated using a rotary evaporator. The concentrated extracts were stored and kept in a controlled temperature for future use (15).

Green Synthesis of Selenium Nanoparticles

SeNPs were synthesized from the prepared plant extracts using phyto-mediated reduction. An aqueous solution of sodium selenite (1–5 mM) was mixed with an equal amount of *C. amboinicus* or *S. trilobatum* extract. The reaction mixture was incubated at 37 °C for 24 h under agitation. A color change from pale yellow to orange red was indicative of nanoparticle synthesis. The nanoparticles were collected by centrifugation at 12000 rpm for 20 min then washed 3 times with deionized water for unbound biomolecules purging. Finally, the nanoparticle powder was dried at 40 °C and further stored at 4 °C in airtight tube until characterization (8, 16, 17).

Characterization of SeNPs

Different physicochemical methods were utilized to synthesize SeNPs and characterize them and to confirm their formation. UV–Vis spectroscopy was conducted to confirm the generation of nanoparticles by using UV–Vis spectroscopy, to enable the detection of the surface plasmonic resonance peaks. FTIR helped determine functional groups that are present and collaborating in the capping and stabilization of SeNPs. SEM was used to determine the morphology and size distributions. The crystalline structure, crystalline nature, was identified by X-ray diffraction (XRD) analysis (18, 19).

In-vitro Antioxidant Properties

DPPH Radical Scavenging Assay

Antioxidant activity of SeNPs produced from *C. amboinicus* (C-SeNPs) and *S. trilobatum* (S-SeNPs) was evaluated using the DPPH assay (20). A methanol-based solution of DPPH (0.1 mM) was prepared and kept in the dark. Multiple dilution of C-SeNPs and S-SeNPs (10–100 µg/mL) were added to 1 mL of DPPH solution and incubated at room temperature for 30 minutes in the dark. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer, with methanol as the blank. Ascorbic acid (10–100 µg/mL) served as a positive control. Radical scavenging activity (RSA, %) was calculated and results were expressed as percentage RSA at each concentration. All experiments were performed in triplicate and results were presented as mean ± standard deviation.

Antibacterial Activities

Ethical and Biosafety Approval

The study protocol involving the handling and analysis of clinical isolates of Staphylococcus aureus was reviewed and approved by the Institutional Biosafety Committee (IBSC), Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India (Approval No. 018/SIMATS/IBSC/Approval/2023). All experimental procedures were conducted in accordance with the biosafety guidelines prescribed by the Department of Biotechnology (DBT), Government of India and institutional biosafety regulations. Appropriate biosafety practices were strictly followed during the handling, cultivation and disposal of pathogenic microorganisms.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of C-SeNPs and S-SeNPs were determined using the broth microdilution method in accordance with the CLSI guidelines

(CLSI, 2020) (21). Stock suspensions of the nanoparticles were prepared at a concentration of 10 mg/mL in Mueller–Hinton broth (MHB) and briefly sonicated before use. Overnight bacterial cultures were adjusted to the 0.5 McFarland standard (approximately 1×10^8 CFU/mL) and subsequently diluted 1:100 in MHB to obtain a final bacterial inoculum of approximately 5×10^5 CFU/mL. Two-fold serial dilutions of the SeNPs were prepared in sterile 96-well microplates to obtain concentrations ranging from 1000 to 1.95 µg/mL. Each well contained 100 µL of MHB and 10 µL of the bacterial inoculum. Column 11 was used as the growth control containing bacteria only, whereas column 12 served as the negative control containing broth alone. The microplates were incubated at 37 °C for 24 h. Following incubation, 30 µL of resazurin solution (0.015% w/v) was added to each well and the plates were further incubated for 30–60 min. Wells that retained a blue color were considered indicative of the absence of bacterial growth, whereas wells that became pink or colorless indicated bacterial viability. The MIC was defined as the lowest concentration of SeNPs that prevented visible bacterial growth and inhibited the resazurin color change. For determination of the MBC, 10–20 µL of the contents from wells exhibiting microbial inhibition at and above the MIC were spot-plated onto Mueller–Hinton agar (MHA) and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of SeNPs that resulted in complete absence of colony growth, corresponding to ≥99.9% bacterial killing. All MIC and MBC assays were performed in triplicate. As MIC and MBC represent discrete endpoint concentrations obtained from two-fold serial dilution assays, the results are presented as the lowest concentration showing inhibitory or bactericidal activity rather than as mean ± SE values.

Results

Synthesis and Preliminary Confirmation

Biosynthesis of SeNPs through aqueous extracts of *Coleus amboinicus* and *Solanum trilobatum* was visually evident with the gradual change of color in the reaction mixture from pale yellow to reddish-brown, due to the reduction of sodium selenite to elemental selenium.

UV–visible Spectroscopy

The specific color change of the solutions from yellow to ruby red, confirmed through the visual observation, indicates the reduction of selenite ions and the successful synthesis of stable SeNPs using plant extracts of *Coleus amboinicus* and *Solanum trilobatum* tubers in an aqueous medium. The confirmation of this visual change is also substantiated by the UV–Vis spectroscopic results that exhibit strong absorption peaks of SeNPs at 281 nm and 270 nm in Figures 1A and 1B. These peaks appear due to the Surface Plasmon Resonance (SPR) effect of SeNPs and indicate that the synthesis of SeNPs has occurred and that nanoscale particles are present. The broadness of the peaks referenced above is indicative of the existence of polydisperse nanoparticles with variation in size and shape of the formed SeNPs which reflects findings reported in the prior literature of nanoparticle synthesized by plants mediated extract due to the function of phytochemicals acting as reducing and capping agents for stabilizing nanoparticles. The rapid formation of SeNPs confirmed by the observation of visual changes made using plant extracts demonstrates a simpler, efficient and eco-friendly method of synthesis compared to chemical methods of synthesis.

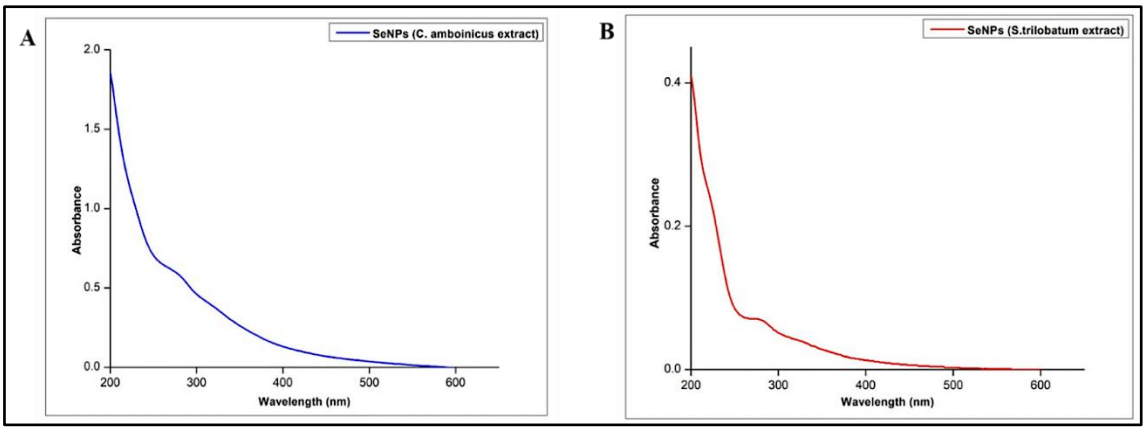


Figure 1: UV–vis Absorption Spectra of SeNPs Synthesized Using Plant Extracts. (A) C-SeNPs Showing a Characteristic Absorption Peak at 281 nm, (B) S-SeNPs Showing a Characteristic Absorption Peak at 270 nm

Note: SeNPs: Selenium Nanoparticles, C-SeNPs: Selenium Nanoparticles Synthesized Using *Coleus amboinicus* Extract, S-SeNPs: Selenium Nanoparticles Synthesized Using *Solanum trilobatum* Extract. The Absorption Peaks Indicate the Formation of SeNPs and Their Surface Plasmon Resonance Characteristics.

FTIR Analysis

The FTIR spectrum of SeNPs synthesized using *Coleus amboinicus* extract in Figure 2A exhibited characteristic peaks at 622, 1050, 1414, 1603, 2859, 2929, 3428, 3829, 4101, 4264 and 4388 cm^{-1}. The peak at 622 cm^{-1} corresponds to C–X stretching, while 1050 cm^{-1} represents C–N stretching of alcohol or phenolic groups, indicating the presence of flavonoids and polysaccharides and C-H binding and characteristics of Se-O vibration. Bands at 1414 cm^{-1} and 1603 cm^{-1} are attributed to C-C stretching and OH stretching of aromatic compounds. Peaks at 2859 and 2929 cm^{-1} correspond to aliphatic C–H stretching and the broad band at 3428 cm^{-1} is associated with O–H stretching vibrations, confirming hydroxyl and amine groups from biomolecules. These functional groups indicate that *C. amboinicus* phytochemicals, such as phenols, terpenoids and proteins, are involved in the bio reduction of Se^{4+} to Se^{0} and in capping/stabilization of the nanoparticles, consistent with previous reports on green-synthesized SeNPs. The FTIR spectrum of SeNPs synthesized using *Solanum trilobatum* extract in Figure 2B displayed several distinct absorption peaks at 581, 624, 670, 784, 1060, 1161, 1266, 1332, 1397, 1516, 1620, 1745, 1843 and 2338 cm^{-1}. These peaks indicate the presence of various functional groups derived from the plant extract. The absorption bands observed between 581–784 cm^{-1} correspond to C–H bending vibrations, while the peak at 1060 cm^{-1} represents C–O stretching vibrations. Peaks in the region of 1161–1397 cm^{-1} are associated with C–N stretching and C–H bending vibrations. The signal that appears at 1620 cm^{-1} corresponds to N–H bending as well as C=C stretching vibrations. The peaks at 1745 and 1843 cm^{-1} are also due to C=O stretching vibrations. Furthermore, there is a sharp peak located at 2338 cm^{-1} that may be related to C≡N stretching or CO_2 overtones. These characteristic peaks provide evidence of biomolecules such as phenols, flavonoids, proteins and other secondary metabolites from *S. trilobatum* extract that serve as reducing and stabilizing agents during the green synthesis of SeNPs.

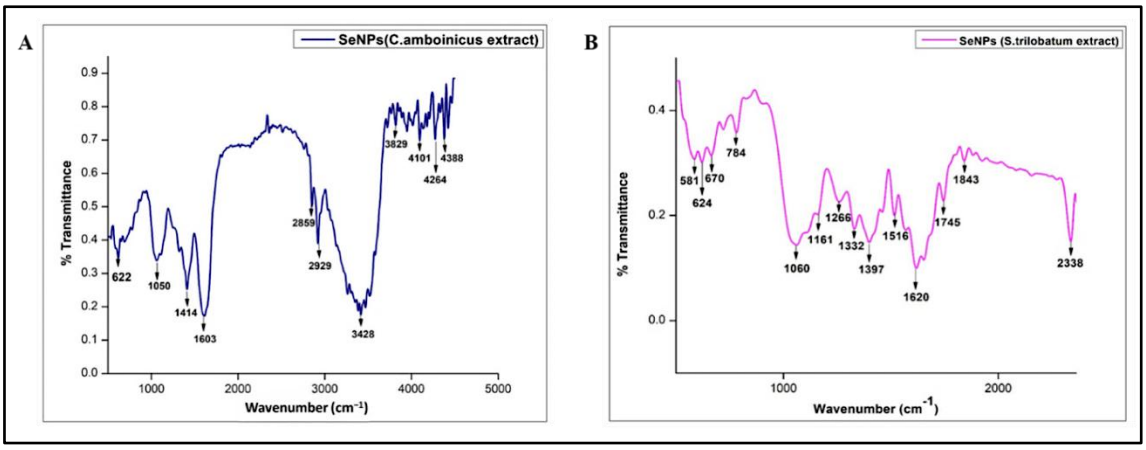


Figure 2: (A) FTIR spectrum of C-SeNPs Showing Characteristic Absorption Peaks Associated with Hydroxyl, Carbonyl, Alkene and C–O Functional Groups, (B) FTIR Spectrum of S-SeNPs Showing Characteristic Peaks Corresponding to C–H, C–O, C=C, C=O and N–H functional Groups

Note: FTIR: Fourier-transform Infrared Spectroscopy, C-SeNPs: Selenium Nanoparticles Synthesized Using *Coleus amboinicus* Extract, S-SeNPs: Selenium Nanoparticles Synthesized Using *Solanum trilobatum* Extract. The Observed Functional Groups Indicate the Involvement of Plant-derived Phytochemicals in the Reduction and stabilization of SeNPs.

In Figure 2A, the prominent absorption peaks at 622, 1050, 1414, 1603, 2859, 2929, 3428, 3829, 4101, 4264 and 4388 cm^{-1} indicate the presence of functional groups such as hydroxyl (–OH), carbonyl (C=O), alkene (C=C) and C–O stretching vibrations, confirming the role of bioactive compounds in the reduction and stabilization of SeNPs. In. Figure 2B, the absorption peaks observed at 581, 624, 670, 784, 1060, 1161, 1266, 1332, 1397, 1516, 1620, 1745, 1843 and 2338 cm^{-1} correspond to functional groups such as C–H, C–O, C=C, C=O and N–H stretching vibrations. These peaks confirm the presence of phytochemical constituents responsible for the reduction and stabilization of SeNPs during green synthesis.

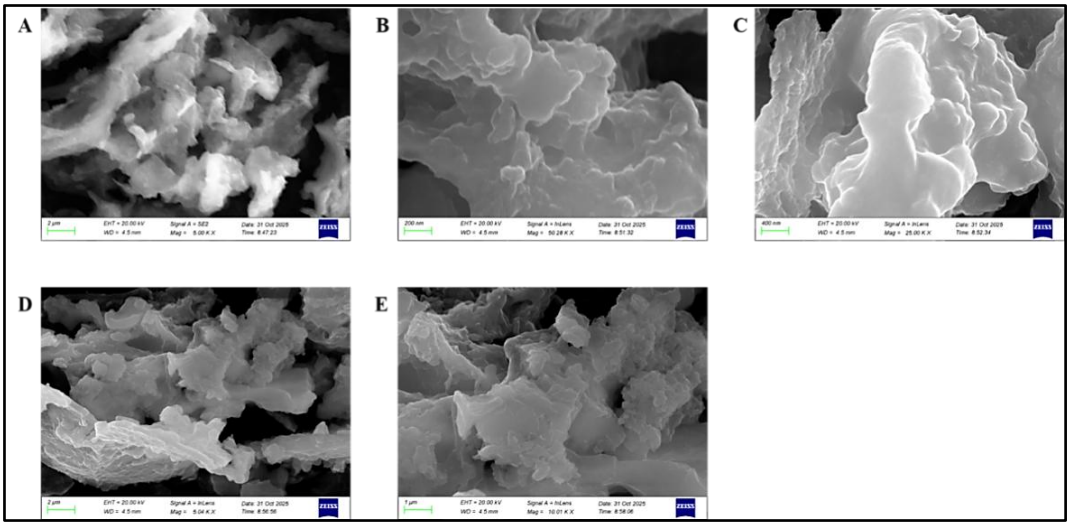


Figure 3: SEM Images of SeNPs Synthesized from the *Coleus amboinicus* Extract Showing Aggregated, Irregular, Spherical to Flake-like Morphologies. (A) Mag = 5.00 K X, (B) Mag = 50.28 K X, (C) Mag = 25.00 K X, (D) Mag = 5.04 K X, (E) Mag = 10.01 K X

Note: SEM = Scanning Electron Microscopy, C-SeNPs = Selenium Nanoparticles Synthesized Using *Coleus amboinicus* Extract.

Scanning Electron Microscopy (SEM)

The SEM micrographs of SeNPs synthesized from the *Coleus amboinicus* extract show that they have both aggregated and irregularly shaped particles with a mostly spherical to flake-like morphology in Figures 3A-3E. At lower magnifications, the SeNPs appear as tightly packed clusters due to natural agglomeration, while higher magnifications showed distinctly nanosized particles that have smoother and well-defined surfaces. The particle size was also found to be in the nanometer range

(about 1µm–200 nm), confirming that SeNPs were successfully synthesized.

The surface morphology shows effective capping and stabilization by the phytochemicals found in the plant extract, indicating that the bioactive compounds served as reducing and stabilizing agents during the synthesis of the nanoparticles. The images of SeNPs synthesized using *Solanum trilobatum* extract taken by SEM reveal irregular flake-like nanostructures to spherical nanostructures in Figures 4A-4F. At increased magnification, the particles were smooth, dense and well-defined keeping with appropriate uniform growth had taken place. Particle size analysis indicates particles are between 1 µM–400 nm thus confirming nano-formation. The observed clustering at lower magnification could be attributed to natural clustering of the particles while drying. Phytochemicals present *S. trilobatum* are acting as reducing and stabilizing agents which keep SeNPs from agglomerating during synthesis.

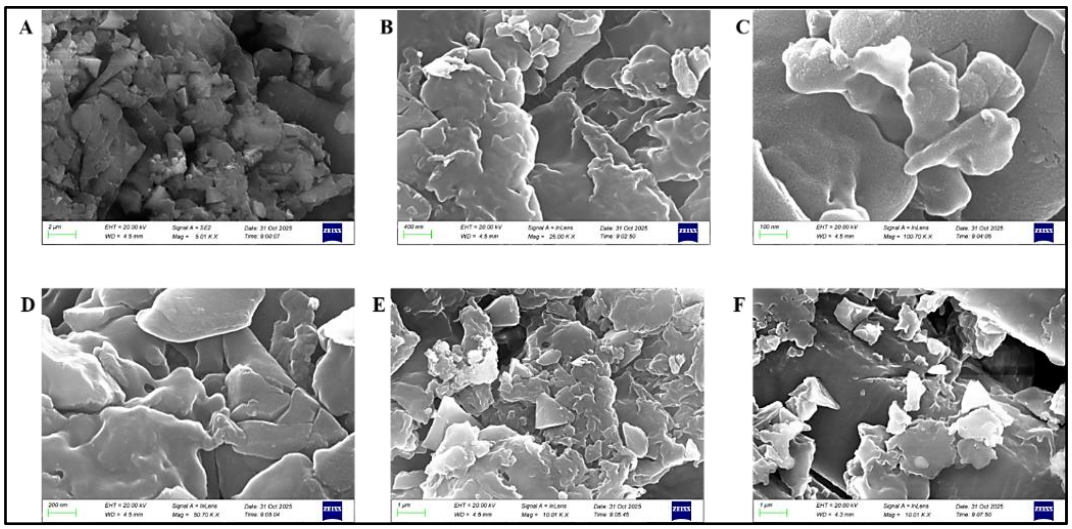


Figure 4: SEM Images of S-SeNPs Showing Irregular Flake-like to Spherical Nanostructures with Relatively Smooth and Well-defined Surfaces. (A) Mag = 5.01 K X, (B) Mag = 25.00 K X, (C) Mag = 100.70 K X, (D) Mag = 50.70 K X, (E, F) Mag = 10.01 K X

Note: SEM = Scanning Electron Microscopy, S-SeNPs = Selenium Nanoparticles Synthesized Using *Solanum trilobatum* Extract. The Observed Morphology and Nanoscale Particle Formation Indicate Successful Synthesis of SeNPs.

X-ray Diffraction (XRD)

Distinct diffraction peaks confirmed the crystalline nature of the synthesized nanoparticles. C-SeNPs and S-SeNPs exhibited reflections at 2θ = The peaks with 23.49, 29.81, 41.86, 43.78, 45.81 and 51.71 correspond to crystalline planes of 100, 101, 110, 102, 111 and 201, corresponding to planes of elemental selenium in Figures 5A and B. The results validate the nanoscale crystalline characteristics of both preparations.

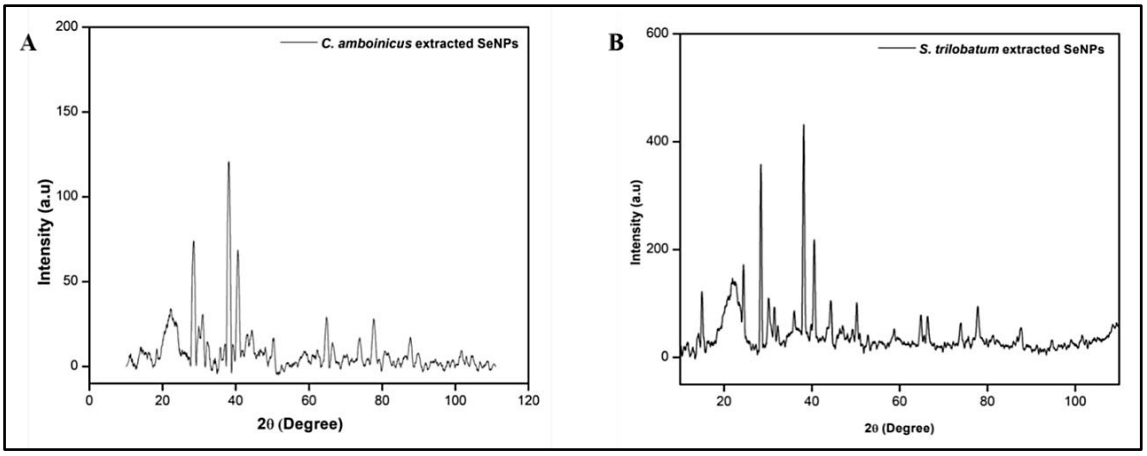


Figure 5: X-ray Diffraction Patterns. (A) XRD Pattern of C-SeNPs, (B) XRD Pattern of S-SeNPs

Note: XRD = X-ray Diffraction, C-SeNPs = Selenium Nanoparticles Synthesized Using *Coleus amboinicus* Extract, S-SeNPs = Selenium Nanoparticles Synthesized Using *Solanum trilobatum* Extract. The Diffraction Peaks Observed at 2θ Values of 23.49°, 29.81°, 41.86°, 43.78°, 45.81° and 51.71° Correspond to the Reported Crystalline Planes of Elemental Selenium.

Antioxidant Activity

The DPPH radical scavenging assay was used to evaluate the antioxidant activity of the synthesized SeNPs at concentrations ranging from 20 to 100 µg/mL in Figures 6A and B. The C-SeNPs showed an increase in DPPH radical-scavenging activity across the tested concentration range, whereas the S-SeNPs exhibited variable scavenging activity across the tested concentrations. The IC50 values were not reliably determined within the tested concentration range because the C-SeNPs did not reach 50% inhibition at the highest concentration tested, while the response pattern of S-SeNPs did not provide a sufficiently consistent concentration-response relationship for reliable IC50 estimation. Therefore, antioxidant activity was compared based on the observed %RSA values across the tested concentration range rather than relying solely on a single concentration.

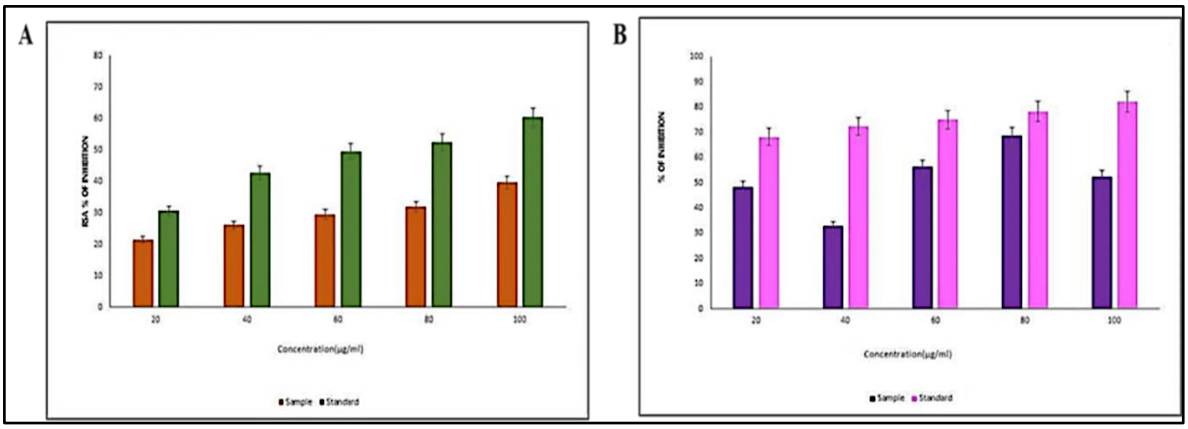


Figure 6: (A) Antioxidant Activity of C-SeNPs, (B) Antioxidant Activity of S-SeNPs Assessed Using the DPPH Radical-scavenging Assay

Note: DPPH = 2,2-diphenyl-1-picrylhydrazyl, C-SeNPs = Selenium Nanoparticles Synthesized Using *Coleus amboinicus* Extract, S-SeNPs = Selenium Nanoparticles Synthesized Using *Solanum trilobatum* Extract, RSA = Radical-scavenging Activity. The Activity Was Evaluated at Concentrations Of 20–100 µg/Ml, with Ascorbic Acid Used as The Standard Reference Antioxidant. Data Are Presented as Mean ± Standard Deviation (N = 3).

In Figures 6A and 6B, the scavenging activity is expressed as percentage radical-scavenging activity (%RSA) at concentrations of 20–100 µg/mL. Ascorbic acid was used as the standard reference antioxidant. Results are presented as mean ± standard deviation (n = 3).

Antimicrobial Activity

Both types of nanoparticles exhibited a dose-dependent antibacterial effect when tested against *Staphylococcus aureus* isolates (S1–S15). This finding was corroborated by the results of the MIC and MBC tests, where the MIC results for S-SeNPs were lower (as low as 3.9–7.8 µg/mL in susceptible strains) than C-SeNPs, which showed generally higher MIC levels of ≥15.6 µg/mL in Tables 1 and 2. In quick summary, *Solanum trilobatum* derived SeNPs had more enhanced antibacterial activity, while *Coleus amboinicus* derived SeNPs had relatively stronger antioxidant properties. Such differences may be due to differences in phytochemical profiles which could affect the nanoparticles surface chemistry and biological activity.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of SeNPs Synthesized from *Solanum trilobatum* (S-SeNPs) Against *Staphylococcus aureus* Clinical Isolates (S1–S15)

Organisms	MIC (Values)	MBC (Values)
S1	500µg/ml	1000µg/ml
S2	3.9µg/ml	7.8 µg/ml
S3	-	-
S4	-	-

S5	15.6µg/ml	31.2µg/ml
S6	-	-
S7	-	-
S8	-	-
S9	500µg/ml	1000µg/ml
S10	7.8µg/ml	15.6µg/ml
S11	-	-
S12	-	-
S13	-	-
S14	-	-
S15	31.2µg/ml	62.5µg/ml

Note: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration. Values Represent the Lowest Concentration of the Test Samples That Inhibited Visible Microbial Growth (MIC) Or Completely Prevented Microbial Growth Upon Subculture (MBC). "–" Indicates No Detectable Antimicrobial Activity at the Tested Concentrations. S1–S15 Represent the Respective Test Samples.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of SeNPs Synthesized from *Coleus amboinicus* (C-SeNPs) Against *Staphylococcus aureus* Clinical Isolates (S1–S15)

Organisms	MIC (Values)	MBC (Values)
S1	-	-
S2	-	-
S3	31.2µg/ml	62.5 µg/ml
S4	31.2µg/ml	62.5
S5	31.2µg/ml	62.5µg/ml
S6	-	-
S7	-	-
S8	-	-
S9	500µg/ml	1000µg/ml
S10	7.8µg/ml	15.6µg/ml
S11	-	-
S12	-	-
S13	-	-
S14	-	-
S15	31.2µg/ml	62.5µg/ml

Note: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration. MIC Represents the Lowest Concentration of the Test Sample That Inhibited Visible Microbial Growth, Whereas MBC Represents the Lowest Concentration That Resulted in Complete Absence of Microbial Growth Upon Subculture. "–" Indicates No Detectable Antimicrobial Activity at the Tested Concentrations. S1–S15 Represent the Respective Test Samples.

Discussion

In this study, SeNPs were produced by using aqueous extracts of *Coleus amboinicus* and *Solanum trilobatum* and proved that both plants can act as an effective green reducing and stabilizing agent. The results of visual observation, in which color changes from pale yellow to reddish-brown and obtaining of SPR peaks at 281 and 270 nm (for C-SeNPs and S-SeNPs respectively) were observed in this study corresponded with that of the previous studies on phytochemistry based processes of reducing sodium selenite to elemental selenium (20, 22). The UV-Vis spectra produced in this study corresponded with what was expected from green synthesized SeNPs (3, 23) and indicated that the process of nucleation and generation of nano sized selenium material was successful. The FTIR results for both forms of nanoparticles showed presence of several functional groups, including OH, C=O, NH, aromatic and aliphatic functional groups. This raises a conclusion that plant polyphenols, flavonoids, terpenoids, saponins and proteins participated in the process of reduction of Se4+ to Se0 and acted as capping agents. Very similar functional groups were reported to be responsible for the enhancement of stability and biological activity of biogenic SeNPs. According to previous research, the noted presence of specific biomolecules in *C. amboinicus* and *S. trilobatum* corroborates *C. amboinicus* and *S. trilobatum* possible role in stabilizing nanoparticles (20, 22, 23). The morphology of both C-SeNPs and S-SeNPs

is shown through SEM imaging, where both show nanoscale spherical to irregular shapes typically seen in plants during biosynthesis. During the process of SEM imaging, the moderate agglomeration can be attributed to natural dehydration of materials together with hydrogen-bonding functional groups, thus similar observations were made in the previous studies within green nanoparticle synthesis (3). The X-ray diffraction (XRD) results confirmed the crystalline nature of the obtained nanoparticles, which is characterized by sharp diffraction peaks on the X-ray diffraction pattern. It is important to mention that sharper peaks observed in S-SeNPs indicate that their crystallinity is higher, thus suggesting that the material has better biological activity due to higher reactivity (4).

Biological studies investigating the synthesized nanoparticles showed different functional properties of the two plant formulations. In this regard, C-SeNPs exhibited slightly higher DPPH radical scavenging activity (~81% at 100 µg/mL), which means available antioxidant properties are higher as compared to S-SeNPs (77%). The difference in this antioxidant activity can be explained by the presence of rosmarinic acid, carvacrol and other compounds known for their antioxidant abilities (9, 10) in the leaves of *C. amboinicus*. The presence of selenium might have enhanced the activity of phytochemicals since selenium is known to have antioxidant properties together with polyphenols (23). The biological experiments performed have demonstrated the role of these nanoparticles. The DPPH test performed showed a concentration-dependent effect, revealing slightly stronger DPPH scavenging properties of C-SeNPs (~81% at 100 µg/mL) as compared to those of S-SeNPs (~77%). This increase in antioxidant properties can be explained by the presence of rosmarinic acid, flavonoids and other compounds found in *C. amboinicus* that possess strong oxidizing abilities.

On the other hand, S-SeNPs showed more excellent antibacterial activity against *Staphylococcus aureus*, as shown by their higher inhibition zones and lower MIC/MBC. The observed outcomes can be attributed to the phytochemicals that *S. trilobatum* contains, including solasodine, steroidal alkaloids, flavonoids and saponins with well-known antibacterial and membrane-disruptive activities (11, 12). The combined action of these phytochemicals with selenium's property of inducing the oxidative stress, affecting the membrane integrity and disturbing the transcription and translation processes likely, led to the fact that they successfully elevate the effectiveness of antibacterial action (2, 6). The difference in MIC/MBC values for C-SeNPs and S-SeNPs suggests the idea that plant phytochemistry does play an important role in influencing nanoparticle chemistry and bioactivity.

The overall findings reveal that different plant species change the physicochemical and biological properties of SeNPs, which supports the previously observed fact that green-synthesized nanoparticles can be tuned for concrete biomedical applications (1, 8). C-SeNPs seem to be more efficient for antioxidant therapy, whereas S-SeNPs may show better properties with respect to their antibacterial activity. Thus, the selective functional behavior of the two nanoparticles makes it evident that we need to pay due attention to understanding phytochemical–nanoparticle interactions while developing new nanomaterials for use in medicine, food, or pharmaceutical industry. However, despite the promising results obtained in the research, the study has several limitations. All the experiments were carried out in vitro and the cytotoxic and biocompatibility of the two types of nanoparticles still need to be evaluated in vivo. *S. aureus* strains were used, while carrying out tests with multidrug-resistant strains and Gram-negative bacteria would make the received results more applicable (24). Future research should focus on the mechanistic pathways involved, such as the quantification of ROS, the assays for membrane integrity, the proteomic reactions and the interactions that occur with standard antibiotics. Furthermore, it would be advisable to perform a systematic comparison of the method of the different extraction methods used in the synthesis of the nanoparticles, considering the different parts of the plants used and the purified products used in the synthesis of the nanoparticles. Overall, this work presents novel comparative data regarding the impact of the phytochemical composition of substances on the functioning of the SeNPs obtained by green synthesis methods.

The observed variations in antioxidant and antibacterial activities of C-SeNPs and S-SeNPs suggest that the two properties are not directly

correlated. The antioxidant activity determined in the DPPH assay mainly measures the capability of surface phytochemical components and those associated with selenium in the reaction with chemically generated free radicals, while the antibacterial activity comprises complex interactions between the nanoparticles and the bacterial cells (25, 26). The interactions may include adhesion to the surface of the bacterial cell, alteration of the membrane structure, interaction with cellular structures and disturbance of cellular redox homeostasis. Hence, a nanoparticle that has a higher radical scavenging ability is not crucially better in terms of its antibacterial ability (27). Variations in the surface chemistry of nanoparticles, including the type and abundance of bioactive compounds that are obtained from plants, may impact their surface properties and how they act when confronted with bacteria. Different FTIR spectra for SeNPs and S-SeNPs indicate differences in functional groups present on their surface, which may be due to different phytochemical compositions of the plant extracts used for their fabrication. This may explain differences in the interactions of the nanoparticles with bacterial membranes or cellular organelles, resulting in the observation of higher antioxidant capacity of SeNPs compared to S-SeNPs (28, 29). As such, it would be appropriate to consider them as separate properties of SeNPs obtained from plants rather than as similar properties. Still, it is worth noting that we did not specifically examine the role of individual phytochemicals on the surface, surface charge, interactions of nanoparticles with membrane, or generation of active oxygen species in this study and this requires further investigation.

Conclusion

This research shows that the synthesis of selenium nanoparticles using plants is an eco-friendly method with specific features confirmed based on studies of the SeNPs' properties. Both *C. amboinicus* and *S. trilobatum* produced SeNPs with specific properties, such as the strong antioxidant properties of the nanoparticles obtained from *C. amboinicus* and the antibacterial efficiency of the nanoparticles obtained from *S. trilobatum* against *Staphylococcus aureus*. The discrepancies in the activity of the SeNPs resulted from the effect of the plant phytochemicals that contribute to the formation and stabilization of nanoparticles forming their biological activity. This study proves the suitability of the plants chosen for the synthesis of SeNPs due to the different but complementary characteristics.

The present study has some limitations. First, all experiments were performed in vitro and therefore may not fully represent the biological interactions that occur when nanoparticles are present in a complex biological system. In addition, the cytotoxicity and biocompatibility of these nanoparticles in mammalian systems remain to be evaluated. Furthermore, only *S. aureus* was tested as a bacterial model organism, limiting the assessment of the antibacterial spectrum of the synthesized SeNPs. Future research should focus on the in vivo safety profile of the nanoparticles and how plant-based bio compounds regulate how the nanoparticles interact with microorganisms in vivo. Future research should also focus on a wider variety of Gram-positive and Gram-negative bacteria (including multi-resistant strains) to study synergistic effects of SeNPs together with conventional antibiotics in terms of possible applications in medicine. In addition, different extraction processes, plant varieties and different phytochemical fractions need to be compared to see how these elements affect the functionality of the generated nanoparticles. Overall, the results of this study imply that plant-derived SeNPs may be used in nanomedicine in a more environmentally friendly way to deal with oxidative damage and antimicrobial resistance. Further research should be conducted to overcome all the shortcomings of this study and find out whether the plant-derived SeNPs are safe, have antimicrobial properties and can be applied in medicine.

Abbreviations

C-SeNPs: Selenium Nanoparticles Synthesized Using *Coleus amboinicus*, FTIR: Fourier Transform Infrared Spectroscopy, MHA: Mueller–Hinton Agar, MHB: Mueller–Hinton Broth, SEM: Scanning Electron Microscopy, SeNPs: Selenium Nanoparticles, SPR: Surface Plasmon Resonance, S-SeNPs: Selenium Nanoparticles Synthesized Using *Solanum trilobatum*, UV–vis: Ultraviolet–visible Spectroscopy, XRD: X-ray Diffraction.

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Author Contributions

Durgadevi Ravi**:** Writing – Review and Editing, Writing – Original Draft, Maghizh Jemima Manavalan: Writing – Review and Editing, Formal Analysis, Bhuvaneswari Gunasekar: Writing – Original Draft, Conceptualization, Writing – Review and Editing, Formal Analysis, Shyamaladevi Babu: Writing – Original Draft, Conceptualization, Writing – Review and Editing, Formal Analysis.

Conflict of Interest

The authors declare no conflicts of interest and assume sole responsibility for both the content and the writing of the article.

Data Availability

This manuscript is a review article, so there is no new data were generated or analyzed during this study; therefore, data availability is not applicable to this manuscript.

Declaration of Artificial Intelligence (AI) Assistance

This manuscript was written by the authors without the use of generative AI or AI-assisted technologies. All content is original and has been created by the authors themselves.

Ethics Approval

The study protocol involving the handling and analysis of clinical isolates of Staphylococcus aureus was reviewed and approved by the Institutional Biosafety Committee (IBSC), Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India (Approval No. 018/SIMATS/IBSC/Approval/2023). All experimental procedures were conducted in accordance with the biosafety guidelines prescribed by the Department of Biotechnology (DBT), Government of India and institutional biosafety regulations. Appropriate biosafety practices were strictly followed during the handling, cultivation and disposal of pathogenic microorganisms.

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