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Original Article

Tinospora cordifolia Stem Extract-mediated Green Synthesis of Selenium Nanoparticles and its Biological Applications

Abhijeet Puri*, Swati Patil

ABSTRACT

Background: According to a literature review *Tinospora cordifolia* possess alkaloids, tannins, flavonoids, glycosides, and steroids with anti-cancer potential. However, there is a lack of scientific evidence on the green synthesis of selenium nanoparticles from the stem of *T. cordifolia* and its biological activities. **Objectives:** This study is intended to synthesize Selenium Nanoparticles using *T. cordifolia* stem extract, characterise them, and evaluate them *in-vitro* for antioxidant and anticancer activity. **Materials and Methods:** The *T. cordifolia* Selenium Nanoparticles (TC-SeNPs) were synthesised by reducing selenious acid with *T. cordifolia* stem extract. TC-SeNPs were characterized by UV–Vis, FTIR, XRD, FE-SEM, EDAX and ICP-AES, further evaluated for *in-vitro* antioxidant activity by DPPH free radical scavenging test and reducing power capacity. The MTT assay of TC-SeNP was carried out *in-vitro* in the human breast cancer cells line (MCF-7) to determine the anticancer activity. **Results:** The UV-spectroscopy of TC-SeNPs showed maximum absorbance at 285 nm, which is the characteristic feature of SeNP. According to DLS, SEM, and TEM images, the size of TC-SeNPs was between 100-200 nm and was stable with a Zeta potential of -23.9mV. XRD analysis confirmed the crystallinity of TC-SeNPs. Selenium in TC-SeNP was verified in EDAX analysis (63.36% weight) and ICP-AES 68.08 ±12.2 µg/ml selenium content. The IC_{50} value 28.62 ± 0.63 µg/mL and EC_{50} (62.50 ± 1.21 µg/mL) values indicated that the TC-SeNPs possess significant antioxidant capacity. MTT assay showed TC-SeNPs were cytotoxic to MCF-7 cell with IC_{50} values (31.29 ± 0.22µg/mL), suggesting that TC-SeNPs display moderate cytotoxicity that could dose-dependently inhibit cell proliferation. **Conclusion:** Thus, experimental evidence provides insight into the successful synthesis of the selenium nanoparticle from the stem of *T. cordifolia,* its potential therapeutic value, and the prospect of developing an anti-cancer TC-SeNPs formulation.

Keywords: *Tinospora cordifolia*, Green synthesis, Selenium nanoparticles, Phytonanoparticles, Antioxidant, Anticancer.

Abhijeet Puri*, Swati Patil

Department of Pharmacognosy, Principal. K M Kundnani College of Pharmacy, Cuffe Parade, Mumbai, Maharashtra, INDIA.

Correspondence

Mr. Abhijeet Puri

Department of Pharmacognosy, Principal. K M Kundnani College of Pharmacy, Cuffe Parade, Mumbai-400005, Maharashtra, INDIA.
Email id: abhijeetp@sjipr.edu.in
ORCID iD:0000-0002-4537-4909

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INTRODUCTION

Natural resources have been extensively utilised to synthesize novel pharmaceuticals in the past two decades. There has been a surge in discovering new plant-based therapeutic compounds in recent years. Phyto Nanoparticles (PNPs) have proved their wide range of applications in biomedicine due to their extraordinary capacity to act as reducing and stabilizing agents.[1] Plant-mediated nanoparticle synthesis is considered a green chemistry technique due to the absence of harmful solvents or reagents. Simple, dependable, cost-effective, and ecologically friendly. Plant systems have garnered considerable attention as viable green alternatives to physical and chemical techniques for PNPs synthesis.[2] The phytoconstituents can function as controlled assemblies for nanoparticle formation and possess essential physical and chemical characteristics for green nanoparticle synthesis. As a result, PNPs synthesised via plant-mediated synthesis are unique nanoparticles with a novel use.[3] Nanotechnology syndicates the physical and chemical properties to create nanoparticles with a specific function. Nanoparticles reveal a wide array of a novel or enhanced features dependent on their size, shape, and orientation.[4] Nanomedicine-based technologies overcome issues associated with conventional medication delivery methods and provide increased safety. Nanomaterial formulations have been shown to increase various phytoconstituents' biological effectiveness and oral bioavailability significantly.[5]

Selenium nanoparticles (Nano-Se) are individual nanoparticles that drew attention of researchers with their exceptional biopotential, better biocompatibility, and low toxicity.[6] Selenium is a critical trace element that demonstrates superior anti-cancer efficacy in addition to disease-protective qualities due to its low toxicity.[7] Selenium plays a substantial role in the biosynthesis of herbs and living systems, and it may operate as an antioxidant enzyme, assisting in inhibiting

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cellular damage instigated by free radicals.[8] It is widely believed that Se compounds exert their anti-cancer activity primarily through their direct or indirect antioxidant capabilities, which help maintain the redox balance inside the cell and protect healthy cells from oxidative damage caused by reactive oxygen species (ROS).[9] Cardiovascular disease, cancer, rheumatoid arthritis, and cystic fibrosis all can be treated with selenium. In large concentrations, selenium oxyanions can be dangerous to humans; however, the toxicity is reduced when selenium is in its elemental form (Se).[10] Selenium nanoparticles are unique because they have a low surface area per unit volume and a high permeability, which boosts their utility when they act as ligands, enhancing affinity en route to their target.[11-12] *Tinospora cordifolia* (*T. cordifolia)* is well-known as "Guduchi" and has played a significant part in Indian traditional medical systems since ancient times. *T. cordifolia* is a critical element of Ayurvedic systems of healing. According to the literature, the stem bark of *T. cordifolia* can be beneficial in treating cancer when combined with milk. According to a literature survey, *T. cordifolia* has a diverse array of phytochemicals. The stems of *T. cordifolia* are primarily composed of alkaloids, glycosides, and steroids, all of these have anti-cancer properties.[13-14] As a result, this research intends to rationalize the fabrication of TC-SeNPs using phytoconstituents from *T. cordifolia* extract. Thus, it was hypothesized to synthesize the TC-SeNPs and characterize them using various techniques, including UV, FT-IR, SEM, DLS, ZP, XRD, EDX, TEM, and ICP-AES. Further, these fabricated SeNPs have also been studied for *in-vitro* antioxidant, antimicrobial, and anti-cancer activity.

MATERIALS AND METHODS

Plant Material

Dr. Jayananda Tosh, Senior Botanist, Research Guide SDSMS, College, Palghar, identified and authenticated *T. cordifolia* stems collected from Palghar District, Maharshtra, India. A sample specimen (V.C./155/2019-20) is deposited in the Pharmacognosy Department at Princ. K. M. Kundnani College of Pharmacy, Mumbai, Maharashtra.

Preparation of extract and Phytochemical Analysis

Fresh *T. cordifolia* stems were cleaned with deionized water to remove dust particles before being dried in the shade. Dried stems (20 g) were powdered separately and placed in a 500 mL deionized water beaker. The mixture was boiled for 15 min or until the aqueous solution turned yellow. The yellow mixture was then allowed to cool to room temperature and filtered with a Buckner funnel and Whatman No. 1 filter paper before being subjected to centrifugation for 5 min at 1500rpm. A portion of the crude extract was suspended in water and subjected to qualitative phytochemical screening.[15]

Synthesis of *Tinospora cordifolia* Selenium nanoparticles (TC-SeNPs)

For the green synthesis of TC-SeNPs, a required 300 mM selenious acid solution was prepared and agitated for 10 min. A measured volume of the aqueous stem extract was added dropwise with constant stirring. Then, 2 mL of 400 mM ascorbic acid was added, which helped initiate the reduction reaction. A small sample of the solution's reaction mixture (1 mL) was subjected to UV–Vis spectroscopy analysis during the interval. The mixture was incubated at room temperature for 24 hr. Upon completion of the incubation, 1 mL of this solution was analysed by UV-Vis spectroscopy. The reaction mixture was then centrifuged at 10,000 rpm for 20 min. After the complete reaction, the pellet was rinsed with deionized water and three times with ethanol before being dried overnight in an oven at the proper temperature. The red TC-SeNPs were suspended in PBS (pH 7.4) by ultra-sonication and centrifuged. The product formed in powder form was stored at 4-5°C and used for further analysis.[16]

Characterization Techniques

Ultraviolet (UV)-Visible analysis

The bio-reduction of selenious acid by aqueous stem extract of *T. cordifolia* was monitored using the colour of the reaction mixture. After synthesising the brick-red selenium nanoparticles, the absorbance was determined at 200 to 600 nm wavelengths using a UV spectrophotometer (Shimadzu UV-1900i). The Fourier Transform Infrared Spectroscopy (FT-IR) spectra of *T. cordifolia* stem extract and TC-SeNPs were determined at wavelengths ranging from 4000–600 cm^{-1}. Numerous reducing and stabilizing functional groups of *T. cordifolia* aqueous stem extract were identified and their involvement in the production of TC-SeNPs, utilising a Jasco FT/IR-4600 spectrometer linked to a single ATR accessory (Jasco ATR PRO ONE). X-Ray Diffractometer (XRD) (Model-D8 Advanced, BRUKER., and Germany) analysis was used to assess the crystallinity of the TC-SeNPs. Scanning Electron Microscopy (SEM) was utilized to investigate the shape, surface topology, and morphology of the produced TC-SeNPs. The elemental composition of TC-SeNPs was determined by energy-dispersive X-ray spectroscopy (EDAX). Scanning Electron Microscope (SEM) (Jeol 6390LA) was equipped with an EDX Detector OXFORD XMXN apparatus. The purity and elemental composition of the sample were determined using the atom percentage of metal in the EDAX analysis. The size distribution and average diameter of the TC-SeNPs were determined using dynamic light scattering (DLS) and zeta potential measurements. The size distribution of TC-SeNPs was determined using a Zetasizer Nano Series (Malvern Pananalytical Ltd., Malvern, UK). The hydrodynamic size of particles was determined using DLS by focussing monochromatic light on the solution, causing a Doppler shift. A wavelength shift occurs when a laser beam strikes moving particles and scatters the light at an angle. Scattering light angle is inversely related to particle size. Zeta potential was measured to calculate the surface charge of the TC-SeNPs. Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (SPECTRO ARCOS, Germany) was used to determine selenium levels at the ppm level qualitatively. A calibration curve for known selenium was created using a standard (Se) solution at 242.80 nm and the selenium concentration of TC-SeNPs was calculated. Three replicate experiments were done, and the mean (ppm) standard deviation was determined.[17-20]

Biological activities of TC-SeNPs

In-vitro Antioxidant Assay

DPPH radical scavenging activity

The DPPH free radical scavenging potential of TC aqueous extract, TC-SeNPs, Selenium (Se), and Standard Ascorbic acid (AA) was assayed at different concentrations (10, 20, 30, 40, 50, and 100 µg/mL). To every 1 ml of test solution, 2 ml of DPPH reagent was added and incubated in the dark for 10 min. The absorbance was measured at 517nm by a UV–Vis spectrophotometer (Shimadzu UV-1900i). The reaction mixture devoid of TC-SeNPs was used as a control, and ascorbic acid was used as a positive control.[21-23] The DPPH free radical scavenging activity was determined using the following formula: % RSA= $[(A - B)/A] \times 100$

Where A is the absorbance of the DPPH solution and B is the absorbance of TC-SeNPs.

Reducing power activity

The various concentrations of TC-SeNPs were analyzed by reducing Fe^{3+} to Fe^{2+} ions. For the reducing power assay, the absorbance was measured

at 700 nm using a UV–Vis spectrophotometer (Shimadzu UV-1900i), and ascorbic acid was used as a positive control.[24]

Antiproliferative activity

The MTT assay was used to evaluate cell proliferation and cytotoxicity of TC-SeNPs. Cell growth and cytotoxic activities were assessed using a colorimetric test technique. In this test technique, the yellow coloured water-soluble tetrazolium dye MTT is reduced to formazan crystals. This creates a purple colour when dissolved in a suitable solvent; the intensity of the colour is related to the number of viable cells, which may be quantified spectrophotometrically at 570nm.[25-28] Without the test agent, cell culture plates were seeded with 200µl cell suspension in a 96-well plate at the required cell density (20,000 cells per well). The cells were then allowed to grow for 24 hr. Appropriate concentrations of the TC, TC-SeNPs, Se and standard Doxorubicin were added to plates. The plates were incubated at 37°C for 24 hr in a 5% CO_2 atmosphere. After the incubation period, the MTT reagent was added to a final concentration of 0.5mg/mL of total volume. Then plates were enclosed in aluminum foil to evade exposure to light and then incubated for 3hr. Later M.T.T. reagent was withdrawn, and 100µL DMSO was added. Moderate stirring in a gyratory shaker improved dissolving and completely dissolved MTT formazan crystals. The absorbance was measured using a spectrophotometer at 570nm and 630nm as reference wavelengths. The IC_{50} value was calculated utilizing a linear regression equation, i.e., Y =Mx+C.

Where Y = 50, M and C values are derived from the cell viability graph.

RESULTS AND DISCUSSION

Phytochemical Screening of *T. cordifolia* extract

Phytochemical screening of aqueous extracts of *T. cordifolia* was carried out to detect the presence of empirical secondary metabolites. Polyphenols, flavonoids, tannins, and saponins were found in the aqueous extract. Polyphenols and pentacyclic triterpenes are worthwhile in antioxidant and anti-cancer activity.[29] The phenolics and flavonoids occurring in phytoextract can bind the exterior of selenium ion in selenious acid to initiate the formation of TC-SeNPs. The hydroxyl groups of phytoconstituents from the phenolics and flavonoids can act as a capping agent.[30-31] These phenolics and flavonoids of *T. cordifolia* extract can be an excellent source of phytochemicals that could reduce selenious acid proficiently during the formation of selenium nanoparticles.[32]

Synthesis of *T. cordifolia* Selenium Nanoparticles (TC-SeNPs)

At applied synthesis conditions, constant stirring of synthesized nanoparticulate dispersion was carried out for 3hr at 30°C in the dark. The colour changes to ruby red due to the synthesis of TC-SeNPs were visually noted, and no further variation in colour was observed after 2 hr.

Characterization Techniques
UV-Visible Spectroscopy

The TC-SeNPs were characterized in a UV spectrophotometer confirm the synthesized TC-SeNPs. The samples were scanned at a speed of 480 nm/min over 200–800 nm wavelengths. UV spectroscopy gave evidence of the formation of Se-NPs after adding selenous acid solution to the *T. cordifolia* extract. It converted from yellow extract color to slightly red, confirming the formation of selenium nanoparticles. The extract's colors and obtained Se-NPs were measured by UV spectrophotometer. The aqueous stem extract of *T. cordifolia* displayed a band at 260. The formation of TC-SeNPs was confirmed by a sharp absorbance (λ_{max}) at 285 nm (Figure 1). This observation of absorbance (λ_{max}) affirms that the TC-SeNPs were completely synthesised from the phytoconstituents of *T. cordifolia* stem extract. The nonappearance of any other peak in the TC-SeNPs specified that nanoparticles were fabricated of pure selenium, with no impurities. The absorption maximum (λ_{max}) of TC-SeNPs was recorded at a wavelength of 285nm after 24hr. UV-Vis spectroscopy is credited to the surface plasmon resonance (SPR) of SeNPs. It is reported that the SPR of Se-NPs causes a maximum absorption between 200 nm to 400 nm due to the formation of Se NPs.[33]

FTIR Analysis

The FT-IR spectrum of *T. cordifolia* and synthesized Selenium NPs revealed several peaks in Figure 2. The shift in peak located around 3,400 indicates the synthesis of selenium nanoparticles by reducing the carbonyl groups present in *T. cordifolia*. Correspondingly, the shift and appearance of a peak at 2,916 and 2,848 cm^{-1} suggested the involvement of the C-H (SP3 hybridised) and O-H (of acid origin) group of stem extract in particle formation. The peak at 1,710 cm^{-1} was shifted to 1,702 cm^{-1} with an increase in height. A broad peak observed in TC-SeNPs at 3321 cm^{-1} corresponds to O-H stretch alcohols and phenols. The absorption peak at 2916 and 2848 cm^{-1} corresponds to C-H stretch alkynes. The strong band at 1443 cm^{-1} is due to aromatic C-C stretching (in the ring). The C-H bending in alkanes is related to the steep peak at 1263 cm^{-1}, while the C-N stretching of amines is attributed to the sharp peak at 1024 cm^{-1}.C-X stretching in alkyl halides causes a band at 809 cm^{-1}, and the weak band at 568 cm^{-1} is the result of C-N-C bending in amines. Absorption peaks at 809,766, 710, 622, and 608 cm^{-1} in SeNP may be due to the partial deuteration of amine. The FT-IR spectrum reveals the presence of various functional groups in the phtyoextract, which could potentially influence the process of nanoparticle reduction and stabilization.[34]

X-Ray Diffraction (XRD) Analysis

The XRD pattern exhibited the crystalline structure's of synthesized TC-SeNPs as described in Figure 3A. The X-Ray pattern displays a comprehensive peak deprived of any sharp Bragg's peaks. Therefore, it can be postulated that the synthesised TC-SeNPs are amorphous, conferring to prior reports.[35] The XRD spectra propose the nanocrystalline nature of TC-SeNPs and counterparts precisely with that of the standard selenium powder, which confirms the synthesis of nanoparticles. The lattice constants calculated are a = 4.363 A° and c =4.952 A°, according to the value from the literature (JCPDS File No.06-0362).[36] The Scherrer's equation has been applied to determine

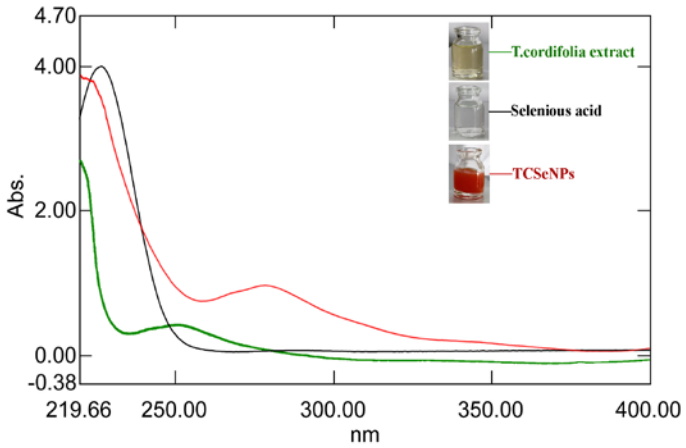

Figure 1: UV-Vis spectrum of *T. cordifolia* extract, TC-SeNPs, and Selenious acid.

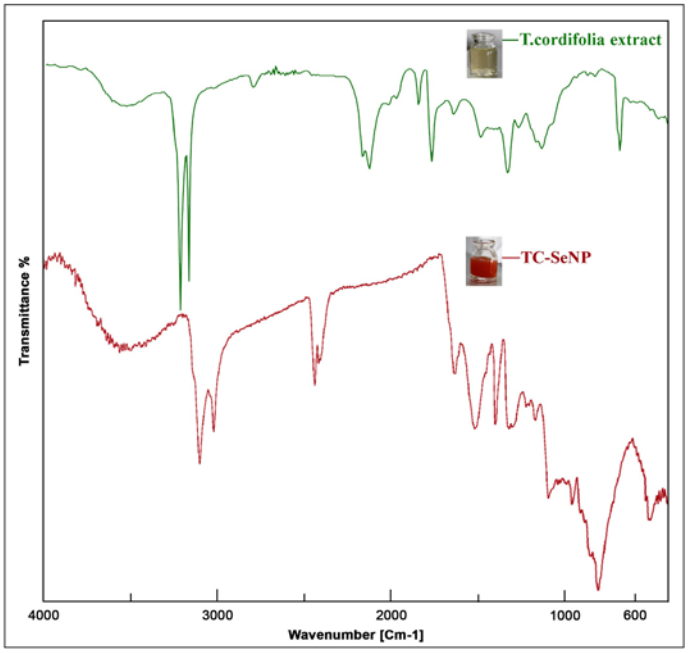

Figure 2: FT-IR spectrum of *T. cordifolia* and Selenium nanoparticles (TC-SeNPs).

the crystallite size of TC-SeNPs, which is observed within the range of 85 nm.

Scanning Electron Microscopy

The dimension of TC-SeNPs is denoted in Figure 3B; SEM images aided us in visualizing the selenium nanoparticles' shape. The synthesized TC-SeNPs were oval with a smooth surface from the SEM images. The TC-SeNPs particle size was around 50-100 nm, properly dispersed with aggregation. Thus, it can be proposed that nanoparticle accretion prevails over the reduction process and primary nucleation of reduced atoms. TC-SeNPs may be related to a more significant number of functional groups of *T. cordifolia* extract bind and nucleate selenious acid ions. The best available metal ions are complexed in a minor quantity for the nucleation process, indicating the metal ion's agglomeration.[37] According to previous reports, the agglomerated nanoparticles demonstrate higher biological activity.[38]

Energy-Dispersive X-ray Spectroscopy Analysis

An EDX analysis of TC-SeNPs was performed to study its elemental composition. This EDX spectroscopic analysis confirmed the occurrence of selenium in sample TC-SeNPs described in Figure 3C. The TC-SeNPs exhibited distinctive absorption peaks of selenium, carbon, and oxygen with a strong signal of the Selenium (Se) atom (63.36%) together with the Oxygen (O) atom (14.16%) and Carbon (C) atom (22.48%). The absence of other elemental peaks and the high selenium concentration in the spectrum prove the purity of the synthesized product's selenium metal. The occurrence of oxygen and carbon peaks in the spectra of the TC-SeNPs confirms the existence of stabilizers composed of alkyl chains.[39]

Transmission Electron Microscopy

As determined by TEM with a specific area of electron diffraction patterns, the green synthesised TC-SeNPs developed agglomerated nanoparticles (amorphous nature). The TC-SeNPs synthesised by biosynthesis were found to be mono scattering and spherical depicted in Figure 3D. The transmission electron microscopy image verified that the TC-SeNPs were spherical and in the nanoscale range. The size

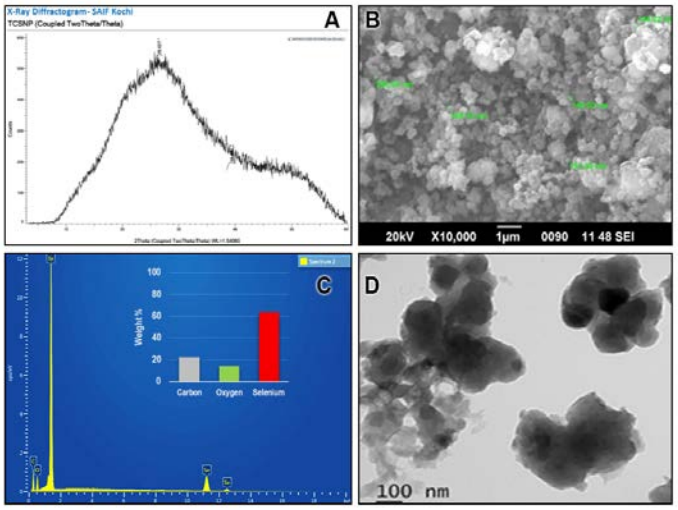

Figure 3: A)XRD pattern, B) SEM of TC-SeNPs, C) EDAX spectrum of TC-SeNPs D) TEM of TC-SeNPs.

distribution of the TC-SeNPs was determined to be within the range of 20–200 nm, with an average size of 140.46 nm. The sizes and shapes of synthesized TC-SeNPs were independent of the plant *T. cordifolia* extract used in synthesis, which may be credited to various polyphenolics possessing reduction potential. Thus, these phytomolecules might force the reduction of Se ions to the nanoscale and correspondingly stabilize the synthesised TC-SeNPs and avoid their aggregation. This implies a strong correlation between the nanoparticles' size and biological activity. Due primarily to its flavonoids and polyphenols, *T. cordifolia's* ability to scavenge free radicals and act as an antioxidant has been demonstrated.[40-41]

Particle Size-distribution analysis

Dynamic Light Scattering (DLS) analysis

The average particle size of TC-SeNP was found to be 168.31nm The TC-SeNP unveiled narrow particle size distribution (z- average =(168.31nm) with PDI value 0.389 depicted in Figure 4A. The PDi value of 0.389 is regarded as a moderate polydispersed system. TEM and DLS measurements of a particle's dimensions are different in practice. DLS measurements include the ligand shell and specify the hydrodynamic size; yet, the numbers reported above from DLS and TEM agree (we can look at the lone metallic core in TEM).[42] As expected, the size indicated by DLS was more significant than that determined by TEM pictures. This is most likely due to the solvation shell formed by the *T. cordifolia* coat on the surface of the selenium core, which contributes to particle diameter only when DLS measurements are taken.[43]

Zeta potential analysis

The zeta potential of TC-SeNPs was -23.9 mV, indicating that the polydispersed suspension of the nanoparticles is more stable (Figure 4B). The zeta potential analysis revealed that the synthesised TC-SeNPs were negatively charged. The negative charge potential value could be determined due to the reducing agent used for oxidising the flavonoid and polyphenolic contents in the *T. cordifolia* stem extract, indicating the presence of repulsive electrostatic force between the green synthesised TC-SeNPs. Previously, if all particles in a suspension had a negative or positive zeta potential, they would tend to reject one another, creating a much-reduced attraction for the particles to aggregate. The excellent stability of TC-SeNPs without forming aggregates may be due to the negative charge on selenium particles.[44-45]

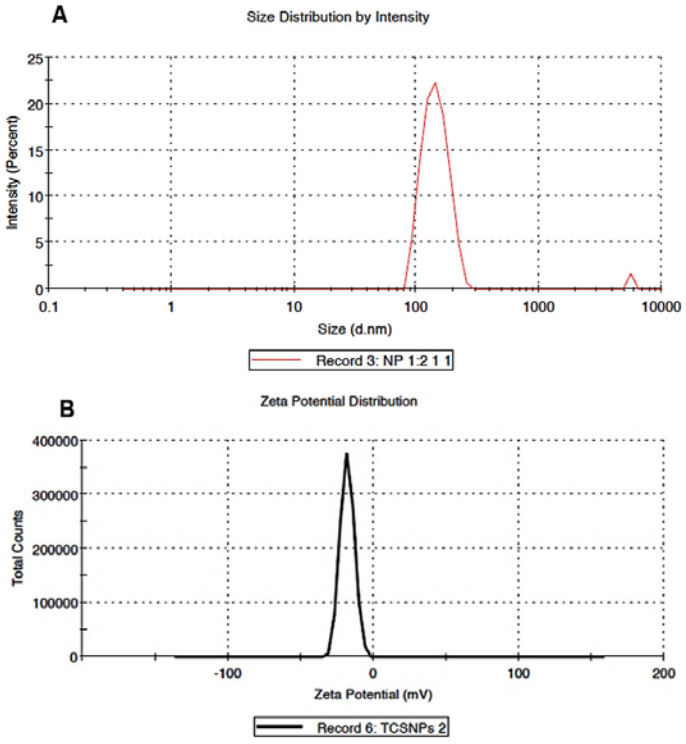

Figure 4: A) DLS analysis of TC-SeNPs B) Zeta potential analysis of TC-SeNPs.

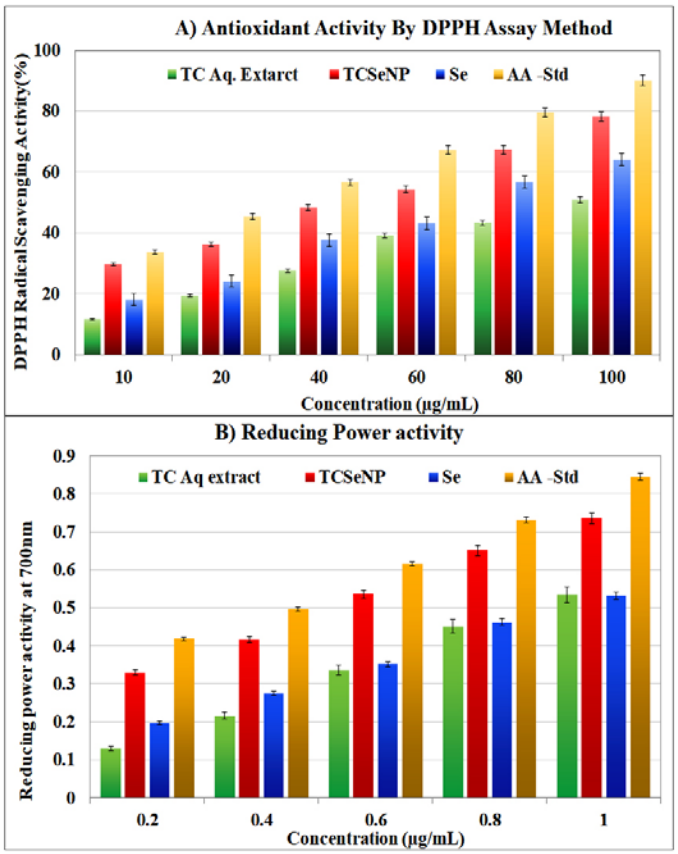

Figure 5: A) DPPH radical scavenging activity and B) Reducing power activity.

ICP-AES—Selenium content estimation

The standard selenium values ranging from 0.1 to 100 mg/mL were used to plot the calibration curve. The calibration graph for selenium was linear at ppm concentrations, with a correlation coefficient of 0.999 for the silver calibration curve. After treatment with nitric acid, the colorless samples indicated that the selenium was solubilized in solution. ICP-AES measurements revealed that the concentration of synthesised TC-SeNPs was 68.8±12.2 µg/mL.

Biological applications

Antioxidant Activity

The substantial antioxidant action of the *T. cordifolia* aqueous extract, synthesised TC-SeNPs, and standard ascorbic acid was determined using DPPH radical scavenging assay. The free radical scavenging capacity of TC-SeNPs was measured spectrophotometrically concerning the change in intensity of DPPH colour from purple to yellow. The percentage of DPPH scavenging activity inclined with increasing in concentrations of all the solutions (10-100 µg/mL). TC-SeNPs showed 78.03% scavenging activity at 100 µg/mL concentration than the Selenium 64.14%, *T. cordifolia* aqueous extract 50.83% and standard Ascorbic acid 90.17%. The IC_{50} value of the TC-SeNPs is 28.62±0.51 µg/mL compared to Selenium 43.72 ±1.21 µg/mL *T. cordifolia* aqueous extract 88.37±0.36 µg/mL and standard (ascorbic acid) IC_{50} value 16.52 µg/mL (Figure 5A). The antioxidant capacity of the TC-SeNPs was significantly higher than that of *T. cordifolia* aqueous extract and moderate to standard (ascorbic acid). Reducing power was greatly associated with antioxidant capacity, and it may probably serve as an important antioxidant property. The colour of the test solution changes from yellow to several shades of green and blue depending on the reducing capacity of each sample in the reducing power assay. Here a tremendous increase in the reducing capacity with the increase in concentration was noted. The EC_{50} values of standard (ascorbic acid) and TC-SeNPs are 43.84±1.20 µg/mL and 81.60±0.20 µg/mL respectively (Figure 5B).

Antiproliferative Activity

MTT assay was applied to investigate the antiproliferative activity of the TC-SeNPs solution and Se with TC aqueous extract and Doxorubicin as the Standard drug. The statistical data of cell cytotoxicity study by MTT against human breast cancer cell lines (MCF-7) suggests that test compounds, namely TC-SeNPs, Se, and Std Drug Doxo, show significant cytotoxic potential properties in comparison to TC. The IC_{50} value for TC 76.48±23 µg/mL, TC-SeNPs 31.28±0.22 µg/mL, Se 40.28±1.31 µg/mL and Std drug 6.41±0.30 µg/mL, Figure 6 a-b-c. respectively states that TC and Se show moderate cytotoxicity among all these compounds, and TC-SeNPs show significant cytotoxicity compared to standard drugs. Antiproliferative activity may be owing to well-characterized TC-SeNPs.

CONCLUSION

Tinospora cordifolia stem extract contains bioactive components such as glycoside, flavonoids, tannins, phenolic, terpenoids, and alkaloids required to synthesize and stabilize TC-SeNPs. The critical finding of this research was the presence and detection of flavonoids and phenolics, which were found appropriate for the biosynthesis of TC-SeNPs. It is evident from FTIR that biosynthesized TC-SeNPs are stable due to the capping of these phytoconstituents. The synthesized TC-SeNPs exhibited highly stable, negative charge, amorphous nature, spherical shape, and nano-size. XRD spectral analysis of TC-SeNPs established an typical absorption spectra of selenium that have high purity and are crystalline, that is identified in the EDX analysis and proved its elemental nature.

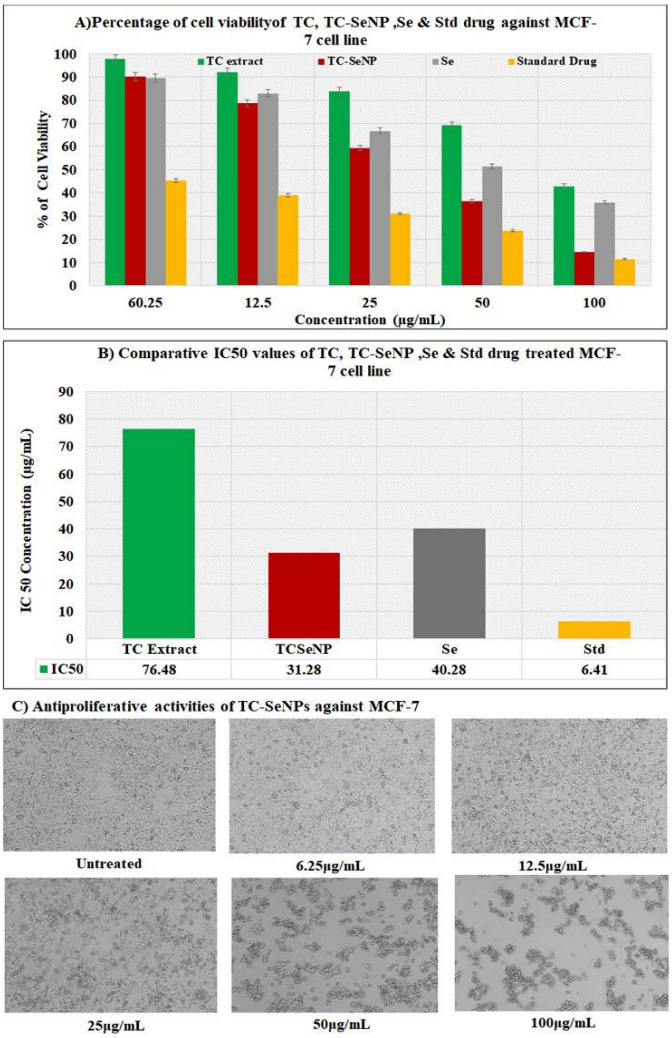

Figure 6: A) Percentage of cell viability of TC-SeNPs B) Comparative IC_{50} values C) Antiproliferative activities of TC-SeNPs against MCF-7 cell lines at various concentrations (6.25-100 µg/mL).

Additional TEM and particle size distribution confirmed the spherical nature of TC-SeNPs. The zeta potential value showed that negative charge highly stable TC-SeNPs. The free radical scavenging by DPPH and reducing power capacity assay of the spherical amorphous TC-SeNPs exhibited good antioxidant activity and can serve as a potent antioxidant. TC-SeNPs have been evaluated for antiproliferative activity against human breast cancer cell lines MCF-7 cell lines by MTT assay revealed that TC-SeNPs serve as a potential anti-cancer activity compared to the standard Doxorubicin. This study suggests that TC-SeNPs possess abundant growth control contrary, unlike cancer cells, which directed their potential in medical applications. In the future, the combination of phytomolecule and nanoparticles can assist an efficient role in multifaceted pharmacological properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TC: *Tinospora cordifolia* extract; **TC-SeNPs:** *Tinospora cordifolia* Selenium Nanoparticle; **DLS:** Dynamic Light Scattering; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDAX:** Energy Dispersive X-Ray; **EC_{50}:** Half Maximal Effective Concentration; **EPR:** Enhanced Permeation and Retention Effect; **FT-IR:** Fourier Transform Infra-Red Spectroscopy; **IC_{50}:** Half Maximal Inhibitory Concentration; **ICP-AES:** Inductive coupled plasma-Atomic emission spectroscopy; **JCPDS:** Joint Committee on Powder Diffraction Standards; **PBS:** Phosphate Buffer Saline; **ROS:** Reactive Oxygen Species; **SEM:** Scanning Electron Microscopy; **EDAX:** Energy dispersive X-Ray; **Se:** Selenium; **SPR:** Surface Plasmon Resonance; **TEM:** Transmission Electron Microscopy; **UV spectroscopy:** Ultra Violet Spectroscopy; **XRD:** X-Ray Diffraction Spectroscopy.

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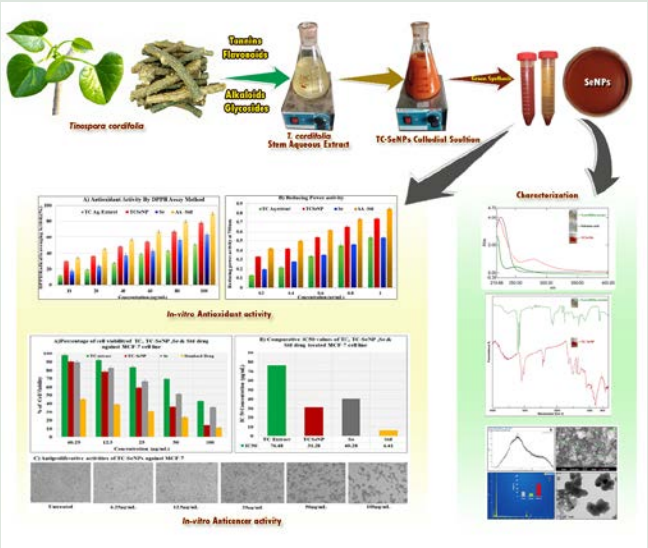
GRAPHICAL ABSTRACT



SUMMARY

Selenium nanoparticles (Nano-Se) provide exceptional biopotential, better biocompatibility, and low toxicity. SeNPs can be synthesized in various ways, including physical, chemical, and biological methods. Plant extracts mediated green synthesis of SeNPs is the most eco-friendly synthesis method compared to physical and chemical processes. In the present study, SeNPs were synthesized by using stem bark aqueous extract of the Tinospora cordifolia. The synthesized SeNPs were characterized by various spectroscopic and microscopic techniques such as U.V-Visible, FT-IR, XRD, SEM,TEM, EDAX, IC-PAES, Particle analyzer, and Zeta potential. The results indicated that the synthesized SeNPs possess considerable structural and physical properties of nanoparticles with potential therapeutic applications, specifically antioxidant and anticancer activity. This rapid green synthesis method is successful in synthesizing and stabilizing biocompatible SeNPs. It offers novel opportunities to formulate and develop well-fabricated biogenic SeNPs that may be manufactured, stored, and commercialized safely worldwide. In the future, the combination of phytomolecules and nanoparticles can play a pivotal role in multidimentional pharmacological applications.

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