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Synthesis, Characterization and Biological Applications of Green Synthesized Se NPs using Locally Isolated Multidrug-Resistant Escherichia Coli

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Abstract

Multidrug-resistant *Escherichia coli* is a leading cause of nosocomial infections globally. The current study aimed to isolate and detect multidrug-resistant *E. coli* for use in the extracellular biosynthesis of selenium nanoparticles (Se NPs) as well as investigate their roles in antibacterial and antioxidant applications. We used Vitek 2 to confirm the species-level identification of the selected bacterial isolate, which had previously undergone classical identification. Different spectroscopy and microscopy analyses were used to characterize the biosynthesized nanoparticles (NPs), such as UV–visible spectroscopy, FTIR, TEM and Zeta analysis. The adsorption peak for Se NPs was observed at 348 nm, which confirmed the successful biogenesis of Se NPs. The presence of proteins linked to NPs that serve as capping and stabilizing agents is indicated by the FTIR spectra. NPs were spherically shaped, negatively charged particles (+48.2 mV) that had an average size of ≈100 nm, assisted in their stability. Se NPs demonstrated strong antibacterial activity against Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Proteus mirabilis*, *E. coli*) in a dose-dependent way. The antioxidant potential of Se NPs was also evaluated compared to ascorbic acid, which indicated their distinguished behavior as antioxidants. This study proposes Se NPs as a safe, effective, and feasible substitute for treating some pathogenic bacterial strains as well as good antioxidants, suggesting that this green synthesis of Se NPs could provide promising medical applications.

Keywords: Green synthesis, Escherichia coli, selenium, nanoparticles, antibacterial, antioxidant

Introduction

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium that dwells in the gut (gastrointestinal tract) of healthy humans and animals (Martinson & Walk, 2020) and avoids dangerous pathogenic microorganisms from colonizing the intestines. However, in some conditions, various strains (types) of *E. coli* can cause illness. Many of the types that cause illness can attach (stick) to your cells and cause watery diarrhea, stomach pain,

and other digestive symptoms (gastroenteritis). Some of them have been reported to survive and develop at high antibiotic concentrations (Haefeli et al., 1984). Three primary methods of antibacterial resistance are (1) blocking access to the target, (2) altering the target of the antibiotic, or (3) directly altering the antibiotic. *E. coli* resistance can easily arise from either single point mutations of antibiotic target genes, of which fluoroquinolone resistance is a classic example, or from the transfer of mobile genetic elements, as has been the case with resistance to third-generation cephalosporins and broad-spectrum penicillin (such as ampicillin or amoxicillin) (Ochoa & Gómez-Duarte, 2016).

Several global health agencies and governments have acted to combat AMR. Several studies reported the potential antibacterial action of different nanoparticles (NPs) to combat the bacterial resistance problems (El-Zahed et al., 2023; Hasanin et al., 2021; Nandana et al., 2021). Nanotechnology is defined as technology performed at the nanoscale with real-world applications. Controlling or modifying matter at the atomic and molecular levels can affect its size. The range is around 1 to 100 nm (Dubey & Dubey, 2021). The underlying science is known as nanoscience. Nanoscale attributes differ from those at larger scales. Their distinctive physicochemical features, including an extraordinarily large surface area, improved reactivity, and higher mechanical strength, distinguish them from bulk materials and contribute to their wide variety of applications (Bera, 2024). These properties enable nanoparticles (NPs) to give novel answers to some of the world's most urgent issues, such as disease treatment, pollution management, and energy efficiency. As a result, NPs are expected to play a critical role in determining the future of technology.

The synthesis of NPs is influenced by factors such as temperature, pressure, time, particle size, shape, preparation cost, and pore size (Demazeau, 2010; Ma et al., 2008). Two approaches that describe the many alternatives for producing nanostructures have been developed. These manufacturing approaches are divided into two categories: top-down and bottom-up. The top-down technique is expensive and unsuitable for soft samples. The top-down method is not ideal for large-scale production but can be used for laboratory experiments. The bottom-up method follows the notion of molecular recognition. Bottom-up approaches synthesize nanostructures by manipulating atoms, molecules, or clusters individually (Patil et al., 2021). These NPs can be manufactured using chemical, physical, and biological means; the biological production of NPs has gained popularity as a sustainable and environmentally benign alternative to standard chemical and physical processes. Chemical and physical methods are less preferable than biological ways because of high temperature conditions, dangerous substances, acidic pH, and highly poisonous and unsafe methods (Salem & Fouda, 2021a). These biological systems play an important role in nanoparticle synthesis by converting metal ions into NPs, frequently producing products with distinct characteristics that differ from those created using conventional methods (Jafir et al., 2023). However, while biological synthesis processes are environmentally favorable, they have hurdles, notably in terms of scalability and speed. The synthesis process usually takes longer, and temperature, pH, the concentration of biological agents, and the presence of other substances can all influence the size, shape, and yield of the NPs generated (Wang et al., 2023). Furthermore, managing the homogeneity of NPs produced by biological processes is a substantial problem, as the complexity of the biological systems involved might result in variances in particle properties (Sharma et al., 2023). Biological synthesis is widely divided into intracellular and extracellular techniques, such as intracellular synthesis, which is the process of creating NPs within living microorganisms or plants' cells. The intracellular process enables the production of NPs in a regulated environment, as the biological system governs particle growth, resulting in NPs that are frequently uniform in size and shape. Extracellular synthesis, on the other hand, occurs outside of live cells, most commonly in the surrounding media. This approach involves mixing biological extracts, such as plant leaves or fungus, with metal salt solutions (Bhatti et al., 2023). This method uses natural agents such as bacteria, fungi, plant extracts, and enzymes to create NPs (Shafqat et al., 2023). Microbial cells are naturally able to thrive in a variety of environments, and they grow quickly and are simple to maintain. Bacteria can be cultured in artificial environments with an optimal growth rate, making them a more viable candidate for NP synthesis than other microbes.

Bacteria have a remarkable ability to decrease heavy metal ions, making them excellent candidates for NPs production. For example, some bacterial species have evolved the ability to use specialized defensive mechanisms to resist stresses such as the toxicity of heavy metal ions or metals. Bacteria are regarded as a possible biofactory for the production of NPs such as Au, Ag, Pt, TiO_2, Se, and so forth. On the other hand, selenium NPs (Se NPs) offer distinct advantages because of their dual function as critical micronutrient carriers and redox homeostasis modulators. When compared to traditional selenium treatments, Se NPs provide a more efficient and environmentally friendly alternative for improving plant resistance while limiting toxicity, especially at low concentrations. Se NPs have received worldwide interest due to their high degree of absorption, biological activity, minimal toxicity, and significant efficacy in inhibiting oxidative damage when compared to their Se-based counterparts (Mal et al., 2017; Zhang et al., 2008).

Se NPs may play a favorable function in biomedicine because of their antioxidant (Bai et al., 2017; Jin et al., 2021), anticancer (Liao et al., 2020; Varlamova et al., 2021), antibacterial, and immune regulatory characteristics. Se NPs have been widely recognized to exhibit a wide range of antibacterial and antifungal action (Lin et al., 2021). Their antibacterial properties may be linked to the overproduction of reactive oxygen species (ROS), which causes cell membrane damage, inhibits amino acid synthesis, and prevents DNA replication (Qin et al., 2025). Se can be found in both crystalline and amorphous polymorphic configurations, and each plays a unique purpose. Se, in its organic form found in selenoproteins, is required in animals for enzyme activity, immunological response, reproduction, and pro- and anti-oxidative characteristics (Constantinescu-Aruxandei et al., 2018; Zhang et al., 2004). These essential characteristics have motivated researchers to assess the potential of Se NPs as a strategy for combating multidrug-resistant bacteria and other microbial diseases.

The current study aimed to isolate a multidrug-resistant *E. coli* and use it to biosynthesize Se NPs, in addition to evaluating their antibacterial and antioxidant properties.

Materials and methods

Sample collection

Under aseptic conditions, 15 samples (10 females and 5 males) were taken in April and May 2024 from private clinics and medical analytical labs in New Damietta City, Kafr Elbattikh, and Faraskour (Damietta Governorate, Egypt, 31.4°N 31.72°E). Urine, stool, and surface exchanges were among the samples. The procedures previously outlined by Santiago-Rodriguez et al. (2015) and Zboromyrska & Vila (2016) were followed while processing the samples.

Isolation and identification of bacterial isolates

Serial dilutions of samples (10^{-2}-10^{-8}) were made and infected using the pour plate method in MacConkey agar plates (Oxoid, Basingstoke, United Kingdom) with 2 mg/l ceftazidime (MCKC). All plates were incubated at 37°C and evaluated after 18, 24, or 48 hours. For 48 hours, each bacterial colony from a distinct bacterial isolate was transferred and subcultured on MacConkey and cystine lactose electrolyte deficient (CLED) agar plates from Oxoid Ltd. in England. The morphological properties of colonies and media, as well as color changes, were then recorded. The bacterial isolates were described and identified using Bergey's Manual of Systematic Bacteriology (Yahr & Parsek, 2006). The morphological and cultural criteria included colony structure, Gram staining, and endospore staining, and the identification was done in a systematic fashion as follows: The following colonial characteristics were recorded: size, form, pigment production, elevation, surface, edge, color, and opacity. Microscopic investigation includes shape, size, Gram stain, acid-fast stain, and endospore production. *E. coli* isolates were chosen, purified, and species-level identification was carried out in accordance with standard recommended standards (Koneman et al., 1997). To confirm the identification results, isolates were subcultured on blood agar plates and analyzed with the Vitek 2 (BioMerieux, Marcy-l'Étoile, France) (Moore et al., 1981). Bacterial isolates were preserved in agar slants for additional research (Glupczynski et al., 2007).

Antibiotic sensitivity test (AST)

The disc diffusion technique was used to assess the AST of bacterial isolates (CLSI, 2000a). Mueller-Hinton agar (MHA, Oxoid Ltd., England) flasks were inoculated with 50 µl of each bacterial suspension (0.5 MacFarland Standard, 1.5×10^8 CFU/ml) independently, and the flasks were subsequently transferred into sterile Petri dishes. Following solidification, various antibiotic discs from various classes were aseptically applied (vanomycin; 30 µg/ml, amoxicillin /clavulanate; 20/10, ampicillin; 30, cefotaxime; 30, ceftazidime; 30, chloramphenicol; 30, doxycycline; 30, levofloxacin; 5, nalidixic acid; 30, and amoxycillin/clavulanate; 20/10). Following a 24-hour incubation period at 37°C, the diameter of the zones of inhibition (ZOI) was measured and recorded in millimeters.

Biosynthesis of selenium nanoparticles

In 100 mL nutrient broth flasks, a 0.5 McFarland standard (1×10^8 cells/mL) from a chosen bacterial isolate was produced for 24 hours at 37 °C. The inoculation flasks were incubated at 37°C and 150 rpm for the entire night. A 0.22 µm syringe filter (Millex GV, Millipore) was used to filter the bacterial metabolites, and the cells were collected using centrifugation at 5000 rpm for 15 minutes after the incubation period. The bacterial metabolites were then transferred into another clean flask. After making 1 mM of Na_2SeO_3 solution and adding it to the bacterial metabolite flasks in a 1:1 (v/v) ratio, the flasks were shaken at 150 rpm at 37°C until the color turned red. The generated Se NPs utilizing selected bacterial isolates were recovered by centrifugation at 30,000 rpm for 20 minutes and lyophilized after being washed four times with distilled water (Mohamed & El-Zahed, 2024).

Characterization of selenium nanoparticles

The biosynthesized Se NPs were examined using two methods: a double beam spectrum UV-Vis spectrophotometer V-760 (JASCO, UK) and Fourier transform infrared spectroscopy (FTIR, FT/IR-4100 type A). Various microscopic approaches are used to characterize the morphology of NPs. Transmission electron microscopy (TEM) is the most widely used technique. TEM and zeta potential measurements (Malvern Zetasizer Nano-ZS90, Malvern, UK) were employed for supplementary analysis.

Antibacterial activity of selenium nanoparticles using the agar well diffusion method

The biosynthesized Se NPs were examined for antibacterial activity against Gram- positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Proteus mirabilis, E. coli*) in comparison to the standard antibiotic, ampicillin, using the Clinical and Laboratory Standards Institute agar well diffusion method (CLSI, 2017). A 0.5 McFarland of the tested bacteria was made and inoculated into sterile, cooled, molted MHA, which was then put into sterile Petri dishes. Se NPs and ampicillin were applied to 5 mm wells in inoculated agar plates at doses of 50, 100, and 150 µg/mL, respectively. Plates were incubated at 37°C for 24 hours before measuring ZOI in millimeters.

Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth dilution method was used to estimate the MIC values of Se NPs and ampicillin (CLSI, 2000). Se NPs and ampicillin at varying doses (0-75 µg/mL) were introduced to sterilized Muller-Hinton broth conical flasks inoculated with 0.5 McFarland of tested bacterial strains. The flasks were incubated at 37 °C for 24 hours. The growth rate of the bacteria was evaluated using a UV-Vis spectrophotometer at 600 nm against an uninoculated broth medium as a blank.

MIC flasks were inoculated into sterile cooled molten MHA media, which was then placed into a sterile petri plate. The inoculated agar plates were kept at 37°C for 24 hours. After incubation, bacterial colonies were counted in colony-forming units per mL (CFU/mL) to determine MBC values (Owuama, 2017).

Antioxidant activity of selenium nanoparticles

Using the *in vitro* DPPH radical scavenging technique, the antioxidant properties of Se NPs were investigated (Turan et al., 2025). The greatest absorbance of DPPH radical solutions occurs at 517 nm. To achieve this, a 1 mM DPPH radical solution was made, and ethanol

was used to bring the control sample's absorbance down to 1.5 ± 0.5. Standard antioxidant ascorbic acid (vitamin C) was used to compare the outcomes. The DPPH radical solution was administered separately to the samples and standards at varying doses (10–110 µg/m1). Following a 30-min incubation period at 25°C, the samples' absorbance values were determined. A sample's ability to scavenge DPPH free radicals is shown by its lowering absorbance at 517 nm. Inhibition of DPPH in percent (I%) was calculated according to % = $(A_{control} - A_{compound}/A_{control}) \times 100$, where $A_{control}$ is the absorbance when the compound is not present, and $A_{compound}$ is the absorbance when the compound is present.

Statistical analysis

The data were statistically analyzed using SPSS version 18. All experiment results were provided as mean ± SD using one-way analysis of variance (ANOVA). A substantial threshold of $p < 0.05$ was used (O'Connor, 2000). Every experiment was done three times.

Results

Isolation, purification, and characterization of E. coli

The procedure successfully isolated 16 Gram-negative, lactose-fermenting, negative citrate, and non-spore-forming bacterial isolates. Two bacterial isolates were identified as *E. coli* strains using conventional recommendations and a Vitek 2 system test. This revealed the presence of two *E. coli* isolates encoded with AUF2 and CUF1 (Table 1; Figure 1). The two *E. coli* isolates were tested for Se NPs production.

Table 1. Biochemical tests for *E. coli* isolates.

Biochemical test	AUF2	CUF1
Citrate utilization	-	-
Catalase	+	+
Oxidase	-	-
Indole production	+	-
Methyl red	+	+
Voges-Proskauer	-	-
Urease	+	-
Nitrate reduction	+	+
H_2S production	-	-
Lactose fermentation	+	+

*+ = Present; - = Absent

AST investigations

Table 2 demonstrates the AUF2 strain's resistance to every antibiotic that has been tested compared to the CUF1 strain. The results show that *E. coli* isolates have a resistance percentage of 100 to ampicillin, cefotaxime, ceftazidime, doxycycline, and vancomycin. The *E. coli* AUF2 isolate has a resistance action against amoxicillin/clavulanate, chloramphenicol, levofloxacin, and nalidixic acid. The obtained results indicated the multidrug resistance behavior for the *E. coli* isolates.

Table 2. Patterns of antibiotic resistance and sensitivity in *E. coli* isolates.

Antibiotic	Concentration (µg/ml)	Antibiotics susceptibility	
		AUF2	CUF1
Amoxicillin/clavulanate	20/10	+	-
Ampicillin	30	+	+
Cefotaxime	30	+	+
Ceftazidime	30	+	+
Chloramphenicol	30	+	-
Doxycycline	30	+	+
Levofloxacin	5	+	-
Nalidixic acid	30	+	-
Vancomycin	30	+	+

Biosynthesis and characterization of Se NPs

The color of the culture media changes from pale yellow at the start of the experiment to brick red at the end of the incubation period. This is utilized to analyze the generation of Se NPs by *E. coli* isolates (Figure 1). The sample's color did not alter in the control studies. According to spectroscopic study outcomes, AUF2 produced Se NPs at larger concentrations and faster rates than the other isolate, CUF1 within 48 hours. The UV-Vis spectrum of AUF2- and CUF1-Se NPs revealed an adsorption peak ranging from 340 to 350 nm, consistent with the characteristics of colloidal Se NPs (Shah et al., 2010).

Se NPs generated by *E. coli* AUF2 were studied using FTIR, zeta, and TEM potential measurements. The capping agent composition of biosynthesized Se NPs was characterized by FTIR spectra, and their shape, size, distribution, and potential were studied by TEM and Zeta studies. Figure 2 shows the FTIR results. The peak at 429.08 cm^{-1} attributed to the successful formation of Se NPs. C-O is represented by the third peak at 863.95 cm^{-1}. The peak with a wavelength at 1012.45 cm^{-1}, and the other peak

with a wavelength cm^{-1} is 1396.21 due to the presence of a C-H bond. The peak at 1629.55 cm^{-1} wavelength corresponds to the protein carbonyl amide bonds. The peak, at 2975.96 cm^{-1} indicates asymmetric tensile vibrations in the C-H groups. The peak at 3449.06 cm^{-1} corresponds to the O-H groups. These moieties hinder Se NP aggregation and increase their stability.

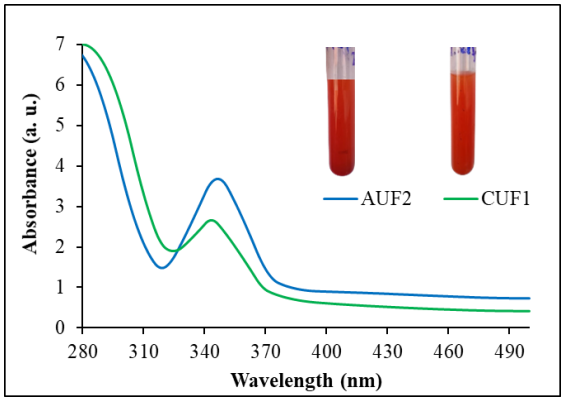

Figure 1. UV-vis spectroscopy and color change investigations were performed during the production of Se NPs using *E. coli* isolates; AUF2 and CUF1.

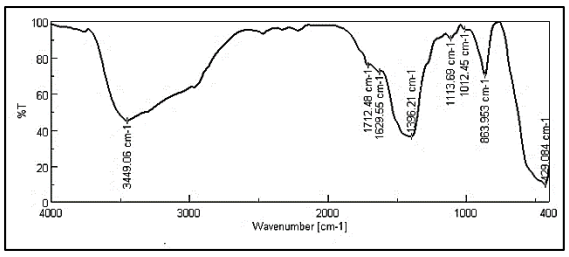

Figure 2. FTIR of the biosynthesized Se NPs.

The zeta potential remains a key indicator of the stability of the NPs' colloidal dispersion. The zeta potential is the measurement of an effective electric charge on the surface of NPs. The NPs with a bigger zeta potential are more stable due to higher electrostatic repulsion between them. The zeta potential measurements revealed the positive charge (+48.2 mV) of the biosynthesized Se NPs, as shown in Figure 3.

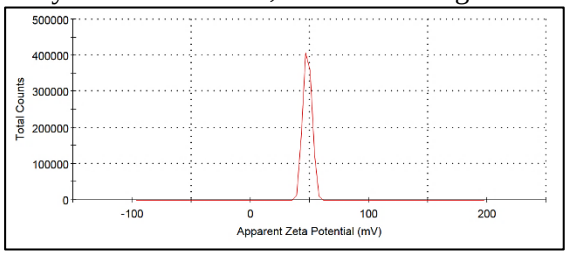

Figure 3. Zeta potential analysis of Se NPs.

The TEM studies are a crucial technique for evaluating and researching the size and shape of NPs. SeNPs' homogeneous distribution, spherical shape, and lack of aggregation were all visible in the TEM micrograph (Figure 4). Se NPs were between 94.15 and 108.43 nm in size.

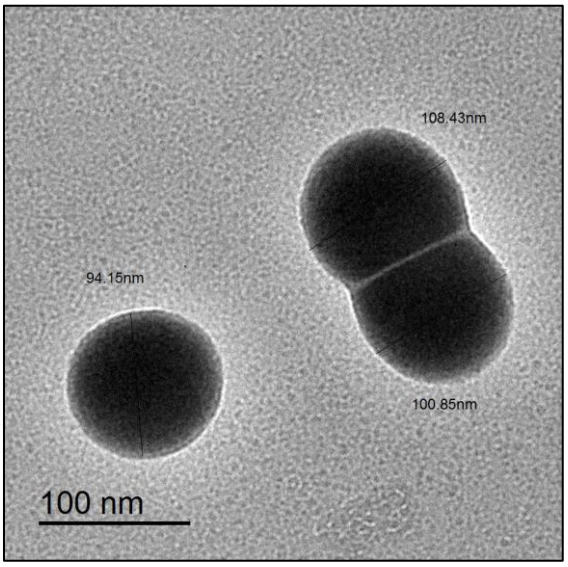

Figure 4. TEM of Se NPs. Scale bar = 100 nm.

The antibacterial action of selenium nanoparticles

Se NPs were bio-produced using *E. coli* AUF2, and their antibacterial activity against different Gram-positive and Gram-negative bacteria was investigated. The antibacterial activity of Se NPs was assessed utilizing the agar well diffusion technique, MIC, and MBC assays, as opposed to traditional antibiotics like ampicillin. *In vitro* testing revealed that biosynthesized Se NPs had a higher antibacterial impact against Gram-negative bacteria than ampicillin (Table 3). The MIC test for Se NPs and ampicillin against Gram-positive bacteria ranged from 5 to 30 µg/mL, while against Gram-negative bacteria ranged from 20 to 70 µg/mL (Figure 5A). The MBC results were consistent with MIC concentrations, confirming the enhanced biocidal effect of the biosynthesized Se NPs against the tested bacteria (Figure 5B).

Table 3. Agar well diffusion of Se NPs against the tested bacteria.

Antimicrobial agents	**Concentration, µg/ml**	**Inhibition zone in mm (mean ± SD)**			
		Gram-negative bacteria		Gram-positive bacteria	
		E. coli	*P. mirabilis*	*B. cereus*	*S. aureus*
Se NPs	50	7±0.14	6±0.18	17±0.06	16±0.14
	100	10±0.16	8±0.21	20±0.03	18±0.14
	150	12±0.06	10±0.14	23±0	20±0.06
Ampicillin	50	-ve	-ve	16 ± 0	-ve
	100	-ve	-ve	20 ± 0	-ve
	150	-ve	-ve	22 ± 0	7 ± 0

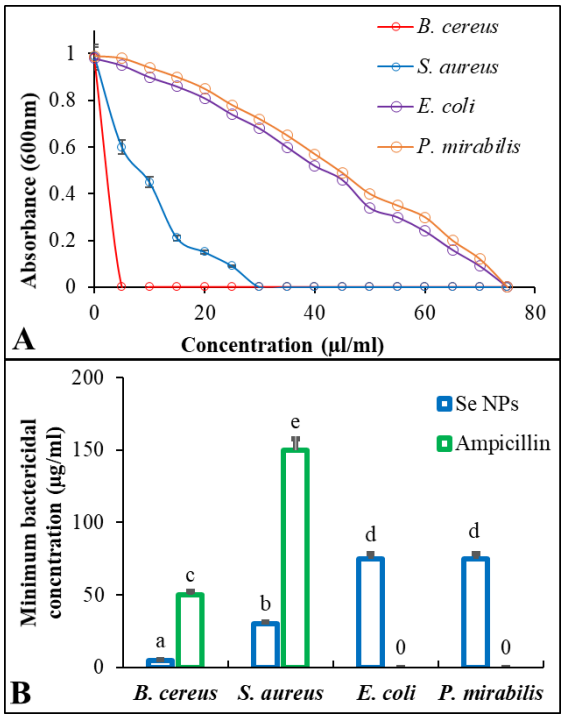

Figure 5. The minimum inhibition concentrations; (A), and minimum bactericidal concentrations; (B), of Se NPs compared to standard; ampicillin.

Antioxidant activity

Using the *in vitro* DPPH radical scavenging technique, the antioxidant properties of the biosynthesized Se NPs were assessed (Figure 6). The findings of the experiment demonstrated that Se NPs had a concentration-dependent scavenging effect and that the radical scavenging ratio rose as the concentrations in the tested range increased. The DPPH radical scavenging activities were 67.5% for Se NPs. In this study, ascorbic acid as a standard antioxidant showed higher antioxidant action than the Se NPs at all concentrations.

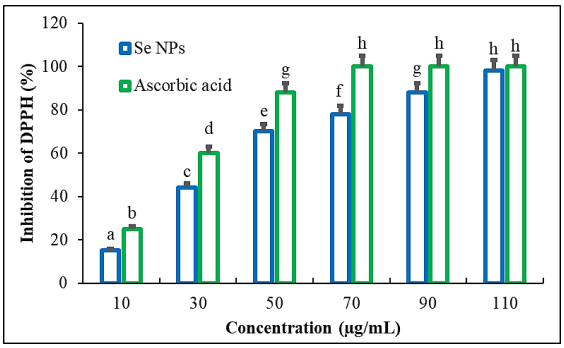

Figure 6. DPPH scavenging activity of Se NPs.

Discussion

Multidrug-resistant *E. coli* is one of the most serious pathogenic bacteria since it causes multiple disorders throughout the body, and the most critical problem is resistance to antibacterial medications. Many *E. coli* strains cause mild illnesses. However, some strains release Shiga toxin, which can cause significant sickness and kidney damage. *E. coli* infections are most commonly found in the gastrointestinal and urinary tracts. Other forms of *E. coli* infections are Bloodstream infections, prostatitis (a prostate infection), pelvic inflammatory disease, or PID, a gallbladder infection (cholecystitis), infections from wounds, pneumonia (infrequent), and meningitis (rare). Symptoms of *E. coli* gastroenteritis are Diarrhea. This is frequently watery and occasionally bloody, stomachaches and cramps, loss of appetite, and a low fever. Watery diarrhea is typically the initial symptom of an *E. coli* infection in the gastrointestinal tract. Symptoms may vary based on the location of infection in your body (İncir & Kaplan, 2025). Although certain types of antibacterial medicines, such as amikacin, gentamicin, ceprofloxacin, amoxicillin/ clavulanic acid, imipenem, ampicillin, cefotaxime, cefepime, and ceftazidime.

Numerous investigations have found that β-lactam antibacterial resistance cases of *E. coli* (Gniadkowski, 2001; Katsanis et al., 1994; Nepal et al., 2017). The current work effectively isolated and identified two multidrug-resistant *E. coli* strains using standard biochemical techniques. An *in vitro* technique and the CLSI criteria were used to determine these bacteria's resistance to various antimicrobial medicines. In addition, this study attempts to identify an alternative green method to combat bacterial resistance concerns, specifically *E. coli* resistance to current commercial medications.

Recently, researchers have tended to synthesize novel active compounds using an environmentally benign approach. Nanomaterials are among the most active substances in agriculture, health, and industry (Sharma et al., 2018). To avoid the detrimental effects of chemical and physical techniques, these materials should be synthesized using green approaches (Salem & Fouda, 2021). Endophytic microorganisms such as fungi, bacteria, and actinomycetes are thought to be attractive sources for the green synthesis of nanomaterials because they secrete large amounts of active metabolites that can be employed as reducing and capping agents (Meena et al., 2021). Green NPs production utilizing bacteria has received more attention than other microorganisms. This is mostly owing to the remarkable secretion of extracellular metabolites, which increases NPs yield and provides great stability to the produced NPs. Bacteria are also known for their ease of handling, high metal tolerance, biomass production, and scalability. The current study looked at the effectiveness of endophytic bacterial strains for Se-NP synthesis. After adding the metal precursor (Na_2SO_3) to the bacterial biomass filtrate, the color changed from colorless to red, progressively increasing, suggesting the creation of Se^0 due to the reduction of $SeO3^{2-}$. The prior combination was kept in the dark for 24 hours to ensure that the metal had been completely reduced and there was no further color change. Recently, Na_2SO_3 was completely reduced by the action of metabolites released by the endophytic bacterial strain, *E. coli*, whereas the Se^0 form was finished after 24 hours of incubation (Fouda et al., 2022). Furthermore, after 24 hours, Se-NPs were produced by reducing Na_2SO_3 with metabolites released by *E. coli*, with no additional color change observed.

The ability of *E. coli* strains to biosynthesize Se NPs was investigated using cell-free supernatants. In comparison to the CUF1 strain, *E. coli* AUF2 showed the highest rate of Se NP synthesis. Se NPs were characterized by UV-visible spectroscopy, FTIR, TEM, and zeta analysis. Se NP biosynthesis was confirmed by an absorbance peak at ≈348 nm. Several research studies have reported distinct absorbance peaks for Se NPs. For example, Hemalatha et al. (2014) analyzed Se NPs with an absorbance peak of 290 nm, but El-Saadony et al. (2021) reported findings at 263 nm and 300 nm, respectively. The absorbance peak shifts to longer wavelengths as particle size rises (Shah et al., 2010). Se NPs only exhibit a continuous absorption peak in the wavelength range above 300 nm when the particle size is 100 nm or bigger. The absorption peak often shifts towards red, with peak intensity decreasing as nanoparticle size rises (Kaur & Bakshi, 2010).

FTIR spectroscopy was utilized to confirm the existence of functional groups that were primarily engaged in the Se NPs bioreduction process. The FTIR spectrum pattern of Se NPs revealed distinct functional groups on the surface, which may be responsible for the reduction of sodium selenite (inorganic form) to organic form Se NPs (Ashengroph & Hosseini, 2021). Stretching and bending bands of various groups (OH, NH, CO, and CN) validated the range of biogenic Se NPs generated by *E. coli* AUF2. The broad absorption bands correlate to the reducing groups (C-O, NH, and C-C) found in bacterial proteins that were responsible for the reduction of sodium selenite into Se NPs. The overall FTIR spectral fingerprint pattern corresponded to the outline of Se NPs synthesized by Alvi et al. (2021), Mehta et al. (2021), and Ramya et al. (2015) using a green synthesis technique. According to prior research, the presence of particular biomolecules may reduce the ability to produce NPs. Polysaccharides have a variety of functions, including hydroxyl groups and a hemiacetal reducing end that can reduce precursor salts. The conversion of polysaccharide hydroxyl groups to carbonyl groups is crucial in the reduction of selenium salts (Mata et al., 2009).

The reducing end of polysaccharides can also be exploited to provide an amino functionality capable of complexing with and stabilizing metallic NPs (Nadkarni et al., 1994).

Carbohydrates with such amino groups bond strongly to the surface of SeNPs, resulting in a hydrophilic surface (Kemp et al., 2009; Park et al., 2011). These protein-loaded Se NPs have a high antibacterial potential and also exhibit antioxidant activity. They play a key function in medications and are widely thought to have significant health advantages (Chen & Dou, 2008; Lamoral-Theys et al., 2010). During the current study, spherical particles of an average size of ≈100 nm were observed. It was a consensus from earlier research (Eszenyi et al., 2011; W. Zhang et al., 2011). These particles are charged species, and their charge is assessed using zeta potential. NPs can survive in colloidal systems with zeta potential values reaching +48 mV, depending on their charge. A low zeta potential, on the other hand, might cause particle aggregation and flocculation as a result of van der Waals attractive forces acting on them (Tso et al., 2010; Ye et al., 2020). The size and shape of the Se NPs were validated using a TEM. Spherical-shaped Se NPs were prepared using the *E. coli* AUF2.

Biosynthesized Se NPs made with *E. coli* AUF2 show better biocidal activity against Gram-positive bacteria with MIC and MBC values of 5-30 µg/mL compared to the traditional drug ampicillin (50-150 µg/mL). El-Saadony et al. (2021) found that LAB-Se NPs had MIC values of 55 µg/mL. Shakibaie et al. (2015) produced Se NPs with *Bacillus* Msh1, which had an MIC of 70 µg/mL against tested microbes.

It was shown that Se NPs are more effective against bacteria that are Gram-positive than those that are Gram-negative. This is explained by a substantial change in the makeup of the bacterial walls. Numerous holes were discovered in the cell walls of Gram-positive bacteria, which facilitate the entry of NPs into the bacterial cells. This increases the NPs' reactivity with bacterial components and fortifies their antibacterial action (Pasquina-Lemonche et al., 2020).

Se NPs primarily exploit oxidative stress to target microorganisms. ROS are created, causing the bacterial cell wall to degrade and release proteins and nucleic acids. ROS also causes crucial respiratory enzymes to malfunction and oxidize key proteins such as glutathione, which kills germs (Sahoo et al., 2023). The obtained results showed that the radical scavenging ratio increased as the concentrations in the measured range increased, indicating that Se NPs exhibited a concentration-dependent scavenging effect. For Se NPs, the DPPH radical scavenging activity was 67.5%. At all doses, ascorbic acid, a common antioxidant, outperformed the Se NPs in this investigation. Se NPs, which are efficient nanometallic compounds, improve cell permeability to NPs by destabilizing bacterial membranes and enhancing the biocidal action against the tested bacteria.

Conclusions

An increasing demand for sustainability initiatives in nanotechnology has resulted in the development of biogenic techniques for the synthesis of Se NPs, which are rapidly replacing traditional chemical syntheses. The current study succeeding in isolating two multidrug-resistant *E. coli* strains that tested for the extracellular biosynthesis of Se NPs. The characterization and bioactivities of Se NPs demonstrated their appropriate composition, survivability against bacterial infections, and antioxidant activity. The findings of this study could have a significant impact because of *E. coli* AUF2's easy culture requirements and the inexpensive manufacturing cost of biologically relevant Se NPs. Given these possible biological effects, the investigated Se NPs have enormous potential for use in the pharmaceutical, biomedical, and food industries, particularly as antibacterial and antioxidant agents.

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الملخص العربي

عنوان البحث: التصنيع والتوصيف والتطبيقات البيولوجية لجسيمات السيلينيوم النانومترية المصنَّعة بطريقة خضراء باستخدام بكتيريا اشريشيا كولاي المعزولة محليًا وذات المقاومة المتعددة للعقاقير

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تُعد بكتيريا اشريشيا كولاي ذات المقاومة المتعددة للعقاقير سببا رئيسيًا لانتشار عدوى المستشفيات. تهدف الدراسة الحالية إلى عزل وتعريف بكتيريا اشريشيا كولاي ذات مقاومة متعددة للعقاقير المختلفة لاستخدامها في التصنيع الحيوي الخارجي لجسيمات السيلينيوم النانومترية، بالإضافة إلى دراسة أدوارها في التطبيقات الضد بكتيرية وكمضادات أكسدة. استُخدم جهاز فايتك ٢ لتأكيد تحديد مستوى الأنواع للعزلة البكتيرية المختارة بعد تحديدها بشكل كلاسيكي. استُخدمت تحليلات طيفية ومجهرية مختلفة لتوصيف الجسيمات النانومترية المصنعة حيويًا، مثل التحليل الطيفي للأشعة فوق البنفسجية والمرئية، وتحليل الأشعة تحت الحمراء، والمجهر الإلكتروني النافذ، وتحليل زيتا. لوحظت قمة امتصاص لجسيمات السيلينيوم النانومترية عند ٣٤٨ نانومتر، مما أكد التكوين الحيوي الناجح لجسيمات السيلينيوم النانومترية. يُشير طيف الأشعة تحت الحمراء إلى وجود بروتينات مرتبطة بجسيمات السيلينيوم النانومترية والتي تعمل كعوامل تغطية وتثبيت للجسيمات. ظهرت الجسيمات النانومترية بشكل كروي واضح، واحتوت شحنة سالبة وصلت الى +٤٨,٢ ملي فولت، كما ظهرت بمتوسط حجم ≈١٠٠ نانومتر. أظهرت جسيمات السيلينيوم النانومترية نشاطًا مضادًا للبكتيريا قويًا ضد البكتيريا موجبة الجرام (باسيلس سيريس، وستافيلوكوكس اوريوس)، والبكتيريا سالبة الجرام (اشريشيا كولاي، وبروتيوس ميرابيلس) بطريقة تعتمد على الجرعة. كما تم تقييم القدرة المضادة للأكسدة لجسيمات السيلينيوم النانوية مقارنةً بحمض الأسكوربيك، مما أشار إلى سلوكها المتميز كمضادات للأكسدة. تقترح هذه الدراسة أن جسيمات السيلينيوم النانومترية كبديل آمن وفعال ومجدٍ لعلاج بعض السلالات البكتيرية المسببة للأمراض، بالإضافة إلى كونها مضادات أكسدة جيدة، مما يشير إلى أن هذا التصنيع الأخضر لجسيمات السيلينيوم النانومترية يمكن أن يوفر تطبيقات طبية واعدة.