Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.13.01.Art061

Green Synthesis of Selenium Nanoparticles using Vaccinium Subg. Oxycoccus for Antioxidant, Anti-Inflammatory, and Cytotoxic Effect

G. Dinesh, T. Lakshmi*, S. Rajeshkumar
Center for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 602 105, Tamil Nadu, India

Abstract

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, lays the groundwork for their versatile application in biological and medicinal contexts.

Keywords: *Anti-Inflammatory Agent, Antioxidant Agent, Biocompatibility, Green Synthesis, Selenium Nanoparticles.*

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 [1]. They enable the administration of high-toxicity treatments with improved safety, such as chemotherapeutic cancer drugs [2]. Nanotechnology also allows for the development of wearable gadgets that can detect changes in vital signs, cancer cell conditions, and infections in the body [3]. By manipulating nanoparticles at the nanoscale, their characteristics can be modified to achieve desired properties for targeted drug delivery, imaging, therapy, and sensors [4]. Nanomaterials have shown promise in improving the efficiency of medical treatment,

Received: 26.09.2024 ***Accepted: 26.11.2024*** ***Published on: 28.03.2025***
****Corresponding Author: lakshmi@saveetha.com***

particularly in drug delivery. They have applications in cancer treatment, coronary artery disease treatment, biomarker detection, and nano antibacterial agents [5].

Selenium nanoparticles have garnered significant attention in nanotechnology and biomedical research due to their unique properties and potential applications. Selenium nanoparticles exhibit notable capabilities in activating the Nrf2-Keap1 signalling pathway, boosting antioxidative selenoproteins expression, and inhibiting mast cell activation, thereby demonstrating effective antiallergic activity [6]. Furthermore, they display anti-inflammatory effects by suppressing the NF-κB and cyclooxygenase-2 pathways, diminishing inflammatory factor expression, and elevating anti-inflammatory markers [7,8]. 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 [9,10].

Green synthesis has emerged as a crucial focus in nanotechnology research, driven by the prospect of developing sustainable and environmentally friendly nanomaterials [5,11]. 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 [12]. 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 green chemistry principles, emphasising waste reduction, energy efficiency, and the use of benign solvents [13].

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 oxycoccos, Vaccinium angustifolium, and Vaccinium myrtillus,* native to cooler regions worldwide, produce small, vibrant fruits rich in antioxidants and vitamin C [14]. 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 fibre, 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 [15,16]. 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 [17]. 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 [18].

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 effects through the Brine Shrimp Lethality

Assay, shedding light on the potential multifaceted applications of these nanoparticles in future biomedical research and applications.

Materials and Methods

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 the 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.

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.

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.

Antioxidant Activity

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-coloured crystalline compounds. The reduction of DPPH radicals into DPPH-H, a colourless 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 *Vaccinium subg. Oxycoccus* extract-mediated selenium nanoparticles (10µg/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:

$$Percentage\ of\ antioxidant\ activity = [(Ac - As) \div Ac] \times 100$$

Where Ac represents the control reaction absorbance, and as is the testing specimen absorbance.

H_2O_2 Assay

In the in vitro hydrogen peroxide radical scavenging assay using *Vaccinium subg. Oxycoccus* extract mediated selenium nanoparticles (SeNPs) as the test substance and the following steps were followed. Hydrogen peroxide (H_2O_2) was prepared as a stock solution at a concentration of 3% (w/v). Horseradish 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-amino antipyrine (4-APA) that changes colour 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, *Vaccinium subg. Oxycoccus* extract-mediated SeNPs were dissolved in an appropriate solvent (Distilled water) to achieve the desired concentration (10µg/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 *Vaccinium subg. Oxycoccus* extract mediated SeNPs.

After incubation, the absorbance of each well was measured at 504 nm using a spectrophotometer, corresponding to the colour 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 colour change was calculated using the formula.

$$Percentage\ of\ Inhibition\ = \frac{(Absorbance\ of\ Control(Absorbance\ of\ Control - Absorbance\ of\ Test\ Solution)}{Absorbance\ of\ Control) \times 100}$$

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 [19,20].

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 (10µg/mL - 50 µg/mL) of green synthesized selenium nanoparticles and standards are dispensed into a 96-well plate in triplicate, approximately 20 µL each. Subsequently, 200 µL 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 Fe^{3+} to Fe^{2+} within the FRAP reagent, resulting in a colour 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.

Anti-Inflammatory Activity

The green synthesized selenium nanoparticles were tested for their anti-inflammatory activity using three assays Bovine serum albumin denaturation assay, Egg albumin denaturation assay, and Membrane stabilization assay.

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 µg/mL. Subsequently, the pH of the solution was adjusted to 6.3. The mixture was then incubated

at room temperature for 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.

The percentage of protein denaturation was determined utilizing the following equation,

$$Percentage\ of\ inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

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 SeNP, 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 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.

The percentage of protein denaturation was determined utilizing the following equation,

$$Percentage\ of\ inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

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 a tris-HCl buffer, human red blood cells (RBCs), phosphate-buffered saline (PBS), centrifuge tubes, and a UV-visible spectrophotometer.

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.

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:

$$Percentage\ of\ inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

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.

Cytotoxic Effect

Brine Shrimp Lethality Assay

The procedure involved dissolving the 2 grams of iodine-free salt in 200 mL of distilled 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 24 hours. Upon completion of the 24-hour incubation period, the ELISA plates were inspected, and

the count of live nauplii was recorded. The percentage of lethal effect was performed using the formula:

$$Percentage\ of\ lethal\ effect = \frac{Number\ of\ dead\ nauplii}{Number\ of\ live\ nauplii} \times 100$$

Result

UV-Visible Spectroscopy

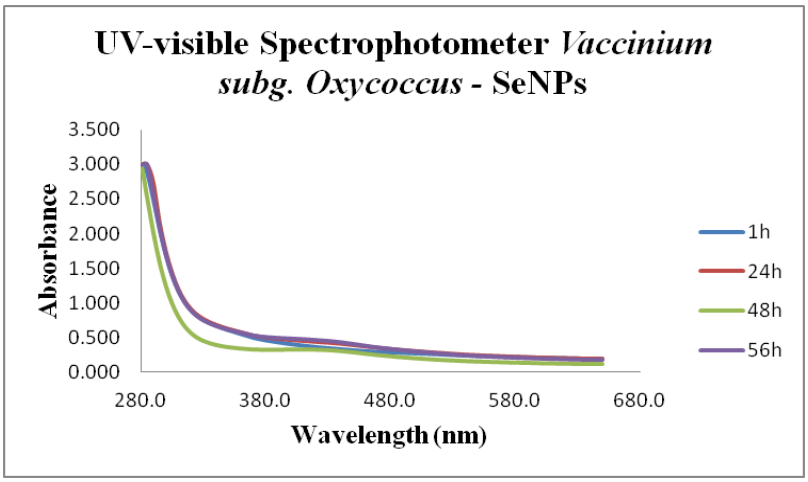

Figure 1. UV-visible spectra of Green Synthesized Selenium Nanoparticles

The UV-visible spectroscopy analysis of selenium nanoparticles synthesized using *Vaccinium* subg. Oxycoccus extract revealed distinct spectral characteristics which are 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 is a green and sustainable synthesis method for selenium nanoparticles, showcasing its potential for various applications in nanotechnology.

Antioxidant Activity:

DPPH Assay

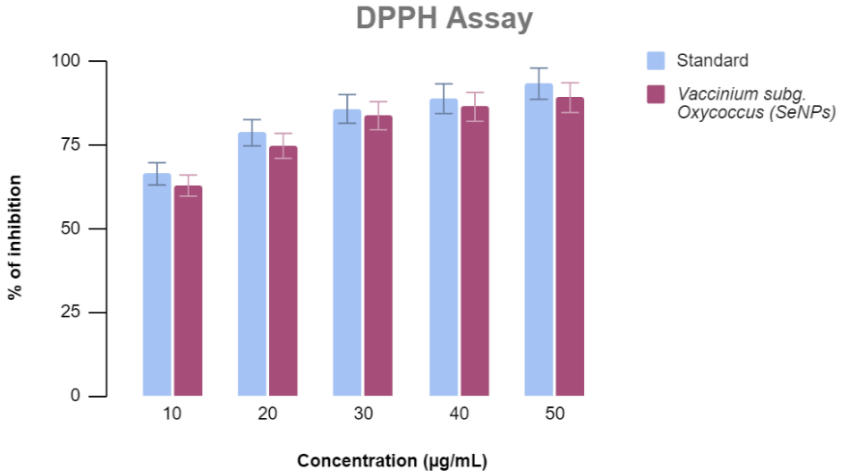

Figure 2. DPPH Assay of *Vaccinium Subg. Oxycoccus* Extract Mediated Selenium Nanoparticles

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 *Vaccinium* subg oxycoccus-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 *Vaccinium* subg. Oxycoccus-mediated SeNPs as the concentration increases. Notably, the DPPH radical scavenging activities of the *Vaccinium* subg. Oxycoccus-mediated SeNPs are generally lower than the standard (ascorbic acid), with a slight increase observed at higher concentrations. This suggests that while the *Vaccinium* subg. Oxycoccus-mediated SeNPs exhibit some level of radical scavenging activity, their effectiveness may be limited compared to the standard. The results indicate that the *Vaccinium* subg. Oxycoccus-mediated SeNPs may have potential applications in areas where radical scavenging is beneficial, such as in developing antioxidants or protecting biological systems against oxidative stress.

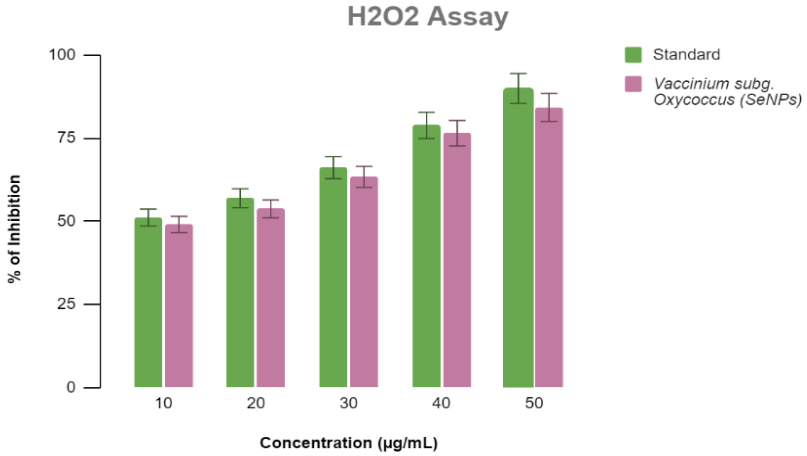

Figure 3. H_2O_2 Assay of *Vaccinium Subg. Oxycoccus* Extract Mediated Selenium Nanoparticles

H2O2 Assay

The hydrogen peroxide (H_2O_2) assay was employed to assess the antioxidant activities of selenium nanoparticles (SeNPs) synthesized through a green approach, utilizing *Vaccinium* subg. Oxycoccus extract is depicted in Figure 3. The H_2O_2 assay result reveals a concentration-dependent reduction in hydrogen peroxide levels for both the standard antioxidant and *Vaccinium* subg. Oxycoccus-mediated SeNPs. As the concentration increases, there is a discernible augmentation in antioxidant activities, suggesting an effective scavenging capacity for H_2O_2 radicals by the SeNPs. At 10µg/mL, the standard antioxidant exhibits an H_2O_2 scavenging activity of 51.1%, while *Vaccinium* subg. 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 50µg/mL, the standard antioxidant achieves a scavenging activity of 89.9%, whereas *Vaccinium* subg. Oxycoccus-mediated SeNPs exhibit a remarkable 84.21% reduction in H_2O_2 levels. These findings underscore the concentration-dependent and commendable antioxidant properties of *Vaccinium* subg—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.

FRAP Assay

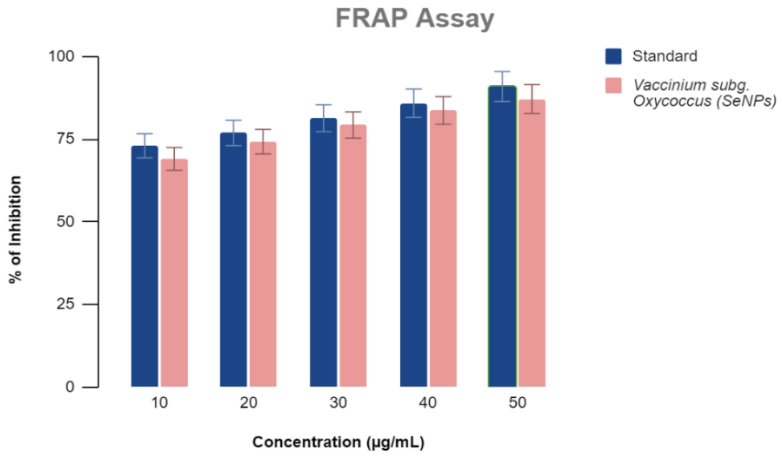

Figure 4. FRAP Assay of *Vaccinium Subg. Oxycoccus* Extract-Mediated Selenium Nanoparticles

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted to assess the antioxidant capabilities of selenium nanoparticles (SeNPs) synthesized through a green approach, utilizing *Vaccinium* subg. Oxycoccus extract (Figure 4). The results cover a range of concentrations from 10µg/mL to 50µg/mL, comparing the performance of the standard antioxidant with *Vaccinium* subg. Oxycoccus-mediated SeNPs. The graph illustrates a concentration-dependent increase in the Ferric Reducing Antioxidant Power for both the standard antioxidant and *Vaccinium* subg. Oxycoccus-mediated SeNPs. At 10µg/mL, the standard antioxidant exhibits a FRAP value of 72.98, while *Vaccinium* subg. 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 *Vaccinium* subg. Oxycoccus-mediated SeNPs demonstrate a notable FRAP value of 87.12. These results underscore the concentration-dependent antioxidant potential of *Vaccinium* subg. Oxycoccus-mediated SeNPs, suggesting their effectiveness in reducing ferric ions and reflecting their capacity to counteract oxidative stress.

Anti-Inflammatory Activity

BSA Assay

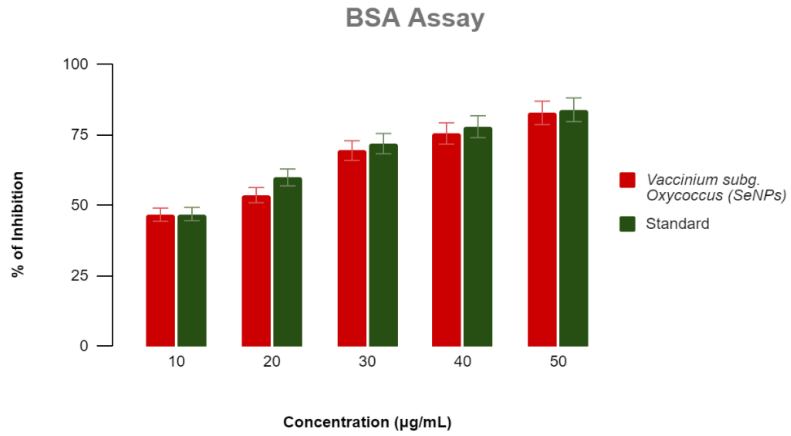

Figure 5. BSA Assay of *Vaccinium Subg. Oxycoccus* Extract Mediated Selenium Nanoparticles

In Figure 5, the Bovine Serum Albumin (BSA) assay showed the denaturation inhibitory effect of selenium nanoparticles (SeNPs) synthesized using *Vaccinium* subg. Oxycoccus extract on protein binding. The results, presented as the percentage of inhibition at concentrations ranging from 10µg/mL to 50µg/mL for both *Vaccinium* subg. Oxycoccus-mediated SeNPs and the standard. The graph illustrates the inhibitory effect of *Vaccinium* subg. 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 an increased inhibitory effect at higher concentrations, reaching 84% at 50µg/mL. These results suggest that *Vaccinium* subg. Oxycoccus-mediated SeNPs possess a notable ability to inhibit BSA binding, and their inhibitory effect increases with concentration.

EA Assay

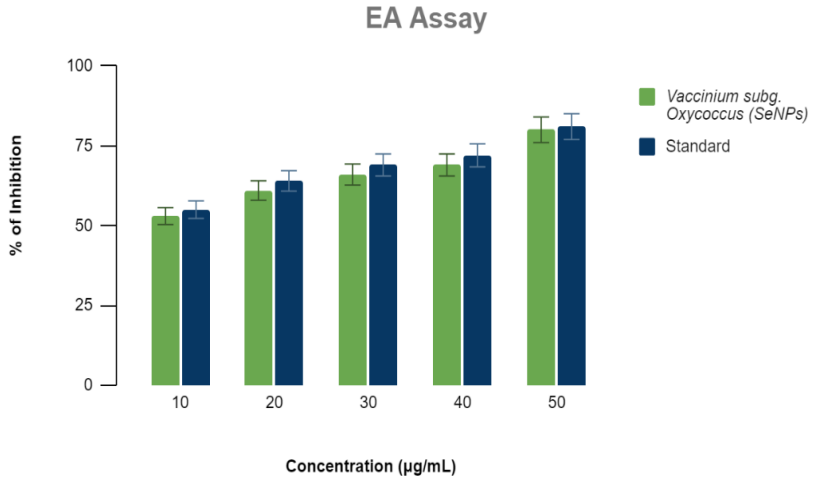

Figure 6. EA Assay of *Vaccinium Subg. Oxycoccus* Extract Mediated Selenium Nanoparticles

The egg albumin denaturation assay conducted in this study aimed to investigate the impact of various concentrations of *Vaccinium* subg. Oxycoccus selenium nanoparticles on the denaturation of egg albumin, which was shown in Figure 6. The concentrations of *Vaccinium* subg. 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 *Vaccinium* subg. Oxycoccus SeNPs in inducing egg albumin denaturation.

The results reveal a clear trend: as the concentration of *Vaccinium* subg. Oxycoccus SeNPs increase and there is a corresponding increase in the percentage of egg albumin denaturation. Notably, the denaturation percentages observed in the presence of *Vaccinium* subg. Oxycoccus 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 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 *Vaccinium* subg. 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 can 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 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 suggest 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.

Membrane Stabilization Assay

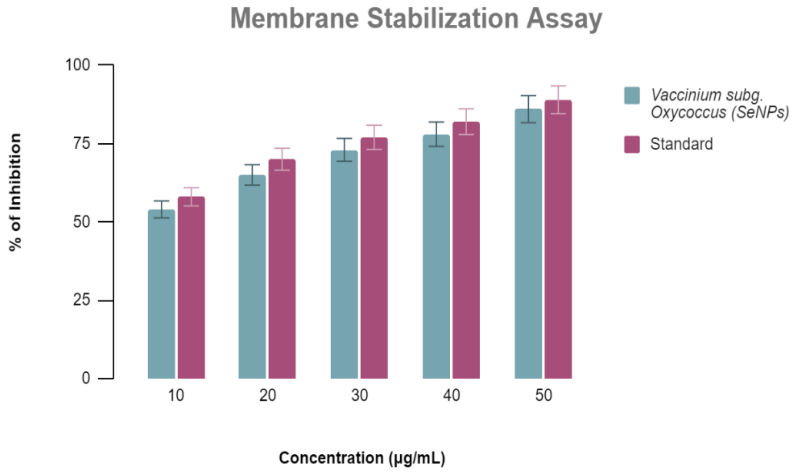

Figure 7. Membrane Stabilization Assay of *Vaccinium Subg. Oxycoccus* Extract-Mediated Selenium Nanoparticles

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 10µg/mL to 50µg/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 reveal 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 10µg/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 20µg/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 30µg/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 40µg/mL concentration, the stability percentage reached 78%, which is equal to the standard's performance. This indicates that at this concentration, the SeNPs can match the standard's membrane stability performance. Finally, at the 50µg/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.

Brine Shrimp Lethality Assay

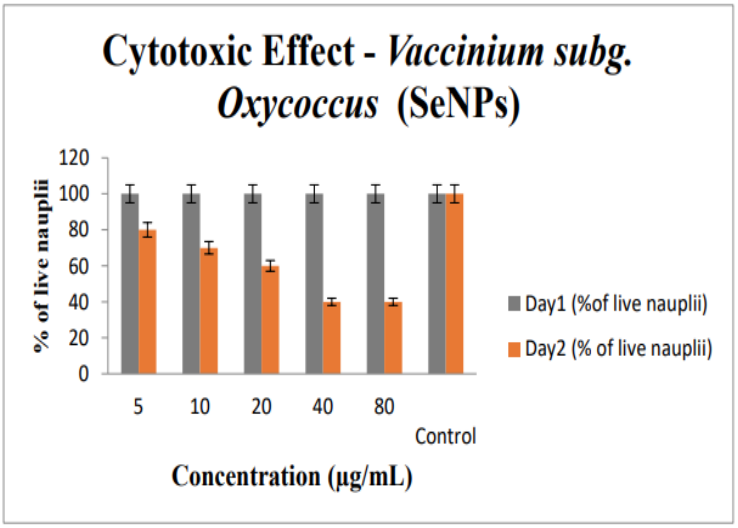

Figure 8. Cytotoxic Effect of Green-Synthesized Selenium Nanoparticles Using Brine Shrimp Nauplii

In investigating the cytotoxic effect of *Vaccinium* subg. Oxycoccus-mediated selenium nanoparticles via the Brine Shrimp Lethality Assay, concentrations spanning from 5 µg/mL to 80 µg/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 µg/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.

Discussion

In this study, the synthesis of selenium nanoparticles (SeNPs) is explored using a green approach involving the extract of *Vaccinium* subg. 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 [3]. 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 [4]. Meanwhile, SeNPs synthesized from *Andrographis alata* display an absorption peak at 274 nm, demonstrating not only antibacterial effectiveness but also potential anti-Alzheimer properties. The SeNPs derived from *Polycladia myrica* showcase antiviral and anticancer effects, with UV-Vis spectra displaying a peak at 380 nm—consistent with *Vaccinium* subg. Oxycoccus extract-based SeNPs[5]. 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 [6]. 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 *Vaccinium* subg. Oxycoccus-mediated selenium nanoparticles (SeNPs) were systematically assessed through DPPH, hydrogen peroxide (H_2O_2), 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 [7]. 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 [8]. 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 [9]. Additionally, SeNPs synthesized from *Acacia catechu* extract exhibited enhanced radical scavenging activity, underscoring the antioxidant potential embedded in SeNP formulations [4]. Lastly, the DPPH assay demonstrated that SeNPs synthesized using *Coccinia grandis* fruit extract possessed a high scavenging capacity, providing additional support for the antioxidant capabilities of green-synthesized SeNPs [10]. These collective findings imply 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 were 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) [11], [12]. These inhibitory effects extend to suppressing reactive oxygen species (ROS) production and elevating glutathione peroxidase (GPx) activity in interleukin-1beta (IL-1β)-stimulated cells [12]. SeNPs also exhibit a regulatory impact on IL-1β-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) [13]. These anti-inflammatory effects are postulated to involve the suppression of the NF-κB p65 and p38/MAPK pathways [14]. 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 LC_{50} value of 168.5 µg/ml [21]. The favourable 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 [22]. 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 Vaccinium subg. Oxycoccus-mediated SeNPs.

In conclusion, this study highlights the green synthesis of selenium nanoparticles using Vaccinium subg. Oxycoccus extract 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.

Conclusion

The comprehensive analysis of *Vaccinium subg. Oxycoccus*-mediated SeNPs reveal a spectrum of promising characteristics with significant implications for biological and medicinal applications. The UV-visible

spectroscopy analysis underscores the efficacy of *Vaccinium subg. Oxycoccus* extract as a green and sustainable synthesis method, leading to dynamic changes in SeNP size and morphology. The DPPH assay highlights the concentration-dependent radical scavenging activities of SeNPs, suggesting potential applications in antioxidant development and protection against oxidative stress. The Hydrogen Peroxide (H2O2) and Ferric Reducing Antioxidant Power (FRAP) assays further validate the concentration-dependent antioxidant properties of SeNPs, showcasing their commendable efficacy compared to standard antioxidants. The Bovine Serum Albumin (BSA) assay and Egg Albumin Denaturation assay demonstrate the inhibitory effect of SeNPs on protein binding and enhancement of the denaturation process, respectively. Moreover, the Membrane Stabilization assay indicates the potential of SeNPs in enhancing membrane stability, presenting further avenues for exploration. However, the Brine Shrimp Lethality assay raises awareness of a concentration-dependent decline in viability, suggesting potential cytotoxic effects on brine shrimp nauplii. This underscores the importance of careful consideration and further investigation into the safety aspects of *Vaccinium* subg. Oxycoccus-mediated SeNPs. Continued exploration of the mechanisms and therapeutic potential of these nanoparticles holds promise for diverse biomedical contexts.

Acknowledgements

The authors are grateful to the Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute Medical and Technical Sciences, Chennai-602105, Tamil Nadu, India, for providing a research facility to carry out the entire research work.

Conflict of Interests

The authors declare that they have no conflicts of interest.

Reference

[1]. Haleem, A., Javaid, M., Singh, R. P., Rab, S., Suman, R., 2023, Applications of nanotechnology in the medical field: a brief review. *Glob Health J.;*7: 70–77. doi:10.1016/j.glohj.2023.02.008

[2]. Pasika, S. R., Bulusu, R., Rao, B. V. K., Kommineni, N., Bolla, P. K., Kala, S. G., et al., 2023, Nanotechnology for Biomedical Applications. Nanomaterials. *Singapore: Springer Nature Singapore;*. 297–327. doi:10.1007/978-981-19-7963-7_11

[3]. Rezić, I., 2022, Nanoparticles for biomedical application and their synthesis. *Polymers* (Basel).;14 (9) 4, 961. doi:10.3390/polym14224961

[4]. Chen, J., Li, L., Song, K., Wang, Z., 2022, Application of different nanomaterials in biomedical field. *Highlights in Science, Engineering and Technology*.;14: 57–64. doi:10.54097/hset.v14i.1593

[5]. Tharani, M., Rajeshkumar, S., Al-Ghanim, K. A., Nicoletti, M., Sachivkina, N., Govindarajan M., 2023, *Terminalia chebula*-Assisted Silver Nanoparticles: Biological Potential, Synthesis, Characterization, and Ecotoxicity. *Biomedicines,* 11. doi:10.3390/biomedicines11051472

[6]. Xie, B., Zeng, D., Yang, M., Tang, Z., He, L., Chen, T., 2023, 1Translational selenium nanoparticles to attenuate allergic dermatitis through Nrf2-Keap1-driven activation of selenoproteins. ACS Nano. 7: 14053–14068. doi:10.1021/acsnano.3c04344

[7]. Devi, M. S., Srinivasan, S., Muthuvel, A., 2023, Selenium nanomaterial is a promising nanotechnology for biomedical and environmental remediation: A detailed review. *Biocatal Agric Biotechnol*. 51: 102766. doi:10.1016/j.bcab.2023.102766.

[8]. Kurup, M., Kumar, M., Ramanathan, S., Chandra, M., 2023, The biogenetic synthesis of metallic nanoparticles and the role they play in anti-

inflammatory drug treatment. *Curr Drug Discov Technol.* 20. doi:10.2174/1570163820666230718123544

[9]. Li, Y., Zhu, S., Luo, J., Tong, Y., Zheng, Y., Ji, L., et al., 2023, The Protective Effect of Selenium Nanoparticles in Osteoarthritis: In vitro and in vivo Studies. *Drug Des Devel Ther.* 17: 1515–1529. doi:10.2147/dddt.s407122

[10]. Arafa, F. M., Mogahed, N. M. F. H., Eltarahony, M. M., Diab, R. G., 2023, Biogenic selenium nanoparticles: trace element with promising anti-toxoplasma effect. *Pathog Glob Health*. 117: 639–654. doi:10.1080/20477724.2023.2186079

[11]. Santhoshkumar, J., Kumar, S. V., Rajeshkumar, S., 2017, Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. *Resour-Effic Technol.* 3: 459–465. doi:10.1016/j.reffit.2017.05.001

[12]. Menon, S., Agarwal, H., Rajeshkumar, S., Jacquline Rosy, P., Shanmugam, V. K., 2020, Investigating the antimicrobial activities of the biosynthesized selenium nanoparticles and its statistical analysis. *Bionanoscience.* 10: 122–135. doi:10.1007/s12668-019-00710-3

[13]. Rajeshkumar, S., Tharani, M., Jeevitha, M., Santhoshkumar, J., 2019, Anticariogenic activity of fresh aloe Vera gel mediated copper oxide nanoparticles. *Indian J Public Health Res Dev.* 10: 3664. doi:10.5958/0976-5506.2019.04158.5

[14]. Zhidkin, R., Zhurbenko, P., Bogomaz, O., Gorodilova, E., Katsapov, I., Antropov, D., et al., 2023, Biodiversity of rolB/C-like natural transgene in the genus Vaccinium L. and its application for phylogenetic studies. *Int J Mol Sci.* 24. doi:10.3390/ijms24086932

[15]. A. Waikar, B., C. Mandave, P., Berries: A new paradigm for nutraceuticals., 2023, Edible Berries - New Insights. *IntechOpen;* doi:10.5772/intechopen.1002226

[16]. Martău, G. A., Bernadette-Emőke, T., Odocheanu, R., Soporan, D. A., Bochiș, M., Simon, E., et al. 2023, Vaccinium species (Ericaceae): Phytochemistry and biological properties of medicinal plants. *Molecules.* 28. doi:10.3390/molecules28041533

[17]. Nurzyńska-Wierdak, R., 2023, Phenolic compounds from new natural sources-plant genotype and ontogenetic variation. *Molecules.* 28. doi:10.3390/molecules28041731

[18]. Priyaa, N. P., Rajeshkumar, S., Lakshmi, T., Roy, A., 2020, Antibacterial Properties of Lodhra Bark (*Symphlocus racemosa*) Mediated Silver Nanoparticles Against Oral Pathogens. *Plant Cell Biotechnology And Molecular Biology.* 1–7.

[19]. Sharma, S. K., Singh, A. P., 2012, In vitro antioxidant and free radical scavenging activity of *Nardostachys jatamansi* DC. *J Acupunct Meridian Stud.* 5: 112–118. doi:10.1016/j.jams.2012.03.002

[20]. Bhatti, M. Z., Ali, A., Ahmad, A., Saeed, A., Malik, S. A., 2015, Antioxidant and phytochemical analysis of *Ranunculus arvensis* L. extracts. *BMC Res Notes.;*8: 279. doi:10.1186/s13104-015-1228-3

[21]. Long, Q., Cui, L-K., He, S-B., Sun, J., Chen, Q-Z., Bao, H-D., et al., 2023, Preparation, characteristics and cytotoxicity of green synthesized selenium nanoparticles using *Paenibacillus motobuensis* LY5201 isolated from the local specialty food of longevity area. *Sci Rep.* 13. doi:10.1038/s41598-022-26396-4

[22]. Vu, T. T., Nguyen, P. T. M., Pham, N. H., Le, T. H., Nguyen, T. H., Do, D. T., et al., 2022, Green synthesis of selenium nanoparticles using Cleistocalyx operculatus leaf extract and their acute oral toxicity study. *J Compos Sci.* 6: 307. doi:10.3390/jcs6100307