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Synthesis, Characterization, and Cytotoxic Evaluation of Selenium Nanoparticles

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Studies on the functional characteristics of Se nanoparticles (SeNP) have become increasingly popular in recent decades because of their strong selectivity, stability, and high biocompatibility. Numerous studies demonstrate SeNP's anticarcinogenic properties. In order to defeat bacteria that are resistant to antibiotics, new strategies need to be developed. A subclass of antimicrobial peptides known as selenium nanoparticles (NPs) can be used either in place of or in addition to well-known antibiotics. SeNPs have been investigated with the Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), zeta potential, and transmission electron microscopy (TEM)., and UV-visible analysis. The antibacterial qualities of SeNPs were confirmed by the generated nanoparticles showed increased antimicrobial activity at different concertation (1000-64) μg/ml In the good diffusion assay, the NPs efficiently suppressed the growth of susceptible SeNPs have zones of inhibition between 5-19 mm against Gram-Negative while against Gram Positive bacteria Staphylococcus epidermidis and Staphylococcus aureus bacteria have zones of inhibition between 6-17 mm , respectively. Our study has shown that combining various antimicrobials with different mechanisms of action led to an extensive variety of inhibition and raised efficacy, particularly against germs that are resistant to several medications. The best anti- human breast cancer cell line MCF7 properties of nanocomposite against the above cell lines was in the case of MCF7 cell line. According to the above findings, the SeNPs may be administrated for the treatment of several types of human breast cancer in humans. Ovarian cancer had increased nanoparticle sensitivity. At a concentration of 25 μL/mL, SeNPs demonstrated excellent cytotoxicity and anticancer activity, making them suitable for use as an anticancer agent.The physicochemical characteristics, experimental setup, and concentrations of SeNPs exploited in this study must all be carefully stated.

Keywords: Ascorbic Acid; Selenium Nanoparticles; X-ray Diffraction; Cell lines (MCF7); GI50 value.

The field of theranostics, which integrates therapy and diagnostics, has advanced significantly due to thanks to developments in nanotechnology, pharmaceutical science, and healthcare. With selenium nanoparticles (SeNPs), which are growing into more and more effective theranostic agents, this is especially so Some studies.[1] Antioxidant, anti-inflammatory, anticancer, antibacterial, antidiabetic, wound healing, and cytoprotective therapies are just a few of the areas in which SeNPs have demonstrated great promise. The review emphasizes SeNPs' versatility and biocompatibility, which are essential for sophisticated disease monitoring, detection,

and individualized care. Particular attention is given to developments in green synthesis methods, highlighting their economical and environmentally beneficial uses in biosensing, imaging, diagnostics, and therapy .[2] Because of its unique bioactivities in nanoforms, selenium (Se), a crucial trace element, stands out among these nanoparticles. Through oxidoreductase activity, a vital enzymatic mechanism, selenium is integrated into selenium-containing proteins like selenocysteine (Sec)"like selenocysteine (Sec), which are crucial for preserving physiological redox equilibrium. Devices based on selenium have been created in the field of medication delivery to deliver drugs to particular areas Researchers like Karthik et al .,[3] The production of selenium nanoparticles (SeNPs) via biological synthesis techniques and their uses in a variety of industries. The environmental-friendly characteristics of plant-based synthesis, which uses ingredients like aloe vera leaf extracts, has drawn research. Other research have employed various species, such as the seeds of Ganoderma lucidum spore oil and Catathelasmaventricosum polysaccharides, for SeNP synthesis, exhibiting a variety of methods. Aloe vera extracts, which are abundant in secondary metabolites, work as natural reductants and stabilizers in SeNP creation Some studies,[4] Selenium has a limited range of therapeutic and dangerous dosages, and even subtoxic concentrations can have negative health effects, despite its critical role in proper biological functioning. Because of their high bioavailability and low toxicity, selenium nanoparticles (NPs) efficiently enhance the delivery and absorption of this trace element without producing toxicity Another investigation. [5] There is a great deal of typological variety in the contentious link between exposure dose and the negative effects of selenium nanoparticles. To improve the risk assessment of these molecules, more research is required to elucidate the absorption, metabolism, and long-term toxicity of selenium nanoparticles Some studies. [6]

We also go over the latest research on the potential of a number of biological materials for the synthesis of selenium nanoparticles. The physical characteristics of the product (size, shape, and stability) will be discussed together with the precursor, extract, procedure, time, temperature, and other synthesis requirements. Future researchers can more accurately synthesize SeNPs and use them in desired applications thanks to the synergy of SeNP synthesis via many Somestudies.[7] Thus, the purpose of this study was to create antibacterial composite resins using crystalline selenium nanoparticles (SeNPs) and evaluate the composite resin's mechanical, physical, and antibacterial qualities following SeNP integration.

MATERIAL AND METHODS

SeNPs' characterization and environmentally friendly manufacture Selenium nanoparticles (SeNPs) were produced by a reduction reaction between sodium selenite and ascorbic acid. 50 mM sodium selenite and stabilizing agents were combined with magnetic stirring for five minutes to produce stabilized SeNPs. The solutions were then gradually supplemented with 1 weight percent ascorbic acid in deionized water. To attain total reduction, the reaction mixtures were shaken for half an hour studies are needed. [8]

Characterization of SeNPs

Characterization is essential in order to fully understand the distinctive properties of nanoparticles. The methods that followed have been utilized to determine the nanoparticles' sizes and shapes: The evaluation of surface structure, particle size distribution, particle or crystal effect, nanoparticle aggregation, surface functionalization, and single-particle assessments can be seen using Field Emission Scanning Electron Microscopy (FE-SEM). A device used in materials research to ascertain a material's crystallographic organization is called an X-ray diffraction machine (XRD).. Fourier-transform infrared spectroscopy (FT-IR, RX I, PerkinElmer, Inc., USA) at a resolution of 4 cm1 and Zeta Potential (ZP) and energy dispersive X-ray (EDX) were used to analyze the functional groups of produced nanostructures in the 4000–400 nm range Auther Salman et al,. [9]

MIC determination

The lowest concentration of SeNPs that restricts bacterial growth was identified (MIC) using Eppendorf tubes as the medium and the broth microdilution technique. After the production in MHB, the microbial inoculum's concentration was lowered to 100 UFC/il. Two-fold serial dilutions of NPs (1000, 500, 250, 125, and 64 ìg/il) were

produced using MHB in six tubes. The tubes were afterward incubated for a full day at 37° C. Following that, results were acquired by looking for indicators of bacterial growth in each tube, making it possible for an estimation of the subMIC Another investigation . [10]

Testing the antimicrobial activity of SeNPs

Natural Products-Based Metallic Nanoparticles possess a variety of bioactivities, such as antimicrobial properties. The microorganism's resistance to multiple drugs and its distinct cell membrane composition limit its bioactivity. Mueller Hinton Agar was used for the Agar Well Diffusion assay. Sterilized inoculating loop or swab were used to spread the test isolates Clinical isolate included gram-negative and gram-positive were obtained from the College of Medicine at Iraqi University were examined. onto Muller-Hinton Agar plates. A sterile cork borer was used to create 5 wells 5 mm, which were then filled with 80 µl of varied concentrations depends on microdilution technique below. Then subsequently incubated for 24 hours at 37 ° C, after which the zones of inhibition were determined the following day Another investigation. [11]

Cytotoxicity assay

The cytotoxic effects were determined with an in vitro 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test. Seven billion cells were introduced in each well of a 96-well plate and incubated overnight to ensure adhesion between cells. After that, cell lines (MCF7) were treated to substances at progressively higher SeNPs concentrations (6.25–100 ìg/ml); three replicate wells were employed for each treatment. After 24 hours of incubation, the media was taken out of the plate, and each well received 20 ìl of MTT solution (5 mg/ml) from Shanghai Macklin Biochemical Co., Ltd. The wells were then incubated for 3 hours at 37ÚC in the dark. After adding 50 ìl of DMSO (Bio Basic Inc.), To dissolve the MTT, it was rattled for a total of ten minutes. A BioTek Instruments, Inc. microplate reader was used for identifying absorbance at 490 nm. The following formula was used to determine the number of living cells from the raw fluorescence data.

$$\text{Viability \%} = \frac{\text{A test} - \text{A blank}}{\text{A control} - \text{A blank}} \times 100$$

Where the absorbance is denoted by "A." GraphPad Prism software version 6 (Dotmatics) was used to create the dose-response curve, and the same curve was used to calculate the growth inhibitory concentration (GI50), which lowers viability by 50% Some studies,[12]

RESULTS

Synthesis of SeNPs

This medium's color shift, which turned red once Se_2O_3 had been reduced to Se0, was the first hint of the synthesis of the SeNPs. After the powdered SeNPs nanoparticles had been produced at 120°C, they were described, and the formation of a white precipitate showed that the nanoparticles were formed. The precipitate turns white after centrifugation. After microwave drying, we were presented with a beautiful white powder.

Characterization of SeNPs

UV–Vis Spectral Analysis and Zeta Potential Analysis

The reduction to Se0 was demonstrated by the UV-Vis spectroscopic research studies. The acquisition of a strong absorption signal at 288 nm validated the production of SeNPs while Na2SeO3. The peak attained 298 nm as the Na2SeO3 concentration grew (Fig. 1a). On the other hand, the zeta potential of He10214 was -51.2 mV, while that of the SeNPs produced by Si10198 was 60.6 mV (Fig. 1b).

Atomic Force Microscopy (AFM) analysis and X-ray analysis

AFM analysis of SeNPs was performed with identify and characterize distributions of nanoparticles. The nanoparticales microstructure was investigated utilizing atomic force microscopy (AFM). Nanoparticles were discovered to have irregular and triangle cluster morphologies, ranging between 0.25 and 23.33 nm in height and 13 to 177 nm in diameter (as verified by a particle size analyzer) according to the three-dimensional AFM. (Fig. 2a). XRD assessing further verified the chemical production of SeNPs (Fig. 2b). The strongest peak was seen at 54 cps, and the peak value for diffraction was roughly 29.5 (2è).

Field emission Scanning Electron Microscope (FESEM) and EDX analysis

Images of SeNPs under an electron microscope appear in Fig. 3.a. The typical lengths

of these spherical particles in 6 and 8 mM of the synthesized Na2SeO3 are around 50–100 nm and 30–100 nm, respective. The spherical shape of the biosynthesised SeNPs was confirmed by FESEM images.The unchanged chitosan nanoparticles are composed of clusters of particles that vary in size

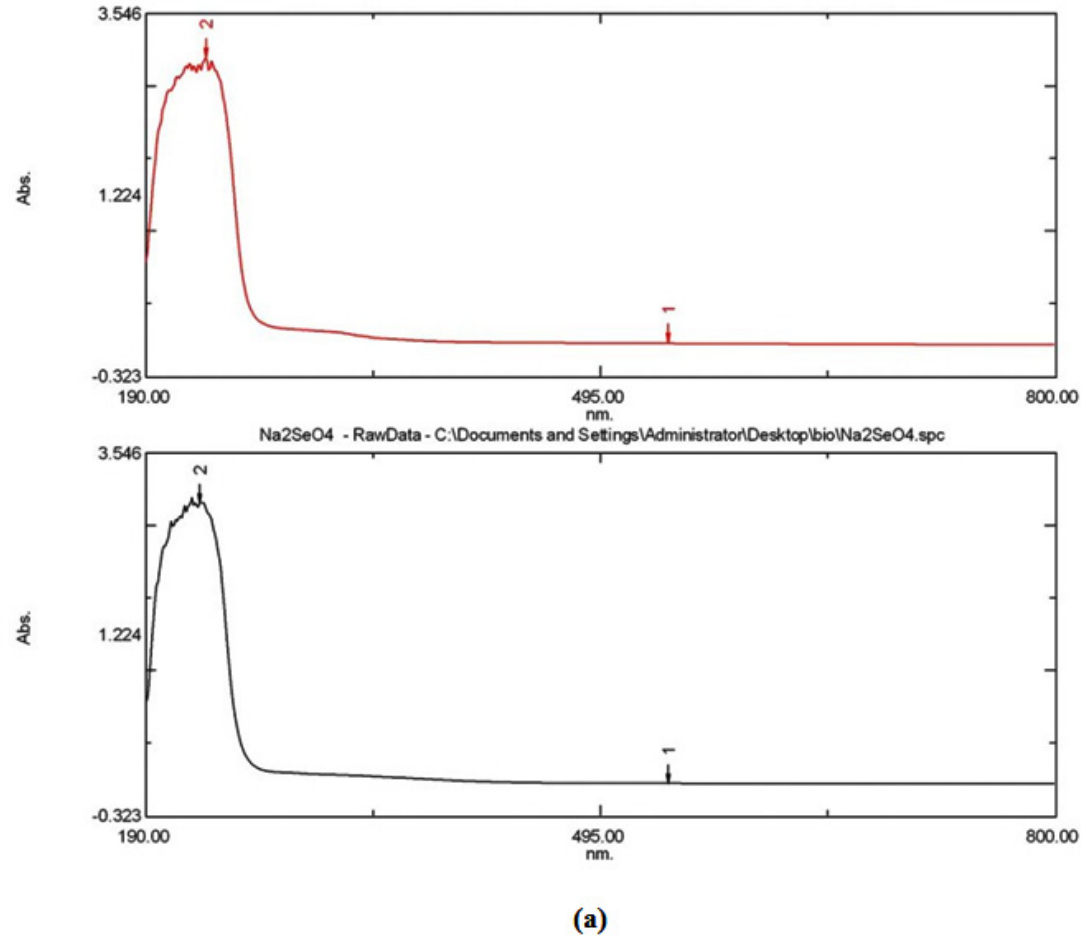


Fig. 1. a) UV analysis b) Zeta potential measurement of the SeNPs of synthesized

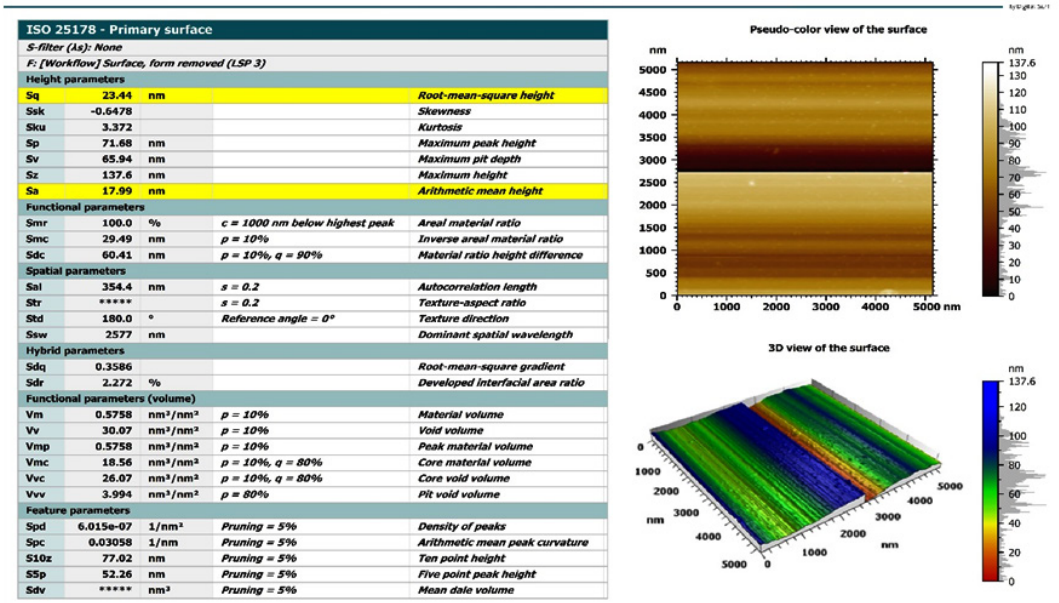
from 10 to 33 nm. The synthesized SeNPs' EDX investigation, as shown in Fig. 3.b

FTIR Analysis

High absorption can be noticed in the FTIR spectra of the unaltered (CH/GL) specimen film at 3293 cm"1 (-OH stretching), 2925 cm"1 (amide A's N-H stretching), 1549 cm"1 (C-C, C bending), 1410 cm"1 (C-O broadening), and 1640 cm"1 (C=O stresses). The carbonyl component (-C=O) of chitosan and the C-H group of gelatin interact in the FTIR a spectrum of the blended composite films (Figure 4).

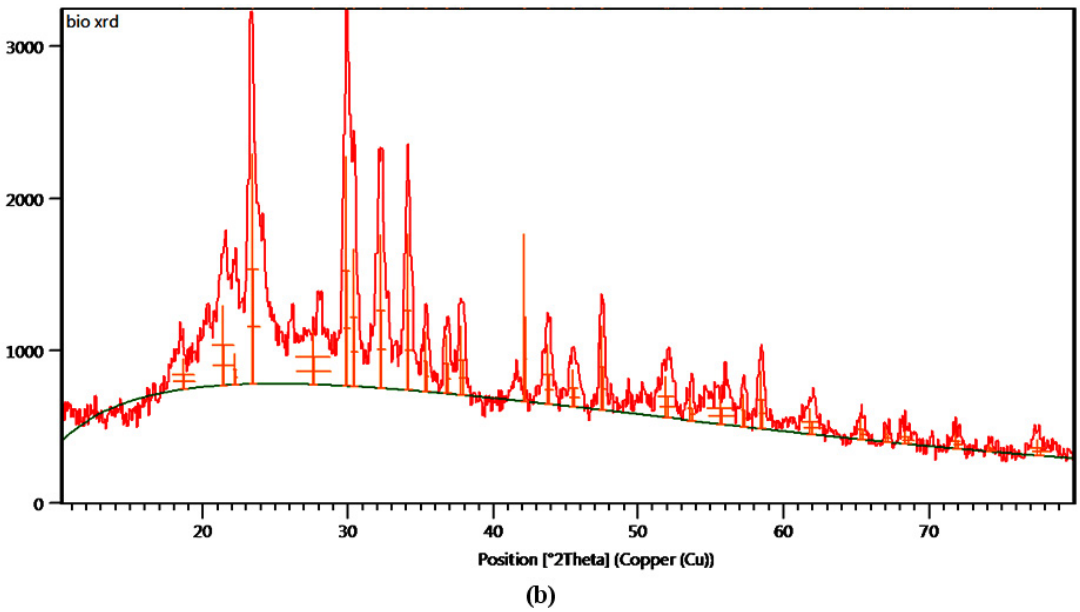
Antibacterial activity SeNPs

Because selenium nanoparticles has antimicrobial action both on its own and when metal nanoparticles bond to this substance,



ISO 25178 - Primary surface				
S-filter (As): None				
F: [Workflow] Surface, form removed (LSP 3)				
Height parameters				
Sq	23.44	nm		*Root-mean-square height*
Ssk	-0.6478			*Skewness*
Sku	3.372			*Kurtosis*
Sp	71.68	nm		*Maximum peak height*
Sv	65.94	nm		*Maximum pit depth*
Sz	137.6	nm		*Maximum height*
Sa	17.99	nm		*Arithmetic mean height*
Functional parameters				
Smr	100.0	%	*c = 1000 nm below highest peak*	*Areal material ratio*
Smc	29.49	nm	*p = 10%*	*Inverse areal material ratio*
Sdc	60.41	nm	*p = 10%, q = 90%*	*Material ratio height difference*
Spatial parameters				
Sal	354.4	nm	*s = 0.2*	*Autocorrelation length*
Str	*****		*s = 0.2*	*Texture-aspect ratio*
Std	180.0	°	*Reference angle = 0°*	*Texture direction*
Ssw	2577	nm		*Dominant spatial wavelength*
Hybrid parameters				
Sdq	0.3586			*Root-mean-square gradient*
Sdr	2.272	%		*Developed interfacial area ratio*
Functional parameters (volume)				
Vm	0.5758	nm³/nm²	*p = 10%*	*Material volume*
Vv	30.07	nm³/nm²	*p = 10%*	*Void volume*
Vmp	0.5758	nm³/nm²	*p = 10%*	*Peak material volume*
Vmc	18.56	nm³/nm²	*p = 10%, q = 80%*	*Core material volume*
Vvc	26.07	nm³/nm²	*p = 10%, q = 80%*	*Core void volume*
Vvv	3.994	nm³/nm²	*p = 80%*	*Pit void volume*
Feature parameters				
Spd	6.015e-07	1/nm²	*Pruning = 5%*	*Density of peaks*
Spc	0.03058	1/nm	*Pruning = 5%*	*Arithmetic mean peak curvature*
S10z	77.02	nm	*Pruning = 5%*	*Ten point height*
S5p	52.26	nm	*Pruning = 5%*	*Five point peak height*
Sdv	*****	nm³	*Pruning = 5%*	*Mean dale volume*

(a)



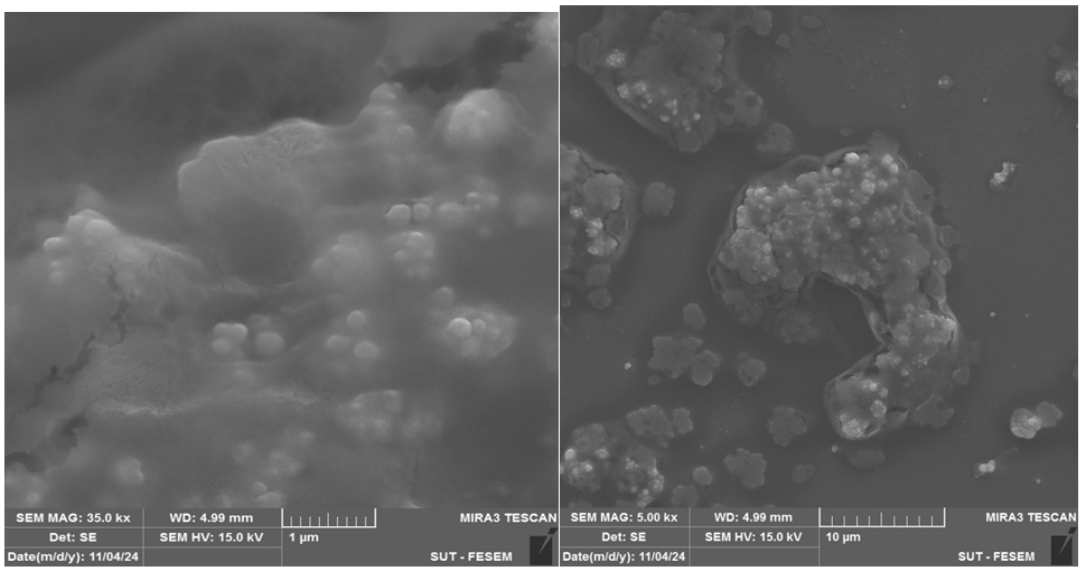
(b)

Fig. 2. a) Atomic Force Microscopy analysis b) X-ray analysis of SeNPs

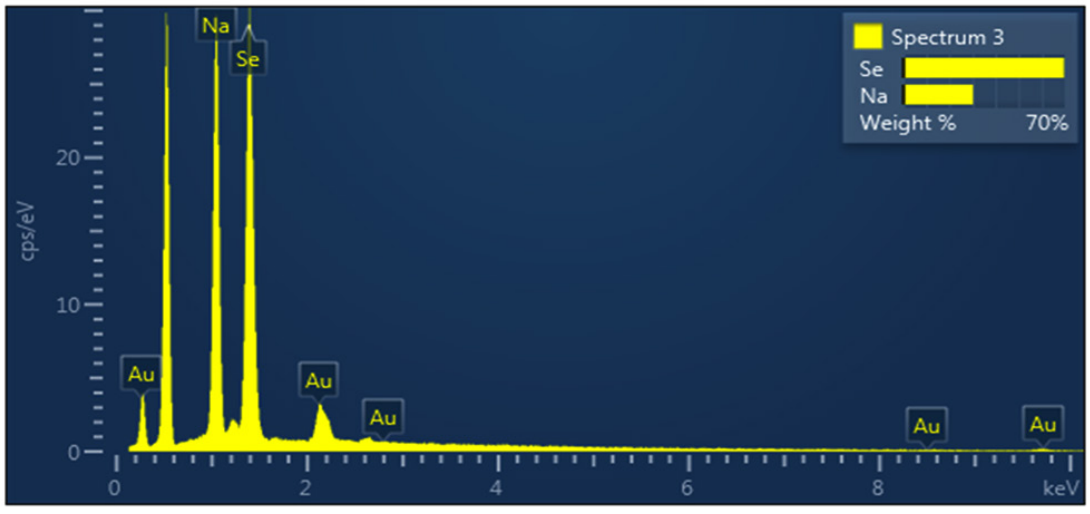
researchers are very interested in SeNPs nanoparticles. It has outstanding antibacterial qualities. Multiple explanations for selenium nanopartical antibacterial effects have been offered by scientists. The antibacterial properties of the targeted metal NPs against four Gram-Positive and negative bacteria were observed are shown Figures 5-a and 5- b respectively. The standard drug kanamycin's zone of inhibition (measured in millimeters) against the Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* were at concentration 1000 µg/ml found 17 mm and 18 mm respectively. On other *staphylococcus aureus and staphylococcus epidermises* at the same concertation range inhibition zone reached 8 and 17 mm at concentration 1000 µg /ml.

The cytotoxic effect of SeNPs nanoparticles on tumor cell lines

The cytotoxic activity of selenium nanoparticles on the human breast adenocarcinoma cell line MCF7 were studied as seen in Table 1 and Figure 6. The results obtained showed that the cell



(a)



(b)

Fig. 3. a) FESEM Images b) EDX analysis confirming selenium element

viability reduced as the concentration of SeNPs increased. The GI 50 value of SeNPs was about 24. 5 µg/ml. These NPs exhibited toxic effects against the MCF7 cell line, and at a concentration of 25 µg/ml, the cytotoxic effects reached 65.4%. SeNPs were tested for *in vitro* cytotoxicity against MCF7 cell lines at concentrations of 6.25–100 ìg/ml; In agreement with our results, several evidence have indicated the cytotoxic potential of SeNPs on different cell lines.

DISCUSSION

All three SeNPs have been determined to be nearly spherical by scanning electron microscopy (SEM) analysis, and the purified SeNPs' EDAX microanalysis demonstrated substantial selenium absorption maxima at 1.37 (SeLa), 11.22 (SeKa), and 12.49 keV (SeKb). Additionally, the elemental compositions in various synthesised SeNPs changed Researchers like. [13] In biogenic SeNPs, for instance, the weight percentage of selenium was 9.26% for Bm-SeNPs(+) and 11.01% for Sm-SeNPs(~).However, ChSeNPs had a greater selenium proportion (31.61%). Additionally, the crystalline makeup of biogenic SeNPs, which were rich in C, O, P, and S, indicate the presence of biological polysaccharides close to the nanostructures. Based on the presence and quantity of C, O, and S, one might believe that the biogenic SeNPs cap is composed of proteins or enzymes, specific cellular residues, and phospholipids found in the membrane (P peaks) [14.] Se-NPs' electrical structure and, in turn, their ability to absorb and scatter light are influenced by their size, the UVvis spectrum is where these impacts are most apparent. This technique aids researchers in analyzing the interactions between NPs and light, yielding crucial data for comprehending NP size and quantum characteristics. Relevant information

Table 1. The cytotoxicity of SeNPs on cell line MCF7

		CV: Control vehicleTR: Technical replicate					
MDA-MB231		CV	6.25 uM	12.5 uM	25 uM	50 uM	100 uM
Compound 1	Raw data	0.361	0.254	0.308	0.201	0.241	0.136
TR1	% Survival	100	70.36011	85.31856	55.67867	66.759	37.67313

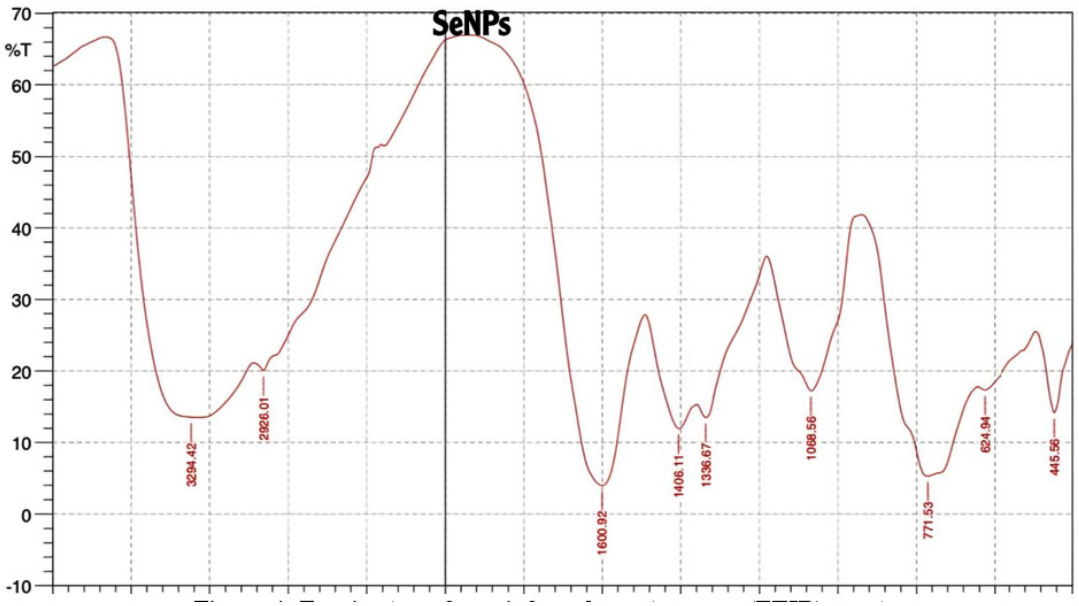


Fig. 4. Fourier-transform infrared spectroscopy (FTIR) spectra

regarding the size, shape, and composition of the particles are provided by this procedure. NPs' small size causes quantized energy levels Another investigation. [15] Studies using UV-vis spectroscopy have demonstrated that Se-NPs absorb light differently due to their differing electrical configurations. To comprehend how light interacts with NPs, surface plasmon resonance data is crucial. Se-NPs have been verified by researchers based on peaks in the ultraviolet (UV) (200–400 nm) Studies by Instructions to Authors.[16] These Se-NPs have a 45–90 nm diameter and a spherical shape.

Particle aggregation takes over during the creation of nanoparticles (NPs), concealing the reduction of precursor atoms and their initial nucleation some studies,[17] XRD may be used to discern distinguish various translucent forms of selenium, particularly the hexagonal and amorphous forms. Peak broadening in the XRD pattern, which is associated with the size of the crystalline structures in the NPs, can be used to estimate the average Se-NP particle size from XRD data. Furthermore, XRD can show which impurities are present in the caused Se-NPs and authenticate the chemical formula and stoichiometry of Se-NPs.

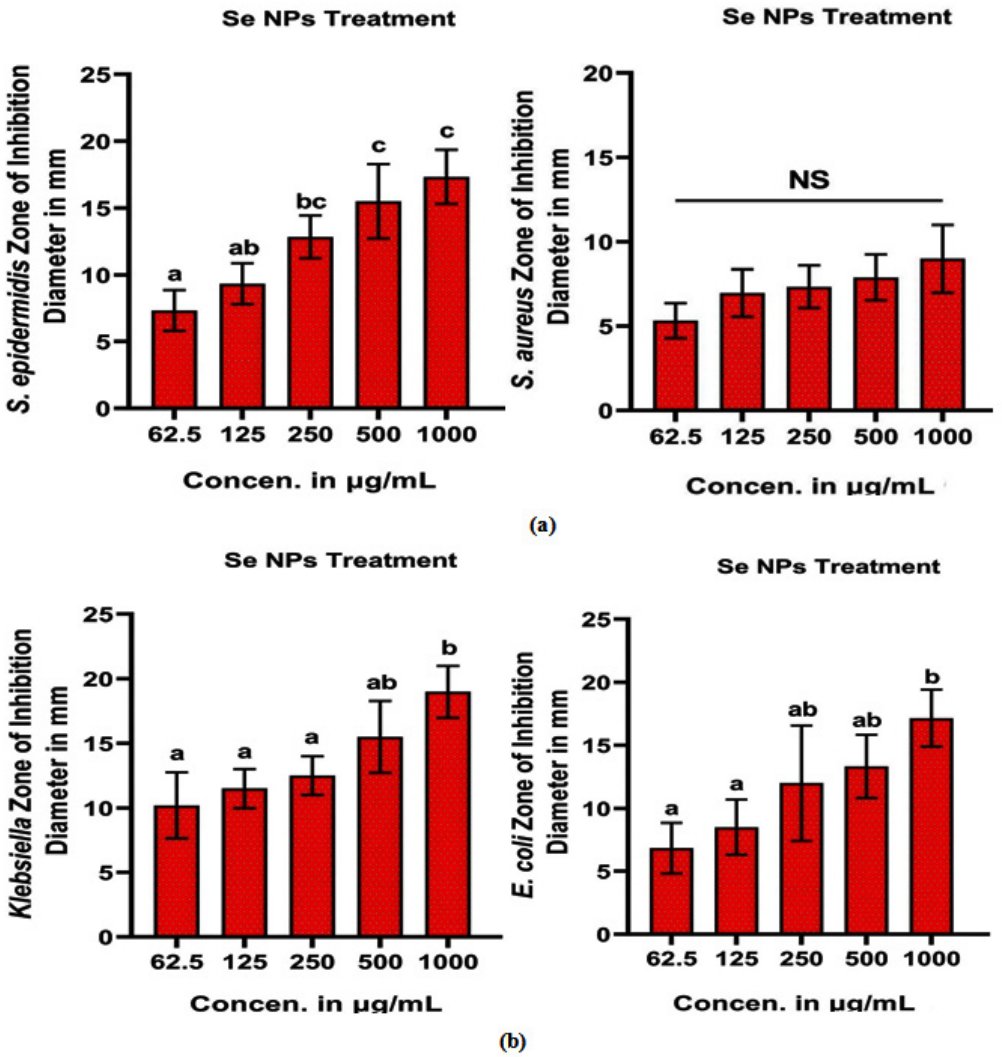


Fig. 5. Mean ± SD Zone of Inhibition in mm treated with SeNPs against a) gram-positive bacteria and b gram-negative at Different Concentrations between 1000, 500, 250, 125, and 62.5 µg/mL.: Standard Deviation, (n = 3)

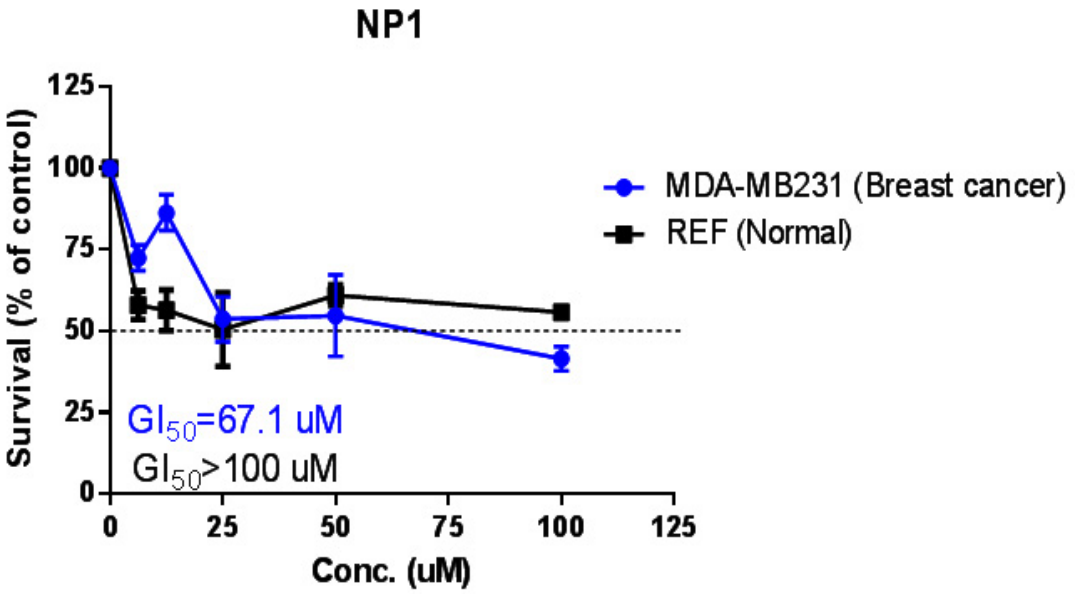


Fig. 6. The cytotoxic effect of SeNPs on MCF7 cells

Some studies, [18]. Examining the cytotoxicity of green produced Se-NPs is essential for determining their appropriateness for biological applications. The green synthesis approach creates Se-NPs from natural and eco-friendly sources, like plant extracts, which are thought to be less hazardous than chemically produced Se-NPs Authors Puri et al ,.[19] Some doses of Se have been shown to be detrimental. Consequently, it is thought that the size, structure, and dosage of Se nanoparticles affect their toxicity. Several procedures have demonstrated that biogenic Se-NPs are less potentially potentially dangerous to animals than sodium selenite. Bano et al. defined that low concentrations of Se-NPs are safe after analyzing the toxicological effects of Se-NPs in animals Another investigation. [20]

CONCLUSIONS

All physiological functions depend on selenium, a necessary element that offers nutritional value. In order to prevent health abnormalities caused by selenium insufficiency, it is crucial to maintain a sufficient supply of selenium. Because nano-selenium is readily available, has low toxicity, SeNPs are an antioxidant, antibiofilm, antibacterial, and anticancer that is also involved in the dietary supplement. SeNPs are less harmful at the right dosage, according to numerous toxicity studies. Future studies on Se-NPs should concentrate on developing characterisation tools, looking for Se-NPs' potential in targeted medication delivery systems and enhancing green synthesis techniques for scalability and sustainability. To guarantee safety, thorough toxicity and environmental effect analyses will be necessary, and in-

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This case report was conducted according to the Helsinki Declaration. The collection and evaluation of the data were confidential according to the health policy of the Iraqi Ministry of Health IN September 22-9-2024 Ref:CSEC/0922/0086

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Author Contribution

Zinah Ayyed Habeeb and Sanaa Khudhur Jameel writing—original draft methodology, investigation, and formal analysis. Mais. Emad. main concepts, data interpretation, supervision, and all reviewed the manuscript.

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