

RESEARCH ARTICLE

Biosynthesis and characterization of selenium nanoparticles using *Ceratonia Siliqua* and evaluation of its antimicrobial properties

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ABSTRACT

This is the research to document the antimicrobial activity of selenium nanoparticles (nanoSe) prepared by an aqueous extract of *Ceratonia siliqua*, which is significant given the potency of nanoSe in medicinal applications. The nanoSe that was produced was characterized employing a variety of conventional methods including powder X-ray diffraction (PXRD), Fourier transforms infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDAX), DLS and z-potential. The PXRD analysis has demonstrated the compatibility of nanoSe with the reference number 00-001-0853. The FTIR spectrum also confirmed the existence of residual organic components in the extract. The FESEM images have revealed the particles were enveloped in the organic materials from *C. Siliqua*. Particles have shown a spherical morphology. The mean hydrodynamic particle size of the biosynthesized nanoSe was approximately 199 nm (size dispersion by intensity). The particles have shown a mean surface charge of -21.88 mV. NanoSe is crucially successful in the suppression of the growth pathogenic bacteria. The project's outcomes highlight the effective antimicrobial properties of the biosynthesized nanoSe, emphasizing the useful applications of metallic nanoparticles such as selenium in future antibacterial applications.

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INTRODUCTION

The special physicochemical characteristics of nanomaterials have drawn substantial interest in biomedical nanomaterials, which develops theranostics for the detection and treatment of microbes (1-5). Biologically created nanoparticles have garnered much interest for their potential as antimicrobial and cancer therapeutics (6-8). These nanoparticles' distinctive optical, physical, electrical, and chemical characteristics, together with their high surface-to-volume ratio, allow them to alter their surface chemistry and combine them with additional imaging or therapeutic substances

to serve as multifunctional nanoparticles (9-13). Many pieces of evidence have been gathered to date supporting the biological action of selenium-containing compounds of various types (14). Researchers have consistently demonstrated that selenium nanoparticles (nanoSe) and other selenium-containing compounds may cause abnormal cells to be eradicated by initiating several signaling pathways and maintaining sustained endoplasmic reticulum stress. Selenium has been used in biomedical treatment for more than a century. NanoSe was used as a therapeutic agent in antimicrobial and antioxidant agents, and significant improvements were noted in

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both the cure rate and side effects. It is also used for antimicrobial resistance, antifungal, injury, drug-induced nephrotoxicity, diabetic disorders, and inflammatory disorders (15-19). NanoSe is less harmful to the liver than selenite (a powerful antioxidant) or other selenium derivatives (20). Hence, nanoSe's lower toxicity and its distinctive physicochemical attributes offer an opportunity to develop new pharmaceuticals. Due to its excellent biocompatibility, stability, and strong selectivity, research on its biological activity has become more popular in recent years (21-23). With the dual role of nanoSe as a material delivery system that has shown both biomedical or protective properties, the design of novel therapeutic agents is not out of reach (24, 25). The antioxidant and antimicrobial mechanism may be related to selenium involvement in oxidation which produces ROS, leading to the eradication of bacteria cells (26, 27). Hence, the ROS generation, caspase-3 activation, upregulation of apoptotic markers (bax, p53, pp38, cytochrome c), downregulation of bcl-2, mitochondrial malfunction, suppression of matrix metalloproteinase-2 expression and disturbance in membrane molecules and F-actin can be the possible reasons for utilize nature of nanoSe (20, 28).

There are several developed ways to produce nanoSe, but two primary ones that stand out are physical and chemical methods. While some physical and/or chemical processes are expensive, demand a lot of energy, or use harmful substances, green and biological approaches provide the most economical and environmentally benign ways to prepare nanomaterials (29-31). Natural resources utilization, such as various herbal extracts, bacteria, fungus, algae, etc., as reducing and stabilizing agents is defined as "biologic approaches for the fabrication of metallic NPs" (32-36). The sizes and shapes of the biosynthesized nanoparticles might vary but factors that affect the diameter size, shape, surface charge, and surface functionalization could also influence cellular uptake and the cytotoxicity of metallic nanoparticles (37-40). The natural sources for the fabrication of metallic NPs may significantly influence the bioactivity of nanoparticles. A range of phenols, flavonoids, etc. may participate in the bio-reduction process and stabilizing of nanoparticles in a plant-mediated synthetic procedure (41-44). Simultaneously, the biomaterials can be conjugated with the nanoparticles, that cause the nanoparticles' surface functioning and donate new functions in

the process (45-48).

Accordingly, it could form a conjugated form of nanoSe and affect the particle sizes and shape. Hence, investigation of the impact of the plant materials on the activity of the nanoSe can produce potentially valuable biological applications. In this study, the morphology, size, and other distinctive physicochemical qualities of the prepared biogenic nanoSe and its antibacterial efficacy were explored.

MATERIALS AND METHODS

Materials and characterizations

Sodium selenite, ascorbic acid, and other materials used were procured from Sigma. Ultrapure deionized water (DI) was used. The *Ceratonia siliqua* was obtained from Azarbijan-Gharbi province, Iran. In order to prepare the required extract, 10 grams of the plant powder were mixed 300 ml deionized water and stirred about 24 h. the final solution was filtered by Whatman paper. The aqueous extraction of *Ceratonia siliqua* was used to prepare the nanoSe particles.

Selenium nanoparticle synthesis

The synthesis procedure was carried out as follows: 900 mg of sodium selenite pentahydrate ($\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$) was dissolved in 200 mL of aqueous extract of *C. Siliqua*, serving as the reaction medium and stabilizing agent. A cold solution of ascorbic acid (17.1 mM) was prepared, ensuring it was well-mixed and chilled before use. The cold ascorbic acid solution was then added to the NaSeO_3 solution while being vigorously stirred, a step performed slowly to ensure proper mixing and reduction. The mixture was stirred continuously for 30 minutes to ensure complete reduction and formation of colloidal crimson nanoSe. The reaction temperature was maintained at approximately 25°C, and the pH of the reaction was kept around 7 for optimal stability and formation of nanoSe. After the reaction was complete, the mixture was allowed to settle for 1 hour. The mixture was centrifuged (10,000 rpm, 15 min) to collect the nanoSe particles. The collected nanoSe particles were washed to eliminate any residual reactants and dried.

Purification of nanoSe particles

The collected nanoSe particles were washed to eliminate any residual reactants by first carefully decanting the supernatant after centrifugation, ensuring the nanoSe pellet at the bottom of the tube

remained undisturbed. Distilled water was then added to the tube, and the particles were gently resuspended by shaking or vortexing. The tube was centrifuged again at 10,000 rpm for 10-15 minutes to re-pellet the nanoSe particles, separating them from any soluble impurities. This washing process, involving the addition of distilled water, resuspension, and centrifugation, was repeated 2-3 times to ensure thorough purification. After the final wash, the supernatant was decanted, leaving behind the purified nanoSe particles, which were then ready for drying.

Characterization techniques

The air-dried nanoSe powder was subjected to Cu Ka (1.54059 Å) radiation using the Philips Panalytical X'pert Pro X-ray diffractometer. The powder was ground with KBr to form pellets for measuring the FTIR between 4000-400 cm⁻¹ using Nicolet Summit PRO FTIR Spectrometer. Field emission scanning electron microscopy (FESEM) and EDAX were employed to examine the size, shape and composition of the nanoparticles by employing Tescan Mira3 FESEM. By using sonication, a diluted sample was created and evenly distributed, and dried afterward. DLS was employed to determine the average hydrodynamic size of the nanoparticles. To prevent "shadowing" of the signal, MilliQ water was used. Using the Zeta Compact from CAD company, the surface charge of the nanoparticles was also determined.

Antibacterial properties

The experiment was performed in three replicates, and Gram(+) bacteria (*Bacillus subtilis* PTCC 1365 and *Staphylococcus aureus* ATCC

25923) and Gram(-) bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027) were employed. Each blank disc was impregnated with nanoSe at a concentration of 300 µg/ml. The plates were incubated at 37 °C for 24 hours. After this period, the diameter of the non-growth region was measured in millimeters. Gentamicin was used as a positive control and sterile distilled water (DW) disc was used as a negative control. In order to determine the MIC and MBC, the micro broth dilution method was used. The nanoSe were prepared with concentrations of 0, 25, 50, 75, 100, 125, and 300 µg/ml. Chloramphenicol was used as a control. After incubation, the first well in which no growth was observed was considered as the MIC. To determine MBC, nutrient agar plates (without nanoSe) were inoculated from the surface of wells without growth and placed in an incubator. For reliability, the tests were repeated 3 times (49).

According to the MBC/MIC ratio, we find out the antimicrobial property. If the MBC/MIC ratio is ≤ 4, the drug is reflected antibacterial; but if the MBC/MIC ratio is > 4, the drug is definite as bacteriostatic (50).

Statistical analysis

Statistical analysis was done by SPSS software (V.21). Mean ± standard deviation was attained using one-way ANOVA and p-values of less than 0.05 (p<05) were deliberated significant.

RESULTS AND DISCUSSION

Physicochemical studies

Powder X-ray diffraction (PXRD)

The PXRD analysis was used to find the

Table 1. Crystallographic parameters of the known selenium structure

Crystal system	Hexagonal
Space group	P3121
Space group number	152
a (Å)	4.34
b (Å)	4.34
c (Å)	4.95
Alpha (°)	90
Beta (°)	90
Gamma (°)	120
Measured density (g/cm ³)	4.81
Volume of cell (10 ⁶ pm ³):	80.74
Z	3

crystallographic parameters associated with prepared nanoSe. The data review has revealed the successful synthesis of selenium nanoparticles. The accepted pattern was compatible with the reference number 00-001-0853. Hence, the nanoSe also shows the same crystallographic parameters as the known selenium structure (Table 1). The

comparison was performed using PANalytical X'Pert HighScore Plus software. In Figure 1, the corresponding 2θ values of the accepted pattern and d-spacing of the accepted pattern (Table 2) have been displayed by using blue lines. The obtained 2θ and d-spacing of the nanoSe indicated the proximity of the PXRD pattern of

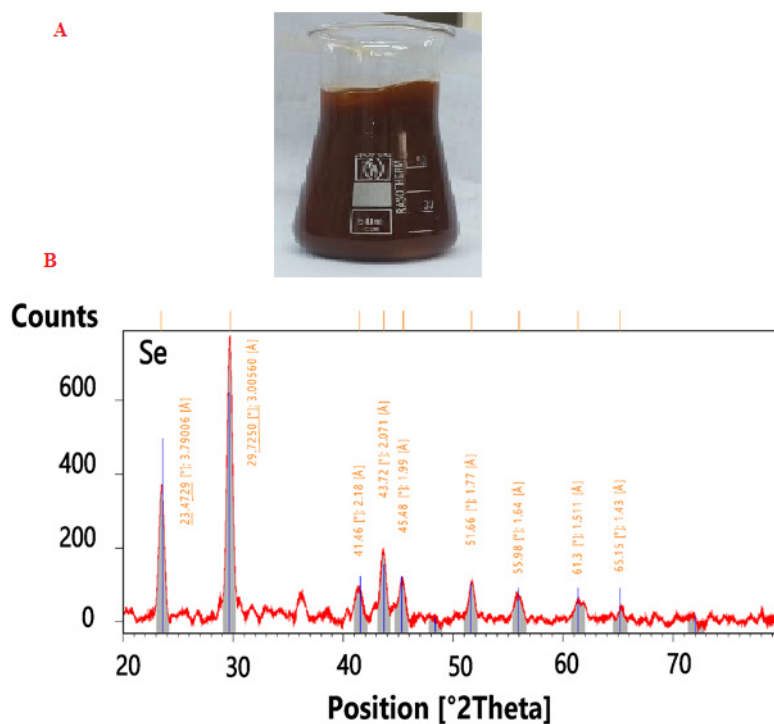


Fig. 1. (A): NanoSe synthesized by *C. Siliqua*; (B): The PXRD pattern of the prepared biosynthesized nanoSe.

Table 2. The known parameter related to the peak list of the known selenium crystals

No.	h	k	l	d [Å]	2Theta[deg]	I [%]
1	1	0	0	3.77	23.58	80
2	1	0	1	3.01	29.655	100
3	1	1	0	2.17	41.584	20
4	1	0	2	2.07	43.694	25
5	1	1	1	2	45.306	20
6	2	0	0	1.88	48.376	2
7	2	0	1	1.77	51.596	17
8	1	1	2	1.64	56.029	15
9	1	0	3	1.51	61.345	15
10	2	1	0	1.43	65.186	15
11	1	1	3	1.31	72.033	3
12	1	0	4	1.18	81.506	3
13	2	1	3	1.08	90.998	2

both nanoSe and accepted reference number.

The most significant peaks of Se were observed at specific 2θ values, which correspond to the crystallographic planes of selenium. These peaks confirmed the presence of nano-sized selenium particles with a well-defined crystalline structure. However, some other low intensity peaks were observed, which suggests that the sample may contain some noise, background signals, or possible impurities. These additional peaks could arise from various sources, such as residual reactants, by-products from the synthesis process, or even instrumental noise.

FTIR Results

The nanoSe spectra (Figure 2) displayed a variety of peaks that could be categorized into several functional groups. A wide peak centered at 3415 cm^{-1} could be assigned to the presence of probable amine and hydroxyl stretching vibrations associated with amines, phenolic chemicals and carbohydrates. The bands at $2921\text{--}2852\text{ cm}^{-1}$ represented the stretching vibrations of aliphatic CH_2 . The peak observed at 1627 cm^{-1} could be related to α , β -unsaturated ketones, or alkenes (cyclic alkenes or conjugated alkene). The alcohol and phenolic OH bending could be identified by the bands at 1409 and 1333 cm^{-1} . The C-O stretching of ether and primary and secondary alcohols might be ascribed to the peaks that appeared in the range of $1150\text{--}1050\text{ cm}^{-1}$. The bands that appeared between $700\text{--}1000\text{ cm}^{-1}$ may be caused by C=C and C-H bending of mono-substituted alkenes (995 cm^{-1}) or multi-substituted ones. The peaks at 775 and 738 cm^{-1} also corresponded to benzene derivatives.

UV-Vis spectrophotometry

The UV-Vis absorption spectrum of nanoSe shows a prominent peak at approximately 258 nm with an absorbance value of around 1.19 (Figure 3). This strong absorption at 258 nm suggests that the nanoSe particles have a significant electronic transition at this wavelength, which is characteristic of their size and electronic structure. The peak indicates the presence of nano-sized selenium particles, as the absorption in the UV region is typically associated with the quantum confinement effect in nanoparticles. This effect occurs when the particle size is smaller than the exciton Bohr radius, leading to discrete energy levels and a shift in the absorption spectrum. Therefore, the UV-Vis spectrum confirms the successful synthesis of nanoSe with distinct optical properties, which could be useful for uses in arenas such as photovoltaics, sensors, and biomedicine.

Field emission scanning electron microscopy (FESEM)

The FESEM images (Figure 4) demonstrated that particles have a nearly spherical morphology. The presence of organic biomolecules led to the agglomeration of particles in the solid phase. The images have revealed high levels of agglomeration. The results from the FESEM images and FTIR spectra both confirmed the residual biomolecule in the composition. Furthermore, the energy-dispersive X-ray spectroscopy (EDX or EDAX) (Figure 5) also investigated the structural composition of the nanoSe, which indicated the presence of 49.86% carbon, 41.01% of oxygen and 9.13% of selenium. It appears that the plant-

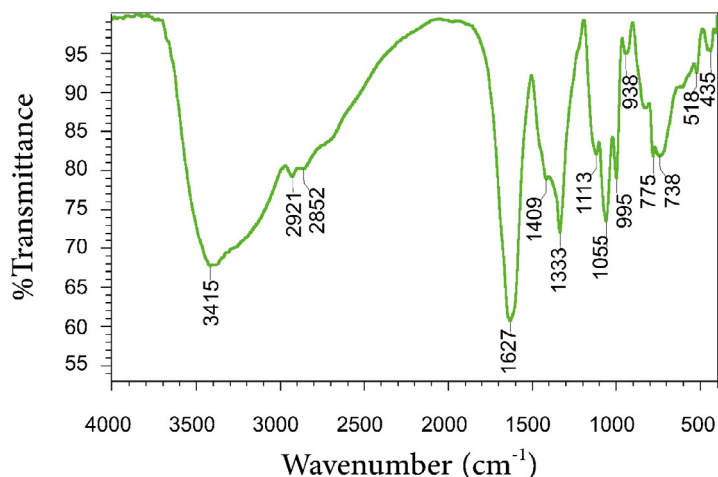


Fig. 2. The FTIR spectrum of the biosynthesized nanoSe containing plant-based organic residues.

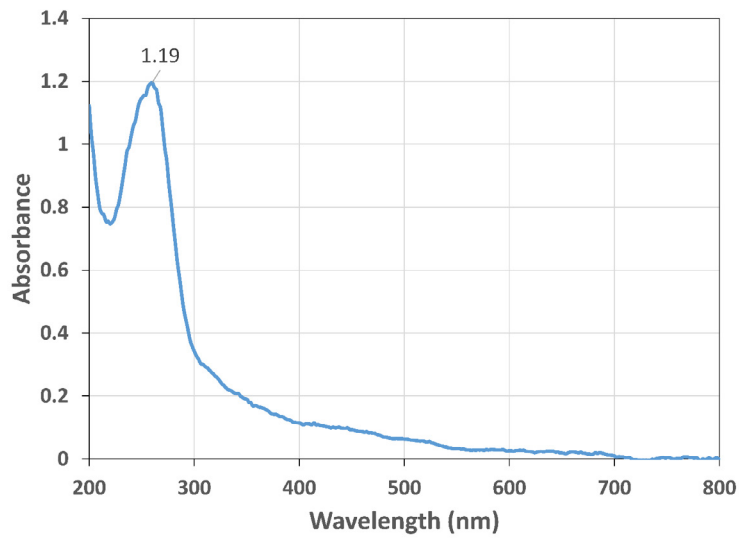


Fig. 3. The UV-Vis of the nanoSe.

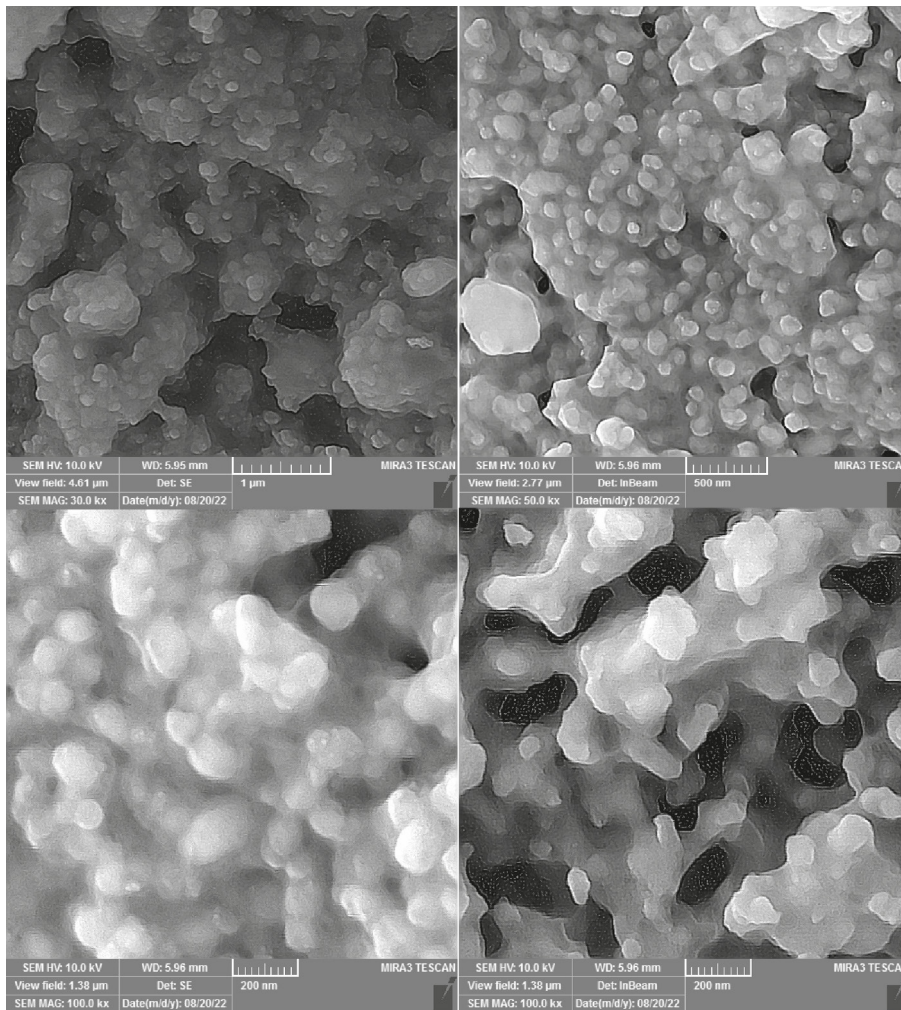


Fig. 4. The FESEM images of the prepared biosynthesized selenium nanoparticles, showing the coated spherical selenium nanoparticle by plant-based residues from the extracts.

based biomolecules were an integral part of the nanoparticle structure.

Dynamic light scattering (DLS) and zeta potential

The DLS results have revealed the hydrodynamic size of 199 nm with an acceptable polydispersity index of 0.1920 by using the intensity distribution. From the solid phase images, it is expected the nanoSe to be highly agglomerated, but the DLS analysis (Figure 6) has shown less agglomeration in the aqueous form at pH = 6.56. The reduced agglomeration of particles in aqueous form at pH = 6.56 can be explained by the interplay between the surface charge of the particles and the electrostatic repulsion forces. At this specific pH, the zeta potential of the particles is sufficiently high. This high zeta potential leads to increased electrostatic

repulsion between the particles, preventing them from coming close enough to aggregate. The results also indicated that the sonication bath could lead to the formation of destabilized colloidal nanoSe, which could be due to its relatively stable surface charge (the mean zeta potential = 21.88 ± 8.39 mV). This could explain the good stability of colloidal nanoSe in an aqueous solution.

Antibacterial study

The average diameter of the non-growth region of *S. aureus* and *B. subtilis* for biosynthesized selenium nanoparticles with carob extract was 15.33 ± 0.1 and 10.33 ± 0.1 mm, respectively; while *E. coli* and *P. aeruginosa* were resistant to nanoparticles and no inhibition growth region was formed around the disc (Figure 7 and Table 3).

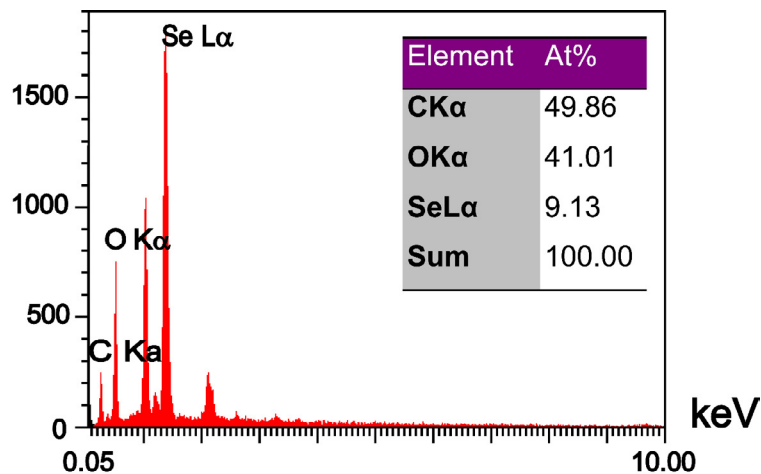


Fig. 5 The EDX of the prepared biosynthesized selenium nanoparticles, which shows its ingredients.

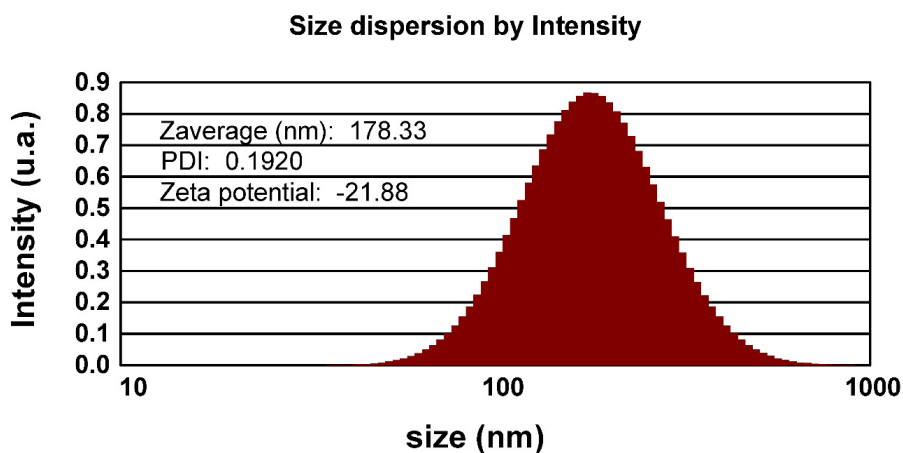


Fig. 6 The size dispersion (by intensity) of the prepared biosynthesized selenium nanoparticles using DLS method.

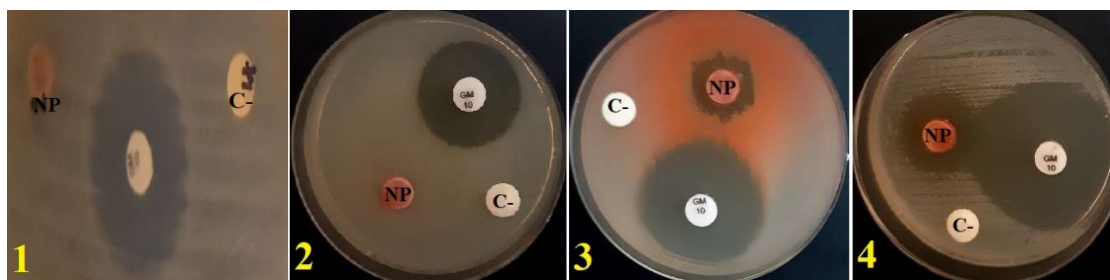


Fig. 7. Antibacterial results of biosynthesized nanoparticles using disk diffusion method. NP: Nano-particle; C-: Negative control; GM: Gentamicin; 1: *E. coli*; 2: *P. aeruginosa*; 3: *B. subtilis*; 4: *S. aureus*.

Table 3. Disk diffusion test. Diameter of non-growth zone (in mm) 4 bacteria.

Material	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
NPs (300 µg/ml)	-	-	15.33±0.1	10.33±0.1
Gentamicin (10 µg) [Control]	21 ±00 mm	20 ±00	27.33±0.2	25.33±0.1

Table 4. MBC, MIC, and MBC/MIC ratio of nanoSe synthesized and control(+) antimicrobial agents (µg/ml) toward 4 bacteria.

Bacteria	NanoSe			Chloramphenicol		
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC ratio	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC ratio
<i>E. coli</i>	-	-	-	75±00	100±00	1.33 (+)
<i>P. aeruginosa</i>	-	-	-	100±00	125±00	1.25 (+)
<i>S. aureus</i>	100±00	150±00	1.5 (+)	100±00	125±00	1.25 (+)
<i>B. subtilis</i>	150±00	175±00	1.16 (+)	100±00	100±00	1 (+)

The average MIC of nanoSe synthesized with carob extract for *S.aureus* and *B.subtilis* is 00±100 and 00±150 µg/ml, respectively, and the average MBC is 00±150 and 00±175, respectively. There was no significant difference in the MIC of nanoSe synthesized with carob extract and chloramphenicol against Gram positive strains (p<0.5) (Table 4).

NanoSe have garnered noteworthy interest for their possible antimicrobial activities, particularly against Gram-positive bacteria. These effects can be attributed to several mechanisms, which disrupt bacterial growth and survival. Here are the key points of discussion regarding the effects of nanoSe on Gram-positive bacteria. NanoSe can attach to the bacterial cell surface, leading to structural changes in the cell membrane. This interaction can cause membrane damage, resulting in increased permeability and ultimately, cell lysis (50, 51). NanoSe can catalyze the production of ROS within the bacterial cells. ROS are very reactive particles that can destruction cell constituents. The oxidative stress induced by ROS is often lethal to bacteria (52). Selenium can bind to thiol

groups in bacterial proteins, leading to protein denaturation or functional inhibition. This can interfere with essential bacterial processes such as enzyme activity and protein synthesis (53). The oxidative stress caused by ROS can also lead to direct damage to bacterial DNA. This impedes replication and transcription processes, which are critical for bacterial growth and reproduction (54). Gram-positive bacteria, characterized by their thick peptidoglycan layer, are generally more resistant to environmental stresses compared to Gram-negative bacteria (55). Numerous studies have demonstrated that nanoSe exhibit strong antibacterial activity against *S. aureus*, a common pathogen responsible for various infections. NanoSe can disrupt the cell wall and generate ROS, leading to cell death (56). NanoSe have been shown to prevent the growth of *B. subtilis* by destructive the cell membrane and inducing oxidative stress. NanoSe can effectively reduce the viability of *Enterococcus faecalis*, which is known for causing difficult-to-treat infections. The nanoparticles interfere with the bacterial cell wall and induce

significant oxidative damage (51). NanoSe have the potential for selective toxicity, meaning they can target bacterial cells while causing minimal harm to mammalian cells. This selectivity is crucial for developing safe antibacterial therapies. The multi-targeted mechanism of action of nanoSe reduces the likelihood of bacteria developing resistance. Traditional antibiotics often target specific bacterial pathways, which can lead to resistance; but nanoSe disrupt multiple cellular processes simultaneously (57, 58).

CONCLUSION

In this study, selenium nanoparticles (nanoSe) in the form of a conjugated system with the organic residues of the plant extract were studied for their antibacterial properties. The physicochemical properties were determined through the conventional method. PXRD has confirmed the successful synthesis of nanoSe, while FESEM has revealed an approximately spherical morphology for the particles. Interestingly, the FESEM image clearly showed the coating of nanoparticles with the extracted biomolecules. Both FTIR and EDX analyses have shown the existence of organic molecules in the composition. The mean hydrodynamic size was also ≈ 199 nm with a partial surface negative charge (-21.88). NanoSe has demonstrated good stability in aqueous solution which could be ascribed to relatively high zeta potential. The biosynthesized nanoSe was used against common pathogenic bacteria which has demonstrated the high inhibition activity of bacteria. The conjugation of the nanoSe with biomolecules from the plant extract may increase the interaction of nanoSe system with bacteria cells, which could result in a synergistic impact on the antibacterial property. Additional work is necessary to completely realize the cytotoxicity and other biological activities of such systems. Although our consequences deliver primary evidence for the antibacterial potential of biogenic nanoSe, more research is necessary to offer conclusive proof of biocompatibility, safety profile, and other pertinent issues before clinical investigations are approved.

CONFLICTS OF INTEREST

None.

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