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Green-Synthesised Selenium Nanoparticles Using Moringa Oleifera Leaf Extract: Characterization And Antibacterial Activity

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Abstract
Selenium nanoparticles have received considerable attention due to their biological and pharmaceutical characteristics. The current study explores the green synthesis of selenium nanoparticles (Se NPs) using Moringa oleifera leaf extract and investigates their physicochemical characteristics and antibacterial efficacy.
The biosynthesized selenium nanoparticles (Se NPs) were characterized using several techniques, including UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and atomic force microscopy (AFM). Additionally, the antibacterial activity of the Se NPs was assessed against Staphylococcus aureus (a Gram-positive bacterium) and Escherichia coli (a Gram-negative bacterium) using the agar well diffusion method and the minimum inhibitory concentration (MIC) method.
UV-Vis analysis confirmed the formation of Se NPs with a peak around 268 nm, while FTIR revealed the involvement of plant phytochemicals in reduction and stabilization. SEM and AFM showed spherical nanoparticles with sizes ranging from 60 to 120 nm and a roughness of 26.6 nm. The results indicated a more potent inhibitory effect against S. aureus, with a MIC of 58.2 µg/ml for both pathogens.
These findings support the potential application of green-synthesized Se NPs as eco-friendly and effective antimicrobial agents for biomedical use.
Keyword: *Selenium Nanoparticles, Characterization MIC, Antibacterial.*

INTRODUCTİON:

Nanotechnology has recently emerged as one of the most active areas of research. The manufacturing and application of nanoparticles have expanded significantly due to rapid advancements in this field. These nanoparticles exhibit unique or enhanced properties that depend on their size, distribution, and morphology. Nanotechnology has multidisciplinary applications and rapidly evolves across various fields, including engineering, biology, chemistry, and physics (Bharadwaj et al., 2021; Hammami et al., 2021). Metal nanoparticles such as selenium (Se), silver (Ag), cerium (Ce), iron (Fe), silicon (Si), titanium (Ti), and zinc (Zn) have garnered significant attention for their antimicrobial properties against various types of microbes. The plant-mediated biological synthesis of these nanoparticles is becoming increasingly valued for its simplicity, eco-friendliness, and broad-spectrum antimicrobial activity (Shakeri et al., 2022). Selenium nanoparticles, in particular, hold a unique position in medicine because they do not exhibit toxic effects on eukaryotic cells, offer improved bioavailability and have no damage to internal organs. Selenium nanoparticles, with small particle size, makes selenium more easily to be absorbed by the body (Serov et al., 2023).
Research published by (Guan et al., 2018) showed that the ionic form of selenium acts is effective in enhancing absorbance in the oral cavity. However, it decreases in bioactivity and increases toxicity compared to selenium nanoparticles (Se NPs). Furthermore, the green synthesised SE NPs by plant extracts were demonstrated to be utilised in drug delivery and other biomedical applications like anticancer, antioxidant and antimicrobial agents (Ikram et al., 2021).
Due to the medical significance and biotechnological applications of selenium nanoparticles, the research focuses on the green synthesis of selenium nanoparticles using Moringa leaves extract, characterization of the selenium nanoparticles and the anti-microbial activity study of selenium nanoparticles against gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli).

MATERİALS AND METHODS

Preparation of Moringa Aqueous Extracts

The air-dried ground plant material was extracted according to the steps explained by (Sultana et al., 2009). A 100 g sample was mixed with the 500 ml solvent aqueous methanol 80 % v/ v (methanol: water) for 8 hours under Soxhlet on a water bath. The extract was filtrated, concentrated and freed of solvent under reduced pressure at 45°C using a rotary evaporator. The dried crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator at 4°C until used (Salem et al., 2022; Sultana et al., 2009).

Biosynthesis of Selenium Nanoparticles

Plant Extract was used for the green biosynthesis of Se-NPs using an eco-friendly method. A combination was prepared by mixing 10 ml of plant extract and 90 ml of 2 mM $NaHSeO_3$. For the control sample, 10 ml of deionised water was added to 90 ml of 2 mM $NaHSeO_3$. Both flasks were incubated in the rotary shaker for 3 h in the dark to obtain a homogenous mixture. The generated Se-NPs were then separated and purified using deionised water and centrifugation. Dried Se-NPs were stored at room temperature for further analysis (8).

Detection and Characterization of Selenium Nanoparticles

The characterization of prepared nanoparticles which mentioned in plant extract selenium nanoparticles were performed using different instruments as shown below:

UV-VIS double beam spectrophotometer

UV-Visible (UV-VIS) double beam spectrophotometer was utilised to measure the absorbance spectra of plant extract selenium nano solution. All spectra were collected in a quartz cell with a 1 cm optical path at room temperature. Deionized distilled water was used as a control. Absorbance measurements were taken in the wavelength range of 200 to 800 nm. The concentrated samples were diluted at 1:10 (Agarwal et al., 2018). The measurements were performed in the Ministry of Science and Technology-Iraq.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

The characterization of functional groups on the surface of plant extract Se NPs was investigated by FTIR analysis (Shimadzu), and the spectra were scanned at a resolution of 4 cm-1 in the range of 400 - 4000 cm-1. By spreading the samples on a microscope slide, the samples were produced according to the standard method explained in (Agarwal et al., 2018) Each sample was prepared as a pellet in potassium bromide (KBr) at a 1: 99 ratio of sample to KBr for FTIR, After that, the sample was subjected to examination (Oh et al., 2019). The measurements were done by the ministry of science and technology-Iraq.

Scanning Electron Microscopy (SEM) Analysis

A scanning electron microscope (SEM) was used to analyze the morphology of the formed nanoparticles. The samples were characterized using a Bruker Scanning Electron Microscope. Samples were spread on a glass slide following standard methods (approximately seven drops on the slide). Subsequently, the samples underwent testing, as noted by (Atangana et al., 2019). A small drop of nanoparticles was placed on carbon coated copper grid and allowed to dry by using the mercury lamp for 5 min. Then readings were taken at a magnification of 5,000x, 10,000x, 20.000x, 50,000x and with steady voltage (Dimitrijević et al., 2013). The measurements were investigated at AlKhora Lab.

Atomic Force Microscopy (AFM)

The surface morphology of the nanoparticles was visualized in Atomic Force Microscope Contact mode under normal atmospheric conditions. Samples of Nanoparticle solution drops on a glass slide (1×2 cm). The samples dried, the slide was dispersed on the AFM sample stage, and analysis was carried out according to the standard procedure explained by (Du et al., 2008). AFM analysis was performed at Baghdad University College of Science, Department of Chemistry.

Estimation of Selenium Ions Concentration of Prepared Green synthesis Se NPs.

The concentration of Se ions for each of prepared green synthesis Se nanoparticles plant Extract SeNPs were estimated using atomic absorption spectrophotometer (AAS) after stabilizing the color change of green selenium nanoparticles solutions (Choudhary et al., 2016).

Investigation of the Antibacterial effect of green synthesized Selenium Nanoparticles

The investigation was conducted on two isolates: Staphylococcus aureus (a Gram-positive bacterium) and Escherichia coli (a Gram-negative bacterium), both of which exhibit multi-drug resistance. The Agar Well Diffusion Method (ADM) was employed for this study. The following steps were followed:

- Wells was created into the agar by a cork borer.

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- 100 µl of Se NPs was transferred into the agar well (concentrations were 116.4,58.2, 29.1 and 14.55 µg/ml).
- The plates were incubated for 24 hours at 37 °C under aerobic conditions.
- After the incubation period, the size of the inhibition zone around each well was measured.

Determination of Minimum Inhibitory Concentration (MIC)

Depending on the (Elshikh et al., 2016) protocol, the MIC experiment was done as follows:

- Bacterial stock cultures of S. aureus and E. coli bacteria were transferred to Nutrient broth media and placed in an incubator at 37 °C for one overnight. The following day, the test required the use of the DensiChek Plus Meter to measure the McFarland standard at a value of 0.5McF.
- The inhibitory effects of the synthesized Se NPs on S. aureus and E. coli were shown using the turbid metric test, which used resazurin. The extract was obtained using a rotary evaporator.
- One 96-well flat-bottom microtiter plate for Se NPs was prepared.
- The test consisted of one vertical row of broth used to check for sterility and three vertical rows for each microorganism.
- Each vertical row received 100µl of Mueller-Hinton broth (MHB).
- The first well contained 100µl of Se NP products at a concentration of 58µg/ml. The first well's mixture was thoroughly mixed.
- Following that, using a separate sterile pipette, 100µl of the first well's mix was transferred to the second well (29.1µg/ml) and well mixed.

RESULTS

Characterization of Se NPs using SEM

The surface structure of the synthesised Se NPs was identified using scanning electron microscopy. The technique was used to determine the size and structure of the synthesised nanoparticles. Se NPs sample for SEM was centrifuged to get the purified form, dried using a hot air oven and mounted on a carbon-coated grid. The sample film was left to dry and tested under the SEM. Figure 1 depicts the SEM results of the Se NPs synthesized from the Moringa leaves extract. The results reveal the particle size ranging from 60 to 120 nm. Also, it showed smooth, clean, and homogeneous, with no signs of surface contamination or mechanical damage particles with a dominant spherical shape.

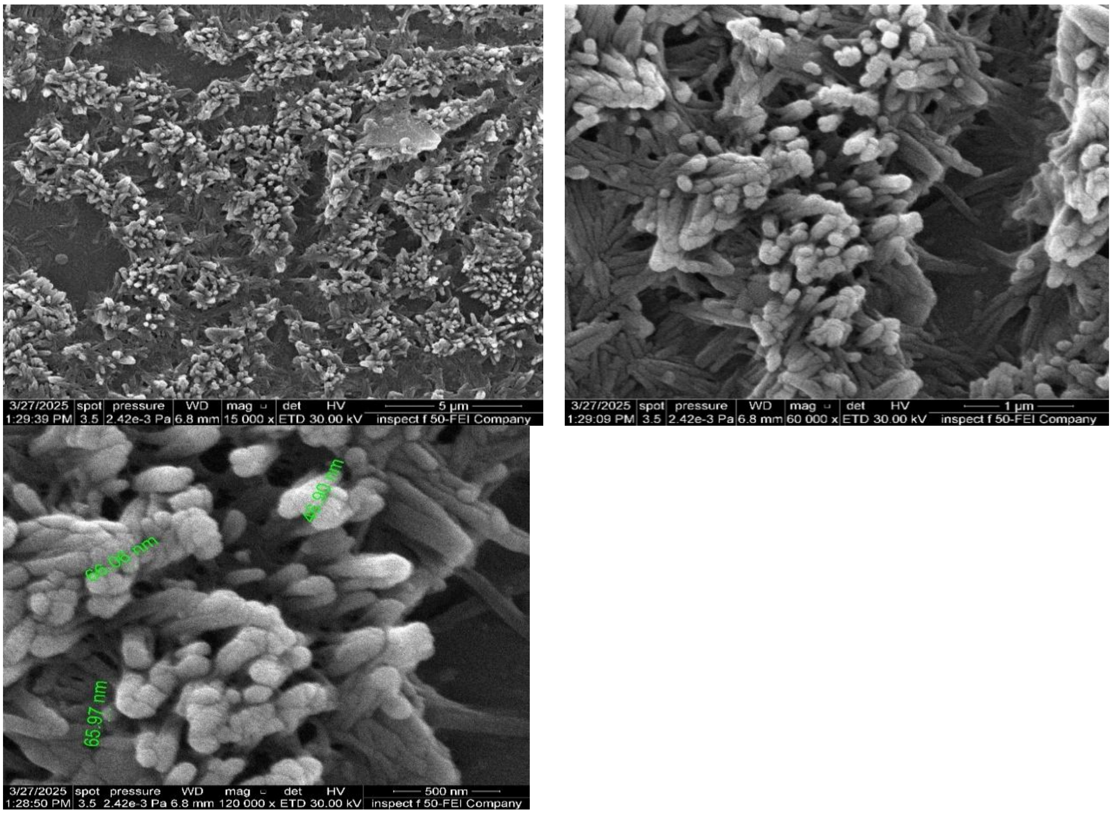


Figure 1. SEM images of Se NPs synthesised from Moringa Leaf extract

Characterization of Se NPs using Atomic Force Microscopy (AFM)
Atomic Force Microscopy (AFM) was utilised to analyse the surface morphology, particle size at the nanoscale, diameter, and roughness of selenium nanoparticles synthesized from plant extraction. The morphology images of the biosynthesized Se NPs are presented in Figure 2, which illustrates the 3D representation of the surface morphology. The particle size of the selenium nanoparticles was measured to be 97 nm.

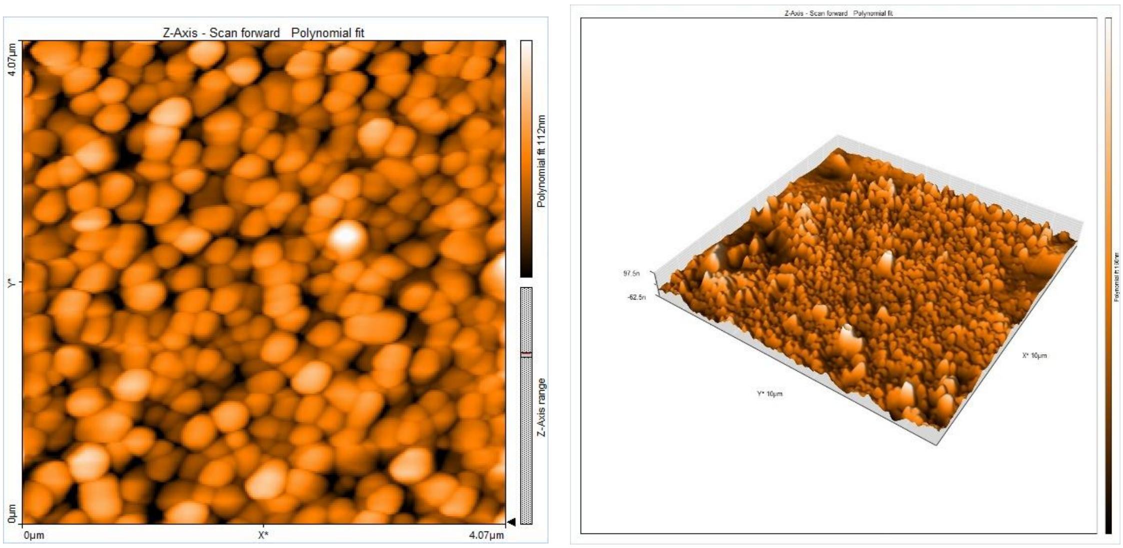


Figure 2. AFM of biosynthesized Se NPs.
Figure 3 shows the particles analysis that determine the volume percentage of dimeter for plant extract and synthesised Se NPs at which the average diameter of synthesised selenium nanoparticles by plants extract was 90.9 nm.

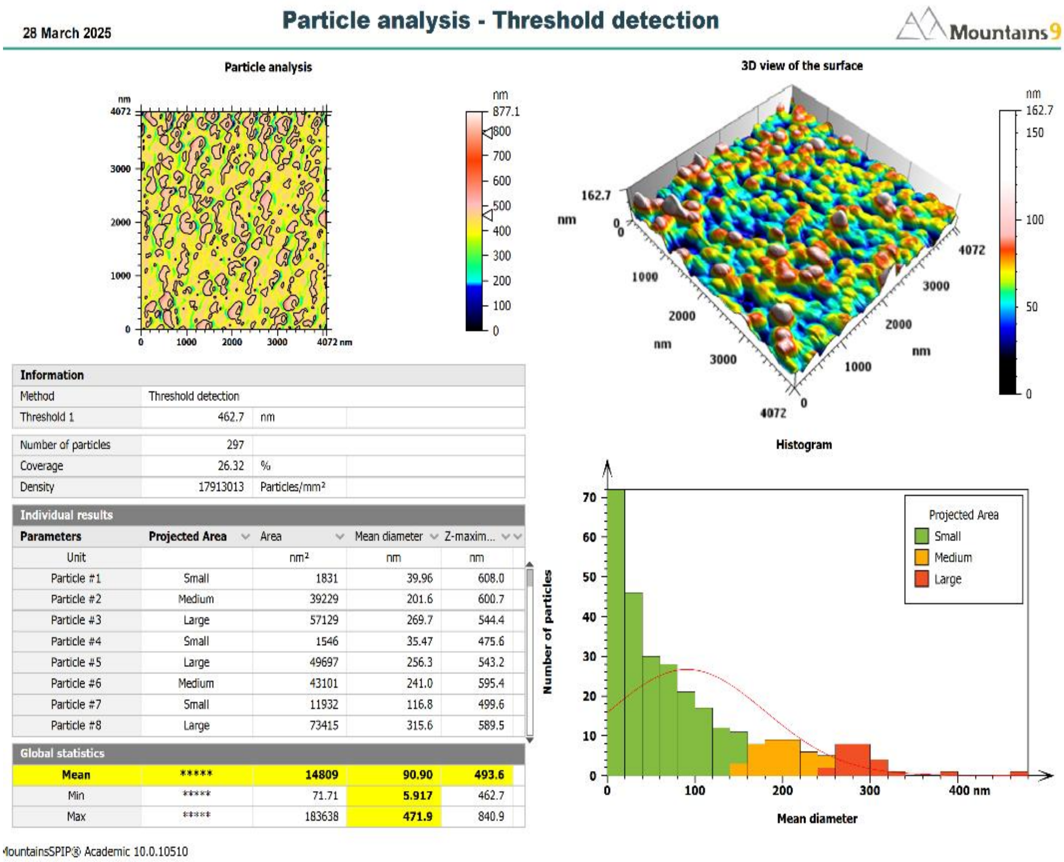
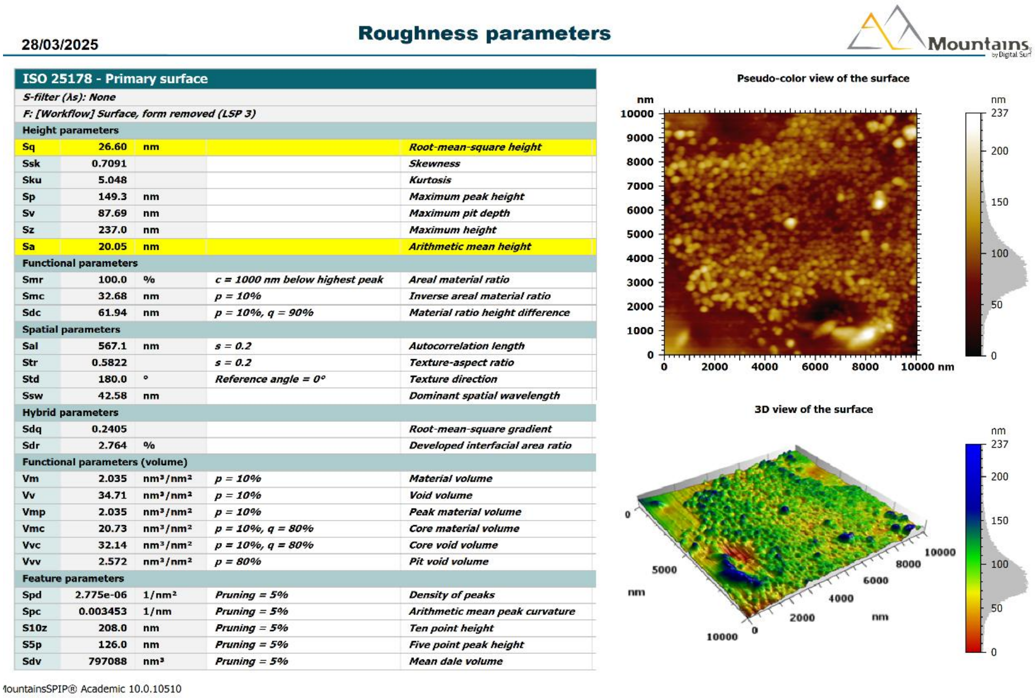


Figure 3. Granularity Distribution and diameter of synthesized Se NPs.
Figure 4. illustrates the surface roughness of the synthesized selenium nanoparticles. It shows that the root mean square (RMS) roughness of the nanoparticles is 26.6 nm.

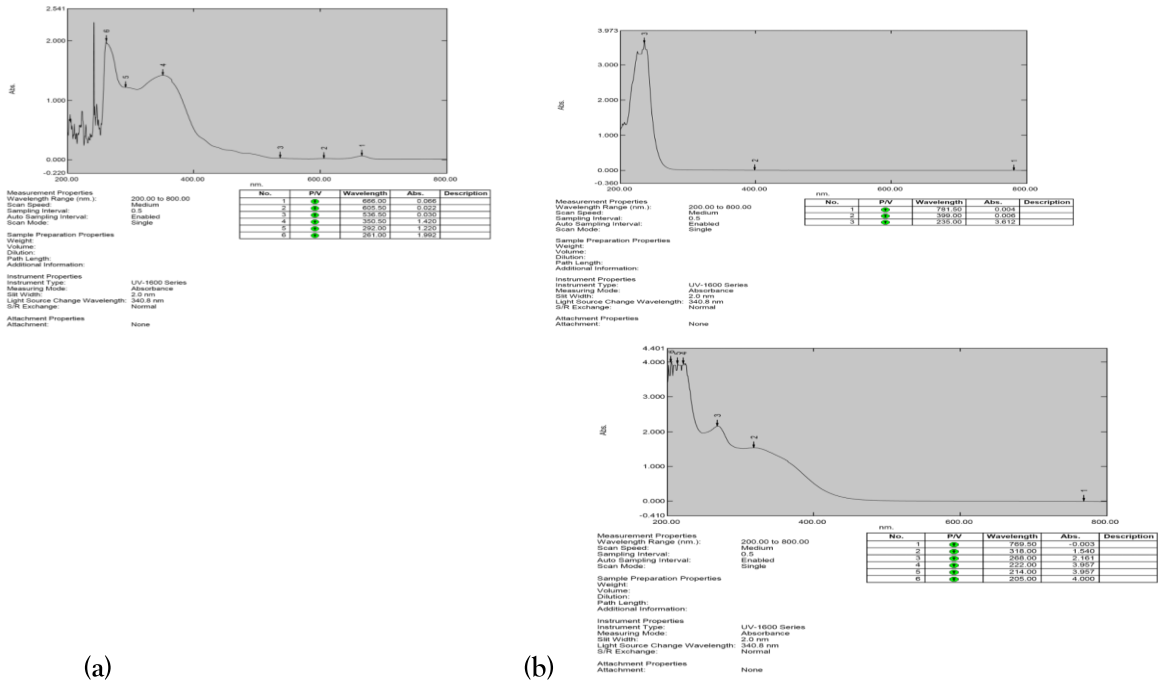


ISO 25178 - Primary surface				
S-filter (λs): None				
F: [Workflow] Surface, form removed (LSP 3)				
Height parameters				
Sq	26.60	nm		Root-mean-square height
Ssk	0.7091			Skewness
Sku	5.048			Kurtosis
Sp	149.3	nm		Maximum peak height
Sv	87.69	nm		Maximum pit depth
Sz	237.0	nm		Maximum height
Sa	20.05	nm		Arithmetic mean height
Functional parameters				
Smr	100.0	%	c = 1000 nm below highest peak	Areal material ratio
Smc	32.68	nm	p = 10%	Inverse areal material ratio
Sdc	61.94	nm	p = 10%, q = 90%	Material ratio height difference
Spatial parameters				
Sal	567.1	nm	s = 0.2	Autocorrelation length
Str	0.5822		s = 0.2	Texture-aspect ratio
Std	180.0	°	Reference angle = 0°	Texture direction
Ssw	42.58	nm		Dominant spatial wavelength
Hybrid parameters				
Sdq	0.2405			Root-mean-square gradient
Sdr	2.764	%		Developed interfacial area ratio
Functional parameters (volume)				
Vm	2.035	nm³/nm²	p = 10%	Material volume
Vv	34.71	nm³/nm²	p = 10%	Void volume
Vmp	2.035	nm³/nm²	p = 10%	Peak material volume
Vmc	20.73	nm³/nm²	p = 10%, q = 80%	Core material volume
Vvc	32.14	nm³/nm²	p = 10%, q = 80%	Core void volume
Vvv	2.572	nm³/nm²	p = 80%	Pit void volume
Feature parameters				
Spd	2.775e-06	1/nm²	Pruning = 5%	Density of peaks
Spc	0.003453	1/nm	Pruning = 5%	Arithmetic mean peak curvature
S10z	208.0	nm	Pruning = 5%	Ten point height
S5p	126.0	nm	Pruning = 5%	Five point peak height
Sdv	797088	nm³	Pruning = 5%	Mean dale volume

Figure 4. Roughness of the Synthesized Se NPs.

Characterization of Se NPs using Ultraviolet Visible Light (UV-visible)

Three samples were analysed using UV visible spectrophotometer. Figure 5 shows the results from UV visible spectrophotometer for three samples which are Moringa plant extract, selenium and synthesised selenium nanoparticles.



(a) (b)

(c)

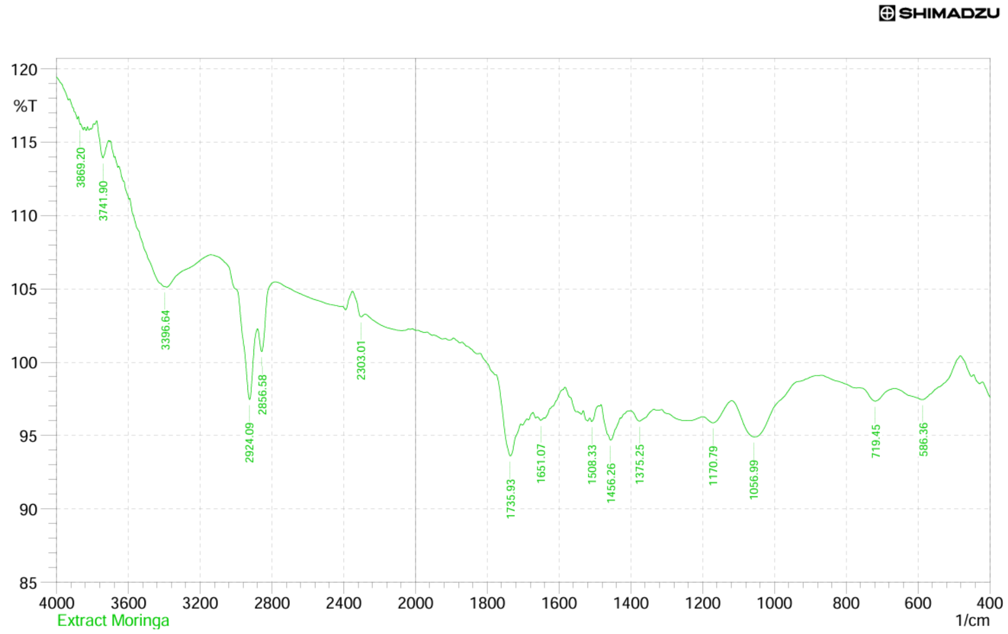
Figure 5. Ultraviolet-visible spectra for (a) Moringa plant extract (b) Se (c) synthesized Se NPs.

Characterization of Se NPs using Fourier Transform Infrared Spectroscopy (FTIR)

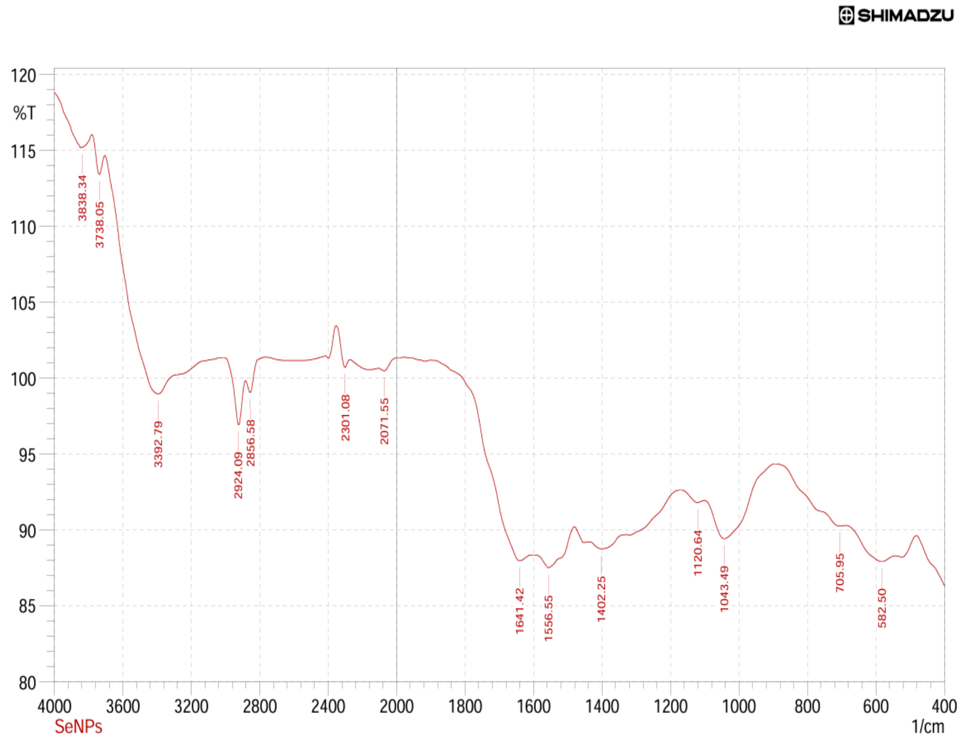
FTIR is a spectroscopy method used to ensure the formation of nanoparticles by giving insights into the vibrational and rational modes of the involved molecules. This technique identifies the functional and potential phytochemical molecules that play a role in reducing and stabilizing selenium nanoparticles.

In Figure 6, FTIR was utilized to characterize (a) Moringa leaf extract and (b) selenium nanoparticles (SeNPs) synthesized using the Moringa extract. FTIR reveals the functional groups involved in reducing and stabilizing selenium nanoparticles through green synthesis.

The FTIR spectrum of Moringa extract (Figure 6 a) revealed a range of absorption peaks indicative of various bioactive compounds typically found in plant-based systems.
The FTIR spectrum of selenium nanoparticles synthesized using the Moringa extract (Figure 6 b) shows several shifts and intensity changes in comparison to the crude extract, which signifies chemical interaction and nanoparticle formation.



(a)

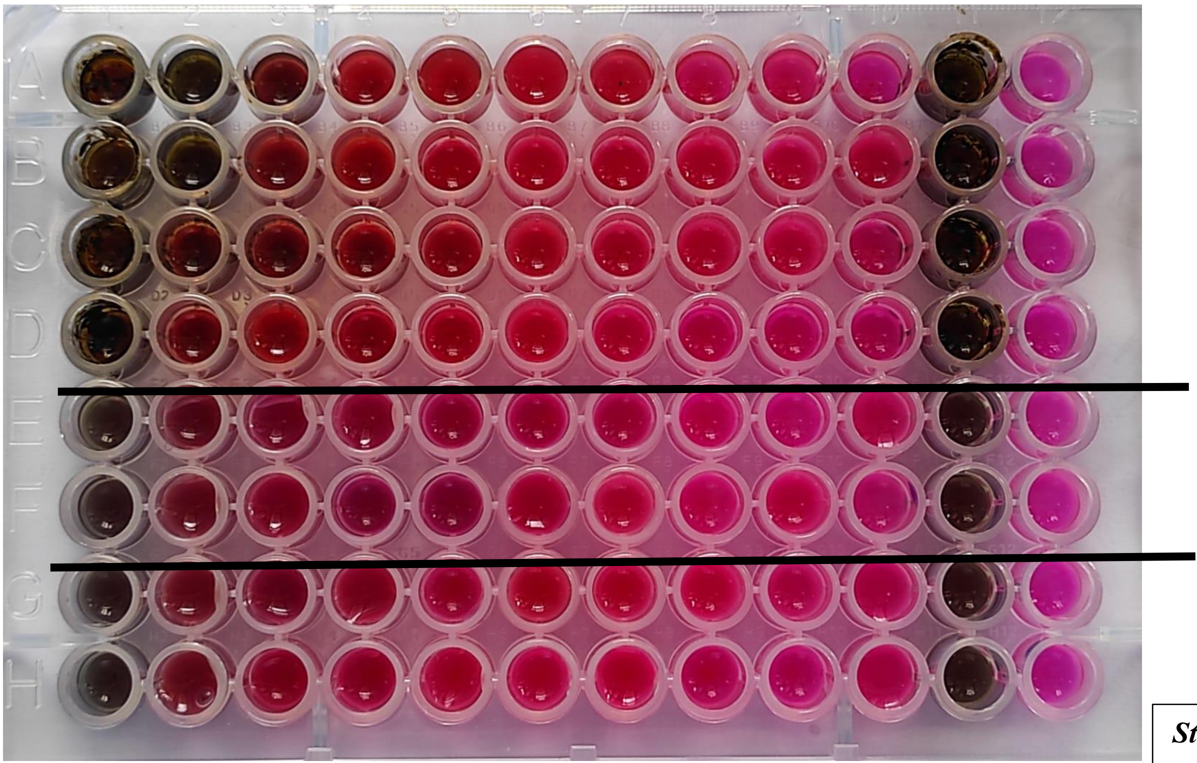


(b)

Figure 6. FTIR Characterization spectrum for (a) Moringa plant extract (b) Synthesized Se NPs

Determine the minimum inhibitory concentration (MIC) of the SN nanoparticles against Pathogenic bacteria

The results from the serial dilutions of selenium nanoparticles (Se NPs) at concentrations of 58.2, 29.1, 14.5, and 7.2 µg/ml were carried out following the methods described by (Khudier et al., 2023). As shown in Figure 7, the change in color indicates that the minimum inhibitory concentration (MIC) against clinical isolates of Staph aureus and E. coli is 58.2 µg/ml for both types of pathogenic bacteria.



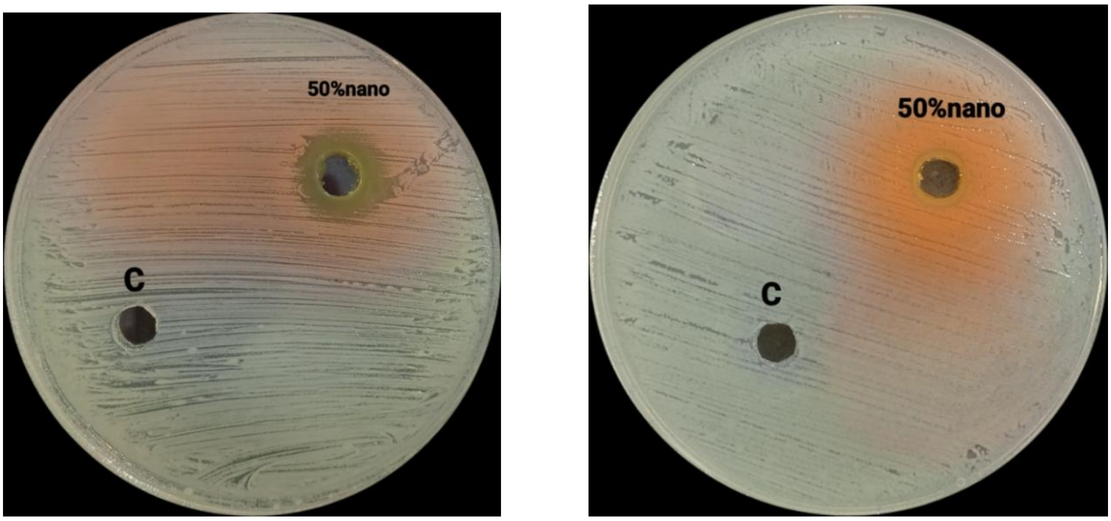
Staph. aureus

E. coli

Figure 7. Flat-bottom microtiter plates for determine MIC of Se NPs against the E. coli and Staph. aureus.

Inhibitory effect of SN NPs on Pathogenic Bacteria

The results present the antibiotic SN NPs against Staph. aureus and E. coli bacteria, observed on two separate plates. The data (Figure 8) indicates that the inhibition zone size for Se NPs (50% nano) is 15 mm on the Staph. aureus plates. On the other hand, on the E. coli bacteria plates, the inhibition zone size is 4 mm for Se nanoparticles (50% nano).



(a) **(b)**

Figure 8. The inhibitory effect of Se NPs (a) Staph. aureus (b) E. coli.

Discussion

Selenium nanoparticles have played a vital role in research related to advancement in drug delivery and new anti-bacterial drugs against multi drug-resistant bacteria. It is essential to verify that the green synthesis procedure was executed correctly to ensure the formation of Se NPs. Characterization techniques are used to confirm this.

The results UV visible spectrophotometer showfor the moringa plant extract, the major peaks at 261.00 nm (Abs = 1.992), 292.00 nm (Abs = 1.220) and 350.50 nm (Abs = 1.420) indicates the presence of phenolic compounds and flavonoids. The Moringa extract contains bioactive compounds with strong UV absorption, useful for acting as reducing and stabilizing agents in nanoparticle synthesis. While, for the selenium salt, the strong peak at 235 nm is typical of selenite (SeO_3^{2-}) ions, reflecting electronic transitions within selenium species. This spectrum confirms the pure selenium salt, likely in its inorganic ionic form, and serves as a control before reduction or synthesis. Furthermore, for the synthesized selenium nanoparticles using plant extract, the appearance of a broad peak around 268 nm with high absorbance indicates formation of selenium nanoparticles (SeNPs).

By comparing the results from the three samples, the shift in peak positions and higher absorbance compared to the selenium salt and plant extract confirms the successful synthesis of selenium nanoparticles using moringa extract.

Similarly, for FTIR (Figure 6), the FTIR spectrum of Moringa extract revealed a range of absorption peaks indicative of various bioactive compounds typically found in plant-based systems.

- A broad and intense absorption band was observed at 3396 cm^{-1}, attributed to O–H stretching vibrations of hydrogen-bonded hydroxyl groups, which are characteristic of phenolic compounds, flavonoids, and carboxylic acids. Additional sharp peaks at 3863 cm^{-1} and 3714 cm^{-1} indicate the presence of free O–H groups, suggesting the abundance of polyphenolic constituents.
- Aliphatic C–H stretching vibrations were confirmed by peaks at 2924 cm^{-1} and 2855 cm^{-1}, corresponding to $-CH_2-$ groups present in long-chain hydrocarbons or fatty acids.
- A strong band at 1753 cm^{-1} is consistent with C=O stretching vibrations of esters or carbonyl-containing compounds, likely derived from lipids or esterified phenolics. The peak at 1651 cm^{-1} can be attributed to C=C stretching or conjugated C=O groups, possibly from aromatic ketones or flavonoids.
- A strong peak at 1056 cm^{-1} is indicative of C–O–C stretching, typical of polysaccharides.

This spectral profile confirms the rich phytochemical composition of Moringa extract, including phenolics, flavonoids, proteins, carbohydrates, and fatty acids.

While, FTIR spectrum of selenium nanoparticles synthesized using the Moringa extract shows several shifts and intensity changes in comparison to the crude extract, which signifies chemical interaction and nanoparticle formation.

- The broad O–H peak remained visible at 3382 cm^{-1}, though with slightly reduced intensity, suggesting partial involvement of hydroxyl groups in the reduction of selenium ions. Peaks corresponding to free O–H groups at 3838 cm^{-1} and 3738 cm^{-1} were also present but diminished in comparison to the plant extract, indicating their consumption during the reduction process.
- Aliphatic C–H stretching peaks at 2924 cm^{-1} and 2855 cm^{-1} were retained, signifying that hydrocarbon chains remain unaffected during synthesis.
- Notably, new or shifted peaks appeared at 2071 cm^{-1} and 2301 cm^{-1}, which may correspond to intermediate organic transformations or possible C≡N groups.
- A key shift was observed in the carbonyl region, with the C=O/C=C stretching band moving to 1641 cm^{-1}, and enhanced amide-related vibrations appearing at 1555 cm^{-1}, confirming the involvement of proteinaceous compounds in capping the SeNPs. Additionally, a new band at 1402 cm^{-1} is suggestive of carboxylate ions (COO^-), possibly indicating deprotonated acids contributing to stabilization.
- The polysaccharide region also exhibited significant changes, with peaks at 1120 cm^{-1} and 1043 cm^{-1}, confirming the presence of C–O–C or C–N functional groups from plant-based biopolymers.
- Importantly, the formation of selenium nanoparticles is confirmed by the appearance of new bands at 705 cm^{-1} and 582 cm^{-1}, which are characteristic of Se–O or Se–Se stretching vibrations. These peaks were not prominent in the original extract and confirm the successful formation of selenium-based nanostructures.

The comparison between the two FTIR spectra illustrates the biochemical changes that occur during the synthesis of nanoparticles. The reduction in the intensity of the hydroxyl and carbonyl peaks suggests that these functional groups are crucial in reducing selenium. Additionally, new peaks in the fingerprint region (700–500 cm^{-1}) indicate the formation of selenium nanoparticles (SeNPs). The increased prominence of amide bands in the SeNP spectrum implies that plant proteins likely function as stabilizing or capping agents.

Consequently, Moringa extract plays a dual role in the green synthesis process: it acts as a reducing agent, facilitating the conversion of selenium salts into elemental nanoparticles, and as a capping agent, providing colloidal stability through interactions with biomolecules. These results are in agreement with the findings of (Banerjee & Rajeswari, 2024) and (Tao et al., 2025).

As the characterisation techniques confirmed the successful formation of Se NPs, the minimum inhibitory concentration (MIC) technique is a key methodology to confirm the activity of the forming Se NPs. The results show the colour change indicates that the MIC against clinical isolates of Staph aureus and E. coli is 58.2 μg/ml for both types of pathogenic bacteria. The finding of the current work agrees with previous research results described by (Huang et al., 2019) who reported the plant green synthesised SeNPs inhibited both S. aureus and E. coli with MIC values of 50–100 μg/mL. Similarly,(Forootanfar et al., 2014) revealed MIC 64 μg/mL for S. aureus and MIC 128 μg/mL for E. coli using fungal-mediated SeNPs. This can be attributed to the existence of phytochemical. The different reported MICs in the literature for various Se NPs can be attributed to differences in nanoparticle size, shape, capping agents, and synthesis methods. The green synthesis approach used in this study likely contributed to enhanced activity through phytochemical surface modifications, as confirmed by FTIR analysis.
Subsequently, Se NPs at a concentration of 58.2 μg/ml, representing the MIC value, were tested using the agar well diffusion method. The findings show that Gram-positive bacteria (S. aureus) were more susceptible to Se NPs than Gram-negative (E. coli), a result consistent with published research by (Guisbiers et al., 2016; Kudaer et al., 2022; Mohammad et al., 2020; T. Mohammed et al., 2020; T. H. Mohammed et al., 2019). The absence of an outer membrane in Gram-positive bacteria may allow selenium nanoparticles (SeNPs) to interact more directly with the peptidoglycan layer, potentially leading to higher susceptibility. In contrast, the outer membrane of Gram-negative bacteria acts as an additional barrier, reducing nanoparticle penetration and antimicrobial effectiveness.
These findings prove the prospect of green-synthesized selenium nanoparticles as effective antimicrobial agents, affirming further exploration for biomedical and pharmaceutical applications.

CONCLUSİON

The successful green synthesis of selenium nanoparticles using Moringa oleifera extract was demonstrated through various characterization techniques, confirming their nanoscale size, morphology, and chemical composition. The involvement of phenolic, flavonoid, and protein-based functional groups in nanoparticle formation was validated through FTIR analysis. The antimicrobial assays revealed significant activity of Se NPs against both Gram-positive and Gram-negative bacteria, with Staphylococcus aureus showing higher susceptibility. The enhanced antimicrobial effect is likely due to the synergistic action of selenium and the bioactive compounds from Moringa extract. This study underscores the potential of biosynthesized Se NPs as promising agents in nanomedicine, particularly for antimicrobial therapies, and highlights the value of plant-mediated nanoparticle synthesis as a sustainable and biocompatible approach.

Authors' Contributions

Afnan A. Al-saeed and Mohsen Hashim Risan developed concept and draft for the paper. Talib S. Al-rubaye contributed in running the experiemnts. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

All authors declared that they have no conflict of interest.

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