RESEARCH PAPER

Biogenic Synthesis and Characterization of Mango Peel-derived Selenium Nanoparticles for its Anti-Bacterial Potential

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ABSTRACT

Current research focuses on the notable antibacterial properties of nanocompounds, including their environmentally friendly production from biological waste. Selenium nanoparticles, or SeNPs, have attracted greater interest in recent years, particularly in nanotechnology. Important biological functions including antioxidant defense are under the control of this component. Examination employing atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and TEM (transmission electron microscopy) are utilized to characterize them. The **λmax** peak in absorption at 280 nm in the UV-visible spectra indicates an abundance of Se-NPs in the specimen in question. In this study, mango peel extract was used to synthesize SeNPs followed by characterization and investigation of their antimicrobial potential. The results showed that the SeNPs were irregular with rod-like, spherical shapes and were detected from 19 nm to 60 nm. It is important to ascertain the antibacterial effect and the potency of antibacterial activity when researching novel SeNPs methods for preparation. The SeNPs exhibited antibacterial action against both gram-positive and gram-negative bacteria, with a greater effect when produced with 8 µg/mL of extract from mango peels. According to prior studies, SeNPs with various shapes and sizes have a range of antimicrobial properties. As a result, while assessing the antibacterial characteristics, this work utilizes the peel of the mango extract as a reduction agent for emerald SeNP synthesis**.** Zinc oxide nanoparticles have been created utilizing mangoes, which is a financially and environmentally good innovation.

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INTRODUCTION

Selenium nanoparticles, or SeNPs, have received increasing attention in recent years. This element is essential for the synthesis of thyroid hormones, immune system function, and the prevention of oxidative damage and infection in cells. A number of dangerous illnesses, including several types of cancer, have been found to be linked to selenium shortage. The synthesis process, kind of reagent used for selenium salt reduction, additions employed, reaction temperature, and reaction duration all affect the characteristics of SeNPs. Different microbial species employ different mechanisms for the manufacture of SeNP, which

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involves many metabolic pathways and distinct enzymes for the reduction process. In addition, it necessitates laborious processes for keeping and storing cell cultures. Green synthesis has been shown to possess advantages over physical as well as chemical approaches in previous research [1]. Plant extract chemicals may act as natural capping agents in addition to being natural reductants, preventing nanoparticle clusters or aggregates or stabilizing their size. The capping agent that is deposited or bonded on the outside of NPs affects their final shape in important parts [2]. Selenium nanoparticles' biological and pharmacological features have been thoroughly investigated, indicating a range of positive effects, including neurodegenerative, anti-inflammatory, anticancer, antiseptic, antidiabetic, and cardiovascular disease prevention. Nanoparticles are particularly helpful in a range of biomedical applications in diagnostics and therapy due to their low toxicity, biocompatibility, and ability to traverse cell membranes for the delivery of drugs [3]. SeNPs have an assortment of intracellular and extracellular mechanisms that reinforce their antimicrobial activity. These mechanisms consist of the creation of reactive oxygen species, penetration of the membrane of the bacteria, disruption of the phospholipids in the cell wall, and activation of proteins leading to bacterial lysis since smaller nanoparticles are capable of passing through the cell wall and membrane, these actions are size-dependent [4]. When compared to the nanoparticles made with grapefruit juice, the bactericidal effectiveness of the biogenic SeNPs created from lemon juice was significantly higher against several strains [5]. The investigation at the scale has been constrained because the biological characteristics of SeNPs have only been examined in model or animal studies. Published research indicates that supplementing selenium in nanoform reduces the likelihood of excess selenium and that rats did not exhibit any appreciable adverse effects from SeNPs [6]. Large volumes of easily available plant extracts are used in the green synthesis approach, which is non-toxic, safe to handle, and highly effective across a range of salinity, pH, and temperature conditions. Furthermore, a commonly accepted and somewhat more effective method for producing NHA for use in biomedical fields is the use of plant materials in a green synthesis process [7]. As members of the Lacertidae family, mangos are referred to as the "King of Tropical Fruits." They are extensively grown in China, Malaysia, the Central South Peninsula, Bangladesh, and India. Over 45 million tonnes of mangos were produced worldwide in 2014, according to the FAO. Peel constitutes 7–24% of the weight of the mango as it is the main result of modern mango processing [8]. Mango peel waste exists in huge amounts throughout the world, but little research has been done on how to create AgNPs using mango peel extract and its high application value. Flavonoids, polyphenolic chemicals, vitamins C and E, and other substances from nature are abundant in mango peels. Mango peels possess a lot of hydroxyl groups, which makes them natural reducing agents [9]. Investigated the techniques, antimicrobial qualities, and mechanisms of antibacterial nanomaterial-containing edible coatings and their successful application on fruits and vegetables. On the other hand, NPs generated from different vegetation showed a range of antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as variations in their morphology [10]. Mango peel extract is utilized in an environmentally friendly renewable method of "green" SeNP synthesis. By creating nanoparticles from plant extracts, these particles may successfully inhibit bacterial growth by catalyzing the breakdown of ethylene. Several studies have been conducted into the use of nanoparticles in the development of composite antibacterial coating materials. Plant extracts are utilized in green processes for SeNP synthesis, which are considered to be low-cost, one-step, and environmentally benign procedures. Chemicals from plant extracts may operate as natural capping agents in addition to their natural reductant function, maintaining the dimensions of nanoparticles and enhancing the physiological properties of SeNPs. This study aims to use a green approach for the synthesis and characterization of Mango Peel-derived Selenium Nanoparticles and study their antibacterial potential towards multidrug-resistant gram-positive and gram-negative bacteria and the use of metal nanoparticles in extending the shelf life of fruit.

MATERIALS AND METHODS

Identification of Microorganisms

Pathogenic gram-positive and negative bacteria isolated from patients with urinary tract infection

(UTI) (*E. Coli, P. aeruginosa Klebsiella spp, S. aureus, S. epidermidis, and S. pyogenes*) were provided by Ibn Sina College of Medicine, Baghdad, Iraq. These isolates were multidrug resistant (MDR). Identification of suspected colonies was carried out via morphological characters and confirmed using the Vitek 2 compact system (BioMerieux/ France) [11].

Collection of Biomaterial

The mango peels were collected, cut into small pieces, and properly washed with distilled water to get rid of any dust particles adhering to the surface. Ten grams of mango peels were added to 100 millilitres of distilled water before being squeezed through a juicer to produce an extract containing compounds from the mango peels; the resulting solution was boiled for thirty seconds at 85 °C [12] (Fig. 1).

Synthesis of Selenium nanoparticles

The SeNPs were prepared using 16.9 grams of sodium selenite (Na_2SeO_3) dissolved in 100 mL as a precursor and mango peel extract as a reducing agent in a mixture consisting of 0.15 g sodium dodecyl sulfate. Heating (at 80 °C) and stirring for one hour was utilized to obtain the nanometer silver colloid. The suspension's color changed from colorless to reddish-brown, confirming the green synthesis of SeNP. [13]. A high-speed centrifuge was used to centrifuge the reaction solution three times at 10,000 r/min for ten minutes. To obtain the SeNP powder, the ensuing precipitate was vacuum freeze-dried.

Characterization of Selenium Nanoparticles

The synthesized mango peel-derived selenium nanoparticles were characterized using According to UV-VIS spectroscopy, Atomic force microscopy AFM, Energy-dispersive X-ray analysis EDX analysis Bruker/Germany, Field emission scanning electron microscope, and A scanning electron microscope 500 nm resolution scanning electron microscope (SEM) [14]. A camera (Hitachi s-3400N, Japan) was used to capture the images (Fig. 2).

Minimum Inhibitory Concentration determination

The MIC of synthesized Se-NPs against tested isolates was investigated via the Broth microdilution method in culture broth via making serial dilutions (4, 8,16,32, and 64 µg/ml). In brief, one millilitre of Se-NP initial solution (1000 mgmL-1) was made. Next, 100 microliters of diluted test material were inserted into the first line of wells. Next, 100 microliters of freshly made Müller Hinton broth (MHB) was added to each well numbered 1 through 10. This is known as a two-fold dilution; well G served as the positive control, and well H was the negative control. Subsequently, 100 microliters were transferred from the first well to the second, and so on until reaching the last well. Müller Hinton broth in well H has been

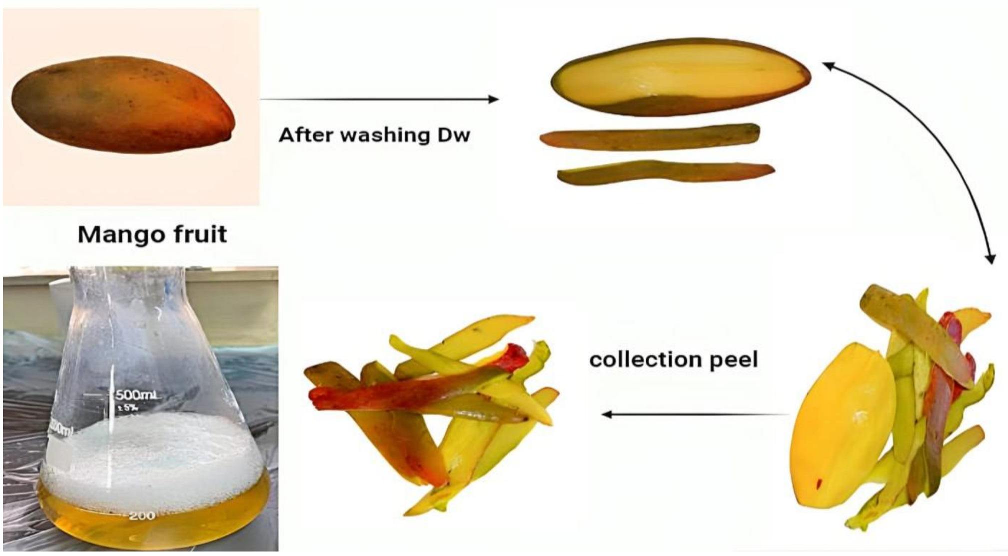


Fig. 1. The process of mango peels Extraction

used as a negative control. Wells were inoculated with 10 microliters of bacterial growth adjusted to McFarland ($1.5 * 10^{8}$ CFU\ml) using densicheck except for the negative control. For 24 hours, the microtiter plate was incubated at 37 C. The growth was then evaluated by utilizing a microtiter plate reader to calculate the absorbance at OD 450 nm. Turbid wells detected bacterial growth, but clear wells showed no growth. The minimum concentration of Se-NPs in which no growth occurs

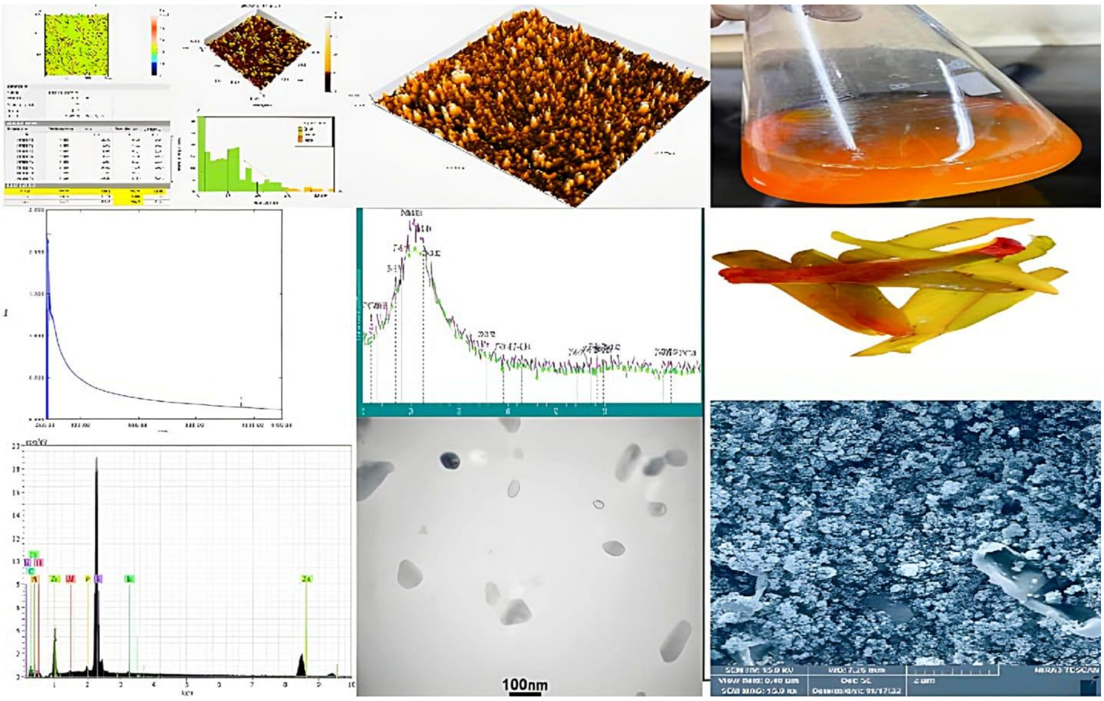


Fig. 2. Characterization of Selenium Nanoparticles

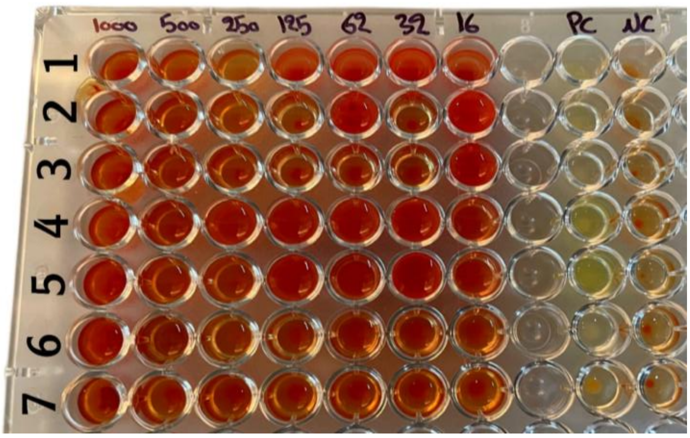


Fig. 3. MIC of the Se-NPs

is termed the MIC [15] (Fig. 3).

Screening of anti-microbial activities by agar well diffusion method (AWD)

The AWD method was used to evaluate the antibacterial potential of Se-NPs against various pathogenic gram-positive and negative isolates *to* determine the minimum inhibitory concentration (MIC) of Se-NPs on different concentrations of Se-NPs (4, 8,16,32 and 64 μgmL^{-1}). The bacteria were spread on the surface of Muller Hinton agar plates, then wells (5 mm in diameter) were cut into the plates with by sterile cork borer, then filled with 100 µl of SeNP solution, and then incubated for 18-24 hr. After incubation, measurements of the diameter of the zones of Inhibition (in millimetres) were done to determine the anti-microbial activity [16].

Statistical Analysis

A one-way analysis of variance ANOVA and Student's t-test were performed to test whether group variance was significant or not. Statistical significance was defined as * $p < 0.05$ or ** $p < 0.01$. All samples analyzed statistically were run in triplicates. Data were expressed as mean ±standard deviation, and statistical significances were carried out using GraphPad Prism version 9 (GraphPad Software Inc., La Jolla, CA).

Group variance was tested for significance using ANOVA and the Student's t-test. The criteria for statistical significance were * $p < 0.05$ or ** $p < 0.01$. All samples analyzed statistically were run in triplicates. Statistical significances were determined GraphPad Prism version 9 (GraphPad Software Inc., La Jolla, CA), and data were reported as mean ±standard deviation.

RESULTS AND DISCUSSION

Biosynthesis of Selenium Nanoparticles:

After being incubated for 24 hours at room temperature, the color of the sodium selenite salt solution changed from yellow to reddish brown due to the addition of mango peel extract. A slight color change that happened 30 minutes after adding fruit peel extracts is shown in Fig. 4.

Characterization of biosynthesized Selenium Nanoparticles

The biggest absorption peak between 200 and 800 nm was seen in biosynthesized Se-NPs. The three extracts' absorbance values, which change somewhat up to 285 nm, indicate that the differences are due to differences in particle size or shape (Fig. 5a). With an average diameter of 37.23 nm, Se-NPs' nanoparticle distributions have been determined and studied using AFM, as shown in Fig. 5b.

SEM-EDX Analysis

The EDX spectrum is mainly used to determine the elemental composition and purity of

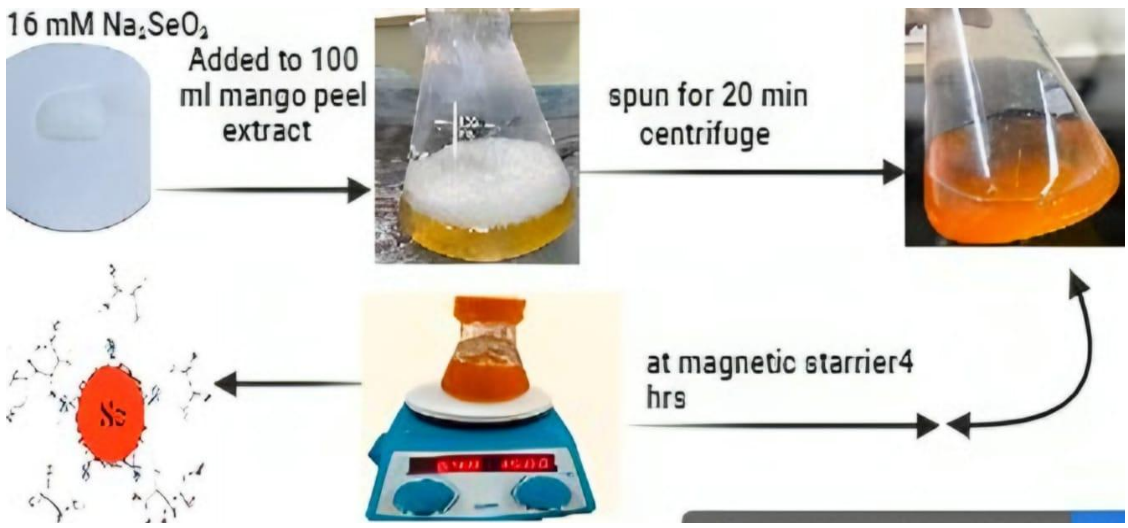
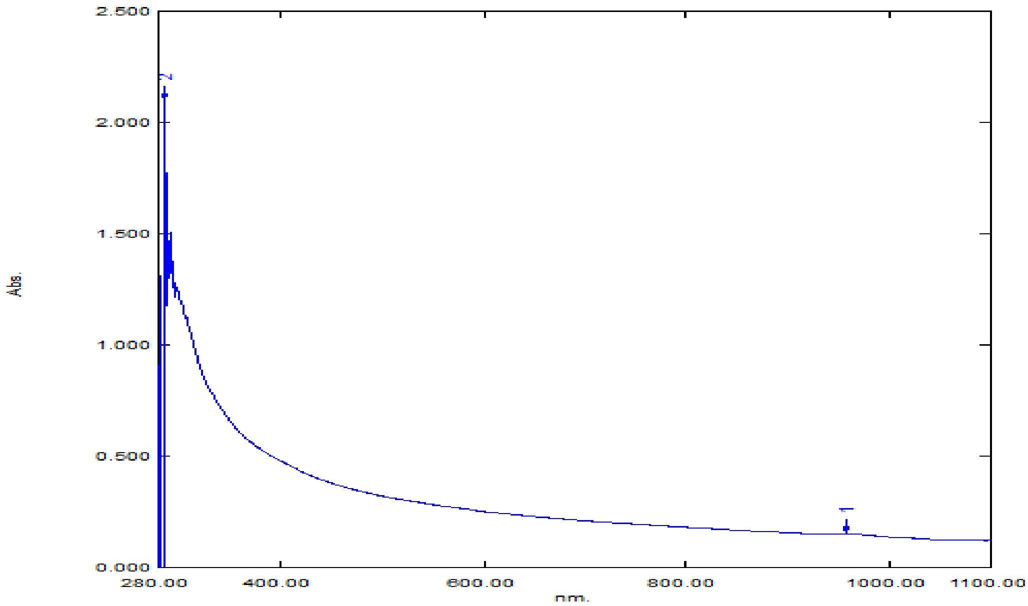
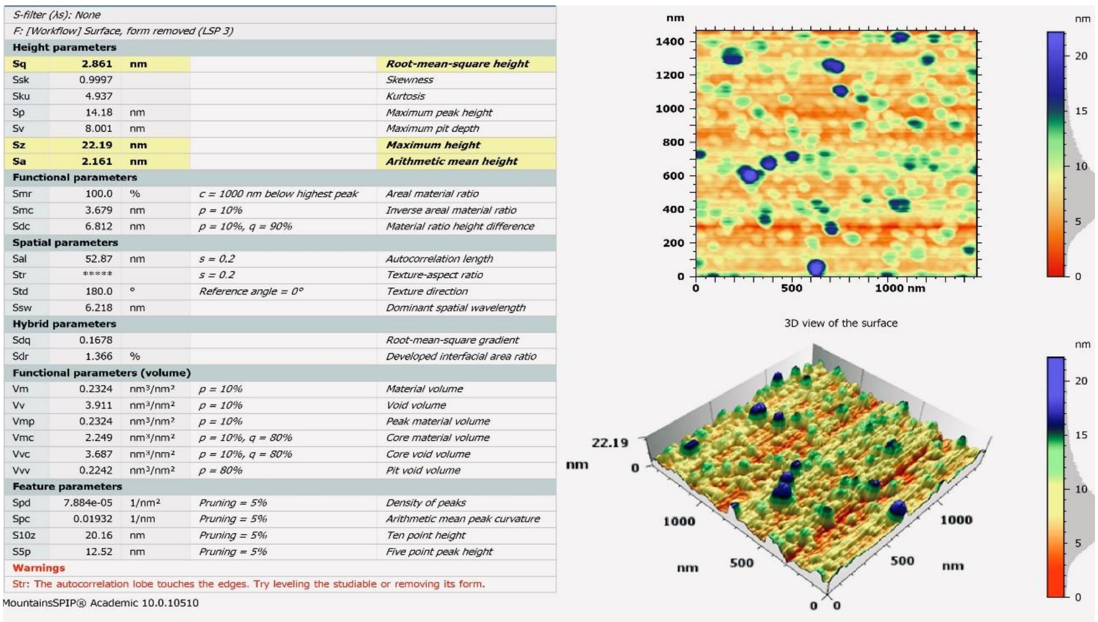


Fig. 4. Synthesis of Se-NPs by a biological method

biogenically formed Se-NPs, as shown in Fig. 6a. A robust Se signal with high atomic percent values at 2 keV showed that Se-NPs were made with an aqueous extract of mango peel. Additionally, a few weak C, O, Al, and P signals were detected; these can be explained through the presence of plant bioactive chemicals on the surface of Se-NPs. SEM micrographs were primarily used for examining the morphological pattern of biosynthesized Se-NPs from Peel extract, as shown in Fig. 6b. These



(a)



S-filter (λs): None				
F: [Workflow] Surface, form removed (LSP 3)				
Height parameters				
Sq	**2.861**	**nm**		***Root-mean-square height***
Ssk	0.9997			*Skewness*
Sku	4.937			*Kurtosis*
Sp	14.18	nm		*Maximum peak height*
Sv	8.001	nm		*Maximum pit depth*
Sz	**22.19**	**nm**		***Maximum height***
Sa	**2.161**	**nm**		***Arithmetic mean height***
Functional parameters				
Smr	100.0	%	*c = 1000 nm below highest peak*	*Areal material ratio*
Smc	3.679	nm	*p = 10%*	*Inverse areal material ratio*
Sdc	6.812	nm	*p = 10%, q = 90%*	*Material ratio height difference*
Spatial parameters				
Sal	52.87	nm	*s = 0.2*	*Autocorrelation length*
Str	*****		*s = 0.2*	*Texture-aspect ratio*
Std	180.0	°	*Reference angle = 0°*	*Texture direction*
Ssw	6.218	nm		*Dominant spatial wavelength*
Hybrid parameters				
Sdq	0.1678			*Root-mean-square gradient*
Sdr	1.366	%		*Developed interfacial area ratio*
Functional parameters (volume)				
Vm	0.2324	nm^3/nm^2	*p = 10%*	*Material volume*
Vv	3.911	nm^3/nm^2	*p = 10%*	*Void volume*
Vmp	0.2324	nm^3/nm^2	*p = 10%*	*Peak material volume*
Vmc	2.249	nm^3/nm^2	*p = 10%, q = 80%*	*Core material volume*
Vvc	3.687	nm^3/nm^2	*p = 10%, q = 80%*	*Core void volume*
Vvv	0.2242	nm^3/nm^2	*p = 80%*	*Pit void volume*
Feature parameters				
Spd	7.884e-05	$1/nm^2$	*Pruning = 5%*	*Density of peaks*
Spc	0.01932	1/nm	*Pruning = 5%*	*Arithmetic mean peak curvature*
S10z	20.16	nm	*Pruning = 5%*	*Ten point height*
S5p	12.52	nm	*Pruning = 5%*	*Five point peak height*
Warnings				
Str: The autocorrelation lobe touches the edges. Try leveling the studiable or removing its form.				

(b)

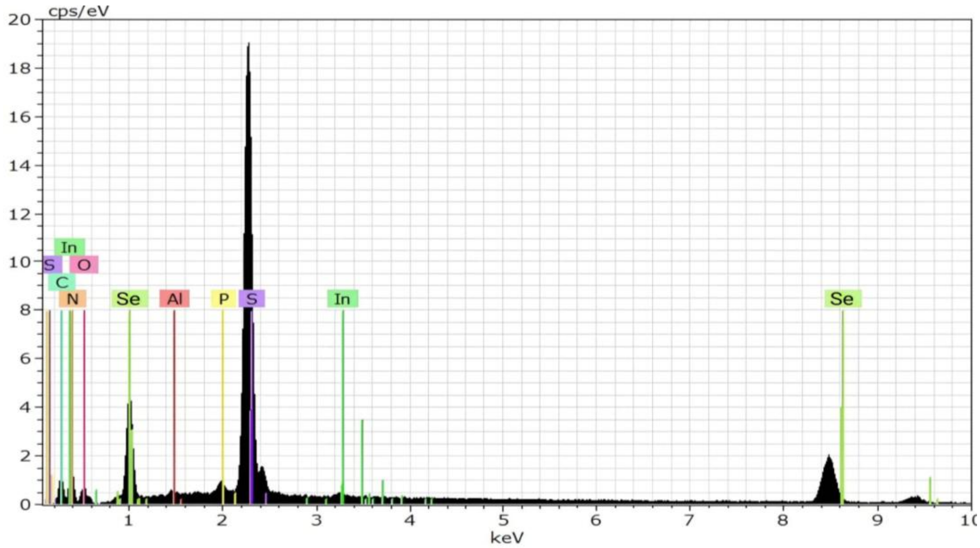
Fig. 5. a) UV-Vis spectroscopy analysis of the selenium nanoparticles b) 3D AFM images of the biosynthesized Se-NPs.

TEM micrographs indicated that the nanoparticles ranged in size from 33 nm and were spherical particles with a mostly face-centered cubic shape as illustrated in Fig. 6c.

The powdery Se-NPs XRD spectra are displayed in Fig. 7, which illustrates that the Se-NPs crystalline structure had formed upon the identification of 20 notable peaks referring to the peaks of diffraction 17.3139, 18.6777, 22.6477, 23.5596, 23.9334, 25.3806, 29.7263, 30.8866, 31.8108, and 72.9431.

Antibacterial Activities of Se-NPs

There have been few reports of Se-NPs' Anti-bacterial action; hence, the data that have been published are indicative of the therapeutic potential of Se-NPs. Six types of bacteria (*E. coli, P. aeruginosa Klebsiella spp, S. aureus, S. epidermidis, and S. pyogenes*). were chosen for the investigation of the antibacterial properties of Se-NPs biosynthesized from mango peel extract. As mentioned earlier, these bacteria



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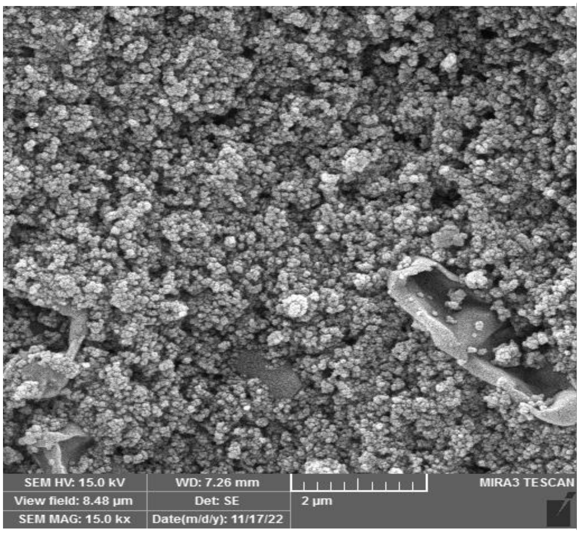
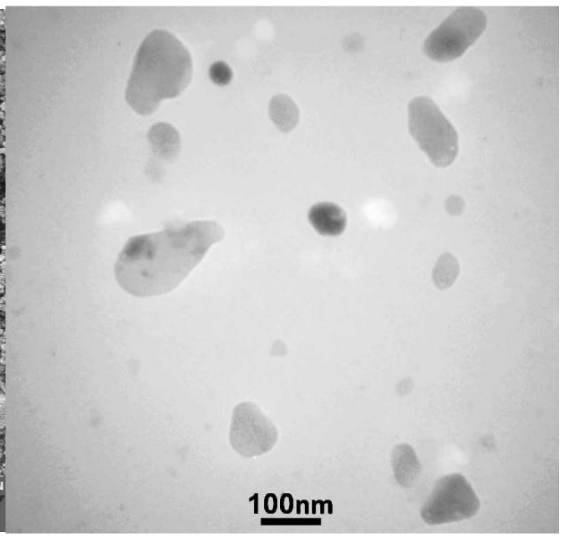




(b) (c)

Fig. 6. Selenium nanoparticles. (a) EDX and, (b) mapping of Se-NPs SEM images and (c) TEM Morphology of the Se-NPs at 100×.

were isolated from patients suffering from urinary tract infections. Fig. 8a shows the inhibited zone diameter (IZD) against tested bacterial *species*. Se-NPs have shown more efficacy against gram-positive bacteria, with the inhibition zone diameter reaching (26.23 mm) in *S. aureus*, while *S. pyogenes* reached (22.4 mm). Further analysis utilizing the MIC method of the diameters of zones of inhibition observed in Fig. 8b revealed substantial variations among the investigated bacterial strains. *S. epidermidis* showed a lower MIC value of 8 µg/ml. The sizes of the zones of inhibition, as determined by the MIC technique, allow one to conclude that the selenium nanoparticles considerably slowed the growth of most species. In general, when comparing Se-NPs to gram-negative *Klebsiella spp,* the anti-bacterial activity against *P. aeruginosa* was higher than that of *E. coli*. It was also shown that there was a significant difference at different concentrations of Se-NPs at ($P< 0.05$) in the dissemination mean zone of the Se-NPs against gram-positive*,* compared to Gram-negative bacteria that was not as significant inhibition zone diameter.

The goal of this investigation was to investigate the use of metal nanoparticles to extend the shelf life of fruit. Despite increased interest in nanoparticles and their potential uses, few practical ways exist to extend the shelf life of fruits. The study focused on the theories that underlie the expansion of metallic nanoparticles' shelf lives, such as those of selenium oxide.

The potential uses of nanoparticles as fruit preservation agents have sparked interest due to their biological characteristics, particularly their antibacterial properties. Many traditional preservation techniques have numerous disadvantages, such as high manufacturing expenses, short shelf lives, unwanted residues, and the inability to adequately store perishable fruits in their natural habitats [17].

Food producers typically discard fruit and vegetable peels, yet these are rich in biologically active compounds, which include flavonoids, polyphenols, antioxidants, carotenoids, and essential oils. etc., that help lower and stabilize metal ions during the green synthesis of nanomaterials [18]. Various NPs have been synthesized using fruit and vegetable peels with different biological properties such as anti-cancerous, antimicrobial, antioxidant, and catalytic efficiency. An essential metric to measure SeNPs is their morphological structure, which is correlated with their optical, electrical, and, occasionally, biological characteristics. TEM allows for an assessment of NP sizes, dispersions that are, and shapes. The findings of the current study support [19], which reported that CuONPs were mostly spherical with a small number of larger ellipsoidal particles. The results demonstrated that SeNPs

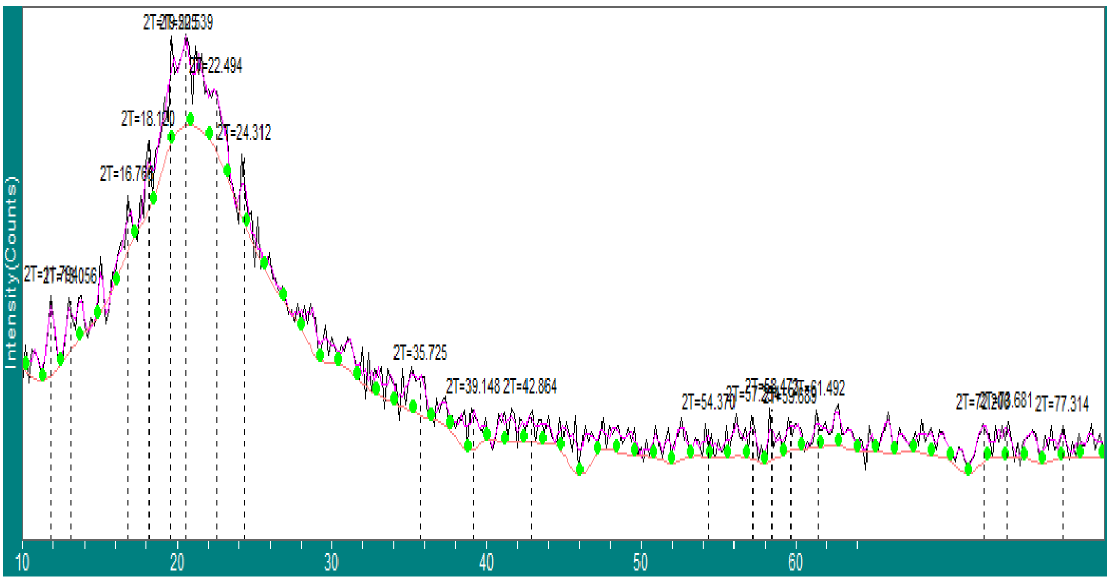
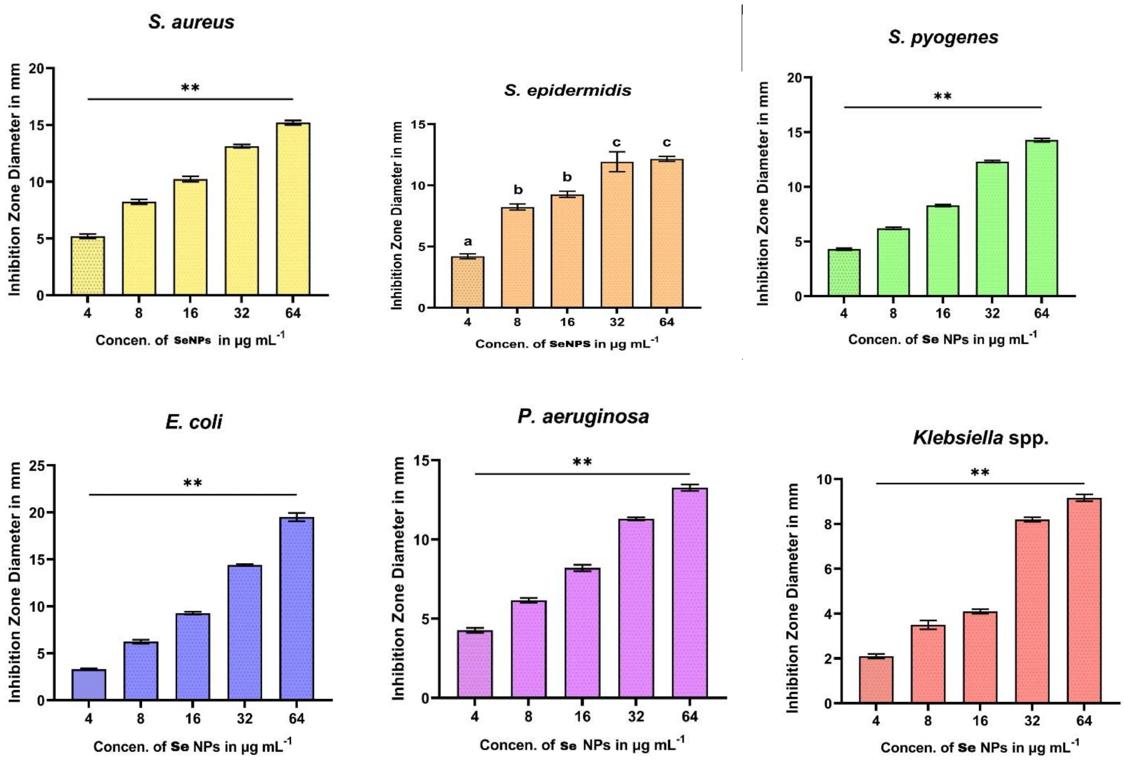
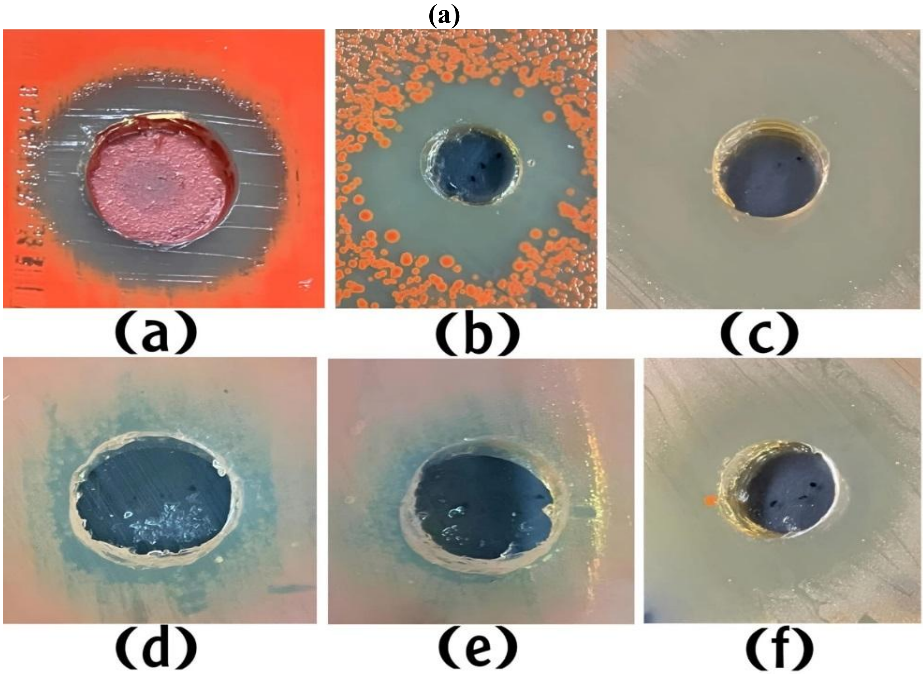


Fig. 7. XRD test of Se-NPs biosynthesized from mango peel extract.



(a)



(b)

Fig. 8. a) Mean ± SD Zone of Inhibition in mm treated with SeNP against gram-positive and negative bacteria at Different Concentrations between 4, 8, 16, 32, and 64 μg/mL.: Standard Deviation, (n = 3) b)The zones of inhibition against Sub-MIC were 8 μg/ml (a) *S. aureus,* (b) *S. epidermidis*, *(c) P. aeruginosa,* (d) *S. pyogenes*, (*e) Klebsiella spp* and (f) *E. coli*. **: $p < 0.05$, NS: Non- Significant, SD: Standard Deviation

with varying morphological characteristics and particle sizes were produced by varying amounts of mango peel extract (MPE), with peel extract serving as a reducing and capping agent in the synthesis of NPs.

Similarly, AgNPs were synthesized with the Peel Extract of Mangoan with an average size of 23.7 nm and were cylindrical and monodispersed [20]. During the experiment, indicator bacteria (both gram-positive and gram-negative) were used to assess the inhibitory effect of various SeNPs obtained from an extract from mango peel on the growth process of the bacteria. The findings demonstrated that the antibacterial activity of the produced SeNPs is enhanced by adding more mango peel extract. Furthermore, the collaboration of particle size, shape, and quantity of particles resulted in excellent NP bactericidal performance [21]. The concentration of the mango peel extract, however, could be having an impact on these features, which consequently would affect the SeNPs' antibacterial activity. Several reducing chemicals, including flavonoid and polyphenolic elements, have been identified in mango peel extract, agreeing with [22] showing that plant-based biomacromolecules might stabilize AgNPs while successfully inhibiting the aggregation-induced production of precipitation. However, a larger concentration of mango peel extract correlated with an increase in the silver ion (Ag^+) reduction rate, which led to AgNP aggregation and precipitated.

The potential of the nanoparticles to stop bacterial growth and protect cells from damage by free radicals was evaluated. The outcomes of this study showed that nanoparticles of selenium created with the help of mango seeds were potent antioxidants and highly successful at preventing the development of the tested bacteria. Other results by [23] show Zinc oxide nanoparticles have been created utilizing mangoes, which is a financially and environmentally good innovation, and synthesized ZnO NPs using mango seed extract.

Green nanotechnology involves the use of medicinal plants to generate metal and other nanoparticles that may be used in the diagnosis and treatment of a range of illnesses. It is being demonstrated that metal nanoparticles made from microorganisms, medicinal plants, and other dietary sources are inexpensive and safe. However, given the strain on global food security and the increasing scarcity of natural resources, the preservation of the environment is a concern.

CONCLUSION

In summary, our research has shed light on the potential uses of biowastes—like the seeds or peels of therapeutic plants—in the biomedical industry for the synthesis of metal nanoparticles. According to this study, SeNPs made from aqueous mango seeds showed significant antioxidant activity and were effective against clinical pathogens that were evaluated. When tested against both Gram-positive and Gram-negative bacterial isolates, the seed-mediated nanoparticles demonstrate excellent antibacterial activity. Green methods for synthesis. SeNPs are considered to be low-cost, one-step, and environmentally friendly processes. The generation of particles with the lowest size and highest degree of stability is the main objective of the synthesis of selenium nanoparticles. Investigating new SeNP preparation methods necessitates determining the antibacterial effect and the strength of antibacterial activity. Previous work showed that selenium nanoparticles exhibit a range of antibacterial effects depending on their size and structure. As an outcome, while assessing the antibacterial qualities, this work utilizes mango peel extract as a reduction agent for green SeNP synthesis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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