Effect of Green Selenium Nanosynthesis on Staphylococcus Aureus

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Abstract: Selenium nanoparticles (SeNPs) represent a promising natural compound for modern medical treatments, offering a potent new weapon against pathogenic bacteria. This study aimed to synthesize SeNPs using sodium selenite (Na_2SeO_3) as a precursor and dried Camellia sinensis (green tea) leaf extract as a reducing and stabilizing agent through an eco-friendly biosynthesis approach. The synthesis was performed via a simple, cost-effective, and environmentally friendly method. The resulting nanoparticles were characterized using UV-visible spectroscopy, field-emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDX). FE-SEM analysis confirmed the formation of spherical SeNPs with varied shapes and an average particle size of 20.5 nm, alongside a narrow size distribution. While the antibacterial evaluation against Staphylococcus aureus showed no significant inhibitory activity against planktonic cells, the SeNPs exhibited a strong antibiofilm effect, inhibiting biofilm formation by 54.7%. These findings highlight the potential of biosynthesized SeNPs as a specific and effective anti-biofilm agent for targeting bacterial biofilms and combating antibiotic resistance.

1 INTRODUCTION

Medicinal plants are valuable natural sources of bioactive compounds, which can be utilized to create pharmaceutical drug [1]. Natural compounds, including chemical compounds, have been found to have potential health benefits and are often found in medical plants[2], [3]. Selenium (Se) is an essential trace element in the human body. Approximately 40–300 micrograms of selenium are required daily as dietary supplements for a typical adult [4]. Lower concentrations of Selenium are necessary to maintain the healthy operation of the human and animal bodies [5]. Researchers found that the evolution of the bacteria was inhibited by the organoselenium [6]. Selenium nanoparticles (SeNPs) have demonstrated exceptional antibacterial effectiveness against the harmful bacteria Staphylococcus aureus in recent times. It has been discovered that elemental Se at the Nano scale has low toxicity and high biological activity [7]. Recently, SeNPs have shown remarkable antibacterial efficacy against the dangerous pathogen *Staphylococcus aureus*. At the nanoscale, elemental Se has been found to have great biological activity and minimal toxicity [8]. These nanoparticles potentially replace the functions of commonly used medications [9], [10]. NP can target a pathogen's cell wall directly without entering the cell. As a result, the bulk of MDR bacteria's defense mechanisms against ineffective treatment drugs are rendered ineffective [11]. SeNPs' properties extend beyond biological applications; they are also widely used in catalysis and oxidation-reduction processes [12].

2 MATERIALS AND METHODS

Camellia Sinensis was dried; all chemicals involved in the experiments are reagent grad. The formula for sodium selenite, an inorganic substance, is Na_2SeO_3, 0.0189 M. In 100 milliliters of deionized water, sodium selenite was produced and dissolved... The anti-bacterial test includes *Staphylococcus aureus*.

2.1 Preparation of Plant Extract

To prepare the *Camellia Sinensis* Extract (green tea), the leaves were washed using deionized water to remove crusts and cut into small pieces. 10 g of it and liquefy it in 100 ml of deionized water and put it on the magnetic jacket for 10 min at a temperature of

60°C and then in the centrifuge at a speed of 4000 rpm for 15 min then filtered with eight filter paper and placed in Cool place. Figure 1.

2.2 Synthesis of Se/Seo NPs by Using Camellia Sinensis L Extract

When the green tea plant extract is heated to 80 degrees Celsius on a magnetic stirrer, the sodium selenite solution is regularly distilled into the extract. The temperature is gradually raised so that it doesn't reach 160 degrees, and after 60 minutes, we observe a change in the solution's color, which indicates the acquisition of nanoscale selenium. Figure 2.

2.3 Characterization of Se/SeO NPs

Nanoparticles can be thought of in a variety of ways. Changing the color of the solution is the first and simplest method. For phase identification and characterization of crystalline metallic nanoparticles, the optical characteristics of Se/Seo NPs were measured using a Spectrophotometer UV-VIS.

The Energy-dispersive X-ray (EDX) spectra show the components in the prepared samples, which the fabricated samples are SeO nanoparticle.

In order to study the morphology and particle size of very large magnifications, the MIRA3 TESCAN Mashhad (MUMS) model (Fill Emission Scanning Electron Microscope) was used to measure the surface morphology characteristics (shape and particle size) of drying solution droplets placed on slides using high-resolution transmission electron microscopy (FESEM). During FESEM study, the electron beam absorbs energy between 10 and 30 kV.

Figure 1: Synthesis of dried leaves extract Camellia Sinensis.

Figure 2: Synthesis of SeO NPs using Na_2SeO_3 salt as a sources of selenium metal by Camellia Sinensis dried leaves extract.

2.4 Antibacterial Activity Test of SeNPs

In this study two isolated used, there are many sample taken from patients and determined by Vitek-2 System which indicated this isolate was *Staphylococcus aureus*. *Invitro* antibacterial activity was determined by agar-well diffusion method [13]. To determine the impact and efficacy of the nanoparticles made in this investigation against Gram-positive bacteria, specifically *Staphylococcus aureus*. The culture media arrived and sterilized the feed for 15 minutes at 121° C and 15 lb/inch of pressure. After being activated in the center of the nutrient broth, the bacteria were cultured for 24 hours at 37° C and stored in the refrigerator until they were needed. Following preparation and sterilization, the nutritional agar was transferred onto sterile Petri dishes.

The zone of inhibition test was active to observe the inhibitory effects of Azithromycin (15μg), Levofloxacin (5μg). Logarithmic phase bacteria (OD600nm 0.5) were diluted to approximately 107 CFU/mL with nutrient broth. The sample disk containing the antimicrobial agent solution was gently placed at the center of the, Mueller-Hinton agar plates and cultured overnight at 37 C. The diameter of the zone of inhibition around the disk is the standard of measure of the antibacterial activity.

2.5 Antibiofilm Activity of SeNPs Against Staphylococcus Aureus

In this study used isolate of *Staphylococcus aureus* which a biofilm-producing and a non-biofilm-producing isolate were used as controls. *Staphylococcus aureus* was determined by the method of [13], Antibiofilm effects of SeNPs and levofloxacin antibiotic, used 96 wells Agar well diffusion methods. Each well of the 96-well microtiter plate filled with 200 μL of the diluted bacterial culture. Incubate the 96-well plate at 37°C for 24-48 hours to permit biofilm formation After incubation period, If the absorbance analysis is higher than the negative control, it shows biofilm formation. After the contents of the microtiter plates were removed, 200 μL of phosphate buffered saline (PBS, pH 7.2) was used to wash the wells. After being emptied, microtiterplates were allowed to air dry. For 15 to 30 minutes, the microtiter-plates were stained with 200 μL of 1% crystal violet per well, which is used for Gram staining. To get rid of extra stain, each well was washed with 200 μL of 95% ethanol solution. Using a microplate reader, measure each well's absorbance at 590 nm. Then, compare the results with both positive and negative controls to verify the establishment of biofilms.

3 RESULTS AND DISCUSSION

3.1 Physical Properties

3.1.1 UV–Visible Spectroscopy and Color Change

A reduction of selenium ions to Se/SeO NPs using green tea extract is indicated by the solution's visual color changing from pale-pink to deep-yellow due to surface vibration of plasmas in Se/SeO NPs. In the UV–vis spectrum, SeNPs displayed surface plasma resonance of a peak centered at roughly 272 nm, which is equivalent to the absorption of Se/SeO NPs. (Figure 3).

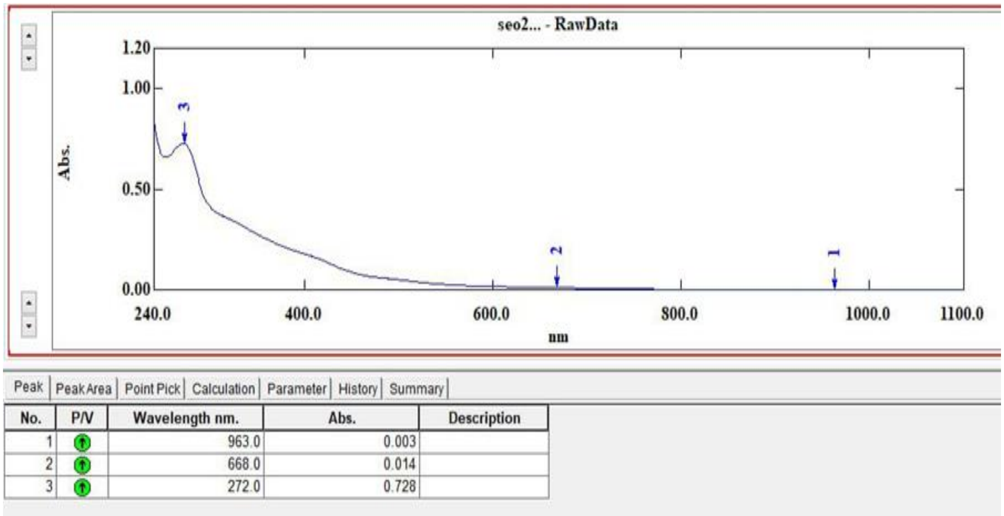
3.1.2 EDX Analysis

The Energy-dispersive X-ray (EDX) spectra show the components in the prepared samples, in which the fabricated samples are SeO nanoparticles. The EDS result in Figure 4, shows the presence of Se and O (Se: 70% and O: 30%).

3.1.3 Field-Emission Scanning Electron Microscope Analysis (FESEM)

The surface morphology for the synthesized nanoparticles was observed using a scanning electron microscope. Figure 5 shows the surface morphology of the SeO (from Na_2SeO_3 salt) nanoparticle respectively, in which the nanoparticles are found to be cylindrical in shape.

With the average size of nanoparticles at 20.5 nm. The results confirm the effect of the anion associated with selenium metal is very clear on the size and shape of the SeO nanoparticles since the different anions gave agglomeration of the nanoparticles and could be related to van der Waals forces among.



No.	P/V	Wavelength nm.	Abs.	Description
1		963.0	0.003	
2		668.0	0.014	
3		272.0	0.728	

Figure 3: Absorbance spectrum as a wavelength function of Se/SeO NPs.

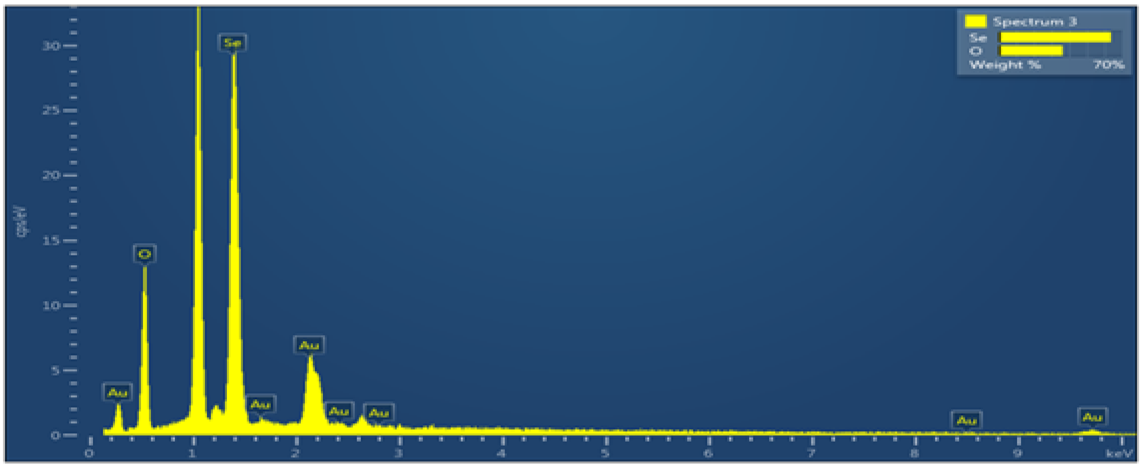


Figure 4: EDS image of SeO nanoparticles, from Na_2SeO_3 salt.

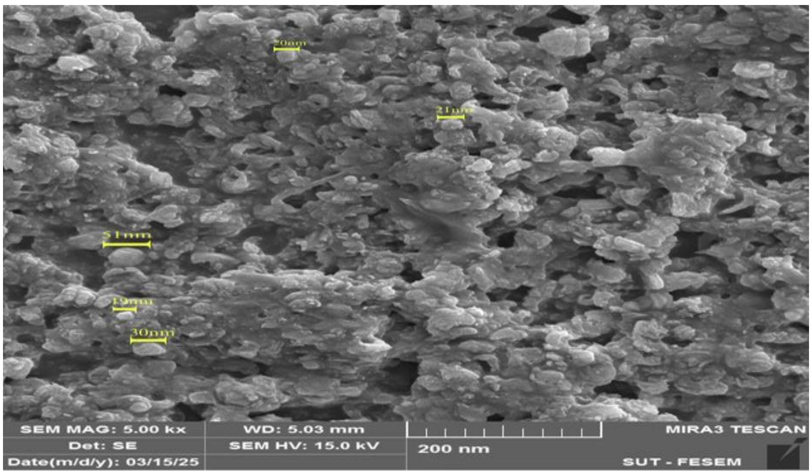


Figure 5: FESEM image of Se/SeO NPs by 200 nm.

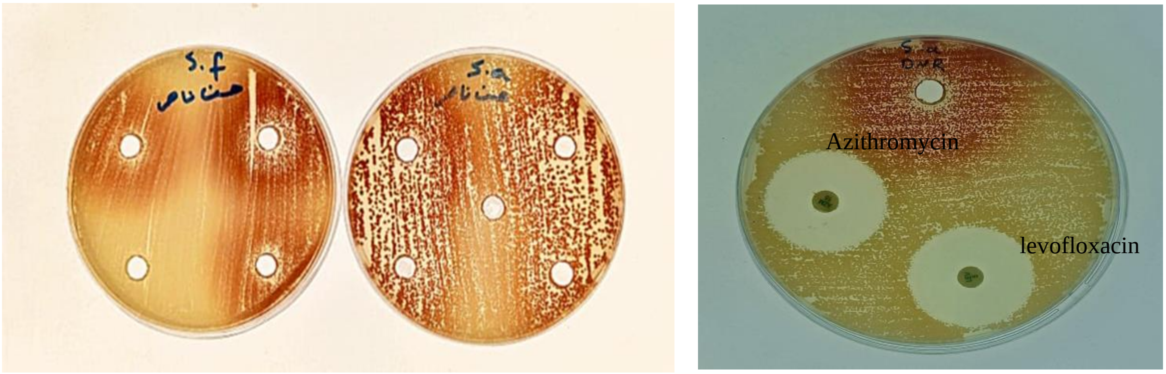


Figure 6: Antibacterial activity assessment of SeNPs against Staphylococcus aureus: a) Antibacterial activity of various concentrations of biosynthesized SeNPs; b) Comparative antibacterial activity of SeNPs, azithromycin, and levofloxacin (reference antibiotics).

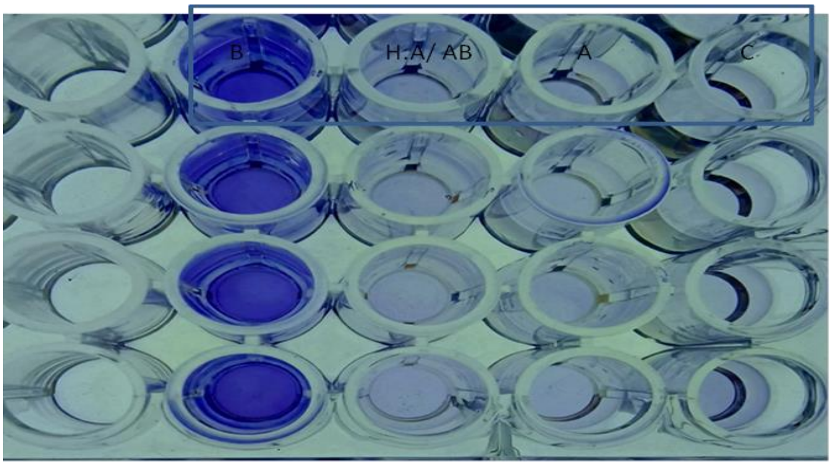


Figure 7: Biofilm activity of the biosynthesized SeNPs against Staphylococcus aureus. B=Biofilm test column, AB=Antibiofilm test line, A= antibiotics, C= Control.

3.2 Biological Properties

3.2.1 Antibacterial Activity of Selenium NPs Using Agar Well Diffusion Method

The effect of Se/SeO NPs has been studied in different concentrations (25, 50, 75, 100) %, Figure 6a Antibacterial inhibition zone.

Measurement is No activity, then compared with two types of antibiotics (Azithromycin and levofloxacin) on the inhibition of bacterial growth of Gram-positive (*Staphylococcus aureus)* as in Figure 6b, the inhibition zone measurement for both Antibiotics were 30 mm.

Since the characteristics of bacterial killing are comparable to those of nanoparticles in terms of volume, stability, and additional concentration to the growth medium, that gives the nanoparticles more time to interact with bacteria, there are a number of theories to explain how nanoparticles inhibit bacteria. A change in the overall structural and optical properties results from an increase in the surface-to-volume ratio, which can occur thousands of times [14].

Bacterial cell membranes of the highest quality have nanoscale stomata or pores. But in order to influence the growth of bacteria by interfering with the regular operation of cells, the nanostructures must penetrate or cross those membranes and settle sufficiently.

3.2.2 Ability of SeNPs to Remove the Established Biofilm

Impacts of SeNPs and levofloxacin antibiotic in eliminating the conventional biofilm (after 24 to 48 hours) were investigated in microtiter plates. According to the acquired results, the SeNPs generated under the investigated conditions exhibited antibiofilm effects against *Staphylococcus aureus*, compared with antibiotic levofloxacin both appeared a strong antibiofilm degrees 54.7, 55% sequentially. Figure 7.

The balance between pathogenic and helpful bacteria in the colon is one factor that distinguishes health from illness. When dysbiosis occurs, it is caused by an imbalance. Conditions like diabetes, obesity, colon cancer, and inflammatory bowel disease can be triggered when the host-microorganism interaction is altered. The formation and accrual of bacterial biofilms upsets this balance and is one of the major causes of chronic infections [15]. Over the past ten years, nanoparticles (NPs) have been the subject of much research as drug carriers in a variety of disciplines, such as neuroscience, immunotherapy, and oncology [16], [17], Additionally, preventing the formation of biofilms is crucial for managing microbial biofilms in a variety of industries, including food processing [18]. Research on selenium nanoparticles' antibacterial properties is rather rare in Iraq. Nevertheless, several studies have found similar results [19]. Selenium nanoparticles, for example, showed strong antibacterial activity against a range of harmful microorganisms in an Iraqi investigation. including *S. aureus* [20]. Even this result is promising but green nanoparticle Selenium need more experimental test to explore its action within others bacteria and tissue culture.

4 CONCLUSIONS

The study concluded that selenium oxide nanoparticles (SeO NPs), synthesized using green tea (Camellia sinensis) leaf extract and Na_2SeO_3 salt using a green, environmentally friendly method, exhibit unique physical properties in terms of size and shape. Their average size was 20.5 nm and they were cylindrical in shape. Although they did not demonstrate any direct antibacterial activity against Staphylococcus aureus when tested using the agar diffusion method, the results demonstrated a strong inhibition and disintegration of the biofilm produced by the same bacteria, reaching 54.7%. These results indicate that these nanoparticles hold promise for biomedical applications, particularly in combating chronic infections associated with biofilm formation. The study recommends further studies to evaluate their effectiveness against other types of bacteria and in more complex biological models.

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