



Original Article

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Biocompatible Withania somnifera Selenium Nanoparticles: Synthesis, Characterization, and Biological Applications

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ABSTRACT

Green synthesis of nanoparticles has evolved as an ecologically friendly and sustainable method for producing nanoparticles using natural sources like botanical extracts. *Withania somnifera*, commonly known as ashwagandha, is a medicinal plant known for its diverse therapeutic properties. This work focuses on the green synthesis, characterization, and applications of selenium nanoparticles (SeNPs). The ecologically sound production of selenium nanoparticles using extracts of *Withania somnifera* extract offers a sustainable and eco-friendly alternative to nanoparticle manufacturing. The synthesized WS-SeNPs exhibit diverse biomedical applications, including antioxidant, antimicrobial, and anticancer activities. The synthesis process involves the reduction of selenium ions from an aqueous solution using *Withania somnifera* extract as a reducing and stabilizing agent. The green synthesis method offers several advantages over conventional chemical synthesis, including reduced environmental impact, cost-effectiveness, and biocompatibility. The synthesized *Withania somnifera* selenium nanoparticles (WS-SeNPs) are characterized using various analytical techniques such as Dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), UV-Visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). These characterization techniques provide insights into the size, morphology, crystalline structure & stability. The ecologically sound production of selenium nanoparticles using extracts of *Withania somnifera* offers a sustainable and eco-friendly approach to nanoparticle fabrication. The synthesized WS-SeNPs exhibit diverse biomedical applications, including antibacterial, and anti-enzymatic activities.

Key words: Green synthesis, Nanomaterials, Biological, Biocompatible, Plant extract

INTRODUCTION

In the synthesis of bio-fabricated nanoparticles, plants play a significant part. Plants produce metabolites that help in the breakdown of precursor chemicals. It also acts as a catalyst and stabilizer throughout the nanoparticle formation process. SeNPs are first formed largely by plants that contain selenium. Sediment rock releases selenium into the soil, that plants take in.

Withania somnifera, commonly known as Ashwagandha has been extensively studied for its pharmacological properties and therapeutic potential in traditional medicine systems like Ayurveda. With the growing interest in

nanotechnology, the synthesis of nanoparticles using natural sources has gained prominence due to their eco-friendly nature and potential biomedical applications. Selenium nanoparticles (SeNPs) have garnered attention in recent years owing to their remarkable antioxidant, antimicrobial, and anticancer properties [1]. Integrating the benefits of *Withania somnifera* with the properties of SeNPs offers a promising avenue for developing novel therapeutic agents with enhanced efficacy and reduced toxicity.

MATERIALS AND METHODS

Preparation of plant extract

The plant was washed with distilled water several times and dried for 24 hours. The plant material was ground using a regular grinder. The extract was prepared using deionized water. The plant extract was made by stirring at 40-45°C for 10 minutes, filtering using Whatman n-1 filter paper, and centrifuging for 15 minutes. The supernatant was collected after filtering.

Biosynthesis of SeNPs

Using a magnetic stirrer set at 600 rpm, 10 ml of botanical extract and 0.14 g of 100 ml of selenic acid were combined and continuously stirred for 15 minutes at a temperature between 40 and 45°C. Next, 10 ml of ascorbic acid was added using a pipette at a temperature range of 40–45°C for 30 minutes, or until the solution's color turned reddish-orange. A change in color of the reaction mixture from colorless or light yellow to red or brown indicates the formation of selenium nanoparticles and can be monitored visually [2]. The reaction is allowed to proceed for a certain time to ensure a complete reduction of selenium ions and the formation of stable WS-SeNPs. After the mixture had cooled, a tiny amount—1 milliliter—was utilized for UV examination. After that, the sediment is centrifuged and repeatedly cleaned with ethanol and water. For four hours, the precipitate was dried at 100°C in an oven. Red Se NP particles were separated, as **Figure 1** illustrates.

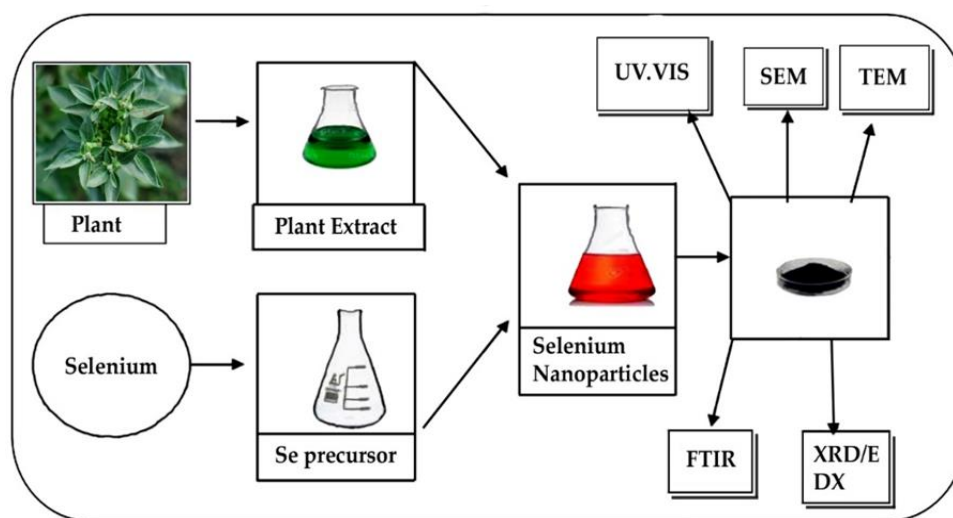


Figure 1. Green Synthesis of *Withania somnifera* SeNPs

Characterization of nanoparticles

FTIR & EDX analysis

The FTIR characterization techniques for WS-SeNPs guide the identification of various peaks associated with functional groups present in *Withania somnifera*-selenium nanoparticles in **Figure 2**. Peaks between 3500 and 3200 cm^{-1} are indicative of the hydroxyl groups' stretching vibrations in polyphenols and other organic compounds. The (C-H) aliphatic stretching vibrations of aliphatic C-H bonds found in organic substances, such as lipids and carbohydrates, are responsible for peaks that emerge between 3000 and 2800 cm^{-1} . Carbonyl (C=O) groups found in ketones, aldehydes, and carboxylic acids are linked to the stretching vibrations of absorption bands detected in the 1750–1650 cm^{-1} range. Peaks in the region of 1300-1000 cm^{-1} indicate the stretching vibrations of C–O bonds present in various functional groups such as alcohols, ethers, and esters [3]. Specific peaks in the range of 750-500 cm^{-1} are observed due to Se-O [4].

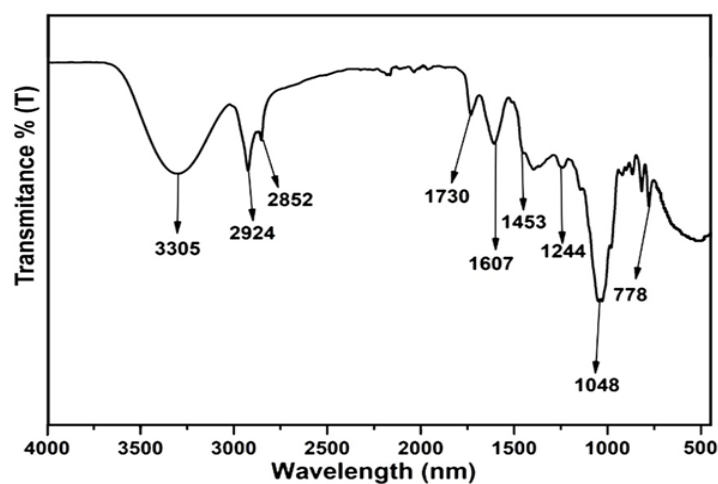


Figure 2. FTIR SPECTRA

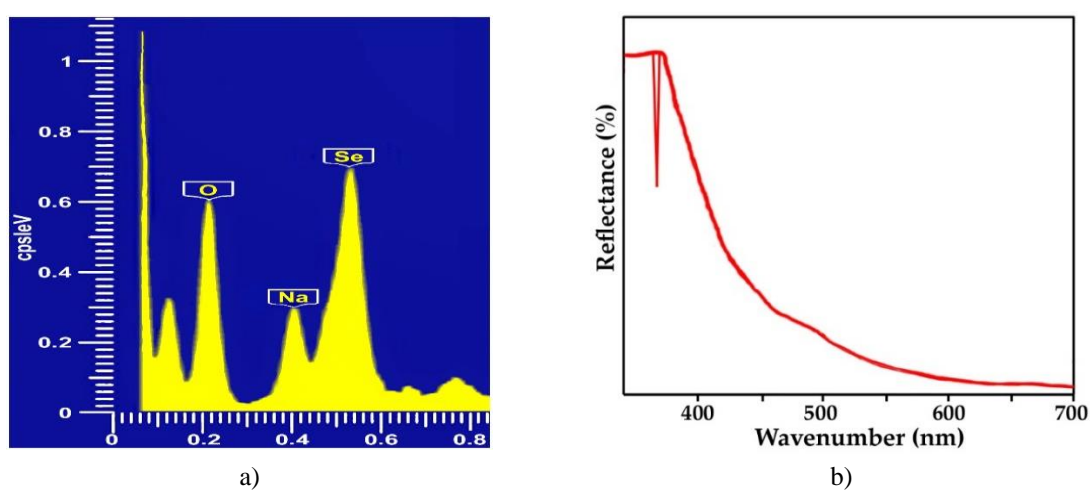


Figure 3. a) EDX Spectra, b) UV-Visible Spectrum

The WS-SeNPs EDX spectrum may be used to determine the elemental makeup and purity of the produced nanoparticles (**Figure 3a**). The successful synthesis of selenium nanoparticles using *Withaniasomnifera* extract is confirmed by the presence of selenium peaks. Because of contaminants or stabilizing agents based on carbon on the sample substrate, peaks in the spectrum corresponding to carbon X-rays may also be seen. If the sample contains contaminants or functional groups that contain oxygen, oxygen X-rays could be seen in the spectrum [5].

UV visible absorption spectra

Using UV-visible spectroscopy, the formation of SeNPs was investigated. The synthesis of Se NPs are confirmed by **Figure 3b**. It shows a plodding increase in absorbance with respect to reaction time at 320 nm. The visual identification of SeNP formation was achieved through the gradual change in color of the reaction mixture from transparent to ruby red. Once the incubation period of 24 hours was up, there was no further color change. This discovery showed that the WS plant produced completely formed Se NPs. Using UV-visible spectroscopy analysis, the absorption maximum (λ_{max}) at 360 nm was discovered after 24 hours and connected to the surface plasmon resonance (SPR) of Se NPs. The creation of SPR of Se NPs was responsible for the UV-visible absorption maxima, which occurred between 200 and 400 nm [6].

SEM & TEM analysis

SEM pictures provide comprehensive details regarding the surface morphology of WS-SeNPs, such as surface porosity, surface coatings, and the existence of organic residues made from an extract from *Withania somnifera*. Depending on the conditions of synthesis, selenium nanoparticles can have a spherical or irregular shape and vary in size in both SEM (**Figures 4a and 4b**) and TEM (**Figures 5a and 5b**) images. The size distribution and packing configuration of the nanoparticles can determine whether they are distributed singly or in clusters [7].

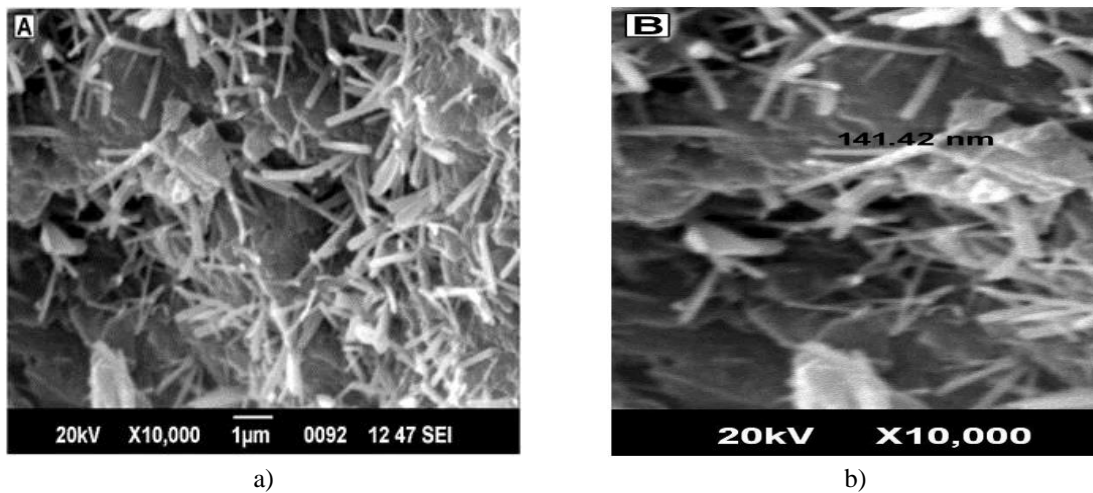


Figure 4. SEM Morphology (a & b)

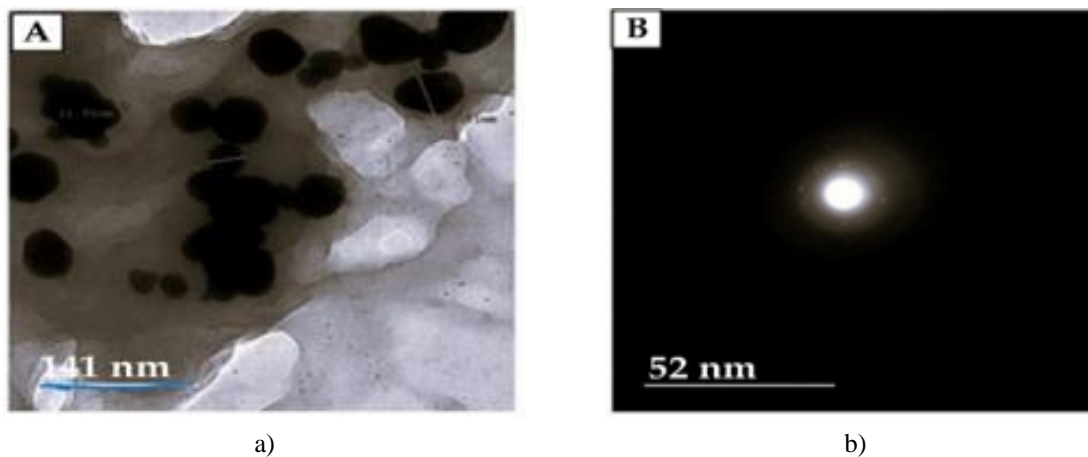


Figure 5. TEM Morphology (a & b)

XRD spectra

Withania somnifera Selenium Nanoparticles' (WS-SeNPs) XRD spectrum offers important information about the crystalline structure, phase composition, crystallite size, and lattice strain of the particles, making it easier to characterize and comprehend their physical characteristics and possible uses. Hexagonal (HCP) and monoclinic (MCP) selenium are common crystallographic phases of selenium nanoparticles, and they show different XRD patterns (Figure 6). Using Scherrer's equation, the prepared Senanoparticle's calculated crystalline size is 48 nm. [8].

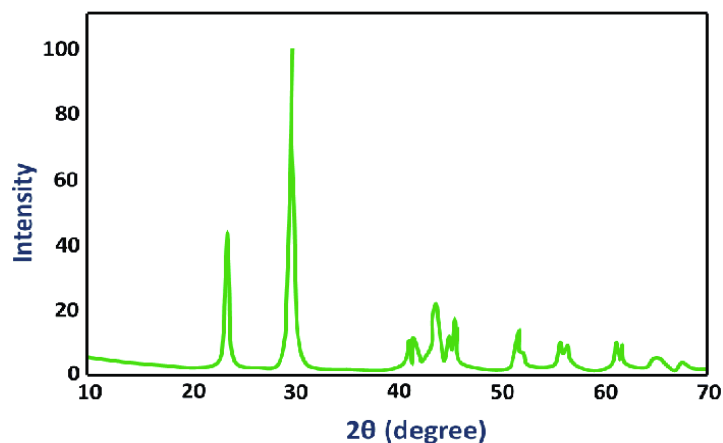


Figure 6. XRD Spectra



Figure 7. Antibacterial Activity (a & b)

Antibacterial activities

Due to their broad-spectrum activity, *Withania somnifera* Selenium Nanoparticles (WS-SeNPs) hold great promise as potent antibacterial agents (**Figures 7a and 7b**). Two pathogenic bacterial strains *E. faecalis* and *S. aureus*, were used to test the antibacterial activity of WS-Se NPs. Ciprofloxacin, an antibacterial medication, served as the positive control. Se NPs demonstrated significant efficacy against *S. aureus* & *Faecalis* with zones of inhibition measuring 13 and 12 mm. Se NPs, which may be an antibacterial agent, cause *S. aureus* to exhibit a larger zone of inhibition (13 mm) (**Table 1**) [9]. To investigate their therapeutic potential and optimize their formulations for clinical use, more investigation and clinical trials are necessary [10].

Table 1. Zone of inhibition (mm) by WS -Se NPs against bacteria.

Concentration SeNPS(ug/ml)	Zone of inhabitation(mm)	
	Gram Positive	Gram Positive
	<i>S. aureus</i>	<i>E. faecalis</i>
100	10 ± 0.22	10 ± 0.22
150	12 ± 0.20	11 ± 0.20
200	13 ± 0.68	12 ± 0.88

Values of a zone of inhibition (mm)

CONCLUSION

Using *Withania somnifera* extract, a green synthesis of selenium nanoparticles offers a sustainable and environmentally advantageous method of producing nanoparticles. The synthesized WS-SeNPs have antibacterial qualities, among other biological applications. These biogenic methods of synthesizing SeNPs are increasingly favored over conventional chemical and physical methods because they make use of living organisms such as plants, microalgae, and other microorganisms. Selenium nanoparticles made by green synthesis could replace antibiotics.

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ETHICS STATEMENT : None

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