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Biogenic Selenium Nanoparticles from *Camptotheca acuminata* Fruit Extract and their Biomedical Applications

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Abstract

Background: Selenium nanoparticles are emerging as promising nanomaterials owing to their unique physicochemical and biological properties, including antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory activities. However, conventional chemical synthesis methods often rely on toxic reagents and harsh reaction conditions, which limit their use in biomedical applicability. Green synthesis using plant extracts provides an eco-friendly, biocompatible, and cost-effective alternative to conventional methods. *Camptotheca acuminata*, a medicinal plant rich in alkaloids, flavonoids, and phenolic compounds, offers suitable phytochemicals for nanoparticle synthesis. **Aim: This** study aimed to establish a sustainable green synthesis of selenium nanoparticles using *C. acuminata* fruit extract and to evaluate their physicochemical characteristics and multifunctional biological activities for potential nanomedicine applications. **Methods:** *Camptotheca acuminate* selenium nanoparticles were synthesized using an aqueous fruit extract of *C. acuminata* as both reducing and stabilizing agent and characterized by UV–visible spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). UV–visible analysis revealed a characteristic absorption band around 310 nm, FTIR confirmed hydroxyl and carboxyl functional groups involved in nanoparticle stabilization, and SEM showed predominantly spherical particles with uniform morphology and sizes ranging from 45 to 90nm. Antioxidant, antimicrobial, anticancer, antidiabetic (α-amylase inhibition), and *in vitro* anti-inflammatory activities were evaluated. **Results:** The green-synthesized CASeNPs exhibited strong antioxidant activity and significant antibacterial effects, producing inhibition zones of approximately 25mm against *Staphylococcus aureus*, 14mm against *Pseudomonas aeruginosa*, and 12mm against *Escherichia coli*. Additionally, CASeNPs demonstrated potent cytotoxicity against A549 lung cancer cells, effective α-amylase inhibition, and notable anti-inflammatory activity. **Conclusion:** Overall *C. acuminata* derived selenium nanoparticles demonstrate excellent multifunctional bioactivities and represent a promising, sustainable nanoplatform for future biomedical and therapeutic applications.

Major Findings: *Camptotheca acuminata* fruit extract enabled green synthesis of stable spherical SeNPs confirmed by UV–Vis, FTIR, and SEM. The CASeNPs showed strong antioxidant and antiinflammatory effects and antimicrobial activity. They also exhibited significant anticancer and antidiabetic indicating promise for nanomedicine applications.

Keywords: Antioxidant Assays, Anti-inflammatory Assays, Antidiabetic Assays, Cytotoxicity *Camptotheca acuminata*, Microbial Inhibition Assay

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1. Introduction

Nanotechnology has greatly advanced medicine and pharmaceutical sciences by enabling the development of materials with improved physicochemical and biological properties[1,2]. Among various nanomaterials selenium nanoparticles have attracted significant attention due to their enhanced bioavailability, strong biological activity, and reduced toxicity compared to organic and inorganic selenium compounds[3,4]. Selenium is an essential micronutrient involved in redox homeostasis, immune modulation, thyroid function, and cellular signaling through its incorporation into selenoproteins[5,6]. However, conventional chemical synthesis methods for Selenium nanoparticles often require harsh conditions and toxic reagents raising environmental and safety concerns[7]. Green synthesis approaches offer eco friendly and cost effective alternatives by employing plant extracts as natural reducing and stabilizing agents[8]. These phytochemical rich extracts containing flavonoids, polyphenols, alkaloids, terpenoids, and proteins, facilitate nanoparticle formation under mild conditions while imparting additional therapeutic properties[9,10]. *Camptotheca acuminata*, known as the cancer tree is a medicinal plant native to China and is renowned for producing camptothecin, a potent topoisomerase I inhibitor with anticancer activity[11,12]. In addition to camptothecin, *C. acuminata* fruits contain diverse bioactive compounds exhibiting antioxidant, anti-inflammatory, and antimicrobial effects[13,14].Despite this rich phytochemical and pharmacological profile, the use of *C. acuminata* fruit extracts in nanoparticle synthesis remains largely unexplored[15,16]. The green synthesis of SeNPs using *C. acuminata* fruit extract offers advantages such as improved biocompatibility and multifunctionality with phytochemical capping contributing to synergistic antioxidant, anticancer, antimicrobial, and antidiabetic activities[17,18].In this study selenium nanoparticles synthesized using *C. acuminata* fruit extract (CASeNPs) were characterized by UV–Visible spectroscopy, Scanning Electron Microscopy (SEM), and FTIR to evaluate their physicochemical properties and potential biomedical applicability.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

The dried fruits of *Camptotheca acuminata* were procured from a commercial distributor in China via their online marketplace (Etsy). Sodium selenite metal salt was obtained from Sigma-Aldrich and used as the inorganic precursor for selenium nanoparticle synthesis.

2.2 Preparation of Selenium Nanoparticles Utilized Fruit

The fruit extract of *was* thoroughly washed with sterile distilled water to eliminate surface contaminants and then shade-dried under ambient conditions. The dried materials were ground into a fine powder using a mixer grinder. To prepare the fruit extract, 1g of material was suspended 100 ml of distilled water and boiled for 15 minutes until a noticeable color change occurred. The resulting mixture was filtered using Whatman No. 1 filter nanoparticle synthesis, 40ml of the fruit extract (acting as the reducing agent) was mixed with a precursor solution prepared by dissolving 0.516 g of sodium selenite in 60 ml of distilled water. The final reaction mixture (100 mL) was incubated in a shaking incubator for three days to facilitate complete reduction of sodium selenite and stabilization of the selenium nanoparticles.

2.3 Characterization of Camptotheca Selenium Nanoparticles

The Selenium Nanoparticles (SeNPs) synthesized from Camptotheca were characterized utilizing various analytical techniques. UV–spectroscopy was performed a Shimadzu UV– spectrophotometer (190–1100nm) to confirm nanoparticle formation. The surface morphology and elemental composition of the SeNPs were examined via field emission scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (ZEISS Sigma 300). Images were acquired at magnifications ranging from 20× to 50×, with a step size of 1–10nm and a scan speed of 1min^{1}. groups associated with the nanoparticles were identified using Fourier-transform infrared spectroscopy (Agilent Cary 630 spectrometer). Spectra were recorded in the mid-infrared range of 4000–650 cm^{1} at high resolution.

2.4 Biomedical Applications

2.4.1 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) Assay

The procedure was carried out based on the free radical scavenging activity of SeNPs, and ascorbic acid standard was measured stable DPPH radical. To 1ml of various concentrations (10, 20, 30, 40, and 50 µg/mL) of SeNPs,1mL of the freshly prepared DPPH (1mm in methanol) solution was added and thoroughly vortexed. The mixture was then incubated at room temperature in the dark for 10 min. The absorbance of the product was measured at 532 nm a Shimadzu UV–spectrophotometer (190–1100nm). A control sample, DPPH (all reagents except the sample), and a blank solution of methanol where the antioxidant activity of the DPPH assay was reported as the percentage of inhibition using the formula:

$$Inhibition(\%) = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ control} \times 100$$

2.4.2 Hydroxyl radical (OH–) Scavenging Activity

Hydrogen peroxide (H_2O_2) scavenging activity of SeNPs was evaluated using ascorbic acid as the standard antioxidant. H_2O_2 was freshly prepared and stabilized in PhosphateBuffered Saline (PBS) to serve as a source of reactive oxygen species. Briefly, 1ml of SeNPs at different concentrations (10, 20, 30, 40, and 50µg/ml) was mixed with 1mlof 6% H_2O_2 prepared in PBS (0.7m). The reaction mixtures were incubated at 37°C in the dark for 5min to minimize photodegradation. After incubation, the absorbance was measured at 532nm using a Shimadzu UV–Vis Double Beam Spectrophotometer (190–1100nm). A H_2O_2 control containing all reagents except the sample and a PBS blank were included in each run. The percentage H_2O_2 scavenging activity was calculated using the following equation:

$$Inhibition(\%) = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ control} \times 100$$

2.5 Anti-inflammatory Activity

2.5.1 Bovine Serum Albumin Denaturation Assay

BSA assay was used to assess the worth of using a Bovine Serum Albumin denaturation assay. A stock solution of BSA was prepared by mixing 0.5g of BSA in 50ml water. For the assay, 1ml of BSA stock was mixed with different concentrations of SeNP (10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL). Then 1ml of phosphate buffered saline (PBS, ph 7.4). The mixture was incubated at 37°C for 20 minutes to allow interaction, followed by heating at 55°C for 10 minutes to induce protein denaturation. After cooling the samples to room temperature, the absorbance was measured at 660nm using a UV– spectroscopy Shimadzu UV-Vis Double Beam Spectrophotometer (190–1100nm). PBS was used as the negative control, and diclofenal as the standard anti-inflammatory agent of SeNPs. The extent of protein denaturation inhibition was quantitatively assessed using the:

$$Inhibition(\%) = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ control} \times 100$$

2.5.2 Heat Induced Denaturation Assay Using Egg Albumin

Selenium Nanoparticles (SeNPs) were evaluated using an Egg Albumin (EA) denaturation assay. A stock solution of EA was prepared by dissolving 50ml of EA in 50 mL of 1XPBS. For the assay, 1ml of the EA were mixed with different concentrations of SeNP (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml) suspension. The mixture was incubated at 37°C for 20 minutes to allow interaction, followed by heating at 55°C for 10 minutes to induce protein denaturation. After cooling the samples to room temperature, the absorbance was measured at 660nm using a UV– spectroscopy Shimadzu UV-Vis Double Beam Spectrophotometer (190–1100nm). PBS as the negative control and served as the standard anti-inflammatory agent in place of SeNPs. The extent of protein denaturation inhibition was quantitatively assessed using the formula provide below:

$$Inhibition(\%) = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ control} \times 100$$

2.6 α-Amylase Inhibitory Activity Assay

Invitro α-amylase inhibition studies were performed as 100µL from the various concentrations of CASeNPs (10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL) in means of different test tubes was taken. Subsequently, 100µl of α-amylase enzyme and 100µl of 2 mM phosphate

buffer (ph 6.9) were added to each. After 10 min incubation, 100µl of 1 % starch solution. The same was repeated with the control samples (400 µl phosphate buffer) without enzyme. Dinitrosalicylic acid reagent was added to the control and test after 10min of incubation. The reaction mixture was kept in a 70°C boiling water bath for 5min and the absorbance was determined at 540nm a spectrophotometer percentage of α-amylase enzyme inhibition was determined the following formula:

$$Inhibition(\%) = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ control} \times 100$$

2.7 Antibacterial Activity

The antibacterial activity of the synthesized Selenium Nanoparticles (SeNPs) was evaluated by the agar well diffusion method against standard reference bacterial strains, including Grampositive *Staphylococcus aureus* (ATCC 25923) and Gramnegative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Mueller–Hinton Agar (MHA) plates were prepared by dispensing 30ml of sterilized medium into sterile Petri dishes and allowing it to solidify, after which 6mm wells were aseptically punched into the agar surface. SeNP suspensions at concentrations of 25, 50, and 100 µg/ml were loaded into wells A, B, and C, respectively; amoxicillin (10 µl) served as the positive control in well D, and sterile distilled water was used as the negative control. The plates were incubated at 37°C for 24h, and antibacterial activity was quantified by measuring the diameter of the Zones of Inhibition (ZOI) in millimeters around each well. All assays were carried out in triplicate and data were reported as mean ± Standard Deviation (SD).

2.8 Culture of A549 and HEk Cells

A549 and HEK cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotic solution. The cultures were maintained at 37°C in a humidified incubator with 5% CO_2, and the culture medium was renewed every three days to sustain optimal cell viability and proliferation.

2.9 Anticancer Activity

2.9.1 MTT Assay for Determination of Cell Viability

This Method of cytotoxic effects of Selenium Nanoparticles (SeNPs) used *Camptotheca acuminata* plant extract were evaluated using the MTT assay. A549 and HEK cell lines were seeded into 96-well plates at a of 5,000 – 10,000 cells per well and incubated at 37°C with 5% CO_2 for 24 to allow for cell attachment. Stock solutions of the test samples (1mg/ml) were prepared in Dulbecco's Modified Eagle Medium filtered through a 0.22µm syringe filter for sterility and diluted to working concentrations of 20, 40, 60, 80, and 100 µg/ml using DMEM.After 24 hours of treatment with the test samples 10 µl of MTT solution (5mg/mL in PBS) was added to each well and the plates were incubated for an additional 3 to 4 hours at 37°C. Following incubation, the medium was carefully discarded and 100µl of DMSO was added to each well to dissolve the purple formazan crystals formed by metabolically active cells. The absorbance of the wells was measured at 570nm. Cell viability was calculated by using the following formula:

$$Cell\ viability(\%) = \frac{OD\ value\ of\ the\ treated\ sample - OD\ value\ of\ the\ blank}{OD\ value\ of\ the\ control\ (untreated\ cell) - OD\ value\ of\ the\ blank} \times 100$$

2.10 Statistical Analysis

All experiments were performed in triplicate, and the data are expressed as mean ± Standard Deviation (SD). Group differences were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post hoc test for multiple comparisons, with $p < 0.05$ considered statistically significant. Statistical analyses were carried out using SPSS software.

3. Results and Discussion

The initial confirmation of Selenium Nanoparticle (SeNP) synthesis using *C. acuminata* fruit extract was evidenced by a pronounced visible color change in the reaction mixture. As illustrated in the aqueous extract of *C. acuminata* initially displayed a light brown coloration. Following the addition of the selenium precursor, sodium selenite (Na_2SeO_3), and incubation under controlled conditions (37°C, 150 rpm, 24 h), the solution gradually transitioned to a characteristic orange red. This distinct visual transformation serves as a hallmark indicator of successful nanoparticle

formation through green synthesis, signifying the reduction of Se (IV) ions to elemental Se (0). The observed color shift arises from the Surface Plasmon Resonance (SPR) phenomenon inherent to Selenium Nanoparticles, modulated by factors such as particle size, shape, and concentration within the colloidal dispersion. Phytochemicals abundant in *C. acuminata* fruit extract including flavonoids, phenolic acids, and terpenoids facilitate bio reduction and stabilization. These bioactive metabolites donate electrons to Se (IV) ions, enabling their reduction to Se (0) and concurrently capping the nascent nanoparticles to impart colloidal stability. This biogenic approach obviates the use of noxious chemical reductants, thereby highlighting the eco-friendliness and sustainability of the protocol.

3.1 UV–Visible Spectrophotometer Analysis

The formation of selenium nanoparticles was further confirmed by UV–spectroscopy. The spectrum (Figure 1) demonstrates a strong absorption peak for the synthesized nanoparticles (SeNPs) while negligible absorbance across the measured spectrum confirms that the peak was not due to any components in the blank medium. The sample showed a broad absorbance band with a sharp decrease starting 390nm and gradually tapering off towards 650nm. This UV-Vis profile is characteristic of nanoparticles which typically exhibit strong absorbance in the UV region due to surface resonance phenomena associated with nanoscale selenium[21,22]. The absence of sharply defined peaks may be attributed to the polydisperse nature or amorphous surface of the nanoparticles synthesized using a biological route. The absorbance intensity of nearly 4.0 units at lower wavelengths suggests a high concentration or efficient synthesis of nanoparticles. This further supports the role of *C. acuminata* fruit extract as a precursor and reducing agent in formation.

3.1.1 Morphological and Elemental Composition Analysis of CASeNPs

The morphology of Selenium Nanoparticles (SeNPs) synthesized using *C. acuminata* fruit extract was investigated by Scanning Electron Microscopy (SEM) coupled with Energy-Dispersive X-ray Spectroscopy (EDX). SEM micrographs recorded at appropriate magnifications (Figure 2 A–C; 5,000×, 10,000×, and 20,000×) revealed that the biosynthesized SeNPs were predominantly spherical to quasi spherical in morphology, with smooth, well-defined surfaces and minimal aggregation[23,24]. The nanoparticles exhibited a relatively uniform distribution with particle sizes ranging from 50–100 nm, which is consistent with values typically reported for plant-mediated selenium nanoparticle synthesis. The limited aggregation observed suggests an effective stabilizing and capping effect exerted by phytochemical constituents of the *C. acuminata* extract, including flavonoids, polyphenols,

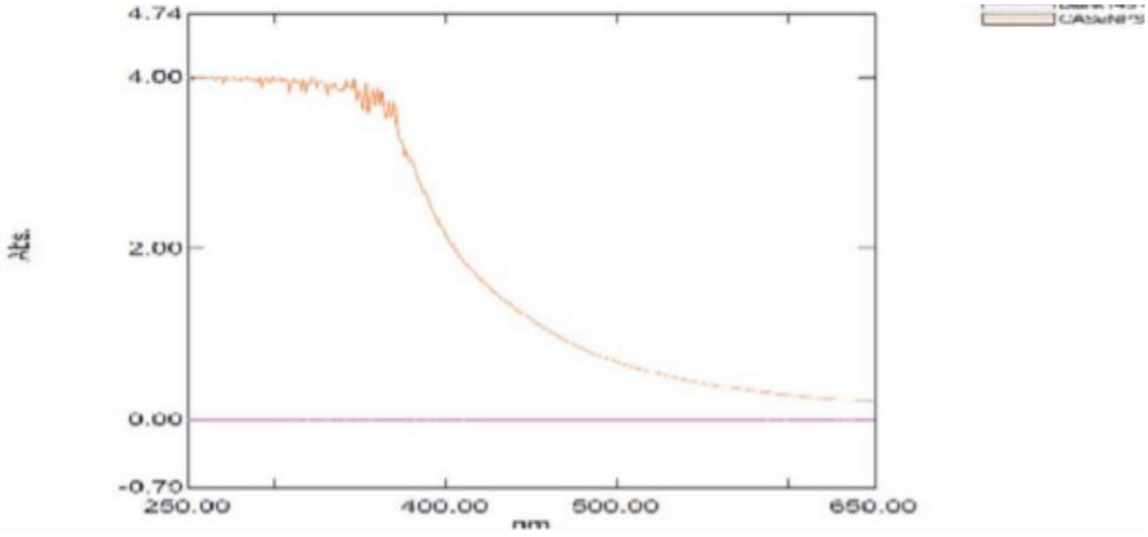


Figure 1. UV spectroscopy; Absorption of Selenium nanoparticles are utilized with *Camptotheca acuminata* fruit extract recorded at 24hr time intervals.

and alkaloids, which function as reducing and stabilizing agents during nanoparticle formation[25,26]. High-magnification images (20,000×) clearly illustrated the nanostructured surface and structural integrity of the particles, confirming successful biosynthesis. In several micrographs, a faint porous or amorphous matrix surrounding the nanoparticles was observed, which is attributed to organic capping layers derived from plant metabolites. These biomolecules likely prevent particle agglomeration and contribute to enhanced colloidal stability of the SeNPs[27]. Elemental composition analysis using EDX (Figure 2D) further confirmed the successful synthesis of selenium nanoparticles. The EDX spectrum exhibited a strong characteristic peak at approximately 1.37kev, corresponding to elemental selenium (Se^0), thereby verifying the effective reduction of selenium ions (Se^{4+}) during the green synthesis process. The dominance of the selenium peak indicates a high selenium content and purity of the synthesized nanoparticles[28]. Minor peaks corresponding to Carbon (C) and Oxygen (O) were also detected, which are attributed to bio-organic residues from the *C. acuminata* extract adsorbed onto the nanoparticle surface, forming a natural capping layer. Importantly, no peaks corresponding to toxic elements or heavy metal contaminants were observed, confirming the environmentally benign and contamination-free nature of the synthesis method. The presence of organic surface functionalization is advantageous, as it enhances nanoparticle biocompatibility and stability, which are critical parameters for biomedical applications. Overall, the combined SEM and EDX analyses confirm the formation of high-purity, stable, nanoscale Selenium Nanoparticles with uniform morphology and clean elemental composition. These physicochemical characteristics support their suitability for downstream biomedical applications, including antioxidant, antidiabetic, and anticancer studies. The results collectively validate the successful green synthesis of SeNPs using *Camptotheca acuminata* fruit extract.

3.2 FTIR Analysis

FTIR was surface chemistry of biosynthesized Selenium Nanoparticles (SeNPs) and to identify the functional groups involved in their reduction and stabilization. The FTIR spectrum (Figure 3) exhibits prominent absorption bands across the 4000–500cm^{-1} range, reflecting the presence of diverse phytochemicals from *Camptotheca acuminata* extract broad absorption peak at 3272.6 cm^{-1} is assigned to O–H stretching vibrations, indicative of hydroxyl groups from alcohols and phenols, which are abundant in plant extracts and may serve as primary reducing agents during nanoparticle synthesis[29,30]. This band may also correspond to N–H stretching from amine groups, suggesting the involvement of proteins or amino acids as stabilizing agents[31].The band at 2922.2 cm^{-1} corresponds to C–H stretching of aliphatic – CH_2 groups, typically originating from lipids or other hydrocarbon chains present in the extract matrix. A minor peak at 2370.6 cm^{-1} is attributed either to Se–H bonding or atmospheric CO_2, which can

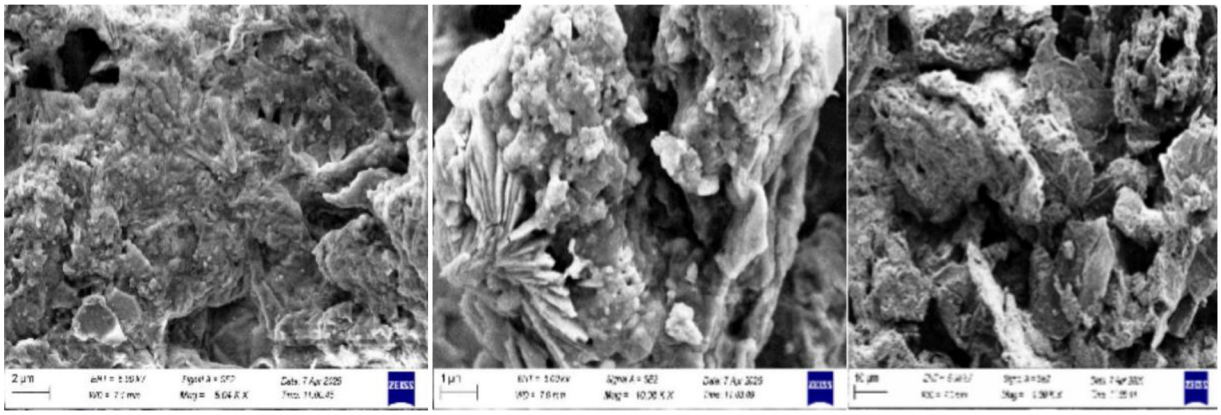


Figure2. SEM with EDX image; Morphological characterization analysis of Selenium nanoparticles synthesized using a *Camptotheca acuminate* fruit extract SEM with EDX image.

occasionally appear in dried FTIR samples[32].Sharp peaks observed at 1602.8 cm^{-1} and 1401.5 cm^{-1} are attributed to C=O stretching (amide I) and N–H bending (amide II) vibrations, respectively, reflecting the presence of proteinaceous capping agents that facilitate nanoparticle stabilization via electrostatic and hydrogen bonding interactions[33,34].Several distinct absorption bands in the region 1300–1000 cm^{-1} (1319.5, 1244.9, 1099.6, and 1032.5 cm^{-1}) are characteristic of C–O and C–N stretching vibrations, which may originate from flavonoids, polyphenols, and aromatic amines. These compounds are not only involved in nanoparticle capping but also contribute to the biological activity of SeNPs by enhancing antioxidant and radical-scavenging functions[35].The peaks at 782.7 cm^{-1} and 723.1 cm^{-1} represent C–H out-of-plane bending of aromatic rings, reinforcing the presence of aromatic phytochemicals.

3.3 Antioxidant Assays

3.3.1 Antioxidant Activity of Selenium Nanoparticles (Senps) Using A Camptotheca acuminata Fruit Extract

The antioxidant of selenium nanoparticles (SeNPs) synthesized using *Camptotheca acuminata* fruit extract was assessed DPPH and H_2O_2 radical scavenging assays. As illustrated in Figures 4 and 5 both assays demonstrated a concentration dependent increase in antioxidant activity for the SeNPs which was comparable to that of the standard antioxidant compound across the tested range (10 to 50µg/ml).

3.3.2 DPPH Assay

The DPPH assay results (Figure 4) show that SeNPs present potent free radical scavenging activity with 68% inhibition at 10µg/mL, rising to 95% at 50µg/ml. The standard showed similar inhibition levels, ranging from 65% 93%. They can be attributed to their ability to a hydrogen or electron to the DPPH radical thereby neutralizing its unpaired electron. The enhanced effect is likely due to the bioactive secondary metabolites from *C. acuminata* that serve as precursors and reducing during nanoparticle synthesis[36]. These compounds not only stabilize the nanoparticles but may also improve their redox potential. The DPPH assay results the robust antioxidant potential of SeNPs, with activity levels comparable to standard antioxidants. This highlights their as natural biocompatible agents for combating oxidative stress and supports their application in pharmaceutical and nutraceutical formulations targeting oxidative damage.

3.3.3 H_2O_2 Assay

The H_2O_2 scavenging activity (Figure 5) further confirmed the antioxidant potential of synthesized SeNPs. At 10µg/ml, SeNPs inhibited 62% of H_2O_2 radicals, increasing to 89% at These values closely aligned with the standard, which also showed a steady

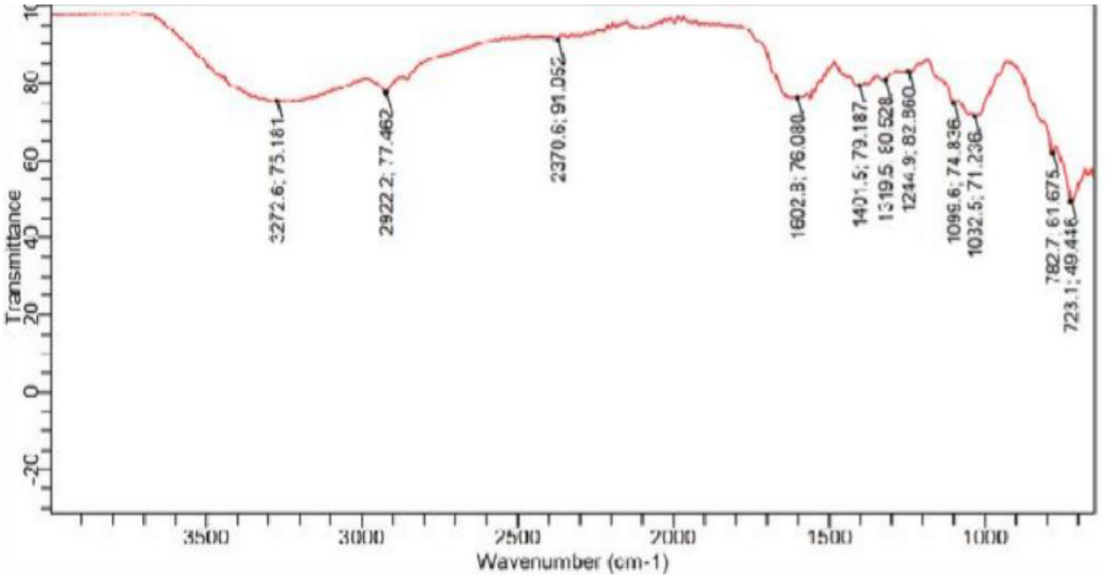


Figure 3. FT-IR spectroscopy; Selenium nanoparticles synthesized using *Camptotheca acuminate* fruit extract.

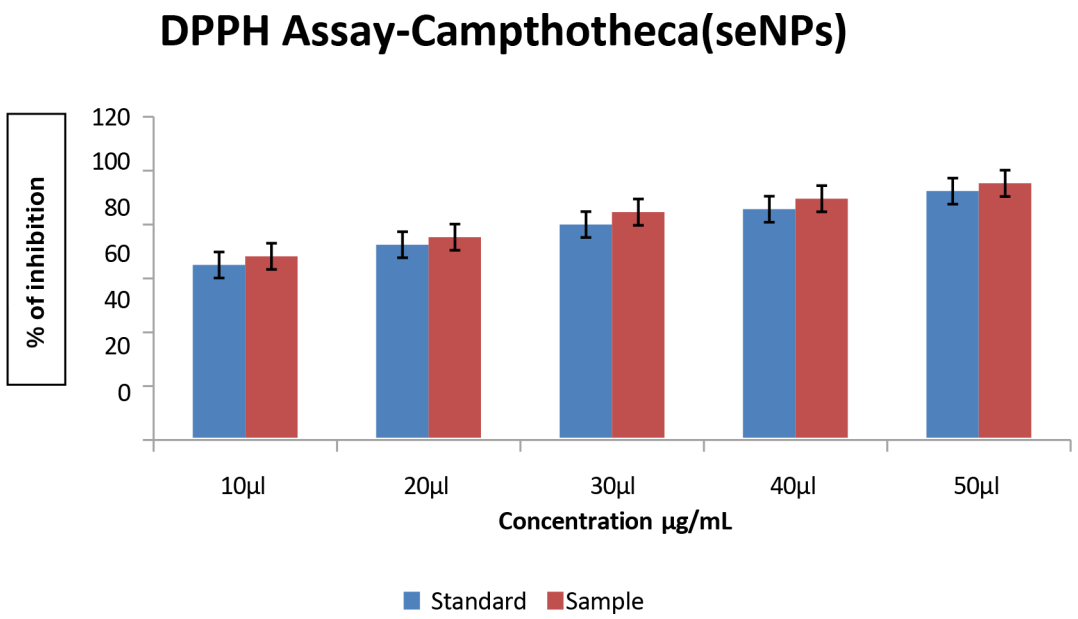


Figure 4. DPPH radical scavenging activity of selenium nanoparticles synthesized using *Camptotheca acuminata* fruit extract (CASeNPs). Data are expressed as mean ± SE. Statistical significance was evaluated at $p < 0.05$ and $p < 0.001$.

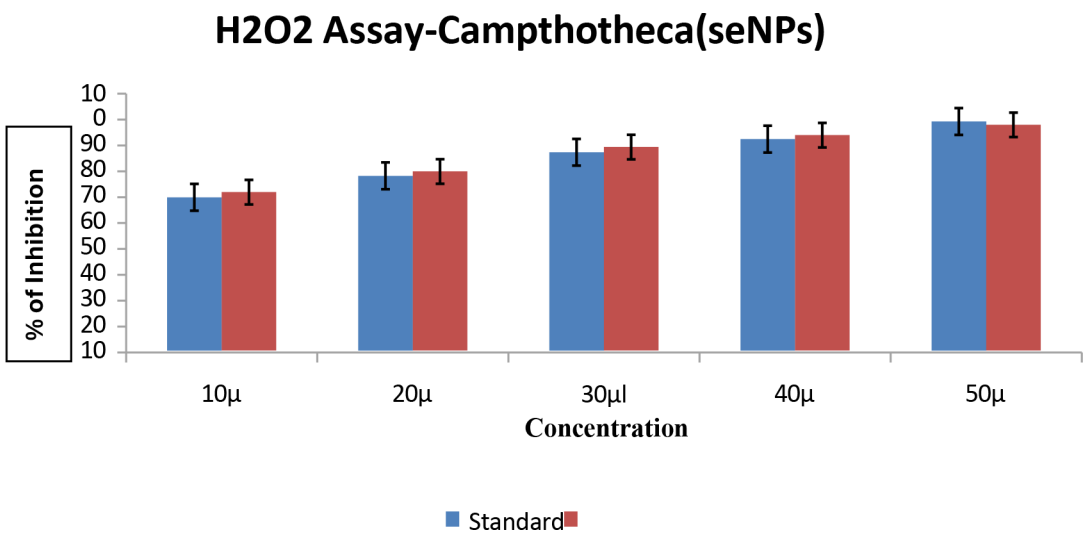


Figure 5. H_2O_2 Assay; Antioxidant activities of Selenium nanoparticles using a *Camptotheca acuminata* fruit extract (H_2O_2).The error bar values are analysed as mean ± SE with Significance differences were indicated as p 0.05 and $p <$ 0.001).

increase in inhibition across concentrations. Hydrogen peroxide is a reactive oxygen that can produce highly reactive hydroxyl radicals in the presence of transition metals through reactions.Therefore, the of SeNPs to neutralize H_2O_2 highlights their potential to reduce oxidative stress, which is implicated in various chronic and degenerative diseases[37]. The remarkable scavenging efficiency of SeNPs can be attributed to which enables it to cycle between different oxidation states and facilitate the neutralization of ROS. Furthermore, the high S/V ratio and nanometric size of SeNPs promote effective with oxidative species enhancing their overall antioxidant performance. In summary the ability of Camptotheca derived SeNPs to effectively scavenge hydrogen peroxide deepens their strong potential to counteract oxidative stress. This supports their applicability in preventing or managing conditions associated with oxidative stress, such as

neurodegenerative, heart diseases, and disorders. The combination of selenium's intrinsic redox activity and the bioactivity of *C. acuminata* phytoconstituents these nanoparticles as promising candidates for therapeutic antioxidant interventions.

3.4 Anti-inflammatory Activity of Camptotheca-derived Selenium Nanoparticles

The anti-inflammatory potential of biosynthesized SeNPs using *C. acuminata* fruit extract using egg albumin and bovine serum albumin denaturation assays which are confirmed in vitro models for screening anti-inflammatory agents[38]. Inflammation is a complex biological response triggered by harmful, protein denaturation plays a significant role.

3.4.1 Egg Albumin Denaturation Assay

These assays were denaturation of egg albumin, which was effectively inhibited by SeNPs in a manner (Figure 6). At a volume of 50 µl the inhibition percentage reached 86.4%, slightly lower than the standard drug's inhibition of 93%. At lower concentrations (10–30 µl) SeNPs exhibited inhibition levels that were comparable or even superior to the standard. The inhibition of albumin denaturation is considered a strong indicator of anti-inflammatory activity as denatured proteins trigger autoimmune responses by exposing new antigenic sites[39,40]. The protective effect of SeNPs suggests they may interfere with protein unfolding and aggregation under thermal stress likely due to their surface interactions and antioxidant capacity which helps stabilize protein structures. The significant inhibition of egg albumin denaturation by *Camptotheca* derived SeNPs highlights their strong anti-inflammatory potential. The results suggest that these nanoparticles can effectively interfere with protein denaturation mechanisms connected with inflammatory conditions further supporting their application as biocompatible, plant-mediated anti-inflammatory agents.

3.4.2 Bovine Serum Albumin Denaturation Assay

Similar were observed in the BSA denaturation assay (Figure 7). SeNPs exhibited dose dependent inhibition achieving 87% inhibition at 50 µl compared to 92% by the standard. These results support the anti-inflammatory potential of SeNPs further revealing their ability to protect protein integrity against thermal stress. The inhibition of BSA denaturation may involve hydrogen bonding and surface interactions that stabilize preventing denaturation[41,42]. The role of phytochemicals from *C. acuminata* in limiting and stabilizing nanoparticles also likely enhances the biological efficacy of the SeNPs by providing additional anti-inflammatory benefits. The findings align with previous reports that highlight the importance of nanoparticle protein interactions in modulating inflammatory responses. By preserving protein

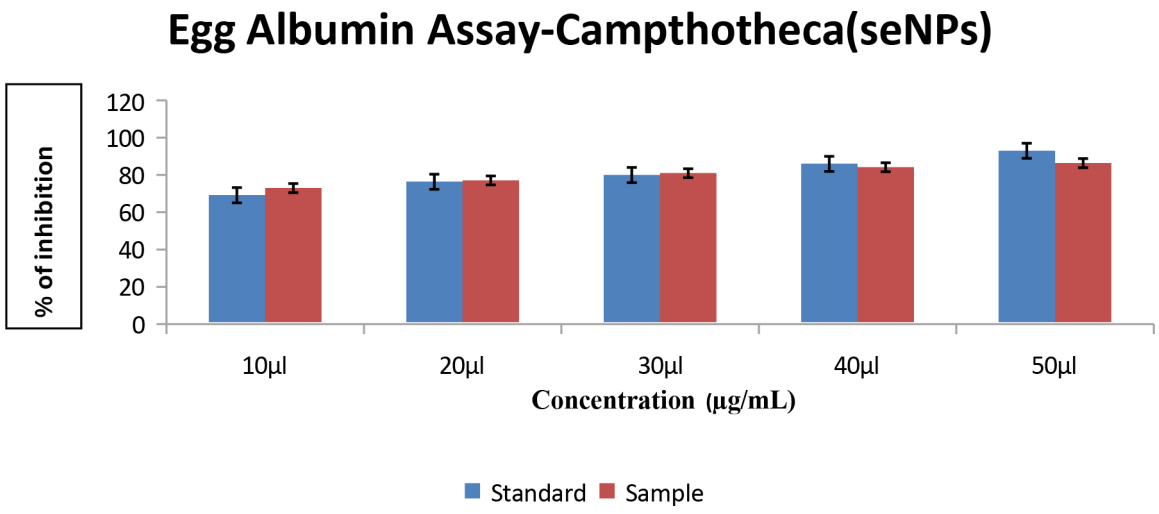


Figure 6. Egg Albumin Assay; Anti-inflammatory activity of selenium nanoparticles synthesized using *Camptotheca acuminata* fruit extract was evaluated by an egg albumin protein denaturation assay. The data are presented as mean ± standard error (SE), and statistically significant differences between treatment groups and controls are denoted at $p < 0.05$ and $p < 0.001$.

integrity under stress SeNPs could potentially reduce inflammation related damage in biological systems. The BSA denaturation assay confirms the anti-inflammatory power of *Camptotheca* derived SeNPs with performance comparable to standard drugs. This result, combined with the biogenic synthesis route and eco-friendly profile, highlights their potential as therapeutic agents in treating inflammatory disorders.

3.5 Antibacterial Activity Assay

The rapid rise of multidrug-resistant bacterial pathogens has intensified efforts to identify novel and effective antimicrobial agents. In this context, Selenium Nanoparticles(SeNPs),particularlythosebiosynthesized using plant extracts, have gained increasing attention due to their distinctive physicochemical properties and favorable biocompatibility[43,44]. In the present study, the antimicrobial activity of SeNPs synthesized using *C. acuminata* leaf extract was evaluated against three clinically relevant bacterial strains: *S. aureus* (Gram-positive), *Escherichia coli*, and *Pseudomonas aeruginosa* (Gram-negative) using the agar well diffusion method. As shown in Figure 8 and Table 1, the synthesized SeNPs exhibited clear concentration-dependent antibacterial activity against all tested strains. *S. aureus* demonstrated the highest sensitivity, with inhibition zones reaching 25.33mm at 100µg/ml of SeNPs. In contrast, *E. coli* and *P. aeruginosa* showed comparatively smaller zones of inhibition at equivalent concentrations. The observed differential susceptibility between Gram-positive and Gram-negative bacteria may be attributed to variations in cell wall architecture[45,46]. Gram-negative bacteria possess an outer membrane enriched with lipopolysaccharides, which acts as an additional barrier to nanoparticle penetration. Conversely, Gram-positive bacteria lack this outer membrane and possess a thicker yet more permeable peptidoglycan layer, potentially facilitating greater nanoparticle interaction and intracellular access[47]. These findings are consistent with previous studies reporting enhanced antibacterial efficacy of SeNPs against Gram-positive organisms[48]. The antimicrobial activity of SeNPs may be mediated through multiple mechanisms, including reactive oxygen species generation, disruption of bacterial membrane integrity, and interference with DNA and protein synthesis however these mechanisms are proposed based on existing literature and were not directly investigated in the present study. Additionally, the phytochemicals derived from *C. acuminata*, such as flavonoids, tannins, and alkaloids, likely contribute to the enhanced antibacterial performance. These bioactive compounds act as reducing and stabilizing agents during nanoparticle synthesis and may synergistically associate with SeNPs to augment their bactericidal activity. Overall, the findings of this study highlight the broad-spectrum antibacterial potential

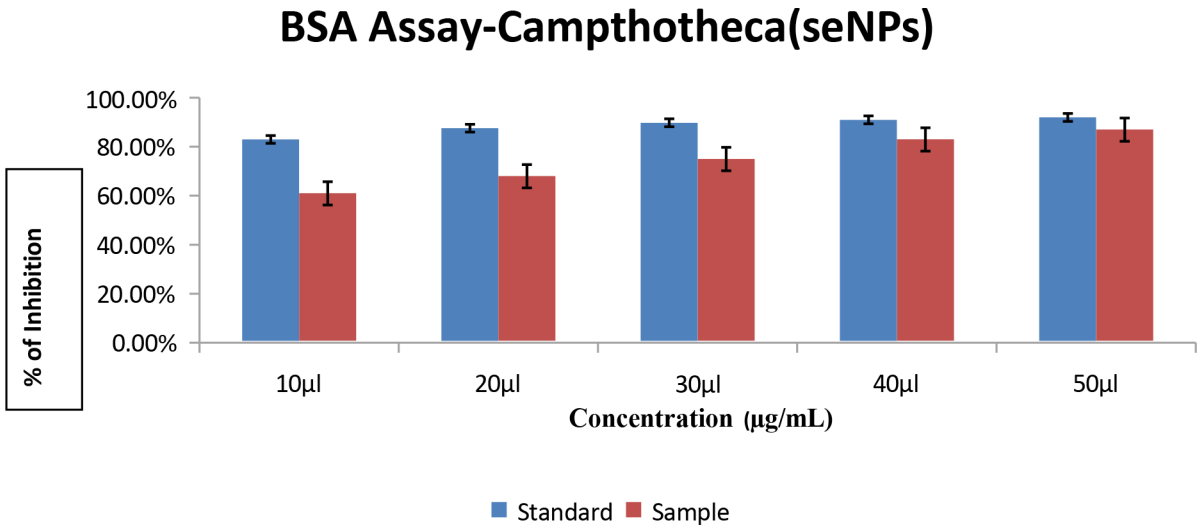


Figure 7. BSA Assay; Anti-inflammatory activity of *Camptotheca acuminata*–derived SeNPs was assessed using the BSA protein-denaturation assay. Results are expressed as mean ± SE, with significant differences indicated at $p < 0.05$ and $p < 0.001$.

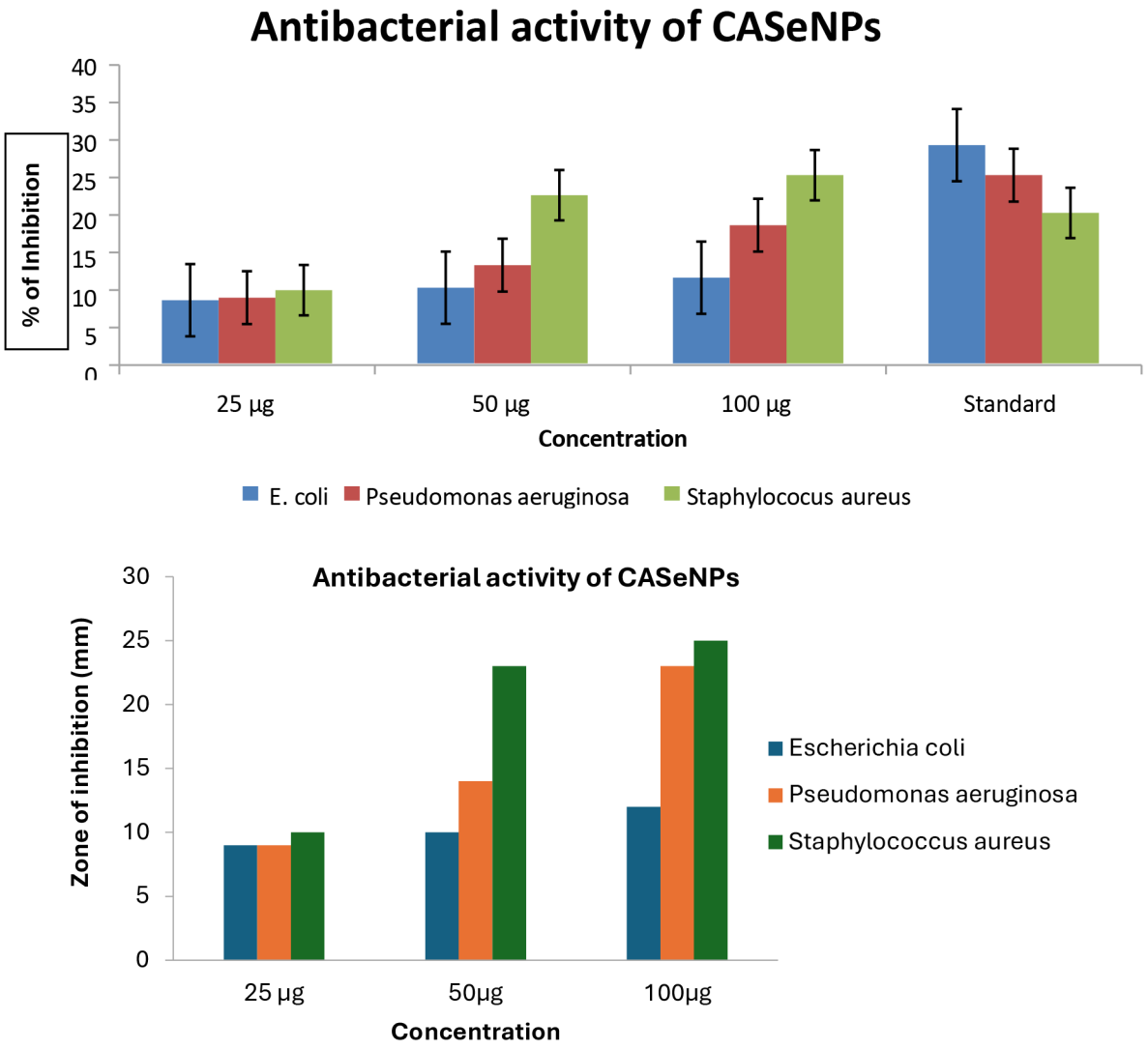


Figure 8. Antibacterial Activity; Antibacterial activity of CASeNPs against Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria was evaluated at concentrations of 25, 50, and 100 µg/mL. Amoxicillin was used as a reference standard and is presented separately. Values represent mean ± SD, and statistical significance was analyzed using one-way ANOVA.

Table 1. Antibacterial activity of *Camptotheca acuminata* derived selenium nanoparticles (CASeNPs) against Gram-positive and Gram-negative bacterial strains at different concentrations (25, 50, and 100 µg/mL), compared with the standard antibiotic amoxicillin. Data are expressed as mean ± SD and analyzed using one-way ANOVA

	Zone of inhibition (mm) (M.S.D)			
Concentration (µg/ml)	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	**P- Value**
25 **µg**	8.66+0.57	9+0	10+0	0.08
50 **µg**	10.33+0.57	13.33+1.15	22.66+0.57	<0.001*
100 **µg**	11.66+0.57	18.66+0.57	25.33+0.57	<0.001*
Standard	29.33+1.15	25.33+0.57	20.3+0.57	<0.001*
	<0.001*	<0.001*	<0.001*	

of *C. acuminata*-mediated SeNPs, particularly against Gram-positive pathogens. Their ability to inhibit bacterial growth through multiple plausible pathways positions them as promising plant-based antimicrobial candidates, especially in the context of escalating antibiotic resistance.

3.6 Antidiabetic Activity of A-Amylase Inhibition Assay

The potential of biosynthesized Selenium Nanoparticles (SeNPs) as antidiabetic agents was explored an *in vitro* α-amylase inhibition assay. This inhibition of is a widely accepted strategy for controlling postprandial hyperglycemia, a key complication Type 2 diabetes mellitus[49]. The SeNPs show dose dependent inhibition of α-Amylase activity with a maximum of 82% inhibition at 50µl, slightly exceeding the standard reference inhibitor, which achieved 80% under the same conditions (Figure 9). This indicates the ability of SeNPs to interfere with the enzymatic hydrolysis of starch into glucose. *α-amylase* is a key digestive enzyme that catalyzes the breakdown of complex carbohydrates. This inhibiting enzyme delays carbohydrate digestion and glucose absorption thereby preventing sharp increases in blood glucose levels after meals[50]. This powerful activity of SeNPs may be attributed to their ability to interact with the active sites of α-amylase potentially through surface-bound phytochemicals inherited from the SeNPs utilized fruit extract. These phytochemicals likely form hydrogen bonds or hydrophobic interactions with amino acid residues in the catalytic domain of the enzyme resulting in conformational changes that reduce enzymatic activity. *Selenium* known for its catalytic redox properties may further impact the enzyme structure through oxidative modulation or direct binding. The results promise the strong antidiabetic potential of SeNPs utilised fruit extract via effective *α-amylase* inhibition. The combination of biogenic synthesis, Phytochemical coating and *selenium* intrinsic properties underscores their promise as a novel plant based therapeutic approach for diabetes control. Further *in vivo* accuracy and mechanistic studies could support their development into functional antidiabetic agents or dietary supplements.

3.7 Cell Viability Assay

3.7.1 Cytotoxicity

oTo assess the biocompatibility and anticancer use of the biosynthesized *selenium* nanoparticles (SeNPs) a cell viability assay was conducted on both Human Embryonic Kidney (HEK) cells and Human lung carcinoma (A549) cells across a range of SeNP concentrations (20 to 100 µg/ml). The data (Table 2, Figure10) revealed a clear concentration dependent cytotoxic response with SeNPs exhibiting selective toxicity toward the A549 cancer cell line while maintaining minimal toxicity in normal HEK cells. HEK cell viability remained consistently high with values ranging from 90.66% at 20µg/ml to 83.13% at 100 µg/ml, suggesting strong biocompatibility and low Cytotoxicity. In contrast A549 cell viability significantly decreased from 65.74% to 46.66% across the same concentration range ($p < 0.001$) indicating a potent anticancer effect[51]. The IC_{50} value calculated using a four-variable logistic (4PL) model was approximately 108.19 µg/ml for A549 cells (Figure 3), expressing the concentration required to reduce cell viability by 50%. This selective Cytotoxicity range with previous findings that SeNPs induce reactive oxygen species mediated oxidative stress a risk to cancer cells due to their altered redox balance, increased metabolic activity and weakened antioxidant defenses[52]. Moreover, the enhanced endocytotic uptake of nanoparticles by malignant cells may contribute to this differential response leading to greater intracellular accumulation, mitochondrial dysfunction, and apoptotic cell death[53]. These mechanistic insights validate earlier reports on the role of SeNPs in triggering apoptotic pathways and DNA damage, particularly in cancer models. Collectively these results highlight the dual advantage of biosynthesized SeNPs' excellent safety profile in normal cells and potent selective Cytotoxicity against the cancer cells. These features underscore their potential utility in targeted nanomedicine applications and provide a promising foundation for future in vivo studies and therapeutic development.

3.7.2 Cell Inhibition Rate

To estimate the biocompatibility and anticancer benefits of the biosynthesized selenium nanoparticles (SeNPs), *in vitro* Cytotoxicity assays were performed

DNA damage, and apoptosis in cancer cells while sparing healthy cells [55]. Additionally the increased endocytic activity in tumor cells may facilitate greater uptake of nanoparticles further enhancing intracellular ROS accumulation and apoptotic signaling. The Phytochemical mediated green synthesis of SeNPs using likely contributes to their enhanced bioactivity. Bioactive plant compounds acting as functional agents may promote cellular uptake and complement the anticancer effects of SeNPs thereby improving their therapeutic potential.

3.8 Limitation

A key limitation of this study is the lack of *in vivo* validation and detailed functional mechanisms. While the *in vitro* assays demonstrate significant bioactivity of the selenium nanoparticles (SeNPs) using these results may not fully translate to complex biological systems due to differences in bioavailability, metabolism, immune responses, and potential toxicity. Additionally, the molecular mechanisms behind the observed activities, such as the pathways involved in *α-amylase* inhibition, anti-inflammatory action, and antimicrobial effects,

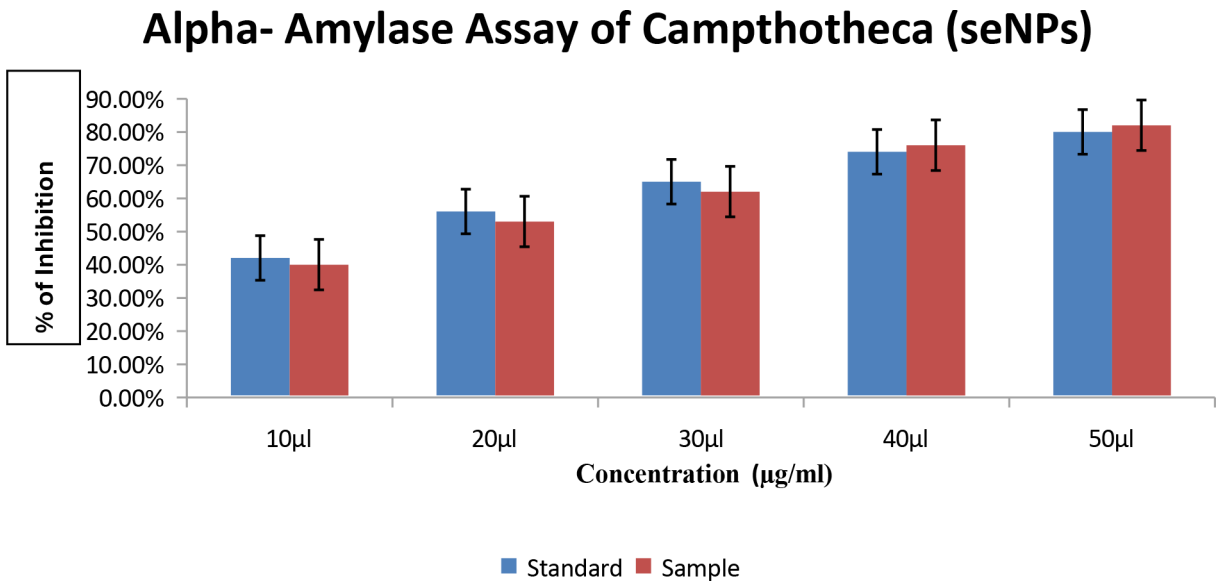


Figure 9. Antidiabetic activity; *Camptotheca acuminate* -mediated sodium selenite nanoparticles (Alpha-Amylase). The error bar values are analysed as mean ± SE with Significance differences were indicated as p 0.05 and p < 0.001).

Table 2. MTT assay results reporting cell viability (%) for HEK and A549 cell lines treated with different concentrations of CASeNPs (20–100 µg/mL) expressed as mean ± SD along with pvalues from oneway ANOVA and independent ttests. Cell viability in HEK cells remains high (~83–91%), whereas A549 cells show lower viability (~47–66%), and all pvalues are reported as <0.001, indicating statistically significant differences between treated groups and between the two cell lines

Concentration (µg/ml)	Cell Viability (%)				p-Value[b]
	HEK cell lines		A549 cell lines		
	Mean	SD	Mean	SD	
20	90.66117	4.092722	65.73711	9.570317	**<0.001***
40	90.16656	5.662169	56.0508	7.572637	**<0.001***
60	87.55803	5.046386	52.32727	5.686914	**<0.001***
80	84.20898	8.426456	47.20022	6.237819	**<0.001***
100	83.12553	0.905914	46.65535	4.469647	**<0.001***
p-Value[a]	**<0.001***		**<0.001***		

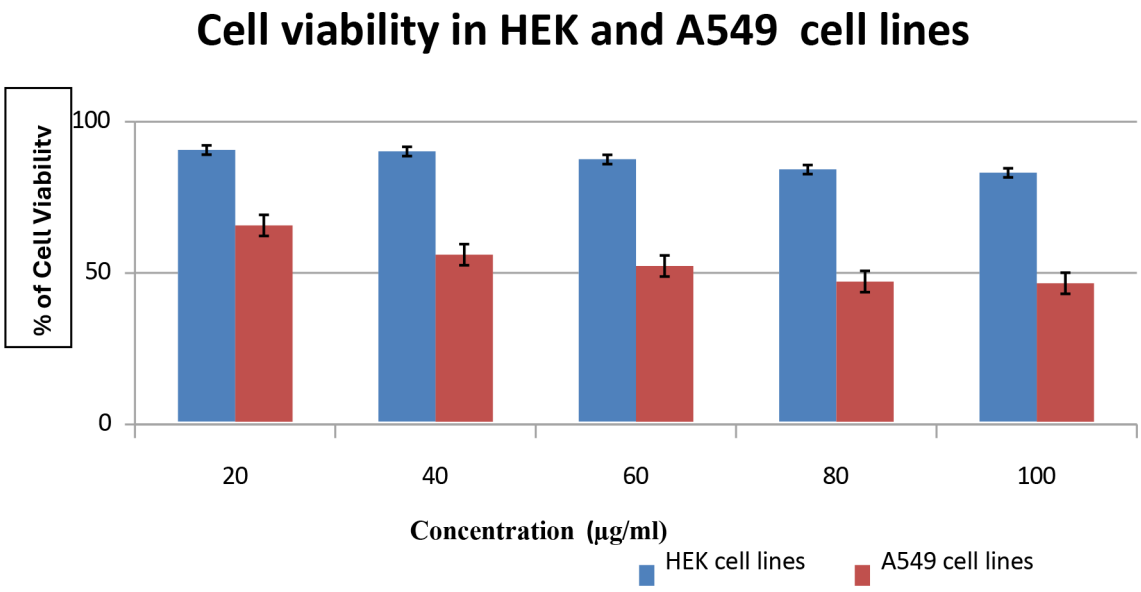


Figure 10. MTT Assay; Comparison of cell viability in HEK cell lines and A549 cell lines between used different concentrations a-One Way ANOVA; b- Independent sample t-test p-value <0.05-statistically significant.

Table 3. MTT assay showing Data represent mean ± SD (n=3). One-way ANOVA showed concentration-dependent effects ($p < 0.001$)^a. HEK cells demonstrate high viability (>83%) across all doses, while A549 shows marked cytotoxicity. IC50: HEK ~108.19 µg/mL; A549 ~20.64 µg/mL.

Concentration (µg/ml)	Cell inhibition (%)				p-Value
	HEK cell lines		A549 cell lines		
	Mean	SD	Mean	SD	
20	9.338829	4.092722	34.26289	9.570317	**<0.001***
40	9.83344	5.662169	43.9492	7.572637	**<0.001***
60	12.44197	5.046386	47.67273	5.686914	**<0.001***
80	15.79102	8.426456	52.79978	6.237819	**<0.001***
100	16.87447	0.905914	53.34465	4.469647	**<0.001***
p-Value	**<0.001***		**<0.001***		

using normal Human Embryonic Kidney (HEK) cells and human lung carcinoma (A549) cells treated with increasing concentrations of SeNPs (20–100 µg/ml). This result exhibits a visible concentration dependent response and marked selectivity of SeNPs toward cancerous cells (Table 3, Figure 11). HEK cells maintain high viability across all concentrations tested, with inhibition values ranging from 9.34% at 20µg/mL to 16.87% at 100 µg/mL indicating low Cytotoxicity and good biocompatibility. In contrast, A549 cells exhibited significantly higher inhibition rates increasing from 34.26% to 53.34% over the same concentration range. Statistical analysis confirmed these differences was highly significant ($p < 0.001$) supporting the selective Cytotoxicity of SeNPs toward malignant cells. To quantify this selectivity IC_{50} values were determined using nonlinear regression analysis (Figure 10). The IC_{50} for A549 cells was approximately 20.64µg/ml whereas for HEK cells it was higher around 108.19µg/ml. This large therapeutic window underscores the preferential Cytotoxicity of SeNPs toward cancer cells, with minimal toxicity to normal cells The observed selectivity is likely attributable to multiple mechanisms[4.] Cancer cells are characterized by heightened metabolic activity impaired redox regulation, and diminished antioxidant defenses, making them more susceptible to oxidative stress induced by SeNPs. SeNPs have been reported to generate ROS, triggering mitochondrial dysfunction,

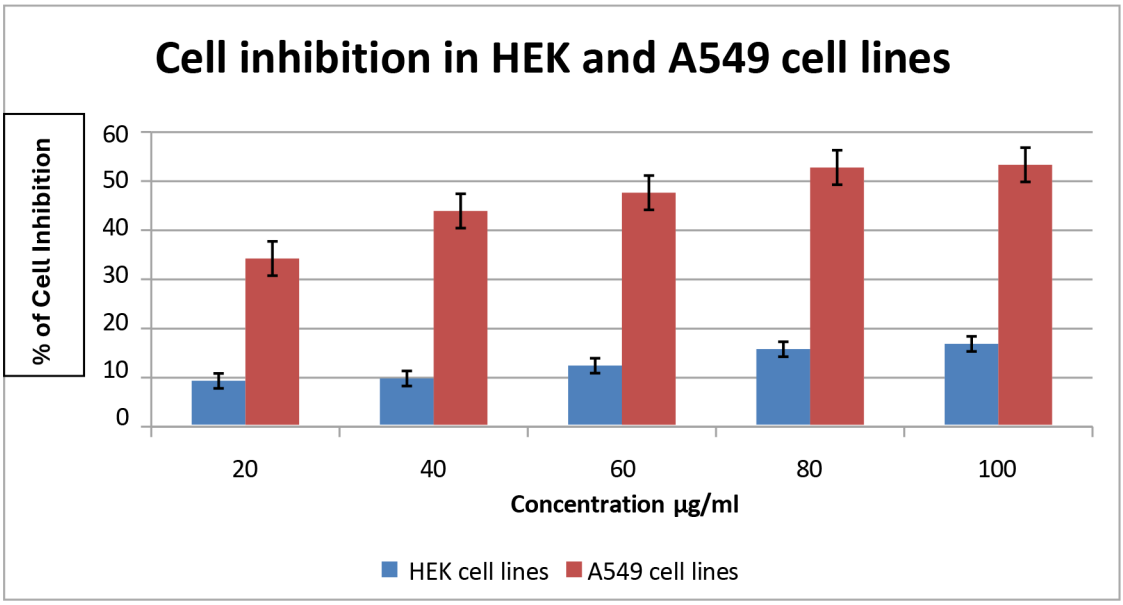


Figure 11. MTT Assay; Comparison of cell inhibition in HEK cell lines and A549 cell lines between different concentrations a-One Way ANOVA; b- Independent sample t-test p-value <0.05-statistically significant.

remain unclear. Without *in vivo* data and mechanistic studies, the therapeutic efficacy, biodistribution, and long-term safety of these SeNPs cannot be definitively established. Therefore, Future research should focus on animal models, pharmacokinetics, and molecular interaction studies to address these gaps and support clinical translation.

4. Conclusion

Camptotheca acuminata–derived selenium nanoparticles (CASeNPs) were successfully synthesized via a green, phytochemical-mediated route and exhibited stable nanoscale morphology. They showed strong antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory activities, with selective cytotoxicity toward cancer cells over normal cells. These findings indicate that CASeNPs are a promising multifunctional nanoplatform, warranting further *in vivo* studies and mechanistic investigations to assess their safety, pharmacokinetics, and clinical translation potential.

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Author Contribution

Performed experiments, Data collection and manuscript, statistical analysis, Yasin Begam Zainudeen; Conceptualization, supervision and revision of the manuscript Dr. Nalini Devarajan; Assisted in experimental design, data validation Uma Mageshwari Karmegam; Conducted nanoparticles data validation and interpretation, statistical analysis and critical revision of the manuscript Dr. Tharani Munusamy. All authors have read and agreed to the published version of the manuscript.