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Green route for selenium nanoparticles synthesis using *Senna spectabilis*: A novel eco-friendly approach

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Abstract

Selenium nanoparticles (SeNPs) were green-synthesised using fresh leaves of *Senna spectabilis* collected from Pookode college campus. Dried leaves were extracted in nanopure water at 60 °C for 45 minutes under continuous stirring. A 100 mM selenious acid solution was added dropwise to the extract, stirred for 3 hours and incubated in the dark for 45 hours. The resulting nanoparticles were collected by centrifugation, washed with isopropyl alcohol and water, and dried at 37 °C. UV-Vis spectroscopy revealed a broad surface plasmon resonance peak at 274 nm, confirming nanoparticle formation. FTIR analysis indicated the participation of phenols, flavonoids, proteins and polysaccharides in reduction and stabilisation. SEM showed irregular, rough-surfaced aggregates (~157 nm), while TEM revealed predominantly spherical particles with sizes ranging from 20-80 nm. SAED patterns confirmed a polycrystalline structure, and EDX analysis identified selenium (36%) as the main element, with carbon, oxygen, and trace sulphur from the leaf extract acting as capping agents. These results demonstrate that *S. spectabilis* leaf extract provides an effective, eco-friendly route for the synthesis of stable SeNPs, with potential applications in biomedical and environmental fields.

Keywords: SeNPs, *S. spectabilis*, green synthesis, selenious acid

Introduction

Nanotechnology is an emerging interdisciplinary field of science and engineering that focuses on the design and application of materials and systems at the nanoscale, typically ranging from 1 to 100 nm. At this scale, structures exhibit novel properties and functionalities that differ significantly from their bulk counterparts, enabling advancements in diverse technological and industrial sectors (Mekonnen, 2021) [15]. Nanoparticles possess an exceptionally small size, a high surface area-to-volume ratio, and distinctive physicochemical features that differentiate them from their bulk counterparts. At the nanoscale, they frequently exhibit improved mechanical strength, greater surface reactivity, reduced melting points, and modified optical and magnetic properties, primarily as a result of quantum confinement and enhanced surface effects (Kumar *et al.*, 2018) [14].

Nanomaterials can be synthesised in two main ways**:** top-down and bottom-up. These techniques are classified based on the phase of the starting material. In the top-down method, the starting material is in the solid state and is broken down into smaller particles, making it more suitable for laboratory use than large-scale production. In contrast, the bottom-up method begins with starting materials in the gaseous or liquid state, where atoms or molecules come together to form stable structures through a process called self-assembly (Patil *et al.*, 2021) [19]. These two approaches form the basis for the various methods of nanoparticle synthesis, which include physical, chemical, and biological methods**.**

Accordingly, nanoparticle synthesis can be broadly classified into physical approaches (mechanical milling, pulse laser ablation, pulsed wire discharge, chemical vapor deposition, laser pyrolysis, and ionised cluster beam deposition), chemical approaches (including sol-gel processing, sonochemical techniques, co-precipitation, inert gas condensation, and hydrothermal methods), and biological approaches, which utilize microorganisms, plant extracts, or algae as eco-friendly reducing and stabilising agents (Ealias and Saravanakumar, 2017) [5]. Green synthesis of nanoparticles has gained considerable attention as a sustainable and environmentally benign approach. Unlike conventional physical and chemical methods that often rely on toxic reagents, generate hazardous by-products, and require high energy input, green synthesis employs biological resources such as plant extracts, microorganisms, and algae. These natural systems act as reducing and stabilising agents, enabling the production of nanoparticles in a safer, cost-effective, and eco-friendly manner while minimising risks to human health and the environment (Aarthye and Sureshkumar, 2021) [1].

Selenium is a trace element that plays crucial roles in both plants and mammals. In mammals and birds, selenium is essential for many biological functions. It is a cofactor for antioxidant enzymes such as glutathione peroxidase, which reduces reactive oxygen species, and is incorporated into selenoproteins that contribute to antioxidant defense, catalysis, anti-inflammatory, antiviral, and antitumor activities. Selenium deficiency can lead to serious health problems, including cardiomyopathy, osteoarthropathy, impaired fertility, weakened immunity, and increased susceptibility to infections, whereas excessive intake can cause toxicity known as selenosis. Because the margin between beneficial and toxic levels is narrow, selenium supplementation requires careful management. Nanotechnology provides a promising approach in this context, enabling the production of selenium nanoparticles with improved bioavailability and reduced toxicity (Garza-García *et al.*, 2022) [8].

Cassia, commonly known as *Senna spectabilis* (Family: Fabaceae; Subfamily: Caesalpinioideae), is native to regions from south-western Mexico to southern tropical America (Irwin & Barneby, 1982) [10]. Since its introduction into the Wayanad Wildlife Sanctuary in the early 1980s, it has become highly invasive, spreading rapidly and occupying nearly 23 percent of the sanctuary area within four decades. Its vigorous growth, prolific regeneration, and ability to colonise open and degraded habitats suppress native vegetation, reduce biodiversity, and pose significant challenges for ecosystem management (Muraleekrishnan *et al.*, 2024) [16]. Recognising its ecological impact, researchers have explored potential ways to utilise the plant. Phytochemical analyses of *S. spectabilis* reveal the presence of tannins, phenols, saponins, flavonoids, steroids, and alkaloids. Studies report that it exhibits antibacterial, antibiofilm, antifungal, anti-inflammatory, anti-hyperalgesic, anticonvulsant, and antioxidant activities, with toxicity assessments indicating its safety (Karau *et al.*, 2013; Jothy *et al.*, 2012) [12, 11]. Leveraging these bioactive compounds, the plant has been successfully used for the green synthesis of selenium nanoparticles, transforming an invasive species into a valuable resource for sustainable nanotechnology applications.

Given its invasive nature and rich bioactive composition, *S. spectabilis* was selected for the green synthesis of Selenium nanoparticles. Green synthesis provides an eco-friendly, cost-effective, and sustainable alternative to conventional chemical or physical methods, avoiding toxic reagents while utilizing the plant's natural compounds. By turning an environmental problem into a valuable resource, this approach not only mitigates the ecological impact of the invasive species but also enables the production of biologically safe nanoparticles with potential applications in medicine, agriculture, and industry.

Materials and Methods

Collection and preparation of S. spectabilis leaf extract

Fresh leaves of *S. spectabilis* used in the study were procured from the Pookode college campus (11°32'08"N 76°01'31" E). Fresh leaves (250 g) were washed with nanopure water (Merck Millipore, USA) and kept it for drying, and dried leaves were transferred to 1000 mL nanopure water. The solution was kept under continuous stirring conditions at a temperature of 60 °C and 250 rpm for 45 minutes using a magnetic stirrer. Later, the solid particles were removed by filtering through Whatman filter paper 1.

Synthesis of selenium nanoparticles using S. spectabilis

A 100 mM solution of selenious acid (Chemika, Australia) was prepared in 900 mL of nanopure water (Merck Millipore). This solution was added dropwise to 900 mL of S. spectabilis leaf extract under continuous stirring at room temperature using a magnetic stirrer (DAN Hot Plate Magnetic Stirrer, India) set at 250 rpm for 3 hours. The reaction mixture was then incubated in the dark for 45 hours to promote nanoparticle formation. After incubation, the synthesised selenium nanoparticles were collected by centrifugation at 10,000 rpm for 10 minutes using a laboratory centrifuge (REMI Equipments, India). The resulting pellet was washed three times, alternating between isopropyl alcohol (Loba Chemie) and nanopure water to remove unreacted compounds and impurities. Finally, the purified selenium nanoparticles were dried overnight at 37 °C in a bacteriological incubator (Labline, India) (Fig.1)

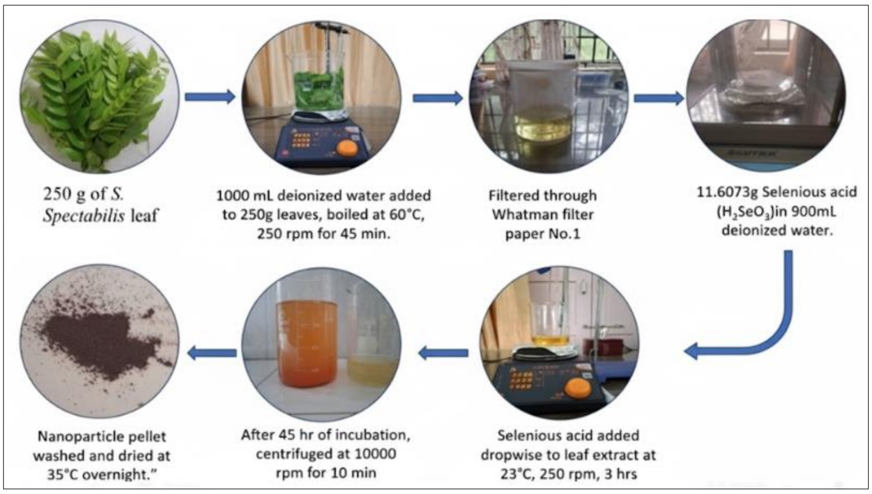

Fig 1: Green synthesis of SeNPs

Characterisation of selenium nanoparticles

The green synthesised selenium nanoparticles (SeNPs) were characterized using UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

Ultraviolet-vision Spectroscopy (UV-Vis spectroscopy)

The nanoparticles were first redispersed in nanopure water (Merck Millipore) to a final concentration of 2 mg/mL using a digital ultrasonic sonicator (Labman Scientific Instruments, India). UV-Vis analysis was performed on the resulting suspension over a wavelength range of 200-600 nm using nanopure water as a blank (ThermoFisher Scientific, USA).

Fourier Tranform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was used to identify the functional groups present on the SeNPs, with spectra recorded from 4000 to 400 cm^{-1} at a resolution of 0.20 cm^{-1} (Thermo Nicolet iS50, USA).

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDAX) analysis

The surface morphology and average particle size of the synthesised nanoparticles were examined using SEM, while their elemental composition was verified through Energy Dispersive X-ray Spectroscopy (EDX/EDAX) (Jeol 6390LA/Oxford XMX N, Japan) spectrum operated at 0-20 KeV.

High Resolution Transmission Electron Microscope (HR-TEM) and Selected Area Electron Diffraction (SAED): HR-TEM was used to examine the morphology, size, and lattice structure of the selenium nanoparticles, while SAED confirmed their crystallinity and crystalline phase (Jeol JEM-2100, Japan).

Result and Discussion

In the current study, freshly prepared leaf extract of *Senna spectabilis* was employed for the green synthesis of selenium nanoparticles (SeNPs). Gradual addition of selenious acid to the leaf extract resulted in a visible colour change from yellow to cloudy orange, indicating nanoparticle formation. This transformation is attributed to the reduction of selenious acid by phytochemicals in the extract, which act as natural reducing and stabilising agents.

UV-Vis spectroscopy (Fig.2) of the synthesised SeNPs showed a broad surface plasmon resonance (SPR) peak at 274 nm, confirming nanoparticle formation. The observed SPR band is consistent with the characteristic absorption range of selenium nanoparticles (260-280 nm) and was slightly red-shifted compared to SeNPs synthesized using *Cassia auriculata* leaf extract, likely due to differences in particle size, surface morphology, and phytochemical composition (Preethi *et al.*, 2025; K. Anu *et al.*, 2020) [21, 3].

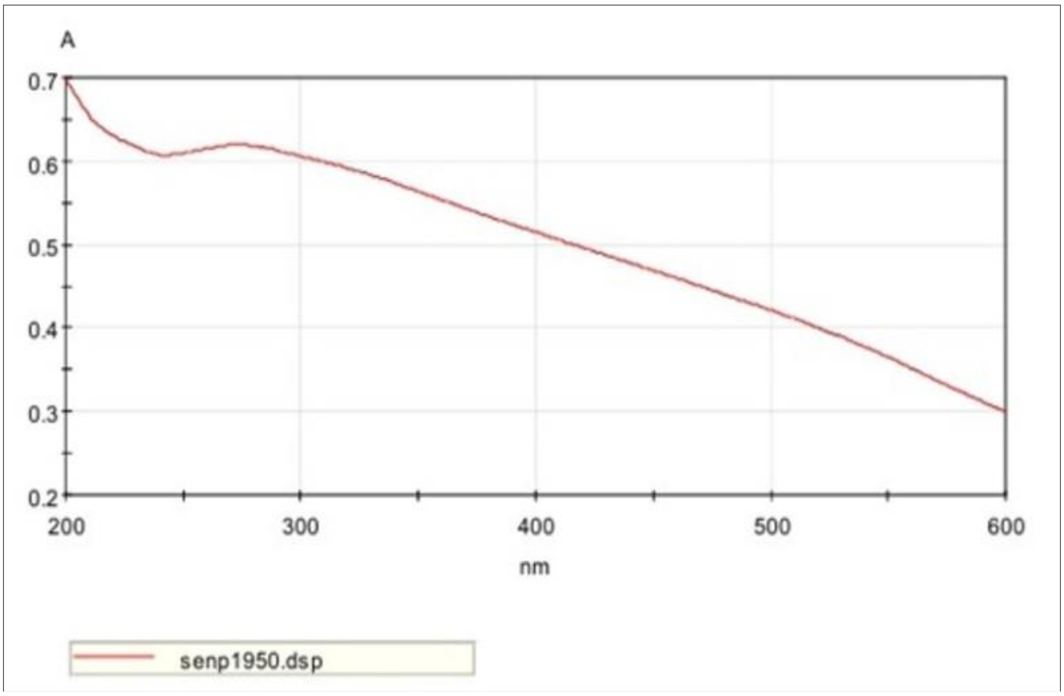

Fig 2: UV-Vis spectra of SeNPs

FTIR analysis (Fig.3) revealed distinct absorption peaks at 3262.66, 2917.12, 1628.25, 1512.23, 1439.93, 1226.50, and 1025.51 cm^{-1}, indicating the involvement of hydroxyl, amine, carbonyl, and aromatic groups from phenols, flavonoids, proteins, and polysaccharides in the reduction and stabilization of SeNPs. The broad band at 3262 cm^{-1} corresponds to O-H and N-H stretching vibrations, while peaks at 1628 and 1512 cm^{-1} represent C=O (amide I) and C=C aromatic stretching, confirming the participation of proteins and phenolic compounds. Peaks at 1439, 1226, and 1025 cm^{-1} are associated with CH_2 bending, O-H deformation, C-O/C-O-C, and C-N stretching, indicating the role of phytochemicals in capping and stabilising the nanoparticles (Pasieczna-Patkowska *et al.*, 2025; Nandiyanto *et al.*, 2019; P.B. Ezhuthupurakkal *et al.*, 2017; Hernández-Díaz *et al.*, 2021) [18, 17, 6, 9].

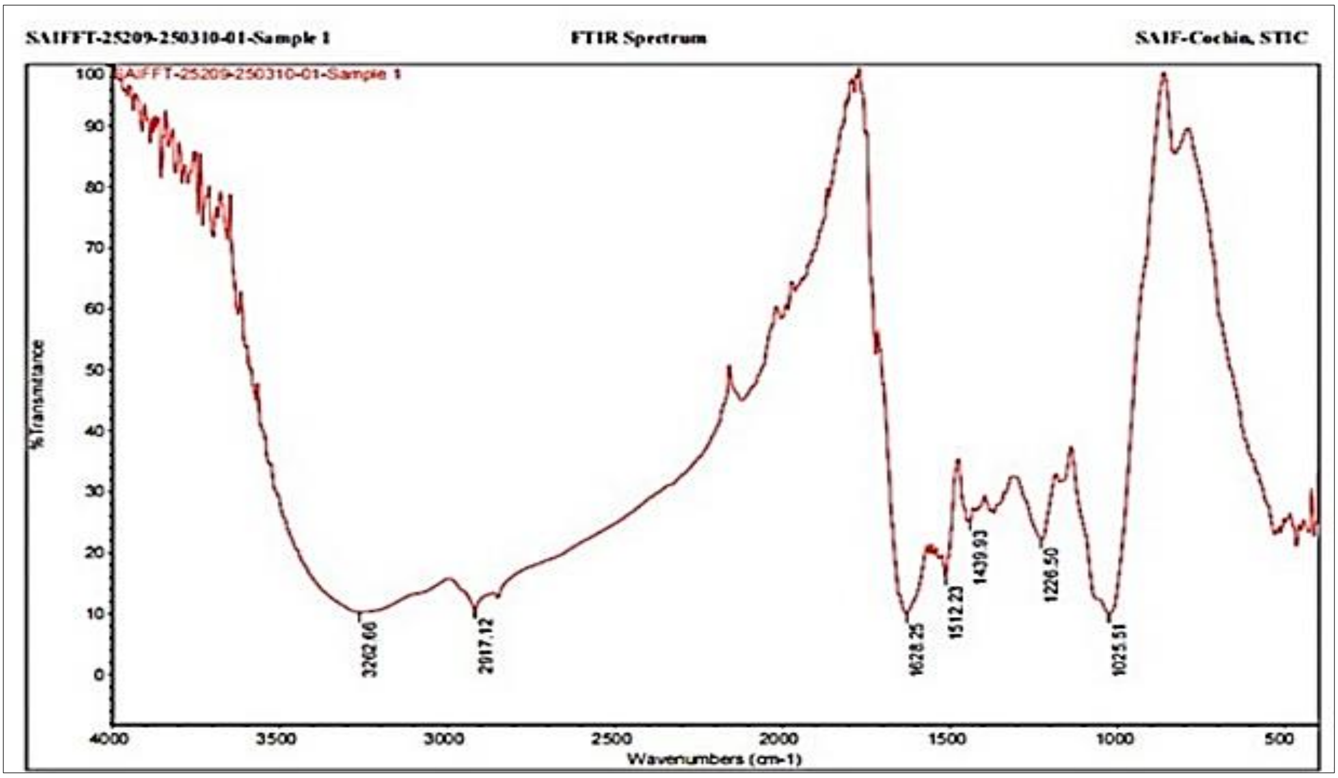

Fig 3: FTIR spectra of synthesised SeNPS

SEM analysis showed irregular, rough-surfaced aggregates with an average particle diameter of 157.12 ± 11.74 nm (Fig. 3. A, B) The formation of these agglomerates may result from the abundance of functional biomolecules in the leaf extract, which facilitate nucleation and partial clustering of selenium ions. Similar observations of clustered SeNPs have been reported using *Pelargonium zonale* extract, where overlapping of nanoparticles made individual boundaries indistinguishable (Filipović *et al.*, 2021; Alagesan & Venugopal, 2019) [7, 2].

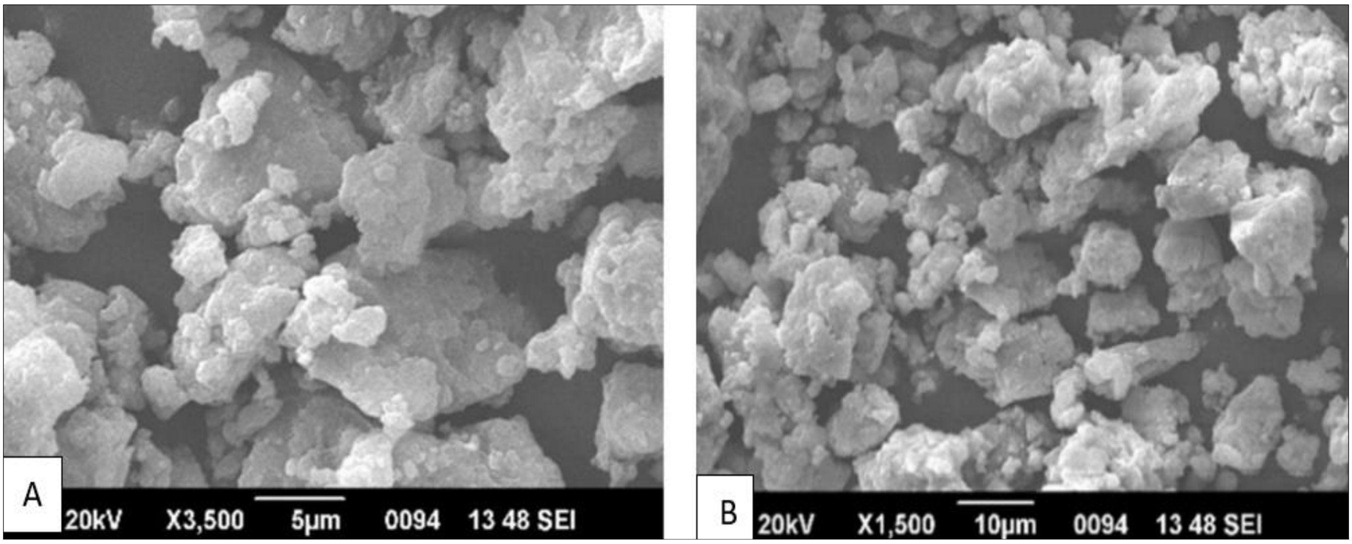

Fig 4: A, B: SEM images of synthesised SeNAPs

EDX analysis (Fig.4) confirmed selenium as the predominant element (36%), with carbon (43.86%), oxygen (19.35%), and trace sulfur (0.78%) derived from phytochemicals adsorbed on the nanoparticle surface, acting as stabilising and capping agents (Dang-Bao *et al.*, 2022; Prasathkumar *et al.*, 2022) [4, 20].

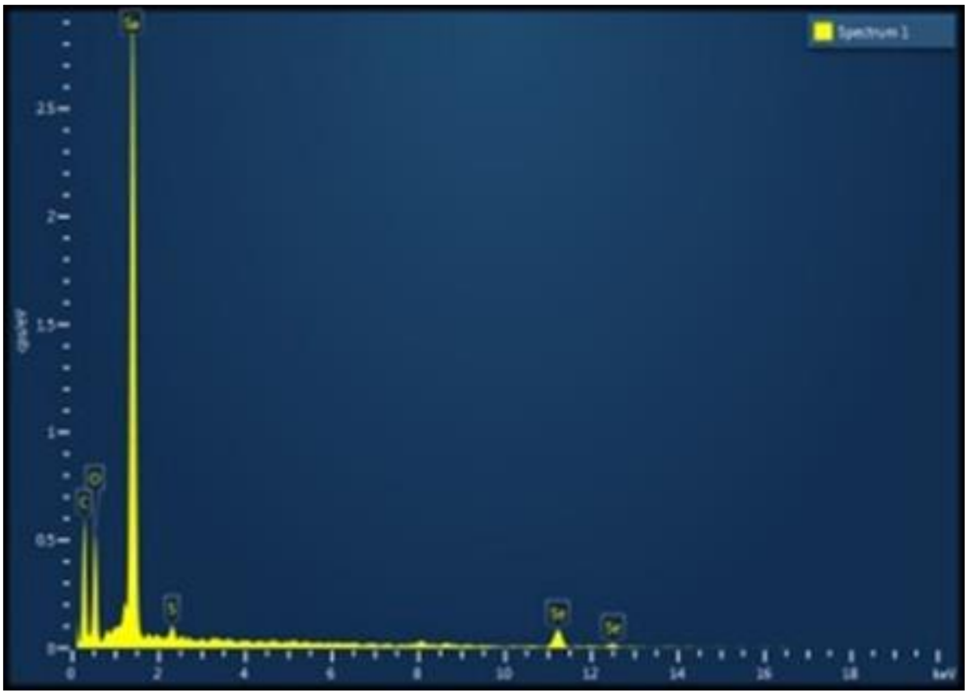

Fig 5: EDX images of SeNP

TEM micrographs (Fig.5.A.B.C) revealed predominantly spherical nanoparticles with occasional oval and irregular shapes, ranging from 20 to 80 nm. The size and morphology variations are influenced by the capping effect of phytochemicals in the *S. spectabilis* extract (Kokila *et al.*, 2017) [13]. SAED analysis (Fig.5.D) exhibited distinct concentric diffraction rings, confirming the polycrystalline nature of the SeNPs, in agreement with previous findings (Vyas & Rana, 2017) [22].

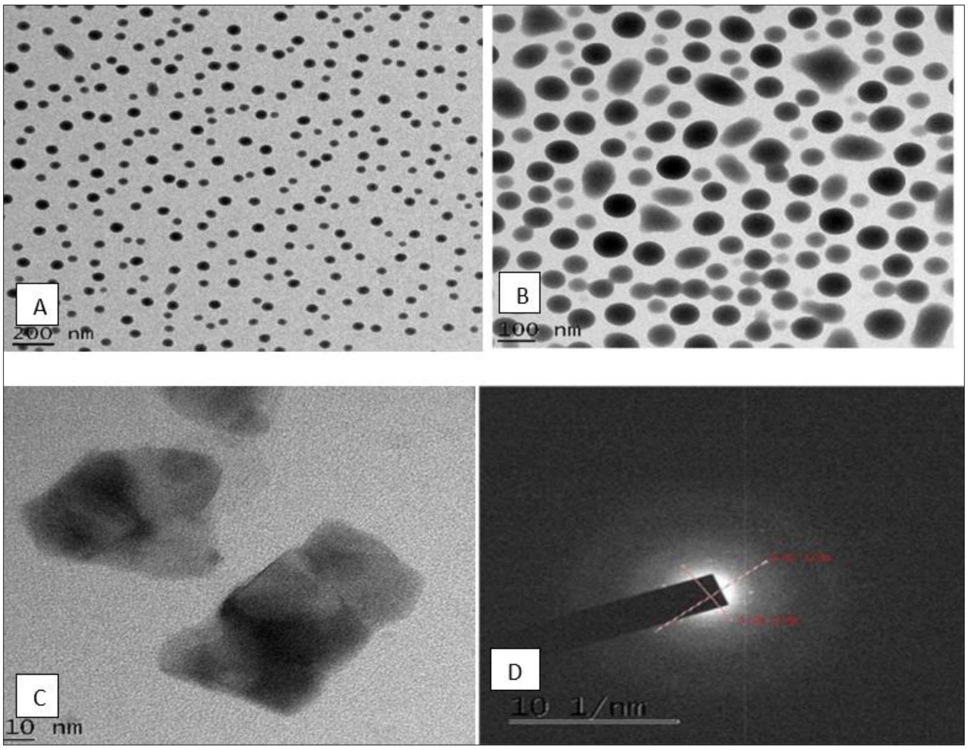

Fig 6: A, B, C: HR-TEM images of SeNPs, D: SAED images of SeNPs

Conclusion

The present study confirms the successful green synthesis of selenium nanoparticles (SeNPs) using *S. spectabilis* leaf extract as a dual reducing and stabilising agent. The characteristic surface plasmon resonance peak at 274 nm observed in the UV-Vis spectrum verified nanoparticle formation. FTIR analysis revealed the active participation of phytochemical functional groups such as hydroxyl, amine, and carbonyl moieties in reduction and stabilisation processes. Microscopic analyses (SEM and TEM) demonstrated predominantly spherical SeNPs with particle sizes ranging from 20 to 80 nm, while EDX confirmed selenium as the principal element and SAED patterns indicated their polycrystalline nature. Overall, the findings highlight *S. spectabilis* as an efficient, sustainable, and eco-friendly source for the synthesis of biocompatible SeNPs with promising potential for future biological and nutritional applications.

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