The Journal of Phytopharmacology 2025; 14(6):493-501
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2025; 14(6): 493-501
November- December
Received: 22-09-2025
Accepted: 01-12-2025
Published: 30-01-2026

doi: 10.31254/phyto.2025.14610

Suneeta Panicker
Assistant Professor, Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India

Venkatesh Gone
Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India

Monika Parmar
Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India

Jyotsna Nivalkar
Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India

Correspondence:
Dr. Suneeta Panicker
Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India
Email: suneetapanicker@gmail.com

Medicinal properties of selenium nanoparticles synthesized using leaf extracts of *Passiflora incarnata* and *Cassia fistula* and mechanistic exploration of antimicrobial effect

Suneeta Panicker*, Venkatesh Gone, Monika Parmar, Jyotsna Nivalkar

ABSTRACT

Background: *Passiflora incarnata* and *Cassia fistula*, both plants are well-known for their medical properties. To enhance these properties, they were used to synthesize Nanoparticles. **Aim and Objectives:** The aim of this project was to explore the medicinal properties of selenium nanoparticles (SeNPs) synthesized using the leaf extracts of *Passiflora incarnata* and *Cassia fistula*, an environmentally friendly and sustainable approach. This study investigated the antimicrobial potential of SeNPs and provided insights into the mechanisms behind their bactericidal activity. **Material and Methods:** The SeNPs were synthesized using aqueous leaf extracts of *P. incarnata* and *C. fistula*, with Polyvinyl Alcohol (PVA) as a stabilizing agent. Further characterization was performed using a UV spectrophotometer, dynamic light scattering (DLS), and zeta potential analysis. Synthesized SeNPs were tested for antioxidant, anti-diabetic, and antimicrobial activities. The potential mechanism underlying antimicrobial action, interactions between SeNPs and cellular biomolecules such as DNA and proteins, were also explored. **Results:** Brick red color formation indicated the synthesis of SeNPs. The biosynthesized SeNPs using P. incarnata exhibited an absorbance peak at 532 nm, and those using C. fistula exhibited an absorbance peak at 500 nm, with a Z-average mean particle size of 648.1 nm and a zeta potential of -1.41 mV. SeNPs displayed promising therapeutic properties, including notable antioxidant, anti-diabetic, and antimicrobial activities against bacterial and fungal pathogens. This study confirmed significant binding of SeNPs, without causing fragmentation of DNA or denaturation of proteins, suggesting that the antimicrobial effect may be mediated through non-destructive interactions that disrupt vital cellular processes. The absence of structural damage to biomolecules implies a unique mode of action that could minimize cytotoxicity to host cells. **Conclusion:** Overall, the results highlight the potential of SeNPs synthesized from *P. incarnata* and *C. fistula* as effective, biocompatible agents with multi-functional biomedical applications.

Keywords: Selenium nanoparticles, *Passiflora incarnata*, *Cassia fistula*, Antioxidant activity, Antidiabetic activity, Antimicrobial activity.

INTRODUCTION

The growing threat of antibiotic-resistant pathogens presents a significant global health concern, largely driven by the overuse and misuse of conventional antibiotics. In response, nanotechnology has emerged as a promising field offering novel solutions to address microbial resistance. Among the various nanomaterials explored, selenium nanoparticles (SeNPs) have garnered significant interest for their strong antimicrobial capabilities, minimal toxicity, and essential biological functions. Selenium is a vital trace element involved in maintaining cellular antioxidant defense and supporting immune system functions [1]. When formulated as nanoparticles, selenium demonstrates improved bioavailability, enhanced reactivity, and targeted activity, positioning SeNPs as potential candidates for antimicrobial therapy [2]. Traditional methods for nanoparticle synthesis, including chemical and physical approaches, often involve high energy consumption and the use of hazardous substances. In contrast, green synthesis techniques utilizing plant extracts present an environmentally sustainable, cost-effective, and scalable alternative. Plant-based synthesis relies on bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, which act as natural reducing and capping agents, facilitating the formation of stable and biologically active nanoparticles [3]. In recent years, several medicinal plants have been employed for nanoparticle synthesis; however, many studies have not clearly justified the selection of specific plant species. The choice is often based on traditional knowledge and the presence of bioactive compounds with known therapeutic effects [4]. In this context, Passiflora incarnata and Cassia fistula represent promising candidates for selenium nanoparticle synthesis due to their rich phytochemical profiles and established pharmacological activities. *Passiflora incarnata*, commonly known as passion flower, is a fast-growing vine belonging to the family *Passifloraceae*. Traditionally used to treat anxiety, insomnia, and infections, this plant

contains diverse bioactive constituents including flavonoids, alkaloids, glycosides, and phenolic compounds. These compounds not only contribute to its medicinal value but also facilitate the reduction of metal ions in green synthesis [5].

Cassia fistula, commonly known as Golden shower, is a member of the *Fabaceae* family and widely used in Ayurvedic medicine. Various parts of the plant including its leaves, seeds, and fruit pulp are known for their laxative, antimicrobial, anti-inflammatory, and antioxidant effects. Its leaves are particularly rich in flavonoids, anthraquinones, and sennosides, which make it suitable for nanoparticle synthesis by promoting reduction and stabilization processes [6].

MATERIAL AND METHODS

Plant Sample Collection

Leaves of *Passiflora incarnata* were collected from Dr. D. Y. Patil ACS College, Pimpri, Pune and *Cassia fistula* leaves were sourced from MIDC, Bhosari, Pune. The leaves of both plants were authenticated by the Botany Department and identified by experts from Dr. D. Y. Patil ACS College, Pimpri Pune.

Preparation of *C. fistula* and *P. incarnata* Leaf Extract by Decoction Method

Fresh leaves were washed with distilled water and dried using blotting paper. 60 g of leaves were ground with a mortar and pestle, mixed with 600 ml of distilled water, and boiled until reduced to 200 ml. The mixture was then filtered using Whatman filter paper to obtain the leaf extract (*P. incarnata* and *C. fistula*, respectively), which was stored at 4°C for further use [7].

Phytochemical Analysis of Prepared Extract

Phytochemical test for Flavonoids, Saponins, Alkaloids, Tannin, Protein, Starch, Reducing Sugars was carried as described earlier [8].

Green synthesis of SeNPs using leaf extract of *P. incarnata* and *C. fistula*

Green synthesis of SeNPs using leaf extract of *Passiflora incarnata* and *Cassia fistula* was carried out as described earlier [9] with slight modifications. Briefly, equal volumes (10 mL each) of 50 mM sodium selenite were added dropwise to the 1% polyvinyl alcohol (PVA) solution under constant stirring while maintaining the temperature at 70°C. PVA was used as a stabilizer. Subsequently, the leaf extract (*Passiflora incarnata* and *Cassia fistula* separately) (40 ml) was added dropwise to the resulting mixture of sodium selenite and PVA with continuous stirring at 70°C. After the complete addition of the leaf extract, the mixture was stirred for an additional 1.5 hrs at 70°C to facilitate nanoparticle synthesis. Samples were collected at 15 min, 30 min, 1 hr, 1.5 hrs to check the efficacy of the synthesized SeNPs. A visible color change from yellowish green to reddish brown indicated the formation of SeNPs.

Characterization of SeNPs

UV–vis spectra analysis

UV–Vis spectrophotometry was used to assess the surface plasmon resonance of the SeNPs. 1 mL sample of the nanoparticle suspension was placed in a quartz cuvette (1 cm path length), and absorbance was recorded between 200 and 800 nm [10].

Dynamic Light Scattering analysis

Dynamic light scattering (DLS) measures variations in scattered light brought on by Brownian motion to estimate the size of nanoparticles in a liquid dispersion. DLS may use the Stokes-Einstein equation to get a hydrodynamic radius since particles get smaller the faster the light intensity changes. This quick, non-invasive, and rather easy method yields the average particle size and size distribution and is useful for describing the stability of nanoparticles over time and in various environments. DLS was used to determine the hydrodynamic size and polydispersity index (PDI) of the SeNPs. Measurements were performed at room temperature using nanoparticle suspensions in distilled water, with samples analyzed in disposable cuvettes and recorded in triplicate for accuracy [11].

Zeta Potential analysis

The zeta potential measurement of nanoparticles indicates the strength of electrostatic repulsion between particles by measuring their surface charge and forecasting stability in a colloidal system. Strong repulsive forces inhibit aggregation and therefore give stability. In order to assess the quality and biological uses of nanomaterials, zeta potential is measured using methods such as electrophoretic light scattering (ELS). Thus, Zeta potential analysis was performed at room temperature in the present study, to assess the surface charge and colloidal stability of the SeNPs. Samples suspended in distilled water were analyzed to evaluate electrostatic interactions and dispersion behavior [10].

Assessment of Antimicrobial activity of SeNPs by Agar well diffusion method

The antimicrobial activity of SeNPs was assessed using the agar well diffusion method against *E. coli*, *S. aureus*, *B. subtilis*, and *Candida* sp., obtained from the Department of Microbiology. Log-phase culture suspensions were spread on sterile Mueller-Hinton Agar (MHA) plates, while *Candida albicans* was spread on sterile Potato Dextrose Agar (PDA) plates. 8mm wells were bored and loaded with 100 µL of SeNPs and appropriate positive controls (amoxicillin for bacteria, fluconazole for fungus). After pre-diffusion at 4°C for 15 minutes, plates were incubated at 37°C for 24 hours, and zones of inhibition were measured [12].

Assessment of Disinfectant activity of SeNPs by EN 13:2024-01 Assay

The EN 13:2024-01 assay was performed to assess the disinfectant activity of SeNPs synthesized from both plant leaves against *S. aureus*. A control group with no SeNPs treatment was included. 100 µL of log-phase *S. aureus* culture was applied as a single drop onto a sterile coin, a non-porous surface, in a Petri dish and dried at 37°C. SeNP solutions (100 µL) were added to the dried cultures in the test groups, while the control group received no treatment. After 30 mins of incubation at room temperature, the coin was rinsed with 500 µL sterile saline and serially diluted up to 10^{-5} which was then plated on sterile Luria agar plates. The plates were incubated at 37°C for 24 hours. Reduction in bacterial count after SeNP treatment was compared with untreated control group.

Mechanistic approach of antimicrobial activity of SeNPs synthesized from *C. fistula*

Effect of SeNPs on DNA structure

DNA isolated from *E. coli* (Gram-negative) was incubated with an equal volume (70 µL) of SeNPs at 37°C for 1 hour, with the DNA concentration determined to be 900 µg/mL. As a negative control, distilled water (70 µL) was used instead of SeNPs. After incubation, samples were loaded onto a 0.8% agarose gel for electrophoresis to assess DNA fragmentation. The band pattern was visualized under a UV transilluminator to evaluate structural changes in the DNA [13].

Effect of SeNPs on Protein structure

The effect of SeNPs on proteins was assessed as described by earlier [14] with slight modifications. A 0.6 mg/mL BSA stock solution was prepared, and 50 µL of BSA was mixed with 50 µL of SeNPs and incubated at 37°C for 1 hour. A negative control consisted of BSA and distilled water. After incubation, loading buffer was added to both test and control samples, which were then loaded onto a 12.5% polyacrylamide gel for SDS-PAGE. Protein bands were stained with Coomassie Brilliant Blue, and structural changes were assessed by comparing the test sample with the control.

DNA-Nanoparticle binding assay

Electronic absorption spectroscopy

DNA was isolated from *Bacillus* (Gram-positive) and *E. coli* (Gram-negative) cultures the concentration of each DNA sample was adjusted 900 µg/mL. Five dilutions (44.5, 89, 178, 267, 356 µg/mL) of each DNA sample were prepared in distilled water. To each dilution, 500 µL of SeNPs was added and incubated at room temperature for 5 minutes. The final volume was adjusted to 1 mL with distilled water, and the optical density was measured using a UV spectrophotometer to assess DNA binding capacity of SeNPs [10].

Protein-Nanoparticle binding assay

Electronic absorption spectroscopy

A 1 mg/mL BSA stock solution was prepared, and five dilutions (50, 100, 200, 300, 400 µg/mL) were made. Each dilution was treated with 500 µL SeNPs and incubated for 5 minutes at room temperature. The final volume was adjusted to 1 mL with distilled water, and optical density was measured using a UV spectrophotometer to assess BSA binding capacity of SeNP [10].

Antioxidant Assay of SeNPs synthesized from leaf extract of *C. fistula* by FRAP assay

The antioxidant activity of SeNPs was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. SeNP (1 mL) was mixed with 2.5 mL phosphate buffer (0.15 M, pH 7.4) and 2.5 mL of 1% potassium ferricyanide, and incubated at 50°C for 20 minutes. Next, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was combined with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm, and results were compared to ascorbic acid (500 µg/mL) as a standard antioxidant [15].

Anti-Diabetic Assay of SeNPs synthesized from leaf extract of *C. fistula* and *P. incarnate*

The α-amylase inhibition assay was used to assess the anti-diabetic activity of SeNPs synthesized from *P. incarnata* and *C. fistula*. SeNPs (200 µL) were mixed with 200 µL of α-amylase (3.75 mg/mL) and incubated at 30°C for 10 minutes. Then, 200 µL of 1% starch solution was added and incubated for 3 minutes. Following this, 200 µL of DNSA reagent was added, and the mixture was heated at 85–90°C for 10 minutes, and then cooled. Absorbance was measured at 540 nm to determine enzyme inhibition. A blank (SeNPs without enzyme) and acarbose (100 µg/mL) served as controls. Greater percentage inhibition reflected stronger α-amylase inhibitory activity, indicating the potential of SeNPs in regulating carbohydrate metabolism [16].

The percentage inhibition of α-amylase activity was calculated using the following formula:

$$\text{α-amylase inhibition (\%)} = \frac{[(AC+-AC-)-(AS-AB)]}{(AC+-AC-)} \times 100$$

Where,

AC^+ = Absorbance of control with 100% enzyme activity (no inhibitor) (Enzyme + starch)

AC^- = Absorbance without enzyme (blank for baseline correction) (Starch + DNSA)

AS = Absorbance of test sample with enzyme (Enzyme + inhibitor + starch)

AB = Absorbance of test sample without enzyme (sample blank) (Inhibitor + starch + DNSA) Acarbose (positive control) = Standard α-amylase inhibitor (positive control) (Enzyme + acarbose + starch)

Negative control = Reaction with solvent instead of test sample (Enzyme + solvent + starch)

RESULTS

Preparation of leaf extract of *P. incarnata and C. fistula* by using decoction method

The leaf extract was prepared using a decoction method and both extracts appeared yellowish brown in color.

Phytochemical analysis of *P. incarnata* leaf extract

The phytochemical analysis of the leaf extract of *P. incarnata* showed the presence of flavonoids, alkaloids, phenol, protein, carbohydrate, terpenoids, reducing sugar, polyphenol, and saponin. In contrast, tannin was not detected; these results are shown in Table 1. These phytochemicals make *P. incarnata* suitable for green synthesis of SeNPs, as they act as reducing agents.

Green synthesis of SeNPs using *P. incarnata* and *C. fistula* leaf extract by decoction method and its characterization

SeNPs were synthesized using *P. incarnata* and *C. fistula* leaf extract by decoction method. The change of color from yellowish brown to reddish brown indicated the formation of SeNPs as shown in Figure 3a and Figure 4a.

UV-vis spectrophotometry analysis was performed where the spectrum was scanned from 200-800 nm and the absorbance peak was obtained at 532 nm and 500 nm for *P. incarnata* and *C. fistula* leaf extract respectively, confirming the formation of SeNPs and the bell shape graph indicates the completion of the reaction (Figure 3b and 4b).

Zeta potential analysis of SeNPs synthesized using *C. fistula* leaf extract

The zeta potential of SeNPs synthesized using *C. fistula* was found to be -1.4mV, as measured by the HORIBA SZ-100 analyzer. This low zeta potential suggests limited electrostatic stability of the colloidal suspension. The electrophoretic mobility corresponding to the zeta potential in Figure 5 was -0.000011cm^2/Vs.

Dynamic light Scattering (DLS) analysis of SeNPs synthesized using *C. fistula* leaf extract:

Dynamic light scattering (DLS) analysis of SeNPs synthesized using *C. fistula* showed a Z- average particle size of 674.1 nm with a polydispersity index (PDI) of 0.658 (Figure 6). The mean particle size was 648.1 nm, indicating a relatively broad size distribution. The SeNPs were found to be monodisperse under standard dispersion conditions. This size range of the obtained SeNPs is larger, indicating that each NPs may consist of multiple crystalline domains or that there is a significant organic coating of metabolites from extract around the inorganic selenium core.

Assessment of Antimicrobial activity of SeNPs by Agar well diffusion method

The antimicrobial efficacy of SeNPs synthesized at various time intervals (15 min, 30 min, 1 hr, and 1.5 hrs) was evaluated against *E. coli, S. aureus, Bacillus sp, Candida sp.* across all tested

organisms, *E. coli* exhibited a larger zone of inhibition with SeNPs synthesized with PVA as a stabilizer. In contrast, the other microorganisms exhibited larger inhibition zones with SeNPs synthesized without PVA.

SeNPs against *Escherichia coli*

The antimicrobial activity of SeNPs synthesized with and without polyvinyl alcohol (PVA) was evaluated against *E. coli*. SeNPs synthesized with PVA exhibited a larger and consistent zone of inhibition (15 mm) at 15 min, 30 min, and 1 hr, indicating enhanced antibacterial activity, in contrast, SeNPs synthesized without PVA showed a slightly lower but constant zone of inhibition (12 mm) across all time intervals as shown in Figure 7. The presence of PVA likely improved NPs dispersion and bioavailability, contributing to increased efficacy.

SeNPs against *Staphylococcus aureus*

The antimicrobial activity of SeNPs synthesized with and without polyvinyl alcohol (PVA) was evaluated against *S. aureus*, where SeNPs synthesized with PVA demonstrated greater inhibition zones measuring 15 mm at 15 min and 1 hr, and 14 mm at 30 min, reflecting improved antibacterial performance, in contrast, SeNPs synthesized without PVA produced comparatively smaller inhibition zones of 12 mm at 15 min, 30 min, and 1.5 hrs, with a slight increase to 13 mm at 1 hr, as shown in Figure 8.

SeNPs against *Bacillus sp*

The inhibitory potential of SeNPs prepared with and without polyvinyl alcohol (PVA) was tested against *Bacillus sp*., where SeNPs synthesized with PVA showed smaller inhibition zones of 12 mm at both 15 min and 30 min and 10 mm at 1 hr, suggesting reduced antibacterial efficiency. In contrast, SeNPs synthesized without PVA exhibited significantly larger inhibition zones of 20 mm at 15 min, 30 min, and 1.5 hrs, with the highest value of 22 mm observed at 1 hr, as evident from Figure 9.

SeNPs against *Candida*

The antifungal activity of SeNPs synthesized with and without polyvinyl alcohol (PVA) was assessed against *Candida sp*., where SeNPs synthesized with PVA showed inhibition zones of 10 mm at 15 min, and 12 mm at both 30 min and 1 hr, indicating moderate activity, in contrast, SeNPs synthesized without PVA exhibited stronger antifungal effects with zones of 15 mm at 15 min and 1 hr, 12 mm at 30 min, and the highest value of 17 mm at 1.5 hrs, as demonstrated in Figure 10.

EN 13697:2024-01 Assay: The European Standard EN 13697:2024-01 assay outlines a quantitative non-porous surface test for assessing the fungicidal and/or bactericidal efficacy of chemical disinfectants. The assay demonstrated that the SeNPs derived from *P. incarnata* and *C. fistula* inhibited the growth of *Staphylococcus*, showing a reduction in CFU / ml compared to the control, which was untreated bacterial suspension as shown in Figure 11 (a) and (b), Table 2. Thus, these SeNPs can be considered as a promising candidate for chemical disinfectants.

Anti-diabetic Activity of SeNPs synthesized from *C. fistula* and *P. incarnate*

The DNSA method assessed the α-amylase inhibitory activity of biosynthesized SeNPs. The goal was to determine whether SeNPs might prevent starch from being broken down by enzymes, which would suggest that they could help regulate postprandial hyperglycemia.

The anti-diabetic assay of SeNPs synthesized from *C. fistula* and *P. incarnata* showed a notable inhibitory effect on the amylase enzyme activity, with both SeNPs treatments exhibiting activity comparable to the positive control (acarbose). Different levels of color intensity are displayed by the tubes following their individual absorbance at 540 nm. *C. fistula* exhibited greater α-amylase inhibition potency (77.91%) than *P. incarnata*- mediated SeNPs

Antioxidant Activity of SeNPs Synthesized from *C. fistula*

The antioxidant assay of SeNPs synthesized from *C. fistula* showed a notable enhancement in absorbance at 700 nm, indicating effective antioxidant activity. The SeNPs exhibited markedly higher antioxidant potential than the negative control, though their activity remained lower than that of the positive control. Furthermore, the SeNPs synthesized with polyvinyl alcohol (PVA) demonstrated improved antioxidant effects relative to those synthesized without PVA, suggesting that the presence of the stabilizing agent contributes to enhanced performance. The efficient but partial antioxidant response of the *Cassia*-mediated SeNPs is demonstrated in Table 4.

Effect of SeNPs on DNA

To investigate the effect of SeNPs on the bacterial DNA structure, the bacterial genomic DNA was incubated with SeNPs. The SeNP treated and untreated DNA samples showed no noticeable shearing in the DNA bands (Figure 12), indicating the absence of structural damage DNA. An intact DNA band with no visible signs of degradation confirmed the integrity of both treated and untreated DNA.

BSA protein was treated with SeNPs to check if the antimicrobial activity was due to interaction of SeNPs with proteins. The BSA sample treated and untreated with SeNPs were loaded on SDS-PAGE to observe any changes in the protein integrity. The band pattern of the treated sample was similar to the untreated, indicating that SeNPs did not affect the protein molecular weight. This suggested that the antimicrobial activity was not due to protein interaction.

In the DNA-NPs binding assay, a general decrease in optical density (OD) was expected to indicate binding since interaction with DNA could lead to complex formation or aggregation, reducing the number of freely suspended SeNPs and thereby lowering OD. The decrease in OD as observed in Table 5, suggested potential binding and partial aggregation with DNA. These observations suggested that SeNPs likely interacted with DNA.

Protein-SeNPs binding assay

In the protein–SeNPs binding assay, the optical density decreased from 1.584 at 50 µg/ml, to 1.22 at 400 µg/ml. As the BSA concentration increased, the OD decreased as seen in Table 6. This decrease indicated that BSA bound to SeNPs, leading to complex formation and aggregation, which reduced the number of free SeNPs in suspension.

Figure 1: a) *Passiflora incarnata* b) *Cassia fistula*

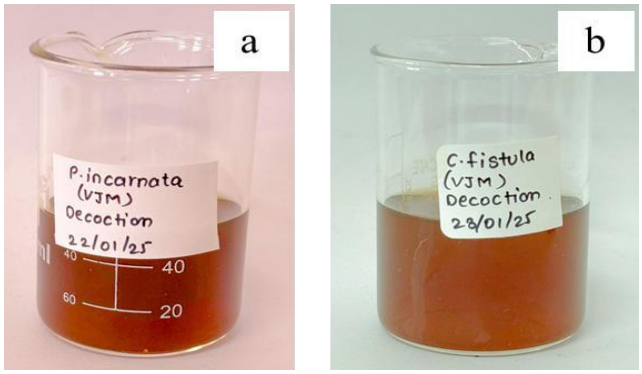

Figure 2: a) Leaf extract of *P. incarnata* b) Leaf extract of *C. fistula*

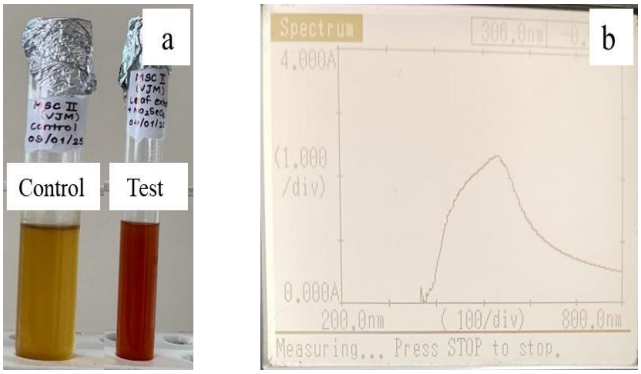

Figure 3: Confirmation and characterization of SeNPs synthesized using *P. incarnata* (decoction extract) a) Visual confirmation of SeNPs synthesis b) Absorbance peak of SeNPs

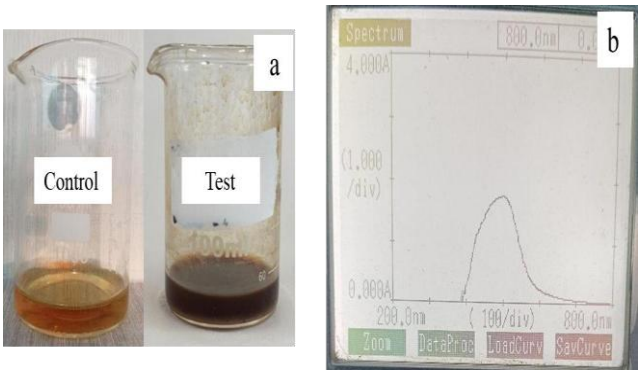

Figure 4: Confirmation and characterization of SeNPs using *C. fistula* (decoction extract). a) Visual confirmation of SeNPs synthesis b) Absorbance peak of SeNPs

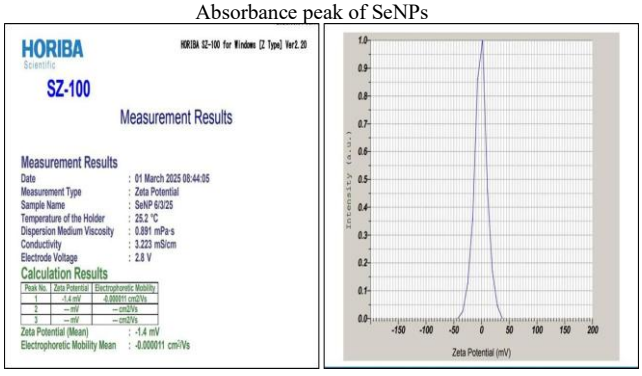

Figure 5: Zeta potential analysis of *C. fistula*

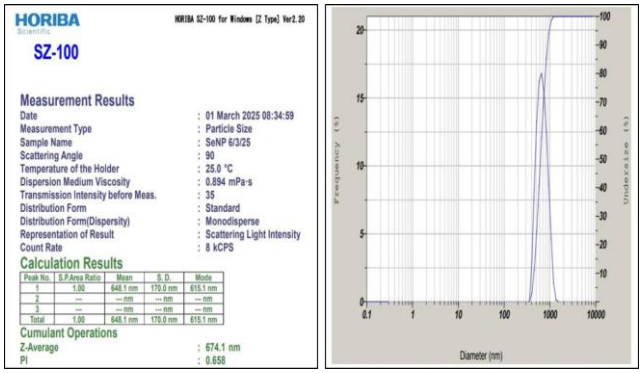

Figure 6: DLS analysis of *C. fistula*

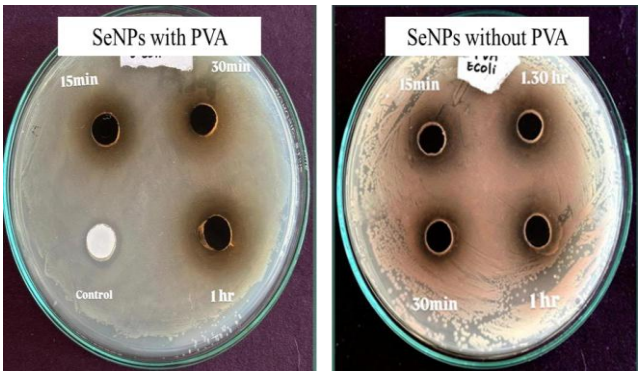

Figure 7: Antimicrobial activity of SeNPs synthesized at different time intervals (15 min, 30 min, 1 hr, 1.5 hrs) against *E. coli*

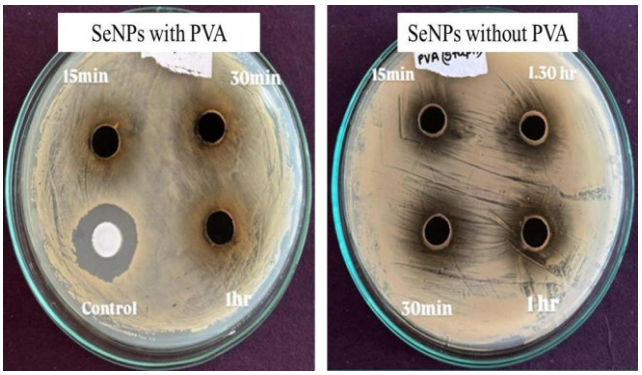

Figure 8: Antimicrobial activity of SeNPs synthesized at different time intervals (15 min, 30 min, 1 hr, 1.5 hrs) against *S. aureus*

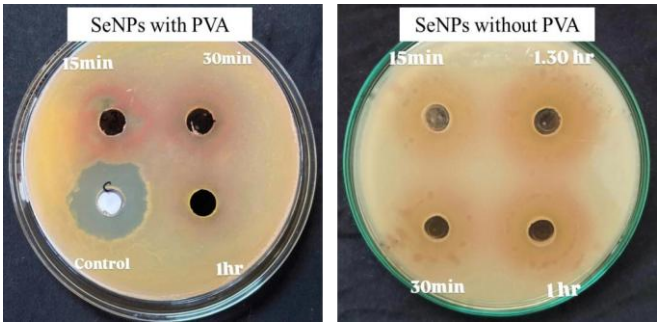

Figure 9: Antimicrobial activity of SeNPs synthesized at different time intervals (15 min, 30 min, 1 hr, 1.5 hrs) against *Bacillus sp.*

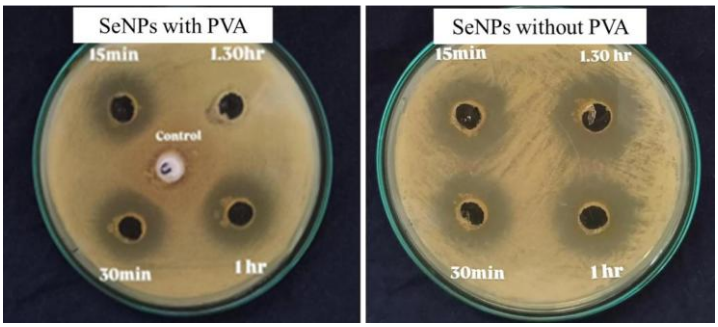

Figure 10: Antimicrobial activity of SeNPs synthesized at different time intervals (15 min, 30 min, 1 hr, 1.5 hrs) against *Candida* sp

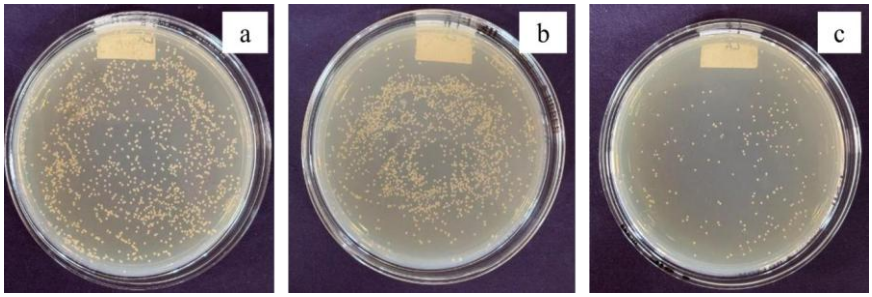

Figure 11: EN 13697:2024-01 assay; (a) Untreated *Staphylococcus* Cell, (b) *P. incarnata* SeNPs treated *Staphylococcus*, (c) *C. fistula* SeNPs treated *Staphylococcus*

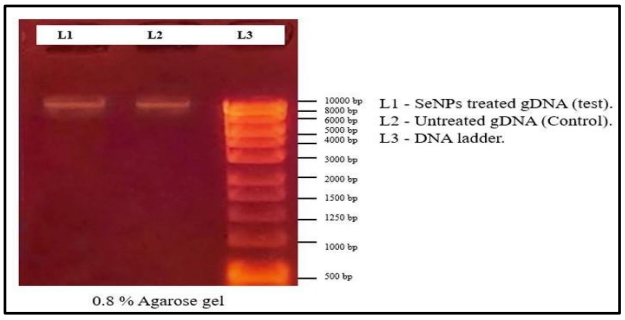

Figure 12: Effect of SeNPs on bacterial DNA

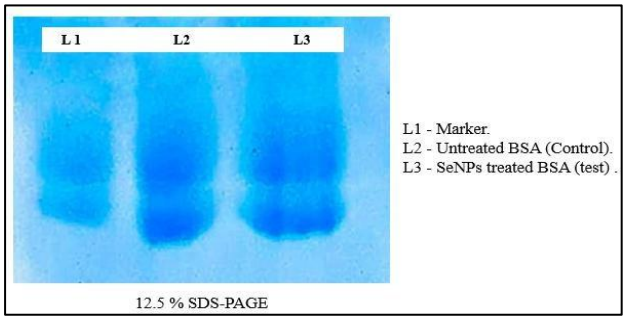

Figure 13: Effect of SeNPs on proteins

Table 1: Phytochemical tests of *P. incarnata* leaf extract

Phytochemical test	Observation	Result
Flavonoid test	Yellow to colorless	Positive
Alkaloid test	Formation of reddish-brown precipitate	Positive
Phenol test	A transient red color	Positive
Protein test	Formation of yellow color	Positive
Carbohydrate test	Formation of blue-black color	Positive
Terpenoids test	Formation of reddish-brown coloration at the junction	Positive
Tannin test	Transient green to black color	Negative
Reducing sugar	Brown to red color	Positive
Polyphenol test	Formation of transient greenish to black color	Positive
Saponin	Formation of stable foam	Positive

Table 2: EN 13697:2024-01 assay with and without SeNPs

EN 13697:2024-01 assay	Number of colonies (CFU/ml)
Control (without SeNPs)	992
P. incarnata SeNPs	827
C. fistula SeNPs	100

Table 3: Anti-diabetic assay of *P. incarnata* and *C. fistula*

Assay of components	OD (540 nm)	% Inhibition
AC+	0.600±0.294	-
AC-	0.170±0.315	-
AB	0.172±0.663	-
Negative Control	0.372±0.604	53.49 %
Positive control (Acarbose)	0.223±0.217	88.37 %
C. fistula SeNPs	0.268±0.599	77.91 %
P. incarnata SeNPs	0.271±0.732	77.0 %

Table 4: Antioxidant assay of *C. fistula*

Antioxidant of *C. fistula*	OD (700 nm)
Negative control	0.255±0.323
Positive control (Ascorbic acid)	2.858±0.214
SeNPs with PVA	0.851±0.371
SeNPs without PVA	0.561±0.389

Table 5: DNA-SeNPs binding assay

Concentration µg/ml	Optical Density
44.5	1.418±0.113
89	1.403±0.188
178	1.384±0.171
267	1.365±0.216
356	1.367±0.152

Table 6: Protein-SeNPs binding assay

Concentration µg/ml	Optical Density
50	1.584±0.308
100	1.508±0.298
200	1.400±0.272
300	1.292±0.319
400	1.223±0.379

DISCUSSION

Green synthesis of selenium nanoparticles (SeNPs) using plant extracts has emerged as a sustainable alternative to chemical and physical methods. In the present study, *C. fistula* and *P. incarnata* extracts were used to synthesize SeNPs, leveraging their phytochemical constituents such as flavonoids, alkaloids, phenols, and reducing sugars. Unlike chemical synthesis, this approach is environmentally friendly and biocompatible [12].

UV-Vis spectra showed peaks at 532 nm for *P. incarnata* and 500 nm for *C. fistula*, aligning with results from SeNPs synthesized using Citrus paradisi (Grapefruit) fresh juice extract which showed the absorbance peaks at 350 nm and 500 nm while SeNPs synthesized using Citrus paradisi (Grapefruit) fresh peels extract displayed absorbance at 345 nm and 550 nm [17].

SeNPs stabilized with PVA showed strong antibacterial activity, particularly against *E. coli*, similar to results from *Cassia javanica*-derived SeNPs [18]. Interestingly, uncoated SeNPs were more effective against *Bacillus* sp., potentially due to microbial reduction of SeNPs into elemental selenium, a phenomenon previously documented by a study [19]. Antifungal activity against *Candida* sp. was also significant and matched reported sensitivities.

FRAP assay results revealed higher antioxidant activity for PVA-coated SeNPs compared to uncoated. These values are comparable to garlic-mediated SeNPs reported by a group [20], emphasizing the stabilizing role of PVA in enhancing redox potential.

SeNPs showed strong α-amylase inhibition of 77.91% for *C. fistula* and 77.0% for *P. incarnata*, close to the standard drug acarbose (88.37%). These results surpassed *Moringa oleifera*-based SeNPs of 61.24% [21] and were comparable to *Mimosa pudica*-based SeNPs of 80% [22], highlighting their therapeutic potential.

No DNA fragmentation was observed, indicating non-genotoxic behavior. UV-Vis and gel electrophoresis results confirmed that SeNPs bind to DNA without causing structural damage, similar to findings by a research group [10]. SDS-PAGE showed no change in BSA protein bands post SeNP treatment, consistent with a study [14] conducted using ZnO nanoparticles (ZnONPs) and supporting safe protein interaction without denaturation.

SeNP–BSA interactions led to changes in optical density, indicating complex formation and aggregation at higher concentrations. Similar patterns were observed by research groups [10, 23] who also investigated SeNPs synthesized by employing vitamin C and yeast extract respectively, demonstrating consistent protein-nanoparticle interaction across green and chemical synthesis routes

CONCLUSION

This study validates the effectiveness of *Cassia fistula* and *Passiflora incarnata* in facilitating the green synthesis of selenium nanoparticles (SeNPs). The UV-Visible spectrophotometric analysis of the SeNPs revealed absorption peaks at 532 nm and 500 nm for SeNPs synthesized using *P. incarnata* and *C. fistula*, respectively, which are within the typical absorbance range for SeNPs and confirmed effective nanoparticle formation. SeNPs synthesized from *Cassia fistula* exhibited a larger hydrodynamic diameter of 674.1 nm, indicating considerable particle aggregation and a broad size distribution. The low zeta potential of SeNPs synthesized from *Cassia fistula* (-1.4 mV) suggested poor colloidal stability, leading to increased aggregation. SeNPs synthesized from *C. fistula* demonstrated promising antimicrobial effects, particularly against *Escherichia coli* and *Staphylococcus aureus*, with enhanced activity observed when SeNPs were stabilized with polyvinyl alcohol (PVA). The antioxidant capacity of SeNPs revealed that SeNPs synthesized with PVA exhibited significant reducing power. Furthermore, the anti-diabetic potential of these SeNPs can provide an alternative to synthetic medications. The genotoxicity of the SeNPs evaluated through DNA fragmentation assays, revealed no DNA degradation in treated samples. Similarly, SeNPs did not induce protein denaturation was demonstrated by SDS-PAGE. Finally, the SeNP–DNA interaction studies revealed that SeNPs could bind to DNA, exhibiting a hypochromic effect without causing significant structural damage.

These results align well with previous reports on plant-based SeNP synthesis and further support the potential of these biologically derived nanoparticles as a multifunctional agent for biomedical use. The green synthesis approach using plant extracts, such as *C. fistula* and *P. incarnata*, provides an eco-friendly and cost-effective route for the development of SeNP-based nanomedicines.

Acknowledgment

Authors acknowledge the Management of Institute for supporting and providing the opportunity to carry out this research work.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

ORCID ID

Suneeta Panicker: https://orcid.org/0000-0002-3228-7718

REFERENCES

1. Rayman MP, Winther KH, Pastor-Barriuso R, Cold F, Thvilum M, Stranges S, et al. Effect of long-term selenium supplementation on mortality: results from a multiple-dose, randomised controlled trial. Free Radical Biology and Medicine. 2018; 127:46-54.
2. Mosallam FM, El-Sayyad GS, Fathy RM, El-Batal AI. Biomolecules-mediated synthesis of selenium nanoparticles using *Aspergillus oryzae* fermented Lupin extract and gamma radiation for hindering the growth of some multidrug-resistant bacteria and pathogenic fungi. Microbial Pathogenesis. 2018;122:108-116.
3. Altammar KA. A review on nanoparticles: characteristics, synthesis, applications, and challenges. Frontiers in Microbiology. 2023;14:1155622.
4. Pyrzynska K, Sentkowska, A. Biosynthesis of selenium nanoparticles using plant extracts. Journal of Nanostructure in Chemistry. 2022;12(4):467-480.
5. Lade BD, Patil AS. Silver nano fabrication using leaf disc of *Passiflora foetida* Linn. Applied Nanoscience. 2017;7(8):501-512.
6. Bharath Kumar N. *Cassia fistula*: A Comprehensive Analysis of its Phytochemical Constituents and Pharmacological Activities. Journal of Emerging Technologies and Innovative Research. 2024;11(6):j688-j693.
7. Alagesan V, Venugopal S. Green Synthesis of selenium nanoparticles Using Leaves Extract of Withania somnifera and Its Biological Applications and Photocatalytic Activities. BioNanoScience. 2019;9(1):105-116.
8. Patil, SV, Shaikh, AZ, Jain, AS, Patil, DR, Shaikh, SR, Pawar, SP. A short review on some medicinal flowers. Research Journal of Pharmacognosy and Phytochemistry. 2024;16(4):254-257.
9. Gunti L, Dass RS, Kalagatur NK. Phytofabrication of Selenium Nanoparticles From *Emblica officinalis* Fruit Extract and Exploring Its Biopotential Applications:

Antioxidant, Antimicrobial, and Biocompatibility. Front. Microbiol. 2019;10:931.
10. Shahabadi N, Zendehcheshm S, Khademi F. SeNPs: synthesis, in-vitro cytotoxicity, antioxidant activity and interaction studies with ct-DNA and HSA, HHb and cyt c serum proteins. Biotechnology Reports. 2021;30:118.
11. Jia Z, Li J, Gao L, Yang D, Kanaev A. Dynamic light scattering: A powerful tool for in situ nanoparticle sizing. Colloids and Interfaces. 2023;7(1):15.
12. Panicker S, Anjum S, Anjum S, Nair A. Antimicrobial nanocomposite of Se, Ag, Cu nanoparticles for enhanced hygiene and comfort. Indian Journal of Pharmacy and Pharmacology. 2024;11(2):96-102.
13. Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Biomedical potential of silver nanoparticles synthesized from calli cells of Citrullus colocynthis (L.) Schrad. Journal of Nanobiotechnology. 2011;9:43.
14. Jung EB, Yu J, Choi SJ. Interaction between ZnO nanoparticles and albumin and its effect on cytotoxicity, cellular uptake, intestinal transport, toxicokinetics, and acute oral toxicity. Nanomaterials. 2021;11(11):2922.
15. Panicker S, Hamdule A. Analysis of medicinal properties of *Vigna aconitifolia* (Matki). Asian Journal of Pharmaceutical and Clinical Research. 2021;14(5):78-81.
16. Wickramaratne MN, Punchihewa JC, Wickramaratne DB. In-vitro alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. BMC Complementary and Alternative Medicine. 2016;16(1):466.
17. Alvi GB, Iqbal MS, Ghaith MMS, Haseeb A, Ahmed B, Qadir MI. Biogenic selenium nanoparticles from citrus fruit have anti-bacterial activities. Scientific Reports. 2021;11(1):4811.
18. Soliman MKY, Amin MAA, Nowwar AI, Hendy MH, Salem SS. Green synthesis of SeNPs from *Cassia javanica* flowers extract and their medical and agricultural applications. Scientific Reports. 2024;14(1):26775.
19. Javed S, Sarwar A, Tassawar M, Faisal M. Conversion of selenite to elemental selenium by indigenous bacteria isolated from polluted areas. Chemical Speciation and Bioavailability. 2015;27(4):162-168.
20. Vyas J, Rana S. Antioxidant activity and green synthesis of selenium nanoparticles using *Allium sativum* extract. International Journal of Phytomedicine. 2017;*9*(4):634-641.
21. Ahamad Tarmizi AA, Nik Ramli NN, Adam SH, Abdul Mutalib M, Mokhtar MH, Tang SGH. Phytofabrication of selenium nanoparticles with *Moringa oleifera* (MO-SeNPs) and exploring its antioxidant and anti-diabetic potential. Molecules. 2023;28(14): 5322.
22. Dhave Prasath VM, Sundar RA, Shanmugam R, Thangavelu L, Muthukumaran D. Anti-diabetic activity and cytotoxic effect of selenium nanoparticles mediated through *Mimosa pudica* leaves extract. Nanotechnology Perceptions. 2023;19(1):1-9.
23. Borowska M, Jiménez-Lamana J, Bierla K, Jankowski K, Szpunar J. A green and fast microwave-assisted synthesis of selenium nanoparticles and their characterization under gastrointestinal conditions using mass spectrometry. Food Chemistry. 2023;417:135864.

HOW TO CITE THIS ARTICLE
Panicker S, Gone V, Parmar M, Nivalkar J. Medicinal properties of selenium nanoparticles synthesized using leaf extracts of *Passiflora incarnata* and *Cassia fistula* and mechanistic exploration of antimicrobial effect. J Phytopharmacol 2025; 14(6):493-501. doi: 10.31254/phyto.2025.14610