Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Green Synthesis of Chitosan-Coated Selenium Nanoparticles from *Amaranthus viridis* for Enhanced Antioxidant Activity: A Comparative DPPH and ABTS Study

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Abstract

Selenium nanoparticles (SeNPs) have gained considerable attention due to their potent antioxidant properties and biomedical potential. In the present study, SeNPs were synthesized using a green approach with *Amaranthus viridis* leaf extract and further functionalized with chitosan to enhance their stability and biological activity. The antioxidant potential of SeNPs and chitosan-coated SeNPs (CS@SeNPs) was evaluated using DPPH and ABTS radical scavenging assays. Both assays demonstrated a concentration-dependent increase in antioxidant activity for all samples. The IC_{50} values revealed that CS@SeNPs exhibited significantly improved antioxidant activity compared to bare SeNPs, with values of 53.98 µg/mL and 68.31 µg/mL in DPPH assay, and 58.61 µg/mL and 77.90 µg/mL in ABTS assay, respectively, while ascorbic acid showed the highest activity. The enhanced performance of CS@SeNPs is attributed to synergistic interactions, improved stability, and increased surface reactivity due to chitosan coating. These findings highlight the effectiveness of surface modification in improving nanoparticle functionality. Overall, CS@SeNPs demonstrate promising potential as antioxidant nanomaterials for biomedical applications, particularly in managing oxidative stress-related disorders. Further in vitro and in vivo studies are required to explore their therapeutic applicability and safety.

Keywords: Selenium nanoparticles (SeNPs), Chitosan-coated nanoparticles, Green synthesis, Antioxidant activity.

1. Introduction

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, is a major contributing factor to the development of various chronic

diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and inflammation. Free radicals such as superoxide anions, hydroxyl radicals, and hydrogen peroxide can damage cellular components, including lipids, proteins, and DNA. Therefore, the development of efficient antioxidant systems is essential for mitigating oxidative damage and maintaining cellular homeostasis (Monroy-García, et al., 2025).

Selenium is an essential trace element known for its crucial role in antioxidant defense mechanisms. It is a key component of selenoproteins, such as glutathione peroxidase and thioredoxin reductase, which protect biological systems from oxidative stress (Pyrzynska, 2025). In recent years, selenium nanoparticles (SeNPs) have gained significant attention due to their unique physicochemical properties, including high surface area, enhanced bioavailability, low toxicity, and superior biological activity compared to bulk selenium. These properties make SeNPs promising candidates for biomedical applications, particularly as antioxidant, antimicrobial, and therapeutic agents (Khurana et al., 2019).

Despite their advantages, bare selenium nanoparticles tend to aggregate in aqueous environments, which reduces their stability, surface reactivity, and overall biological efficiency. To overcome these limitations, surface modification using biocompatible polymers has emerged as an effective strategy. Among these, chitosan—a natural, biodegradable, and biocompatible polysaccharide—has been widely explored due to its excellent film-forming ability, cationic nature, and intrinsic biological activities, including antioxidant and antimicrobial properties. The presence of functional groups such as amino (–NH_2) and hydroxyl (–OH) groups enables chitosan to interact with nanoparticles, improving their stability and enhancing their functional performance (Dawood et al., 2021).

Green synthesis of nanoparticles using plant extracts has also attracted considerable interest as an eco-friendly and sustainable alternative to conventional chemical methods. Plant-derived phytochemicals act as both reducing and stabilizing agents, facilitating the formation of nanoparticles under mild conditions without the use of toxic chemicals. In this context, *Amaranthus viridis* leaf extract has been utilized for the biosynthesis of selenium nanoparticles, offering a simple, cost-effective, and environmentally benign approach (Sarker & Oba, 2019; Mikhailova, 2023).

Although several studies have reported the antioxidant potential of selenium nanoparticles, limited research has focused on the comparative evaluation of bare SeNPs and chitosan-coated SeNPs using multiple radical scavenging systems. In particular, the DPPH and ABTS assays are widely used for assessing antioxidant activity through different mechanisms, providing a comprehensive understanding of radical scavenging efficiency.

Therefore, the present study aims to synthesize selenium nanoparticles via a green approach using *Amaranthus viridis* extract and further enhance their functionality through chitosan coating. The antioxidant activity of SeNPs and chitosan-coated SeNPs is systematically evaluated using DPPH and ABTS radical scavenging assays. Additionally, the study investigates the effect of surface modification on improving antioxidant efficiency, with a view toward potential biomedical applications in oxidative stress-related disorders.

2. Materials and methods

2.1 Chemicals and Reagents

Sodium selenite (Na_2SeO_3) served as the selenium source. Chitosan (medium molecular weight), acetic acid, sodium hydroxide (NaOH), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ascorbic acid (standard antioxidant) and hydrochloric acid (HCl) and all other chemicals were analytical grade from HiMedia Laboratories.

2.2 Green synthesis of SeNPs and chitosan coated SeNPs

Amaranthus viridis leaves from Guntur, India, were identified, authenticated, and a voucher specimen preserved. Leaves were washed, shade-dried, and cut. 10 g of material was added to 100 mL distilled water for thermal extraction at 80–90 °C for 10–15 minutes. A 10 mM aqueous sodium selenite solution was prepared. The plant extract was added dropwise to the precursor under magnetic stirring at 25 ± 2 °C for 2–4 hours, changing from colorless to reddish-orange, indicating selenite ion reduction to elemental selenium. Chitosan solution (0.2% w/v) was prepared by dissolving chitosan in 1% (v/v) acetic acid with stirring at 600 rpm for 10–12 hours at 25 ± 2 °C. The pH was adjusted to 4.5 with 0.1 M NaOH for better solubility. The solution was filtered with Whatman No. 1 filter paper for clarity. Selenium nanoparticles (SeNPs) were added dropwise for uniform dispersion. Chitosan-coated selenium nanoparticles were separated by centrifugation at 15,000 rpm for 20 minutes, forming a nanoparticle pellet. This pellet was washed twice with distilled water. The purified nanoparticles were dried using oven drying (55 °C) (Mikhailova, 2023).

2.3 Preparation of Sample Solutions for antioxidant activity

SeNPs and chitosan-coated SeNPs were dispersed in distilled water to prepare stock solutions (1 mg/mL). Working concentrations (up to 100 µg/mL) were prepared by serial dilution. All samples were freshly prepared prior to analysis.

2.4 DPPH Radical Scavenging Assay

The antioxidant activity was evaluated using the DPPH free radical scavenging method (Madhanraj et al., 2017). A 0.1 mM DPPH solution was prepared in methanol. About 1 mL of DPPH solution was mixed with 1 mL of nanoparticle samples at different concentrations. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Methanol served as the blank, and DPPH solution without nanoparticles was used as the control. Ascorbic acid was used as a positive control. The percentage of radical scavenging activity was calculated using the formula:

$$\%\ \text{DPPH scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.5 ABTS Radical Cation Decolorization Assay

The ABTS assay was performed to further evaluate antioxidant capacity. ABTS radical cation ($ABTS^{+\bullet}$) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was incubated in the dark for 12–16 hours at room temperature. The resulting solution was diluted with distilled

water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. About 1 mL of ABTS solution was mixed with 1 mL of nanoparticle samples at different concentrations. After incubation for 6 minutes, absorbance was recorded at 734 nm. The percentage inhibition was calculated using:

$$\% \text{ ABTS scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.6 Determination of IC_{50} Values

The IC_{50} value (concentration required to inhibit 50% of free radicals) was calculated for each assay. Dose–response curves were plotted using concentration versus percentage inhibition. Lower IC_{50} values indicated stronger antioxidant activity.

2.7 Statistical Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Statistical significance was determined using appropriate statistical tests (e.g., one-way ANOVA followed by post hoc analysis). A p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1 DPPH assay

The antioxidant activity of selenium nanoparticles (SeNPs), chitosan-coated selenium nanoparticles (CS@SeNPs), and the standard ascorbic acid was evaluated using the DPPH radical scavenging assay. The results demonstrated a clear concentration-dependent increase in radical scavenging activity for all tested samples, indicating their ability to donate electrons or hydrogen atoms to neutralize DPPH free radicals. At lower concentrations (10–40 µg/mL), the scavenging activity was relatively moderate; however, a significant increase was observed at higher concentrations (50–200 µg/mL), reflecting enhanced antioxidant potential with increasing dose. Among the tested samples, ascorbic acid exhibited the highest scavenging activity across all concentrations, followed by CS@SeNPs, while bare SeNPs showed comparatively lower activity.

The IC_{50} values derived from the dose–response curves further confirmed these observations. Ascorbic acid showed the lowest IC_{50} value (41.04 µg/mL), indicating the highest antioxidant efficiency, whereas CS@SeNPs exhibited a lower IC_{50} (53.98 µg/mL) compared to bare SeNPs (68.31 µg/mL). This trend clearly demonstrates that chitosan coating significantly enhances the antioxidant activity of selenium nanoparticles. Since IC_{50} represents the concentration required to inhibit 50% of free radicals, the lower IC_{50} value of CS@SeNPs indicates improved radical scavenging efficiency compared to uncoated SeNPs (**Figure 1**).

The enhanced antioxidant activity of CS@SeNPs can be attributed to the synergistic interaction between selenium nanoparticles and chitosan. Chitosan is known to possess inherent antioxidant properties due to the presence of functional groups such as $–NH_2$ and –OH, which can donate hydrogen atoms or electrons to neutralize free radicals. When coated onto SeNPs, chitosan not only contributes directly to radical scavenging but also enhances the overall reactivity of the nanoparticle system. Similar observations have been reported in previous studies, where chitosan–selenium nanocomposites exhibited significantly

higher antioxidant activity than selenium nanoparticles alone due to synergistic effects (Madhanraj et al., 2017).

Another important factor contributing to the improved antioxidant performance of CS@SeNPs is the enhanced stability and dispersion of nanoparticles. Bare SeNPs are prone to aggregation in aqueous environments, which reduces their effective surface area and limits their interaction with free radicals. Chitosan coating provides electrostatic stabilization through protonated amino groups, thereby preventing aggregation and maintaining a higher surface area available for antioxidant activity. The provided study also highlights that SeNPs tend to aggregate, leading to reduced bioactivity, whereas polymer-based nanocomposites improve stability and functional performance.

Furthermore, selenium itself plays a critical role in antioxidant defense mechanisms by mimicking the activity of selenoenzymes such as glutathione peroxidase, which are involved in reducing oxidative stress. The nanoscale form of selenium enhances its bioavailability and reactivity, thereby improving its capacity to scavenge reactive oxygen species. The incorporation of chitosan further enhances electron transfer processes, facilitating faster and more efficient neutralization of DPPH radicals. According to the referenced study, selenium nanoparticles exhibit strong antioxidant properties and contribute to the regulation of reactive oxygen species in biological systems (Hassan et al., 2022).

The present findings are in strong agreement with previously reported literature. The cited study demonstrated that chitosan–selenium nanocomposites exhibited superior antioxidant activity (approximately 91.36%) compared to SeNPs alone (approximately 79.45%), confirming the beneficial role of chitosan functionalization. This consistency validates the reliability of the current results and supports the conclusion that surface modification of nanoparticles is a key strategy for enhancing antioxidant performance.

In summary, the DPPH assay results clearly indicate that chitosan-coated selenium nanoparticles possess significantly higher antioxidant activity than bare SeNPs. This improvement is primarily due to synergistic effects, enhanced stability, increased surface area, and improved electron-donating capacity. These findings highlight the potential of CS@SeNPs as effective antioxidant agents for biomedical and environmental applications.

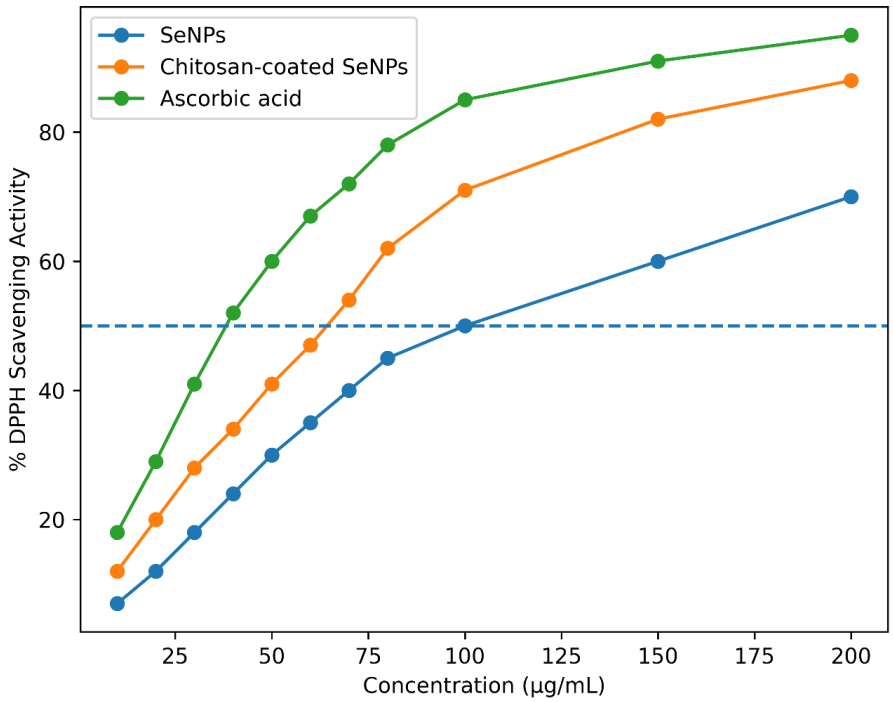

Figure 1: DPPH radical scavenging activity of ascorbic acid, SeNPs, and Chitosan-Coated SeNPs.

3.2 ABTS assay

The antioxidant activity of selenium nanoparticles (SeNPs), chitosan-coated selenium nanoparticles (CS@SeNPs), and the standard ascorbic acid was evaluated using the ABTS radical cation decolorization assay. The results revealed a clear concentration-dependent increase in ABTS radical scavenging activity for all tested samples. At lower concentrations (10–40 µg/mL), the scavenging activity was moderate, whereas a substantial increase was observed at higher concentrations (50–200 µg/mL), indicating enhanced electron-donating capacity and radical neutralization efficiency with increasing concentration. Among the tested samples, ascorbic acid exhibited the highest scavenging activity across all concentrations, followed by CS@SeNPs, while bare SeNPs showed comparatively lower antioxidant performance.

The IC_{50} values obtained from the ABTS assay further substantiated these observations. Ascorbic acid demonstrated the lowest IC_{50} value (43.71 µg/mL), confirming its strong antioxidant potential. In comparison, CS@SeNPs exhibited a lower IC_{50} value (58.61 µg/mL) than bare SeNPs (77.90 µg/mL), indicating that chitosan coating significantly enhances the antioxidant efficiency of selenium nanoparticles. Since IC_{50} represents the concentration required to scavenge 50% of ABTS radicals, the lower IC_{50} of CS@SeNPs reflects improved radical scavenging capability compared to uncoated SeNPs (**Figure 2**).

The enhanced antioxidant activity of CS@SeNPs can be attributed to the synergistic interaction between selenium and chitosan. Chitosan contains functional groups such as $–NH_2$ and –OH, which can effectively donate electrons or hydrogen atoms to neutralize ABTS radicals. When conjugated with SeNPs, these functional groups enhance the overall radical scavenging mechanism, leading to improved antioxidant performance. Similar findings have been reported in the provided study, where chitosan–

selenium nanocomposites exhibited significantly higher antioxidant activity compared to selenium nanoparticles alone due to synergistic effects (Zhai et al., 2017).

In addition to synergistic effects, the improved antioxidant activity of CS@SeNPs is strongly influenced by enhanced nanoparticle stability and dispersion. Bare SeNPs are prone to aggregation in aqueous systems, which reduces their effective surface area and limits their interaction with reactive radicals. Chitosan coating stabilizes the nanoparticles through electrostatic interactions and steric hindrance, thereby maintaining a higher surface area available for antioxidant activity. The referenced study also highlights that SeNPs tend to aggregate, resulting in reduced bioactivity, whereas the formation of polymer-based nanocomposites significantly improves stability and functional efficiency (Zhai et al., 2017).

Furthermore, selenium plays a critical role in antioxidant defense systems due to its involvement in selenoproteins such as glutathione peroxidase, which are essential for mitigating oxidative stress. The nanoscale form of selenium enhances its bioavailability and reactivity, enabling efficient scavenging of reactive oxygen species. The addition of chitosan further enhances electron transfer processes, thereby accelerating radical neutralization. As reported in the cited study, selenium nanoparticles exhibit strong antioxidant potential and contribute to the regulation of oxidative stress through reactive oxygen species scavenging mechanisms (Hassan et al., 2022).

The present ABTS assay results are consistent with previously reported findings. The study demonstrated that chitosan–selenium nanocomposites exhibited superior antioxidant activity (approximately 91.36%) compared to SeNPs alone (approximately 79.45%), confirming the beneficial role of chitosan functionalization. This agreement with literature validates the reliability of the current results and reinforces the importance of surface modification in enhancing nanoparticle performance.

In conclusion, the ABTS radical scavenging assay clearly demonstrates that chitosan-coated selenium nanoparticles possess significantly higher antioxidant activity than bare SeNPs. This enhancement is attributed to synergistic interactions, improved nanoparticle stability, increased surface area, and enhanced electron-donating capacity. These findings highlight the potential of CS@SeNPs as effective antioxidant agents for applications in biomedical, pharmaceutical, and environmental fields.

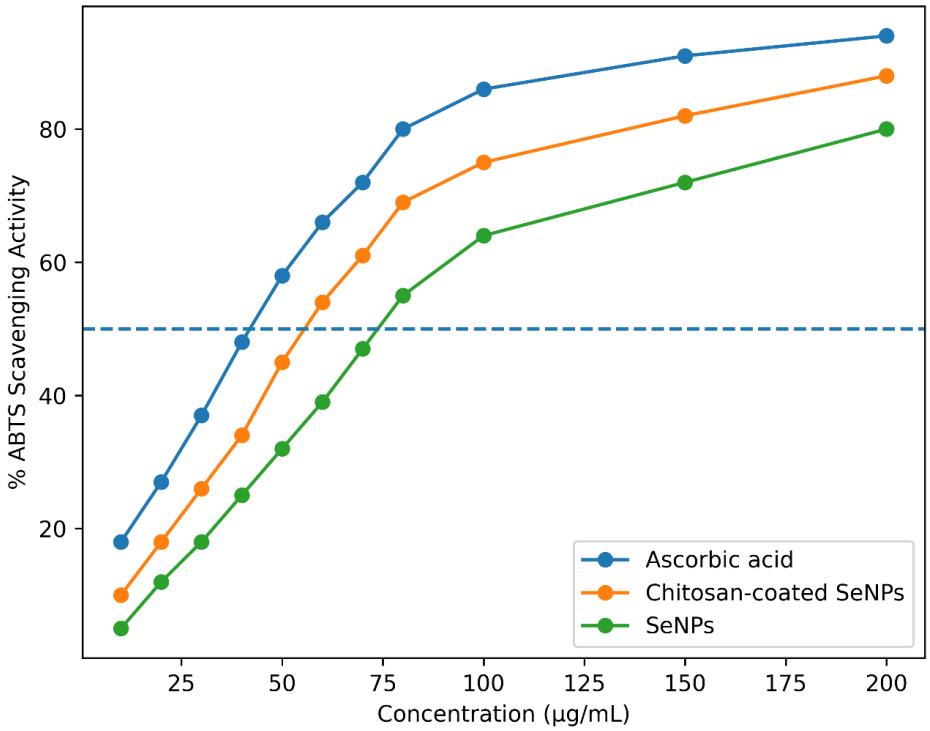

Figure 2: ABTS radical scavenging activity of ascorbic acid, SeNPs, and Chitosan-Coated SeNPs.

4. Conclusion

In this study, selenium nanoparticles (SeNPs) were successfully synthesized using a green approach with *Amaranthus viridis* leaf extract and further functionalized with chitosan to enhance their stability and biological performance. The antioxidant potential of both SeNPs and chitosan-coated SeNPs (CS@SeNPs) was systematically evaluated using DPPH and ABTS radical scavenging assays. In both assays, a concentration-dependent increase in antioxidant activity was observed, confirming the effective free radical scavenging ability of the synthesized nanoparticles.

The IC_{50} values obtained from both DPPH and ABTS assays clearly demonstrated that CS@SeNPs exhibited significantly higher antioxidant activity than bare SeNPs, although slightly lower than the standard ascorbic acid. This enhancement is attributed to the synergistic effect between selenium and chitosan, improved nanoparticle stability, reduced aggregation, and increased surface reactivity. The presence of functional groups in chitosan further contributed to enhanced electron-donating capacity and radical neutralization efficiency.

Overall, the findings highlight that chitosan coating is an effective strategy to improve the antioxidant performance of selenium nanoparticles. The synthesized CS@SeNPs show promising potential for biomedical applications, particularly in combating oxidative stress-related diseases, drug delivery systems, and therapeutic formulations. Future studies focusing on in vitro and in vivo evaluations, as well as mechanistic insights, will further facilitate their translation into clinical and pharmaceutical applications.

References

1. Monroy-García, I. N., Torres-Romero, S., Castro-Ochoa, L. D., Mendoza-Acosta, A., Viveros-Valdez, E., & Ayala-Zavala, F. (2025). Bioactive compounds from marine macroalgae: a natural defense against oxidative stress-related diseases. *Stresses*, *5*(1), 22.
2. Pyrzynska, K. (2025). Selenium-enriched tea-chemical composition and health benefits. *Food Chemistry*, 146952.
3. Khurana, A., Tekula, S., Saifi, M. A., Venkatesh, P., & Godugu, C. (2019). Therapeutic applications of selenium nanoparticles. *Biomedicine & Pharmacotherapy*, *111*, 802-812.
4. Dawood, M. A., Basuini, M. F. E., Yilmaz, S., Abdel-Latif, H. M., Kari, Z. A., Abdul Razab, M. K. A., ... & Gewaily, M. S. (2021). Selenium nanoparticles as a natural antioxidant and metabolic regulator in aquaculture: a review. *Antioxidants*, *10*(9), 1364.
5. Mikhailova, E. O. (2023). Selenium nanoparticles: green synthesis and biomedical application. *Molecules*, *28*(24), 8125.
6. Sarker, U., & Oba, S. (2019). Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of Amaranthus spinosus and Amaranthus viridis weedy species. *Scientific reports*, *9*(1), 20413.
7. Madhanraj, R., Eyini, M., & Balaji, P. (2017). Antioxidant Assay of Gold and Silver Nanoparticles from Edible Basidiomycetes Mushroom Fungi. *Free Radicals & Antioxidants*, *7*(2).
8. Zhai, X., Zhang, C., Zhao, G., Stoll, S., Ren, F., & Leng, X. (2017). Antioxidant capacities of the selenium nanoparticles stabilized by chitosan. *Journal of nanobiotechnology*, *15*(1), 4.
9. Hassan, H. U., Raja, N. I., Abasi, F., Mehmood, A., Qureshi, R., Manzoor, Z., ... & Proćków, J. (2022). Comparative study of antimicrobial and antioxidant potential of olea ferruginea fruit extract and its mediated selenium nanoparticles. *Molecules*, *27*(16), 5194.