

Phytochemical-Assisted Fabrication of Selenium Nanoparticles: An Eco-Biogenic Approach to Antioxidant Therapy

Hemalatha Punati¹, Sri Venu Madhav Tippabhotla², and Sudhakar Poda³

^{1,2} *Ph.D. student, Department of Biotechnology, Acharya Nagarjuna University, Guntur – 522 510, Andhra Pradesh*

³ *Associate professor, Department of Biotechnology, Acharya Nagarjuna University, Guntur – 522 510, Andhra Pradesh*

Abstract—Selenium nanoparticles (SeNPs) were synthesized using an eco-friendly, phytochemical-assisted approach with *Phyllanthus acidus* fruit extract (PFA). The formation of PFA-SeNPs was confirmed by the appearance of a brick-red color and a characteristic surface plasmon resonance peak at 290 nm in the UV-visible spectrum. Scanning electron microscopy revealed that the PFA-SeNPs were irregular in shape, with sizes ranging from 50 to 150 nm, and tended to form loose clusters. The zeta potential of -25.09 mV indicated moderate colloidal stability due to the capping effect of anionic biomolecules from the plant extract. PFA-SeNPs exhibited significant antioxidant activity in a dose-dependent manner, as demonstrated by their ability to scavenge DPPH and ABTS radicals. The nanoparticles also showed promising broad-spectrum antibacterial efficacy against both Gram-positive and Gram-negative bacteria, with inhibition zones increasing with higher concentrations. The enhanced bioactivity of PFA-SeNPs is attributed to the synergistic effect of elemental selenium and the phytochemicals adsorbed on the nanoparticle surface. These findings highlight the potential of PFA-SeNPs as a green and multifunctional nanomaterial for antioxidant and antibacterial applications, with implications for biomedical and therapeutic fields.

Index Terms—Selenium nanoparticles, Eco-friendly, Antioxidant activity, Antibacterial activity.

I. INTRODUCTION

Green synthesis of selenium nanoparticles (SeNPs) has emerged as a compelling approach to obtain biocompatible and antioxidant-active agents, while aligning with principles of green chemistry. The literature emphasizes that plant- and microorganism-mediated reduction of selenite/selenate to elemental

selenium enables production of SeNPs under mild conditions. These methods often yield particles with favorable stability, bioactivity, and reduced cytotoxicity compared to conventional chemical routes (1).

Green synthesis employing plant extracts or microbial systems avoids hazardous reducing agents and high-energy conditions, yielding biocompatible SeNPs with tunable size and morphology. This consensus is reflected in multiple studies that demonstrate successful SeNPs production using diverse biological reducing agents under ambient or mild conditions. The methodological diversity (garlic, green tea, *Vitis vinifera*, *Ocimum gratissimum*, Aloe vera, citrus extracts, and bacteria) underscores the broad scope of feasible green routes. These approaches are positioned within green chemistry paradigms due to non-toxic reagents, ambient processing, and potential cost-effectiveness (2).

Plant extracts provide reducing and capping/stabilizing phytochemicals (polyphenols, flavonoids, sugars, proteins) that mediate the reduction of selenite to elemental selenium, often yielding stable dispersions with favorable antioxidant profiles. A consistent outcome across green SeNPs studies is their capacity to scavenge free radicals and to enhance cellular antioxidant defenses. These features underpin strong antioxidant activity and capacity to mitigate oxidative stress in diverse biological contexts, including cellular, reproductive, microbial, and therapeutic settings. The evidence supports that green SeNPs offer a versatile and sustainable platform for antioxidant delivery and

oxidative stress modulation with broad biomedical and industrial relevance (3).

II. MATERIALS AND METHODS

A. Chemicals and reagents

Sodium selenite, ethanol, DPPH, potassium persulfate, ABTS, acetonitrile, ascorbic acid, and tetracycline, and ultrapure distilled water were obtained from Sigma-Aldrich (Bengaluru, India). All other chemicals used in this study were of analytical grade and obtained from HiMedia (Mumbai, India). The glassware and plasticware used in this study were purchased from Borosil and Tarsons Products (Mumbai, India), respectively.

B. Green synthesis and characterization of PFA-SeNPs

Five grams of *P. acidus* fruit extract was boiled in 100 mL water at 75 °C for forty-five minutes. The filtered extract mixed with 1 mM sodium selenite solution turned reddish-brown after twelve hours, indicating PFA-SeNPs synthesis (4). The suspension was centrifuged at 15000 rpm, rinsed with water, and dried at 45 °C to obtain powdered PFA-SeNPs. UV-vis spectroscopy monitored sodium selenite conversion to PFA-SeNPs, using water as reference. PFA-SeNPs spectra were obtained at 200–800 nm scanning rate (Bartosiak et al., 2019). ATR-FTIR spectra were recorded using a Fourier spectrometer with Zn-Se ATR cell and DTGS Detector. Spectra were collected at 16 scans, 4 cm⁻¹ resolution. The PFA-SeNPs' zeta potential was measured using Zetasizer Nano S (Zetara Instruments, UK) following Qiu et al. (2018).

C. Antioxidant efficacy

To assess the antioxidant potential of PFA-SeNPs, we used the method by Dumore & Mukhopadhyay, with modifications (5). A 100 µL reaction mixture was prepared with different quantities of PFA-SeNPs and 50 µL of 0.1 mM DPPH in methanol. The mixtures were incubated for 30 min at 37°C with shaking at 150 rpm. Absorbance was measured at 519 nm using a microplate reader. A mixture without PFA-SeNPs served as negative control, while ascorbic acid (25-150 µg/mL) with DPPH was used as reference. Results were expressed as EC₅₀, representing PFA-SeNPs concentration needed to reduce DPPH radical absorbance by 50%.

PFA-SeNPs' ability to neutralize ABTS free radicals was assessed following Dumore & Mukhopadhyay, with modifications (5). The ABTS working solution was prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate equally and kept dark for 12–16 h. For testing, 135 µL ABTS working solution was mixed with 15 µL PFA-SeNPs (up to 150 µg/mL) in a microplate and stored dark for 30 min. Absorbance was measured at 750 nm using Synergy H1. The negative control used 135 µL ABTS solution with 15 µL ethanol, while reference control used ascorbic acid (25–150 µg/mL) with ABTS solution. Results were expressed as EC₅₀ values, showing PFA-SeNPs concentration needed to reduce ABTS radicals' absorbance by 50%.

D. Statistical analysis

Experiments were conducted in six replicates (n = 6), with results shown as means ± SD. Statistical analysis used one-way ANOVA and Student's t-test, performed using GraphPad Prism v10 (USA).

IV. RESULTS AND DISCUSSION

A. Synthesis and Characterization

Selenium nanoparticles (SeNPs) were successfully synthesized using the extract from *P. acidus* fruit. When a sodium selenite (Na₂SeO₃) solution was added to the fruit extract in water, the initially pale yellow mixture gradually turned brick red after a few hours of incubation (Fig. 1). This distinct brick-red color is a recognized visual sign of the formation of elemental SeNPs, resulting from the reduction of selenite ions (Se⁴⁺) to selenium (Se⁰) by the bioactive compounds in the fruit extract. The observed color change is attributed to the surface plasmon resonance (SPR) phenomena of nanosized selenium particles, which occur when bioactive molecules in the extract—such as phenolics, flavonoids, tannins, and other reducing agents—reduce selenium ions and stabilize the resulting nanoparticles. Supporting our findings, Shahbaz et al. observed a similar color transition from light green to brick red in their reaction mixture, confirming the formation of SeNPs and highlighting the importance of the concentration of active components in the plant extract for this synthesis (6). Other studies have noted similar transitions during the biosynthesis process, which aligns with these color changes (7).

The extract from *P. acidus*, which is abundant in phenolic and flavonoid compounds, likely served as both a reducing and stabilizing agent, aiding in the transformation and capping of the SeNPs. The continuous nucleation and growth of SeNPs is indicated by the deepening brick-red hue over time. The absence of precipitation or phase separation suggests that the colloidal suspension remained stable. Although the brick-red hue initially suggests the formation of SeNPs, additional characterization studies are crucial to definitively verify the synthesis, morphology, and stability of these nanoparticles.

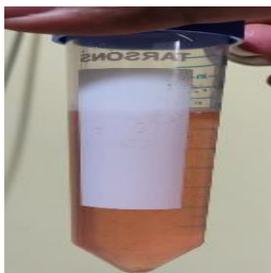


Figure 1: Formation of *P. acidus* fruit extract-mediated selenium nanoparticles (PFA-SeNPs) indicated by the appearance of a brick-red color after incubation of *P. acidus* fruit extract with sodium selenite.

The initial verification of *P. acidus*-mediated selenium nanoparticles (PFA-SeNPs) formation was achieved using UV-visible spectrophotometry, a common method for observing nanoparticle synthesis by examining surface plasmon resonance (SPR) characteristics. As depicted in Figure 2, the UV-Vis absorption spectrum of the produced SeNPs exhibited a distinct peak at approximately 290 nm, indicative of the SPR band typical of elemental-Se nanoparticles. This peak signifies the successful conversion of selenite ions (Se^{4+}) to selenium (Se^0) nanoparticles, facilitated by the phytochemicals in the *P. acidus* fruit extract. The presence of this peak, coupled with the lack of significant absorbance beyond 320 nm, indicates that the nanoparticles were well-dispersed and free from large aggregates, suggesting effective stabilization by plant biomolecules. The relatively sharp and symmetrical peak suggests a narrow size distribution of SeNPs (8).

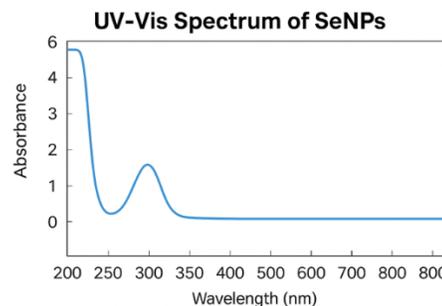


Figure 2: UV-visible absorption spectra of PFA-SeNPs

The surface morphology and structural characteristics of the PFA-SeNPs synthesized through plant mediation were analyzed using scanning electron microscopy (SEM). The micrograph indicates that the SeNPs primarily exhibit irregular shapes and tend to cluster together, forming loosely packed groups of nanoscale particles. A closer examination revealed rough and granular surface textures, suggesting the presence of biomolecular capping layers originating from phytochemicals in the plant extract. The observed aggregation may be attributed to hydrogen bonding and van der Waals forces between the biomolecules surrounding the nanoparticles.

When magnified 20,000 times, the particles were estimated to measure between 50 and 150 nm, although they often clustered into larger groups. This structure aligns with previously documented biosynthesized SeNPs, where plant metabolites serve as reducing and stabilizing agents, managing the nucleation and growth of selenium nanocrystals in the process. The observed morphology and distribution validated the successful creation of nanoscale selenium particles through an environmentally friendly method using plant extract components.

In the realm of plant-assisted synthesis of SeNPs, numerous studies have emphasized the value of SEM analysis for verifying the successful synthesis and characterizing the produced nanoparticles. For instance, Gharbavi et al. employed SEM to verify the spherical shape of SeNPs created using *Vaccinium arctostaphylos* fruit extract, demonstrating a relatively consistent nanoparticle size distribution (9). This emphasizes the role of plant extracts in reducing metal ions and stabilizing and shaping the resulting nanoparticles. This size range is particularly beneficial

because it can affect the bioavailability and effectiveness of nanoparticles against bacterial strains.

The zeta potentials of the PFA-SeNPs synthesized using the plant extract were measured to assess their surface charge and colloidal stability. The zeta potential was approximately -25.09 mV, suggesting that the nanoparticles had a moderately negative surface charge. This negative charge results from the adsorption of anionic biomolecules, including phenolic compounds, flavonoids, and proteins, present in the plant extract used for their synthesis. These biomolecules provide functional groups, such as hydroxyl, carbonyl, and carboxyl groups, which stabilize the nanoparticle surface via electrostatic repulsion and steric hindrance, thus preventing aggregation.

A zeta potential exceeding ± 20 mV typically indicates moderate stability, whereas values exceeding ± 30 mV suggest that the colloids are highly stable. Therefore, the observed value implies that the synthesized SeNPs possessed excellent colloidal stability in a water-based suspension. The negatively charged capping agents from the plant extract not only improve particle dispersion but also affect the biological interactions and the biocompatibility of the nanoparticles (10).

B. Antioxidant activity

The antioxidant properties of SeNPs synthesized using *P. acidus* fruit extract (PFA-SeNPs) were assessed using DPPH and ABTS radical-scavenging assays. Figure 3 illustrates that the PFA-SeNPs demonstrated a dose-dependent enhancement in radical-scavenging activity, highlighting their significant antioxidant potential. At the lowest concentration tested (25 $\mu\text{g/mL}$), PFA-SeNPs scavenged approximately 10–12% of DPPH and ABTS radicals. As the concentration increased, the scavenging capacity also increased, achieving over 75–85% inhibition at 250 $\mu\text{g/mL}$. This evident concentration-dependent reduction in the percentage of free radicals underscores the effective radical neutralization of PFA-SeNPs. Statistical analysis indicated significant differences ($p < 0.05$) in antioxidant activity among the treatments, with mean values marked by different letters denoting statistically distinct groups. Both assays exhibited similar patterns, although the DPPH values were slightly higher than the ABTS values at

the same concentrations, suggesting a robust hydrogen-donating and electron-transfer capability of the PFA-SeNPs.

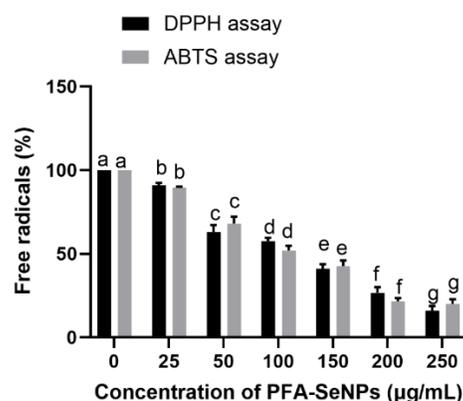


Figure 3: The free radical scavenging activity of PFA-SeNPs was determined using DPPH and ABTS assays. Values are represented as mean \pm standard deviation ($n = 6$). Bars labelled with different letters (a–g) are significantly different at $p < 0.05$ according to one-way ANOVA followed by Tukey's post hoc test.

Extensive research on the antioxidant activity of SeNPs synthesized using plant extracts supports our report. SeNPs synthesized using the leaf extract of *Morinda citrifolia* exhibited significant antioxidant properties, with free radical inhibition ranging from 66.7% to 83.7% (11). These nanoparticles also showed promise for antibacterial and antiproliferative applications, indicating their diverse bioactivities.

PFA-SeNPs demonstrated antioxidant capabilities, indicating that the biogenic synthesis process not only facilitated the reduction of selenium ions but also integrated bioactive phytochemicals from the extract onto the nanoparticle surface. These phytoconstituents, primarily phenolics, flavonoids, and tannins, likely served as capping agents, stabilizing the SeNPs and enhancing their redox properties synergistically. The robust antioxidant activity of the PFA-SeNPs can be attributed to the synergistic interaction between elemental selenium and phytochemicals attached to the nanoparticle surface. Selenium, an essential trace element, naturally participates in redox reactions through its role in enzymes such as glutathione peroxidase, while polyphenolic compounds from *P. acidus* provide additional electron-donating and radical-scavenging capabilities.

The strong antioxidant properties of PFA-SeNPs underscore their potential for use in the biomedical and therapeutic fields. The overproduction of reactive oxygen species (ROS) is linked to oxidative stress, which can lead to cellular damage, inflammation, aging, and the onset of chronic illnesses such as cancer, diabetes, and cardiovascular diseases. By effectively neutralizing free radicals, PFA-SeNPs may help reduce oxidative damage and restore redox balance. Additionally, the green synthesis of PFA-SeNPs suggests that they are likely biocompatible, environmentally friendly, and less toxic than nanoparticles produced using chemical methods. Their phytochemical coating may improve cellular absorption and bioavailability while reducing the adverse effects associated with inorganic selenium compounds.

CONCLUSION

Selenium nanoparticles (PFA-SeNPs) were synthesized using an eco-friendly approach with *Phyllanthus acidus* fruit extract. The formation of PFA-SeNPs was confirmed by UV-visible spectroscopy, scanning electron microscopy, and zeta potential measurements. PFA-SeNPs exhibited significant dose-dependent antioxidant activity, as demonstrated by their ability to scavenge DPPH and ABTS radicals.

ACKNOWLEDGMENT

The authors are thankful to Acharya Nagarjuna University for their support and encouragement.

REFERENCES

- [1] Hussain, A., Lakhan, M. N., Hanan, A., Soomro, I. A., Ahmed, M., Bibi, F., & Zehra, I. (2023). Recent progress on green synthesis of selenium nanoparticles—a review. *Materials Today Sustainability*, 23, 100420.
- [2] Alagesan, V., & Venugopal, S. (2019). Green synthesis of selenium nanoparticle using leaves extract of *Withania somnifera* and its biological applications and photocatalytic activities. *Bionanoscience*, 9(1), 105-116.
- [3] Abbasi, A. J., Anas, M., Elahi, M., Khan, A., Khattak, W. A., Saleem, M. H., ... & Quraishi, U. M. (2025). Restoring wheat productivity and nutrient balance under cadmium stress through reducing toxicity, metal uptake, and oxidative damage using selenium nanoparticles. *Journal of Trace Elements in Medicine and Biology*, 89, 127644.
- [4] Ali, B. M. H., & Almashhedy, L. A. (2023, April). Green Synthesis Optimization and Characterization of Selenium Nanoparticle Using Aqueous Extract of Peel *Solanum Melongena* L. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1158, No. 10, p. 102007). IOP Publishing.
- [5] Dumore, N. S., & Mukhopadhyay, M. (2020). Antioxidant properties of aqueous selenium nanoparticles (ASeNPs) and its catalysts activity for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reduction. *Journal of Molecular Structure*, 1205, 127637.
- [6] Shahbaz, M., Akram, A., Raja, N. I., Mukhtar, T., Mehak, A., Fatima, N., ... & Abasi, F. (2023). Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease. *PLoS One*, 18(2), e0274679.
- [7] Alvi, G. B., Iqbal, M. S., Ghaith, M. M. S., Haseeb, A., Ahmed, B., & Qadir, M. I. (2021). Biogenic selenium nanoparticles (SeNPs) from citrus fruit have anti-bacterial activities. *Scientific Reports*, 11(1), 4811.
- [8] Pradhan, S., Palai, S., Dash, J. R., Patra, R., Sahoo, P. R., Behera, P. C., & Parija, S. C. (2023). Characterisation of selenium nanoparticles and *Phyllanthus niruri* Hook. f. selenium nanoparticles with histopathological investigation of their effects on cadmium-induced gastric toxicity in wistar rats. *Ann. Phytomed*, 12(1), 367-375.
- [9] Gharbavi, M., Mousavi, M., Pour-Karim, M., Tavakolizadeh, M., & Sharafi, A. (2022). Biogenic and facile synthesis of selenium nanoparticles using *Vaccinium arctostaphylos* L. fruit extract and anticancer activity against in vitro model of breast cancer. *Cell Biology International*, 46(10), 1612-1624.
- [10] Bhiri, N., Masquelez, N., Nasri, M., Nasri, R., Hajji, M., & Li, S. (2025). Synthesis, Characterization, and Stability Study of Selenium Nanoparticles Coated with Purified

Polysaccharides from *Ononis natrix*.
Nanomaterials, 15(6), 435.

- [11] Nagalingam, M., Rajeshkumar, S., Balu, S. K., Tharani, M., & Arunachalam, K. (2022). Anticancer and antioxidant activity of *Morinda citrifolia* leaf mediated selenium nanoparticles. *Journal of Nanomaterials*, 2022(1), 2155772.