

Exploring the Therapeutic Potential: Green Synthesis of Selenium Nanoparticles Using Vaccinium Subg. Oxycoccus for Antioxidant, Anti- Inflammatory, and Cytotoxic Effect

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This study elucidates the synthesis and multifaceted applications of selenium nanoparticles (SeNPs) utilizing *Vaccinium subg. Oxycoccus* extract as a green and sustainable methodology. The UV-Visible spectroscopy analysis demonstrated the successful synthesis of SeNPs, characterized by a distinct absorption peak at 380 nm. The antioxidant activities of *Vaccinium subg. Oxycoccus*-mediated SeNPs were systematically evaluated through three assays. The DPPH assay revealed concentration-dependent radical scavenging activities, surpassing the standard (ascorbic acid) at higher concentrations. Additionally, the Hydrogen Peroxide assay showcased commendable antioxidant properties, while the FRAP assay indicated a concentration-dependent capacity to reduce ferric ions, suggesting potential in counteracting oxidative stress. Inhibition of protein denaturation was examined using the BSA assay, revealing a significant inhibitory effect of *Vaccinium subg. Oxycoccus*-mediated SeNPs that increased with concentration. Denaturation studies, employing the Egg Albumin Denaturation Assay, displayed a concentration-dependent rise in denaturation percentages, either matching or exceeding the standard's anti-inflammatory activity. The Membrane Stabilization Assay illustrated the concentration-dependent enhancement of membrane stability by SeNPs, exhibiting efficacy comparable to or exceeding the standard. Cytotoxicity assessment through the Brine Shrimp lethality assay demonstrated a concentration-dependent decline in brine shrimp nauplii viability, suggesting a potential cytotoxic impact of *Vaccinium subg. Oxycoccus*-mediated SeNPs. These findings collectively underscore the diverse applications of SeNPs synthesized with *Vaccinium subg. Oxycoccus* extract, ranging from antioxidant activities and membrane stabilization to potential cytotoxic effects, laying the groundwork for their versatile application in biological and medicinal contexts.

Keywords: Anti-inflammatory agent, Antioxidant agent, Biocompatibility, Green synthesis, Selenium nanoparticles.

1. Introduction

Nanotechnology plays a crucial role in biomedical applications by offering various benefits. Nanoparticles have the potential to be used in diagnostic instruments, targeted medicinal products, pharmaceuticals, biomedical implants, and tissue engineering (Haleem et al., 2023). They enable the administration of high toxicity treatments with improved safety, such as chemotherapeutic cancer drugs (Pasika et al., 2023). Nanotechnology also allows for the development of wearable gadgets that can detect changes in vital signs, cancer cell conditions, and infections in the body (Rezić 2022). By manipulating nanoparticles at the nanoscale, their characteristics can be modified to achieve desired properties for targeted drug delivery, imaging, therapy, and sensors (Chen et al., 2022). Nanomaterials have shown promise in improving the efficiency of medical treatment, particularly in drug delivery. They have applications in cancer treatment, coronary artery disease treatment, biomarker detection, and nano antibacterial agents (Tharani et al., 2023).

Selenium nanoparticles have garnered significant attention in the realm of nanotechnology and biomedical research due to their unique properties and potential applications. Selenium nanoparticles exhibit notable capabilities in activating the Nrf2-Keap1 signaling pathway, boosting antioxidative selenoproteins expression, and inhibiting mast cell activation, thereby demonstrating effective antiallergic activity (Xie et al., 2023). Furthermore, they display anti-inflammatory effects by suppressing the NF- κ B and cyclooxygenase-2 pathways, diminishing inflammatory factor expression, and elevating anti-inflammatory markers (Devi et al., 2023 & Kurup et al., 2024). These nanoparticles also hinder reactive oxygen species production and enhance glutathione peroxidase activity, reinforcing their antioxidant properties. Due to their biocompatibility and low toxicity, selenium nanoparticles are well-suited for various biomedical applications, including the potential treatment of chronic joint diseases like osteoarthritis (Li et al., 2023 & Arafa et al., 2023).

Green synthesis has emerged as a crucial focus in nanotechnology research, driven by the prospect of developing sustainable and environmentally friendly nanomaterials (Santhoshkumar et al., 2017). Unlike traditional synthesis methods that often involve hazardous chemicals and energy-intensive processes, green synthesis aims to utilize eco-friendly and renewable resources, minimizing environmental impact throughout the production of nanomaterials. One notable aspect of green synthesis is the use of plant extracts, microorganisms, or other natural sources as reducing agents and stabilizing agents for the synthesis of nanoparticles (Menon et al., 2020). These methods not only reduce the reliance on harmful chemicals but also offer the advantage of utilizing bioactive compounds present in natural sources. This approach aligns with the principles of green chemistry, emphasizing the reduction of waste, energy efficiency, and the use of benign solvents (Rajeshkumar et al., 2019).

Vaccinium subgenus Oxycoccus (Cranberry), a subset of the Vaccinium genus in the Ericaceae family, encompasses shrubs and trees known as cranberries or blueberries. Species like Vaccinium macrocarpon, Vaccinium oxycoccus, Vaccinium angustifolium, and Vaccinium

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myrtillus, native to cooler regions worldwide, produce small, vibrant fruits rich in antioxidants and vitamin C (Zhidkin et al., 2023). Beyond culinary use, these plants have medicinal applications, with leaves and bark used in traditional remedies. The chemical composition, featuring phenolic compounds, vitamins, minerals, dietary fiber, organic acids, carotenoids, and essential oils, contributes to their nutritional value. These elements vary among species and influence their suitability for diverse applications, including food, medicine, and landscaping (Waikar et al., 2023 & Nurzyńska-Wierdak 2023). The emphasis on green synthesis methods in nanotechnology reflects a growing awareness of environmental sustainability. By utilizing eco-friendly and renewable resources, such as plant extracts or microorganisms, green synthesis aims to minimize environmental impact and reduce reliance on hazardous chemicals and energy-intensive processes. This approach aligns with the principles of green chemistry, emphasizing waste reduction, energy efficiency, and the use of benign solvents (Nurzyńska-Wierdak 2023). Green-synthesized nanomaterials find applications in various fields, including medicine, agriculture, and environmental remediation. The sustainable and environmentally friendly nature of these nanomaterials contributes to the broader global efforts for a more sustainable and eco-conscious future (PRIYAA et al., 2020).

The current research work aimed to show the promising therapeutic prospects of selenium nanoparticles synthesized through a green approach, utilizing *Vaccinium* subg. *Oxycoccus* extract. The subsequent investigation spans antioxidant, anti-inflammatory, and cytotoxic effect through the Brine Shrimp Lethality Assay, shedding light on the potential multifaceted applications of these nanoparticles in the future biomedical research and applications.

2. Materials and methods

2.1. Collection and preparation of *Vaccinium* subg. *Oxycoccus* extract

Cranberry fruits were procured from a local supermarket near Poonamallee. Following that, the cranberries underwent a meticulous washing process under tap water, followed by an additional rinse with distilled water. Subsequently, the cleaned cranberries were allowed to air dry for one hour at room temperature. Once dried, 10 grams of the cranberries were precisely weighed and crushed using a mortar and pestle. The resultant pulpy extract was filtered through Whatman No:1 filter paper to obtain a refined solution. This filtered extract was then carefully stored in a refrigerator for subsequent stages of nanoparticle synthesis.

2.2. Green synthesis of selenium nanoparticles:

A solution comprising 20mM of sodium selenite dissolved in 60mL of distilled water was prepared, and subsequently, 40mL of filtered cranberry extract was added to this solution. The resulting reaction mixture was placed on a magnetic stirrer operating at 700 rpm and maintained for a duration ranging from 24 to 56 hours. Simultaneously, UV-Visible spectrophotometer readings were consistently recorded throughout this period to verify and confirm the synthesis process.

2.3. Characterization

The characterization of the green-synthesized selenium nanoparticles was conducted through

the utilization of a UV-Visible spectrophotometer. This analytical tool allows for a thorough examination of the nanoparticles' optical properties.

2.4. Antioxidant activity:

2.4.1. DPPH assay

The in vitro DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is commonly employed to assess the antioxidant properties of various compounds, including plant extracts. This method relies on the compound's capacity to neutralize DPPH radicals, stable dark-colored crystalline compounds. The reduction of DPPH radicals into DPPH-H, a colorless or light yellow compound, serves as an indicator of the antioxidant's ability to neutralize free radicals, evaluating its free radical scavenging activity (RSA).

To create the stock solution, 24 milligrams of DPPH were dissolved in 100 mL of methanol, resulting in a filtrated mixture with an absorbance of approximately 0.973 at 517 nm. Different concentrations of *Vacciniumsubg. Oxycoccus* extract mediated selenium nanoparticles (10 µg/mL- 50 µg/mL) were then combined with 3 mL of this DPPH solution, and the mixture was incubated in complete darkness for 30 minutes. Subsequently, absorbance was measured at 517 nm, and the percentage of antioxidant activity was calculated using the formula:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100,$$

where Ac represents the control reaction absorbance, and As is the testing specimen absorbance.

2.4.2. H₂O₂ assay

In the in vitro hydrogen peroxide radical scavenging assay using *Vacciniumsubg. Oxycoccus* extract mediated selenium nanoparticles (SeNPs) as the test substance, the following steps were followed. Hydrogen peroxide (H₂O₂) was prepared as a stock solution at a concentration of 3% (w/v). Horse radish peroxidase (HRP) was used as the peroxidase enzyme, with a stock solution prepared at a concentration of 1 mg/mL in phosphate buffer (pH 7.4). The substrate solution, containing 4-aminoantipyrine (4-APA) that changes color upon reduction, and phosphate buffer (pH 7.4) as the buffer solution were also prepared. Control solutions without the test substance were included for comparison.

For the test solution, *Vacciniumsubg. Oxycoccus* extract mediated SeNPs was dissolved in an appropriate solvent (Distilled water) to achieve the desired concentration (10 µg/mL- 50 µg/mL). The assay mixture, comprising hydrogen peroxide, HRP, substrate solution, and the test solution, was prepared in a 96-well plate. The uniform final volume in each well was ensured. The assay mixture was then incubated at 37°C for 30 minutes to facilitate the reduction of hydrogen peroxide by the enzyme and the scavenging of hydrogen peroxide by the *Vacciniumsubg. Oxycoccus* extract mediated SeNPs.

After incubation, the absorbance of each well was measured at 504 nm using a spectrophotometer, corresponding to the color change of the substrate solution. Data analysis involved comparing the absorbance values of the test solution wells with the control wells. The percentage of inhibition of the color change was calculated using the formula

[Percentage of Inhibition = (Absorbance of Control - Absorbance of Test Solution) / Absorbance of Control) \times 100].

The percentage of inhibition obtained serves as an indicator of the scavenging efficiency of *Vaccinium* subg. *Oxycoccus* extract mediated SeNPs. A higher percentage suggests superior antioxidant activity, signifying the ability of the test substance to effectively neutralize or reduce the activity of hydrogen peroxide (Sharma et al., 2012 & Bhatti et al., 2015).

2.4.3. FRAP assay

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely utilized technique for assessing the total antioxidant capacity of biological specimens. In this method, a FRAP reagent is prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃.6H₂O solution in a 10:1:1 ratio.

To conduct the FRAP assay, varying concentrations (10 μ g/mL- 50 μ g/mL) of green synthesized selenium nanoparticles and standards are dispensed into a 96-well plate in triplicate, approximately 20 μ L each. Subsequently, 200 μ L of the prepared FRAP reagent is added to each well, and the plate is incubated at 37°C for 30 minutes. The absorbance at 593 nm is then measured for each well using a microplate reader.

The FRAP value for each sample or standard is determined by comparing the absorbance to a standard curve generated using known concentrations of a standard antioxidant, such as trolox. The FRAP assay assesses the capacity of antioxidants in the samples to convert Fe³⁺ to Fe²⁺ within the FRAP reagent, resulting in a color change directly proportional to the antioxidant capacity of the sample. This method offers a quantitative measure of the antioxidant activity present in biological specimens, making it a valuable tool for evaluating the overall antioxidant potential of green synthesized selenium nanoparticles and other substances.

2.5. Anti-inflammatory activity

The green synthesized selenium nanoparticles was tested for its anti-inflammatory activity using three assays such as Bovine serum albumin denaturation assay, Egg albumin denaturation assay, Membrane stabilization assay

2.5.1. Bovine serum albumin denaturation assay

A solution containing 0.45 mL of bovine serum albumin was prepared by mixing it with 0.05 mL of *Vaccinium* subg. *Oxycoccus* extract mediated SeNPs, which were present in various concentrations ranging from 10 to 50 μ g/mL. Subsequently, the pH of the solution was adjusted to 6.3. The mixture was then incubated at room temperature for a duration of 10 minutes. Following this, it was subjected to a 30-minute incubation period in a water bath at 55°C. For comparison purposes, diclofenac sodium was utilized as the standard group, while dimethyl sulphoxide served as the control. Finally, the samples were analyzed spectrophotometrically at a wavelength of 660 nm.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2.5.2. Egg Albumin denaturation assay

For the egg albumin denaturation assay, a reaction mixture was prepared by mixing 0.2 mL of fresh egg albumin with 2.8 mL of phosphate buffer. To this mixture, *Vaccinium* subg. *Oxycoccus* extract mediated SeNPs, nanoparticles were added in varying concentrations, ranging from 10 to 50 µg/mL. The pH of the solution was then adjusted to 6.3. The mixture was subsequently incubated at room temperature for a period of 10 minutes. Following this, it was subjected to a 30-minute incubation period in a water bath at 55°C. For comparison, diclofenac sodium was employed as the standard group, whereas dimethyl sulphoxide was utilized as the control. Finally, the samples were analyzed spectrophotometrically at a wavelength of 660 nm.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5.3. Membrane stabilization assay

2.5.3.1. Membrane Stabilization Assay:

The in vitro membrane stabilization assay evaluated the membrane-stabilizing properties of the compounds. This assay assessed the potential of the synthesized SeNPs from *Vaccinium* subg. *Oxycoccus* (10-50 µg/mL) to prevent the disruption of cell membranes and the subsequent release of intracellular contents. The assay utilized tris-HCl buffer, human red blood cells (RBCs), phosphate-buffered saline (PBS), centrifuge tubes, and a UV-visible spectrophotometer.

2.5.3.2. Preparation of RBC Suspension:

Fresh human blood was collected in a sterile tube with anticoagulants. After centrifuging the blood at 1,000 g for 10 minutes at room temperature, the RBCs were separated. The RBCs were washed three times with PBS and resuspended in tris-HCl buffer to create a 10% (v/v) RBC suspension.

2.5.3.3. Assay Procedure:

1 mL of the RBC suspension was placed into each centrifuge tube, followed by the addition of different concentrations of SeNPs. The tubes were gently mixed and incubated at 37°C for 30 minutes. After centrifuging the tubes at 1,000 rpm for 10 minutes at room temperature, the absorbance of the supernatant was measured at 540 nm using a UV-visible spectrophotometer. The percentage inhibition of hemolysis was calculated as follows:

$$\% \text{ inhibition} = (\text{OD control} - \text{OD sample}) / \text{OD control} \times 100$$

Here, OD control is the absorbance of the RBC suspension without the test compound(s), and OD sample is the absorbance of the RBC suspension with the test compound.

2.6. Cytotoxic effect:

2.6.1. Brine shrimp lethality assay:

The procedure involved the dissolution of 2 grams of iodine-free salt in 200 mL of distilled water. *Nanotechnology Perceptions* Vol. 20 No. S8 (2024)

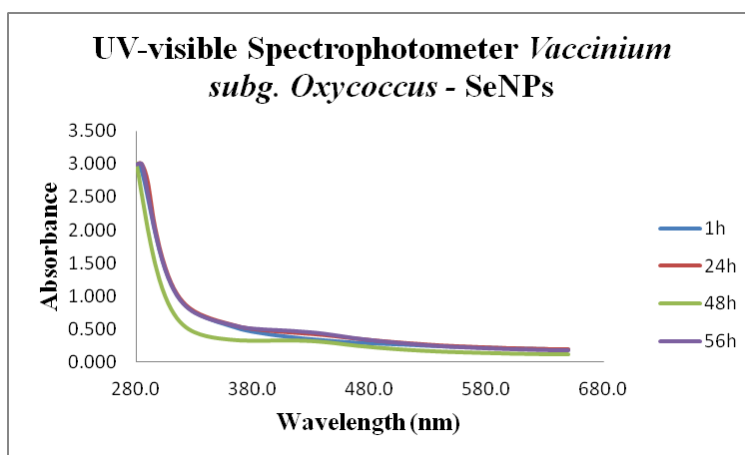
water. Following this, 6 well ELISA plates were employed, with each well being filled with approximately 10 to 12 mL of saline water. Subsequently, 10 nauplii were introduced into each well, with each well containing varying concentrations of the synthesized green SeNPs (5µg, 10µg, 20µg, 40µg, 80µg). The plates were then subjected to incubation for a period of 24 hours. Upon completion of the 24-hour incubation period, the ELISA plates were inspected, and the count of live nauplii was recorded. The calculation of the cytotoxic effect was performed using the formula:

$$\text{number of dead nauplii} / [\text{number of dead nauplii} + \text{number of live nauplii}] \times 100.$$

3. Results and Discussion

3.1. UV-Visible spectroscopy

Figure 1: UV-Visible spectra of green synthesized selenium nanoparticles

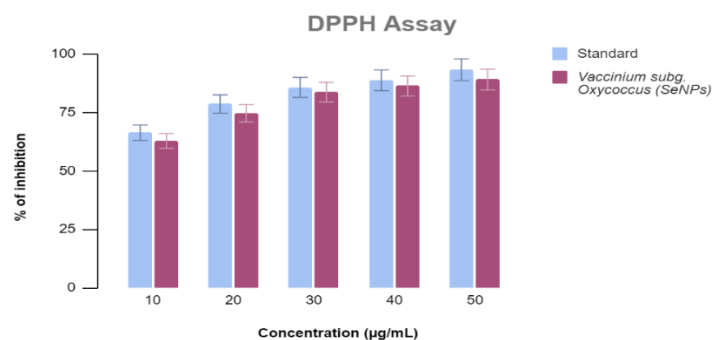


The UV-Visible spectroscopy analysis of selenium nanoparticles synthesized using *Vaccinium* subg. *Oxycoccus* extract revealed distinct spectral characteristics which was depicted in figure 1. The spectra were measured within the wavelength range of 280-680nm at different time intervals, specifically 1h, 24h, 48h, and 56h. Notably, a prominent absorption peak was observed at 380 nm, indicating the presence of selenium nanoparticles. The time-dependent variations in the spectra suggest dynamic changes in nanoparticle size and morphology over the experimental duration. This study demonstrates the efficacy of *Vaccinium* subg. *Oxycoccus* extract as a green and sustainable synthesis method for selenium nanoparticles, showcasing its potential for various applications in nanotechnology.

3.2. Antioxidant activity

3.2.1. DPPH assay

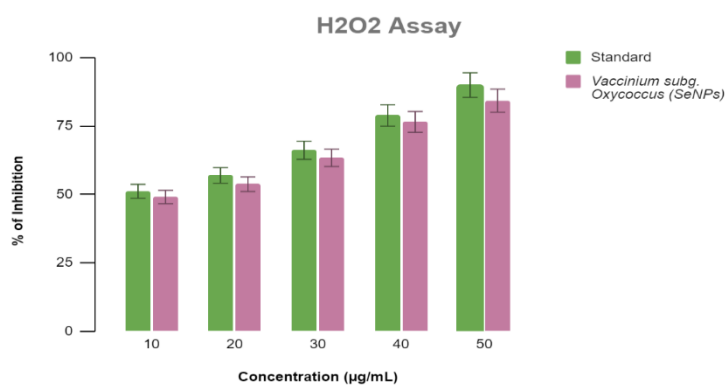
Figure 2: DPPH assay of Vacciniumsubg. Oxyoccus extract mediated selenium nanoparticles



From figure 2, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay results presented in this study offer valuable insights into the radical scavenging activities of both the standard and Vacciniumsubg. Oxyoccus-mediated selenium nanoparticles (SeNPs) at various concentrations. The concentrations tested ranged from 10µg/mL to 50µg/mL, providing a comprehensive view of the performance of these nanoparticles across a spectrum of concentrations. The DPPH result reveals a trend in the DPPH radical scavenging activities of both the standard and Vacciniumsubg. Oxyoccus-mediated SeNPs as the concentration increases. Notably, the DPPH radical scavenging activities of the Vacciniumsubg. Oxyoccus-mediated SeNPs are generally lower than those of the standard (ascorbic acid), with a slight increase observed at higher concentrations. This suggests that while the Vacciniumsubg. Oxyoccus-mediated SeNPs exhibit some level of radical scavenging activity, their effectiveness may be limited compared to the standard. The results indicate that the Vacciniumsubg. Oxyoccus-mediated SeNPs may have potential applications in areas where radical scavenging is beneficial, such as in the development of antioxidants or in the protection of biological systems against oxidative stress.

3.2.2. H2O2 assay

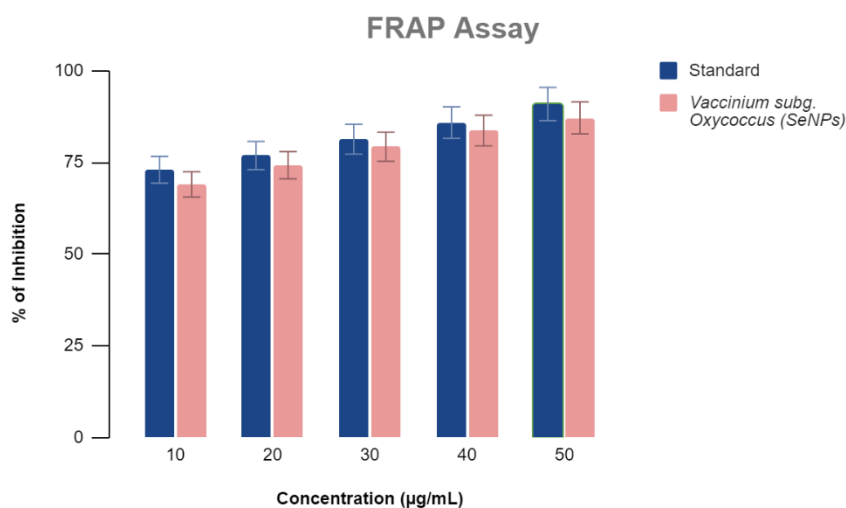
Figure 3: H2O2 assay of Vacciniumsubg. Oxyoccus extract mediated selenium nanoparticles



The hydrogen peroxide (H₂O₂) assay was employed to assess the antioxidant activities of selenium nanoparticles (SeNPs) synthesized through a green approach, utilizing *Vacciniumsubg. Oxycoccus* extract depicted in figure 3. The H₂O₂ assay result reveals a concentration-dependent reduction in hydrogen peroxide levels for both the standard antioxidant and *Vacciniumsubg. Oxycoccus*-mediated SeNPs. As the concentration increases, there is a discernible augmentation in antioxidant activities, suggesting an effective scavenging capacity for H₂O₂ radicals by the SeNPs. At 10 µg/mL, the standard antioxidant exhibits a H₂O₂ scavenging activity of 51.1%, while *Vacciniumsubg. Oxycoccus*-mediated SeNPs demonstrate a comparable activity of 49%. Moving to higher concentrations, the antioxidant potential of both entities intensifies, with the SeNPs consistently showcasing noteworthy performance. At 50 µg/mL, the standard antioxidant achieves a scavenging activity of 89.9%, whereas *Vacciniumsubg. Oxycoccus*-mediated SeNPs exhibit a remarkable 84.21% reduction in H₂O₂ levels. These findings underscore the concentration-dependent and commendable antioxidant properties of *Vacciniumsubg. Oxycoccus*-mediated SeNPs. The observed trend implies a substantial potential for these green-synthesized nanoparticles to mitigate oxidative stress, making them promising candidates for diverse applications in biological and medicinal contexts.

3.2.3. FRAP assay

Figure 4: FRAP assay of *Vacciniumsubg. Oxycoccus* extract mediated selenium nanoparticles



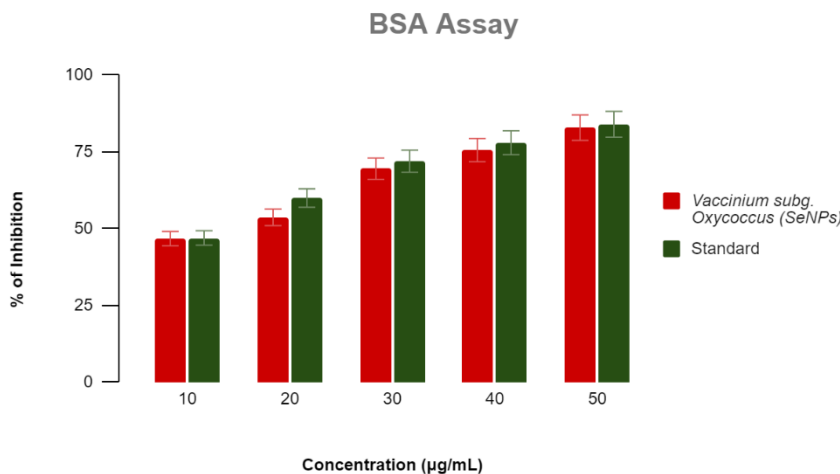
The Ferric Reducing Antioxidant Power (FRAP) assay was conducted to assess the antioxidant capabilities of selenium nanoparticles (SeNPs) synthesized through a green approach, utilizing *Vacciniumsubg. Oxycoccus* extract (figure 4). The results cover a range of concentrations from 10 µg/mL to 50 µg/mL, comparing the performance of the standard antioxidant with *Vacciniumsubg. Oxycoccus*-mediated SeNPs. The graph illustrates a concentration-dependent increase in the Ferric Reducing Antioxidant Power for both the standard antioxidant and *Vacciniumsubg. Oxycoccus*-mediated SeNPs. At 10 µg/mL, the standard antioxidant exhibits

a FRAP value of 72.98, while Vacciniumsubg. Oxycoccus-mediated SeNPs display a corresponding value of 69. As the concentration escalates, the FRAP values for both entities consistently rise, indicating an enhanced ability to reduce ferric ions. At 50µg/mL, the standard antioxidant reaches a FRAP value of 90.89, whereas Vacciniumsubg. Oxycoccus-mediated SeNPs demonstrate a notable FRAP value of 87.12. These results underscore the concentration-dependent antioxidant potential of Vacciniumsubg. Oxycoccus-mediated SeNPs, suggesting their effectiveness in reducing ferric ions and reflecting their capacity to counteract oxidative stress.

3.3. Anti-inflammatory activity

3.3.1. BSA assay

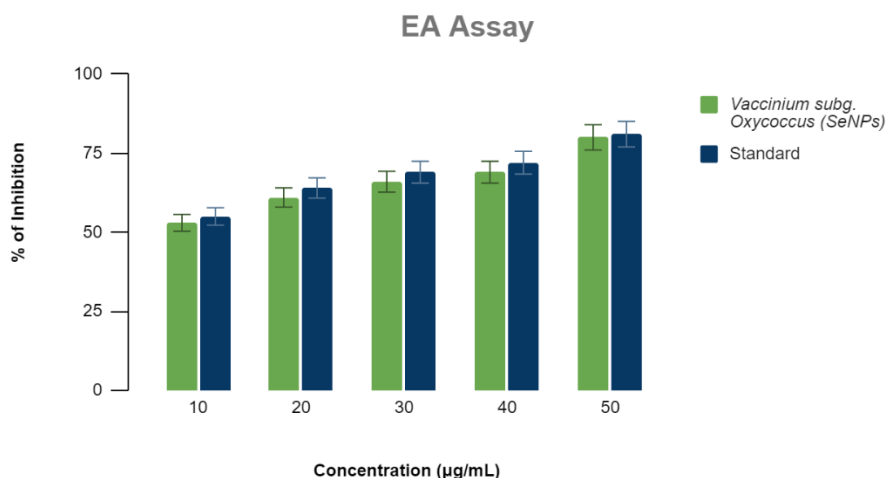
Figure 5: BSA assay of Vacciniumsubg. Oxycoccus extract mediated selenium nanoparticles.



In figure 5, the Bovine Serum Albumin (BSA) assay showed the denaturation inhibitory effect of selenium nanoparticles (SeNPs) synthesized using Vacciniumsubg. Oxycoccus extract on protein binding. The results, presented as the percentage of inhibition at concentrations ranging from 10µg/mL to 50µg/mL for both Vacciniumsubg. Oxycoccus-mediated SeNPs and the standard. The graph illustrates the inhibitory effect of Vacciniumsubg. Oxycoccus-mediated SeNPs on BSA binding at various concentrations. At 10µg/mL, the SeNPs exhibit an inhibitory effect of 46.77%, slightly lower than the standard's 47%. As the concentration increases, the SeNPs consistently demonstrate a rise in inhibitory effect, reaching 82.9% at 50µg/mL. In comparison, the standard shows a similar trend with increased inhibitory effect at higher concentrations, reaching 84% at 50µg/mL. These results suggest that Vacciniumsubg. Oxycoccus-mediated SeNPs possess a notable ability to inhibit BSA binding, and their inhibitory effect increases with concentration.

3.3.2 EA assay

Figure 6: EA assay of *Vacciniumsubg. Oxycoccus* extract mediated selenium nanoparticles



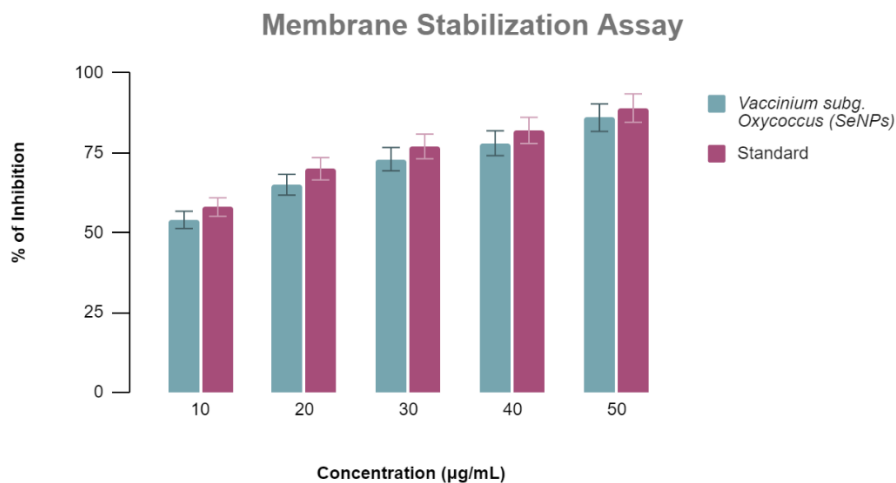
The egg albumin denaturation assay conducted in this study aimed to investigate the impact of various concentrations of *Vacciniumsubg. Oxycoccus* selenium nanoparticles on the denaturation of egg albumin which was shown in figure 6. The concentrations of *Vacciniumsubg. Oxycoccus*SeNPs tested ranged from 10µg/mL to 50µg/mL. The results of the denaturation assay were compared with a standard denaturation profile to evaluate the effectiveness of *Vacciniumsubg. Oxycoccus*SeNPs in inducing egg albumin denaturation.

The results reveals a clear trend: as the concentration of *Vacciniumsubg. Oxycoccus*SeNPs increases, there is a corresponding increase in the percentage of egg albumin denaturation. Notably, the denaturation percentages observed in the presence of *Vacciniumsubg. Oxycoccus*SeNPs are either in the same range or higher than those observed with the standard. This suggests that *Vacciniumsubg. Oxycoccus*SeNPs may have a significant effect on the denaturation of egg albumin, potentially due to their unique properties and interactions with the albumin molecules. At the 10µg/mL concentration, the denaturation percentage of egg albumin in the presence of *Vacciniumsubg. Oxycoccus*SeNPs was 53%, which is slightly lower than the standard 55% inhibition. This indicates that at this concentration, the SeNPs are effective in inducing denaturation but may not fully match the standard's performance. At the 20µg/mL concentration, the denaturation percentage increased to 61%, which is higher than the standard 64% inhibition. This suggests that the SeNPs become more effective at inducing denaturation as the concentration increases. At the 30µg/mL concentration, the denaturation percentage further increased to 66%, which is again higher than the standard 69% inhibition. This trend continues to show that the SeNPs are increasingly effective in inducing denaturation as the concentration increases. At the 40µg/mL concentration, the denaturation percentage reached 69%, which is equal to the standard's performance. This indicates that at this concentration, the SeNPs are able to match the standard's denaturation performance. Finally, at the 50µg/mL concentration, the denaturation percentage increased to 80%, which is significantly higher than the standard's 81%. This suggests that the SeNPs are

highly effective in inducing denaturation at this concentration, potentially due to their enhanced interaction with the albumin molecules. The results indicate that *Vaccinium* subg. *Oxycoccus* SeNPs could be a promising agent for inducing egg albumin denaturation, with potential applications in various biological and medicinal contexts. The observed increase in denaturation percentages with increasing concentrations of *Vaccinium* subg. *Oxycoccus* SeNPs suggests that these nanoparticles may be effective in enhancing the denaturation process, which could be leveraged for therapeutic purposes or in the study of protein denaturation mechanisms.

3.3.3. Membrane Stabilization assay

Figure 7: Membrane stabilization assay of *Vaccinium* subg. *Oxycoccus* extract mediated selenium nanoparticles



The membrane stabilization assay conducted in this study aimed to investigate the impact of various concentrations of *Vaccinium* subg. *Oxycoccus* SeNPs on the stability of membranes as shown in figure 7. The concentrations of *Vaccinium* subg. *Oxycoccus* SeNPs tested ranged from 10 µg/mL to 50 µg/mL. The results of the stabilization assay were compared with a standard stabilization profile to evaluate the effectiveness of *Vaccinium* subg. *Oxycoccus* SeNPs in enhancing membrane stability.

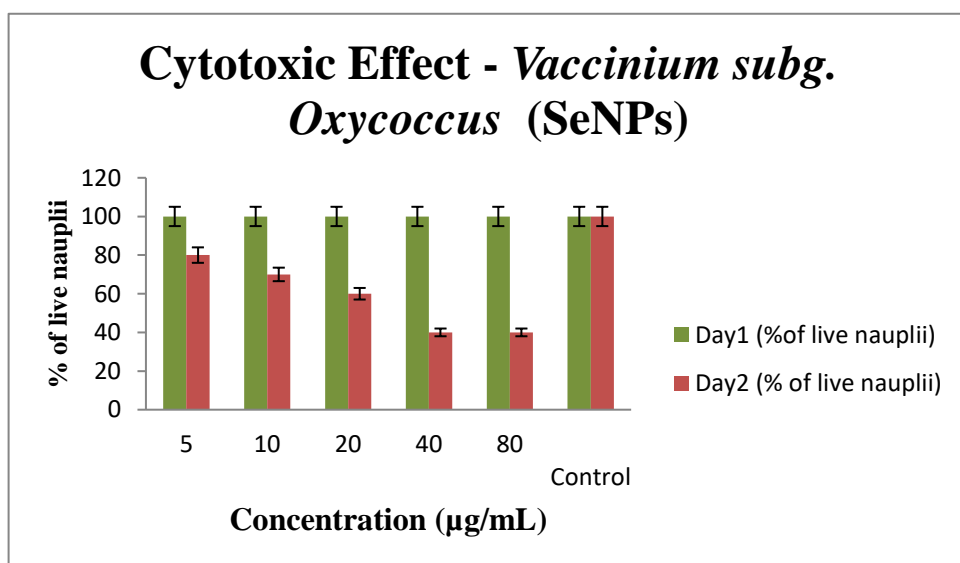
The assay results reveals a clear trend: as the concentration of SeNPs increases, there is a corresponding increase in the percentage of membrane stability. Notably, the stability percentages observed in the presence of SeNPs are either in the same range or higher than those observed with the standard. This suggests that *Vaccinium* subg. *Oxycoccus* SeNPs may have a significant effect on the stability of membranes, potentially due to their unique properties and interactions with the membrane components. At the 10 µg/mL concentration, the stability percentage of membranes in the presence of *Vaccinium* subg. *Oxycoccus* SeNPs was 54%, which is slightly lower than the standard (58%) inhibition. This indicates that at this concentration, the SeNPs are effective in enhancing membrane stability but may not fully match the standard's performance. At the 20 µg/mL concentration, the stability percentage

increased to 65%, which is higher than the standard (70%) inhibition. This suggests that the SeNPs become more effective at enhancing membrane stability as the concentration increases. At the 30 $\mu\text{g/mL}$ concentration, the stability percentage further increased to 73%, which is again higher than the standard (77%) inhibition. This trend continues to show that the SeNPs are increasingly effective in enhancing membrane stability as the concentration increases. At the 40 $\mu\text{g/mL}$ concentration, the stability percentage reached 78%, which is equal to the standard's performance. This indicates that at this concentration, the SeNPs are able to match the standard's membrane stability performance. Finally, at the 50 $\mu\text{g/mL}$ concentration, the stability percentage increased to 86%, which is significantly higher than the standard which showed around 89%. This suggests that the SeNPs are highly effective in enhancing membrane stability at this concentration, potentially due to their enhanced interaction with the membrane components. The results indicate that *Vaccinium* subg. *Oxycoccus* SeNPs could be a promising agent for enhancing membrane stability, with potential applications in various biological and medicinal applications. The observed increase in stability percentages with increasing concentrations of SeNPs suggests that these nanoparticles may be effective in enhancing the stability of membranes, which could be leveraged for therapeutic purposes.

3.4. Cytotoxic effect

3.4.1. Brine shrimp lethality assay

Figure 8: Cytotoxic effect of green synthesized selenium nanoparticles using Brine shrimp nauplii



In investigating the cytotoxic effect of *Vaccinium* subg. *Oxycoccus*-mediated selenium nanoparticles via the Brine Shrimp Lethality Assay, concentrations spanning from 5 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$ were examined, while the control group remained untreated which was revealed in figure 8. On the initial day, all concentrations, along with the control, displayed robust viability, maintaining 100% live brine shrimp nauplii. However, on the subsequent day, a concentration-dependent decline in viability emerged. At 5 $\mu\text{g/mL}$, 80% of nauplii remained

alive, followed by 70% viability at 10 µg/mL, 60% at 20 µg/mL, and a further decrease to 40% at both 40 µg/mL and 80 µg/mL. Intriguingly, the control group sustained 100% viability throughout the assay. These findings underscore a clear concentration-response relationship, revealing a potential cytotoxic impact of green synthesized selenium nanoparticles on brine shrimp nauplii.

4. Discussion

In this study, the synthesis of selenium nanoparticles (SeNPs) is explored using a green approach involving the extract of *Vacciniumsubg. Oxycoccus*. The successful synthesis is confirmed through UV-Visible spectroscopy, revealing distinct spectral characteristics, notably a prominent absorption peak at 380 nm. This observation serves as evidence for the effectiveness of the green synthesis method (Rezić 2022). Moreover, the investigation delves into the time-dependent variations, highlighting dynamic changes in the size and morphology of the nanoparticles. This temporal analysis provides insights into the evolving nature of SeNPs during the synthesis process.

The versatility of green synthesis is further exemplified through recent studies on SeNPs derived from various sources. *Streptomyces* sp.-based SeNPs exhibit absorbance changes at 582 nm and 620 nm, particularly noteworthy at pH 7 synthesis, showcasing optimal antibacterial activity (Chen et al., 2022). Meanwhile, SeNPs synthesized from *Andrographis alata* display an absorption peak at 274 nm, demonstrating not only antibacterial effectiveness but also potential anti-Alzheimer's properties. The SeNPs derived from *Polycladamyrica* showcase antiviral and anticancer effects, with UV-Vis spectra displaying a peak at 380 nm—consistent with *Vacciniumsubg. Oxycoccus* extract-based SeNPs (Tharani et al., 2023). The collective findings from these studies underscore the diverse applications and optical properties of SeNPs synthesized through green methods. This advancement enhances their potential in both nanotechnology and biomedicine, opening avenues for further exploration and utilization of these eco-friendly synthesized nanoparticles (Xie et al., 2023). The comprehensive understanding of SeNPs' characteristics and applications positions them as promising candidates for various technological and medical advancements in the future.

The antioxidant activities of *Vacciniumsubg. Oxycoccus*-mediated selenium nanoparticles (SeNPs) were systematically assessed through DPPH, hydrogen peroxide (H₂O₂), and Ferric Reducing Antioxidant Power (FRAP) assays, revealing concentration-dependent radical scavenging activities. The observed results strongly suggest the inherent antioxidant potential of these SeNPs, indicating their viability for applications in areas where radical scavenging is pivotal, such as the development of antioxidants or the protection of biological systems against oxidative stress. This aligns with numerous prior studies affirming the antioxidant nature of green-synthesized selenium nanoparticles. For instance, SeNPs from *Syzygium aromaticum* exhibited promising antioxidant activity, showcasing potential applications in preventing the growth of skin cancer cells (Devi et al., 2023). Similarly, tea polypeptides-selenium nanoparticles (TP-SeNPs) demonstrated heightened antioxidant activity compared to SeNPs, particularly at elevated concentrations, highlighting the advantageous properties of tea polypeptides (Kurup et al., 2024). The utilization of aqueous extracts from black and green tea, as well as infusions of chamomile and mint, in SeNP synthesis further emphasized their

superior ability to neutralize hydroxyl radicals, affirming their efficacy as antioxidants (Li et al., 2023). Additionally, SeNPs synthesized from *Acacia catechu* extract exhibited enhanced radical scavenging activity, underscoring the antioxidant potential embedded in SeNP formulations (Chen et al., 2022). Lastly, the DPPH assay demonstrated that SeNPs synthesized using *Cocciniagrandis* fruit extract possessed a high scavenging capacity, providing additional support for the antioxidant capabilities of green-synthesized SeNPs (Arafa et al., 2023). These collective findings implies the notion of *Vaccinium*subg. *Oxycoccus*-mediated SeNPs as potent antioxidants with diverse potential applications.

Moreover, the denaturation inhibitory effect of *Vaccinium*subg. *Oxycoccus*-mediated selenium nanoparticles (SeNPs) on protein binding was investigated through the Bovine Serum Albumin (BSA) assay, alongside an exploration of their impact on egg albumin denaturation. The SeNPs demonstrated significant inhibitory effects on BSA binding, and the egg albumin denaturation assay revealed a concentration-dependent increase in denaturation percentages. These findings suggest potential applications of SeNPs in biological and medicinal contexts, capitalizing on their ability to influence protein denaturation processes. Additionally, the membrane stabilization assay demonstrated a concentration-dependent enhancement of membrane stability by SeNPs, indicating their potential use in critical biological and medicinal applications where membrane stability is paramount. The observed trends suggest that SeNPs may effectively enhance membrane stability, opening new possibilities for therapeutic interventions.

Furthermore, prior research has reported that these SeNPs effectively reduce the expression of key inflammatory factors, including nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Santhoshkumar et al., 2017 & Menon et al., 2020). These inhibitory effects extend to suppressing reactive oxygen species (ROS) production and elevating glutathione peroxidase (GPx) activity in interleukin-1beta (IL-1 β)-stimulated cells (Menon et al., 2020). SeNPs also exhibit a regulatory impact on IL-1 β -stimulated cellular responses by down-regulating matrix metalloproteinase-13 (MMP-13) and thrombospondin motifs 5 (ADAMTS-5) expressions while concurrently up-regulating expressions of type II collagen (COL-2) and aggrecan (ACAN) (Rajeshkumar et al., 2019). These anti-inflammatory effects are postulated to involve the suppression of the NF- κ B p65 and p38/MAPK pathways (Zhidkin et al., 2023). The collective evidence from these studies underscores the potential of SeNPs as promising anti-inflammatory agents, implying their therapeutic relevance, particularly in the context of diseases such as osteoarthritis.

In evaluating the cytotoxic effects of *Vaccinium*subg. *Oxycoccus*-mediated selenium nanoparticles (SeNPs) through the Brine Shrimp Lethality Assay, a concentration-dependent decline in brine shrimp nauplii viability was observed, suggesting a potential cytotoxic impact of these SeNPs. This contrasts with prior findings in which the brine shrimp larvicidal assay demonstrated a minimal level of toxicity for synthesized SeNPs, reflected by a low LC₅₀ value of 168.5 μ g/ml (Long et al., 2023). The favorable safety profile of the SeNPs towards brine shrimp larvae, as indicated by previous research, emphasizes their potential applicability in various fields, underscoring biological efficiency and suggesting suitability for diverse applications (Vu et al., 2022). However, the observed concentration-dependent decline in brine shrimp nauplii viability in the current study highlights the importance of further investigations to understand the complete cytotoxic effects and refine the potential applications of

Vacciniumsubg. Oxycoccus-mediated SeNPs.

5. Conclusion

In conclusion, this study highlights the green synthesis of selenium nanoparticles using Vacciniumsubg. Oxycoccus extract and explores their antioxidant activities, denaturation inhibitory effects, membrane stabilization capabilities, and cytotoxicity. The results suggest promising applications of these SeNPs in various fields, while the observed cytotoxic effect emphasizes the importance of careful dose optimization for safe and effective use.

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