RESEARCH PAPER

Dedect Anti-Bacterial Activity of Selenium Nanoparticles Synthesized by Serratia Marcescens

Hawraa Khalaf Abbood *, Rashid Rahim Hateet

Department of Biology, College of Science, University of Misan, Iraq

ARTICLE INFO

Article History:
Received 05 September 2025
Accepted 27 October 2025
Published 01 January 2026

Keywords:
Fe-SEM
Na_2SeO_3
Selenium nanoparticles
Serratia marcescens
TEM

ABSTRACT

The main purpose of the present study is to test antibacterial activity of selenium nanoparticles (SeNPs) bio-synthesized by some pathogenic bacteria. The bacteria were isolated from urine sample and identified by PCR technique and their genotype was determined by 16s rRNA sequencing analysis and compared with NCBI, GenBank which were identified as Serratia marcescens. The color change of the mixture of bacterial filtrate with 2mM sodium selenate (Na_2SeO_3) salt was attributed to the biosynthesis of SeNPs after incubation for 48h at 37 °C. The synthesized SeNPs were characterized by some physicochemical tests that included ultraviolet radiation (Uv-visible) at a wavelength of 298nm and infrared radiation FTIR. The X-ray diffraction (XRD) result indicated that the resulting material is selenium nanoparticles. The size and shape of the particles were determined by Field Emission scanning and transmission electron microscopy (Fe-SEM,TEM) where the sizes ranged from (40-64 nanometer) in spherical shapes, while the EDX profile results confirmed that the bacterial filtrate contains the element selenium completely. Recently the anti-bacterial activity of SeNPs was tested against two types of bacteria (Staphylococcus aureus) and (Escherechia coli) And with inhibitory diameters (20mm,24mm) based on the antibiotic gentamicin as a control at a concentration of 1000 mg/mol with diameter (20mm).

How to cite this article
Abbood H., Hateet R. Dedect Anti-Bacterial Activity of Selenium Nanoparticles Synthesized by Serratia Marcescens. J Nanostruct, 2026; 16(1):1091-1101. DOI: 10.22052/JNS.2026.01.097

INTRODUCTION

Nano-technology is the cluster of techniques involved in design, synthesis characterization, and application of materials, devices and systems by manipulating size and shape at nanometer structure. At nanometer level, individual molecules and interaction between them becomes important in comparison with the macroscopic properties of the material [1]. Nanoparticles (NPs) are materials whose basic unit lies in one dimension or three dimensions within the nanoscale range (1-100 nm) [2]. In general, the size of a NPs spans the range between (1 and 100 nm). Metallic nanoparticles have various physical and chemical properties from bulk metals (e.g., lower melting points, specific optical properties, higher specific surface areas, specific magnetizations and mechanical strengths,) properties that might prove attractive in different industrial applications [3].

Particularly, essential dietary micro-nutrient, selenium (se) found in the form of Se NPs, is relatively a new member of drug nano-carriers in medicine [4] because Se NPs exhibited strong anti-oxidative [5] and anti-bacterial activity

* *Corresponding Author Email: qqaaww2710@gmail.com*

[6] Selenium nanoparticles' antimicrobial, antioxidant, anti-cancer, and biofilm inhibitory effects have recently been revealed [7]. It has been suggested by different researchers, that impetus to conduct research on Selenium NPs is greatly desired owing to their tremendous applications in the bio-medical fields due to their excellent Antibacterial activity, sometimes even more effective than silver nanoparticles (AgNPs) [8,9].

The biological method of NPs synthesis depends on the ability of microbial cells and enzymes to bioreduct, Bacteria, Fungi and plants have recently received excellent attention due to their ability to synthesize metallic nanoparticles (NPs) in a safe and environmentally friendly way[10].For example, AuNPs were successfully synthesized by the fungus (*Penicillum citrinum* (MEBPOO1))[11] and a study [12] also indicated the synthesis of AuNPs from *Allium ampeloprasum* (AA) leaf extract . The study also showed the production of AgNPs from Actinomycetes [13]. *Serratia marcescens* is, gram negative, an opportunistic, nosocomial pathogen which belongs to family, Enterobacteriaceae. He named the organism in honour of the Italian physicist (Serratia) who invented the steam-boat and marcescens, which is the latin word for 'decaying', as the bloody discolouration on cornmeal disappeared quickly [14]. Currently, most pathogens have become drug-resistant due to the increasing doses and continuous use of a wide range of antibiotics [15]. Therefore, anti-infection agents have been developed to treat a wide range of non-resistant diseases. A variety of metal NPs and their oxides have been developed that have been documented as common anti-microbial agents [9]. Selenium NPs have also found a role in medical applications as an anti-microbial agent to protect implanted clinical devices from bacteria.Tran et al incorporated selenium NPs into common polymers such as polyvinyl chloride, polyurethane, and silicon, which are generally used in bio-medical fields. They observed that the anti-bacterial activity was directly related to the concentration of selenium (se) as a coating material with various polymer [9]. The goal of study was to isolate and identify bathogenic bacteria *Serratia marcescens* from urine. Biosynthesis of SeNPs which were characterized using physico-chemical tests such as (UV-spectroscopy, XRD, Fe-SEM, TEM, EDX, FTIR, Zeta potenial), and Study of the anti-bacterial activity of the SeNPs bio synthesized against pathogenic bacteria.

MATERIALS AND METHODS

Collection and isolation of Bacteria

In this study, one bacteria was isolated from urine. The samples were collected from the laboratory of Children and Maternity Hospital in Maysan / Iraq, and were molecularly diagnosed using the (PCR) technique. The isolation method was as follows: A loop full of urine samples were streaked on Blood agar and MAC agar, and incubated at 37°C for 24 h.Then, the inoculated culture plates were observed for presence of any bacterial growth. On the basis of morphological and cultural characteristics, individual colonies were selected, purified onto nutrient agar medium, and then incubated at 37°C for 24 h [16].

Moleculer identification of bacteria

different methods for determining DNA sequence have been used using a universal primer (Macrogen/Korea) Table 1.

Genomic DNA was extracted from isolates using DNA Kit (Presto' Mini g DNA Bacteria Kit, Geneaid, Taiwan[16] . The isolation strain was identified genotypically by 16s rRNA sequencing analysis and then compared with NCBI GenBank [17].

Biosynthesis of SeNPs

Biosynthesis of SeNPs was carried out according to [18] Singh *et al*. with some modifications, The

Table 1. Primer kite that used in study.

No	Gene	Primers Sequence	Product size Annaling temp.
1	16 S rRNA	F- AGAGTTTGATCMTGGCTCAG R- TACGGYTACCTTGTTACGACTT	1500 bp 56 C

isolates has been inoculated in a flask with 200 ml of sterile nutrient broth (N.B). The culture was centrifuged at 6000 rpm for 20 minutes using a Table Top Centrifuge (Gemmy, Taiwan) after 48h of incubation at 37°C in an orbital shaker (130 rpm) to get the supernatant.50 mL of Sodium selenate (Na_2SeO_3) solution (2mM) was added to the 100ml culture supernatant. Then, flask was incubated at 37°C for 48h at 130 rpm. The supernatant was visually noticed for any development in color during the bio-synthesis of SeNPs.

Characterization of the Bio-synthesized SeNPs
UV–Visible spectrophotometer

To prove the fabrication of SeNPs, the culture supernatant was examined by a Dual Beam UV-1800 Spectrometer (Shimadzu, Japan) with a wavelength range from 200 to 800 nm [19].

The X-ray diffraction (XRD) analyses

The X'pert Pro X-ray diffract meter (PANalytical, Netherlands) was employed to measure the XRD of SeNPs bio-synthesized by *S. marcescens* [20].

Transmission electron microscopy (TEM)

TEM was used to analyze the distribution, size and shape of SeNPs. [20].

Field Emission Scanning Electron Microscopy (Fe-SEM)

The morphology traits of the bio-synthesized SeNPs were observed by Fe-SEM (MIRA3 LMU TESCAN, Czech) [21].

Zeta potential analysis

The zeta potential analysis was utilized to assess the stability of SeNPs bio-synthesized by *S. marcescens* using a zeta potential analyzer instrument (HORIBA Scientific SZ-100, Japan) [22].

Fourier transform infrared (FTIR) spectroscopy

FTIR spectrum of the bio-synthesized SeNPs wer recorded on a FTIR instrument mode Nicolet 6700 spectrometer at a resolution of 4 cm-1 attachment [23].

Anti-bacterial activity of bio-synthesized SeNPs

The anti-bacterial activity of bio-synthesized SeNPs was evaluated against both gram-negative (*Escherechia coli*) and gram-positive (*Staphylococcus aureus*) according disc difusion mehtod. These bacteria isolated from bathogenic bacteria identified by VITIk-2 at child and Martinty hospital in Misan,Iraq. on nutrient agar, pure colonies of bacteria were grown at 37°C for 24h,

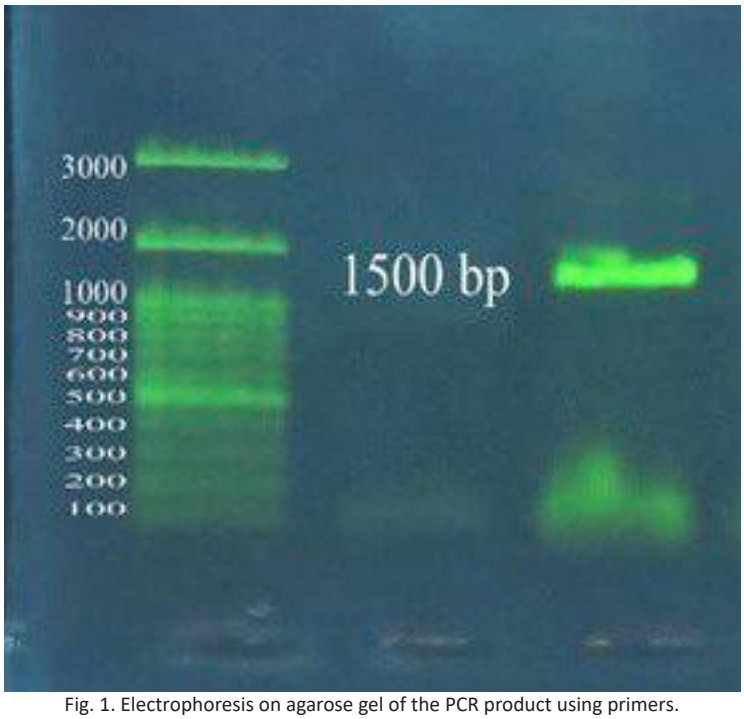


Fig. 1. Electrophoresis on agarose gel of the PCR product using primers.

and the turbidity was adjusted to (0.5) McFarland standard using (D.W) sterile distilled water. Then, each type of bacteria was uniformly swabbed onto MHA plates and put SeNPs discs. After that plates were incubated.Petri dishes were evaluated for the inhibitory zone measure in milli-meters (mm) after incubation for 24 h at37°C [24]. Using gentamicin (10 µg) as a references.

RESULTS AND DISCUSSION

After incubation period, the isolates bacteria was grown on culture media, then purified on transfer media and were identified genotipically [25].

The DNA pathogenic bacteria isolates collected and isolated from urine was extracted, and the results using the polymerase chain reaction

Fig. 2. color chang of supernatant *S. marcescens* A) without salt B) with salt.

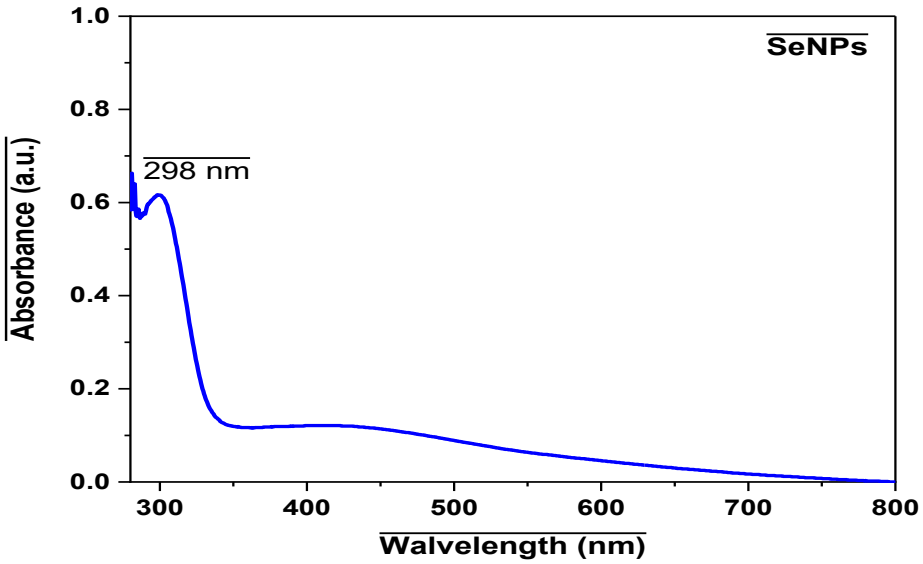


Fig. 3. UV-Vis spectroscopy of SeNPs synthesized by *S. marcescens*.

technique showed that the primers amplified the gene sequence and the locations of the amplified bands appeared between (1000-1500pb) Fig. 1 The results of the analysis of the nitrogenous base sequence of the genetic material DNA of the bacterial species showed their identification and comparison with the isolates present in GenBank NCBI. The results of PCR showed that the studied isolate is 99.98% identical to *Serratia marcescens* and new isolates were obtained and registered in the Gene Bank.NCBI**.**

The bio-synthesis of SeNPs was first affirmed by noticing the change in color of *S. marcescens* supernatant treated with (Na_2SeO_3) solution. A time dependent color change was observed in supernatant after incubated at 37ºC for 48h, as shown in Fig. 2. Firstly, the initial light yellow color of solution gradually changed into white orange with time. The orange color of supernatant was due to the excitation of surface plasmon (SPR) vibrations of the SeNPs. This means that the formation of bio-synthesis (SeNPs) was only due to the bacteria and its protein [26].

The chang color of the distributed SeNPs was due to the absorption of the (SPR) of SeNPs. Metallic bio-NPs, as a result, metallic bio-NPs have

Table 2. Bands and Functional group of SeNPs.

wavenumber (cm^{-1})	Bands	Functional group
3326	N-H	Aliphatic primary amine
2173	N=C=S	Isothiocyanate
1637	C=C	Alkene
1367	S=O	Sulfonamide
1241	C-O	Amine
1161	S=O	Sulfonamide

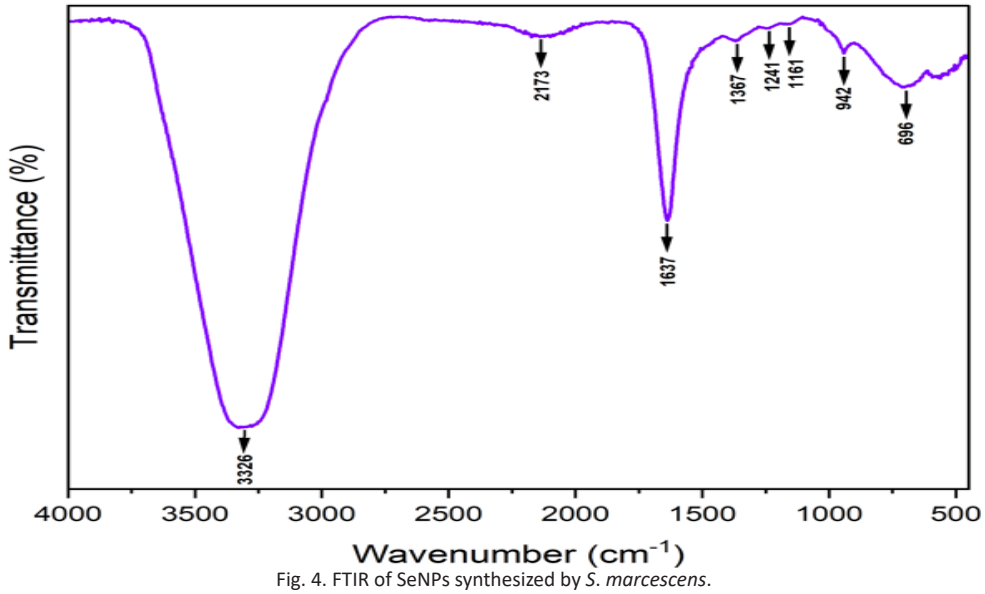


Fig. 4. FTIR of SeNPs synthesized by *S. marcescens*.

a distinctive optical absorption spectrum in the UV visible region (200-800nm). The optical absorption spectrum used in this study was at a wavelength of 298nm. Fig. 3.

FTIR spectroscopy was performed here to identify the functional groups and bands, which serve as a unique identifier of SeNPs. The Table 2 shows the presence of fuctional groups at the indicated peaks. Fig. 4 showed FTIR peaks of SeNPs .These results may indicate the contribution of proteins and other molecules in the bioreduction process and stability of nanoparticles [20].

Fig. 5 shows the crystal structure study and evaluation of the XRD of orange-produced SeNPs. The XRD spectrum of the SeNPs synthesized from the filtrate (*S. marcescens*) showed four Bragg reflections (101) (110) (201) (022) at 2 theta angles (31) (45) (56) (66) respectively and were characterized as crystalline particles with a hexagonal structure, referring to the JCPDS data

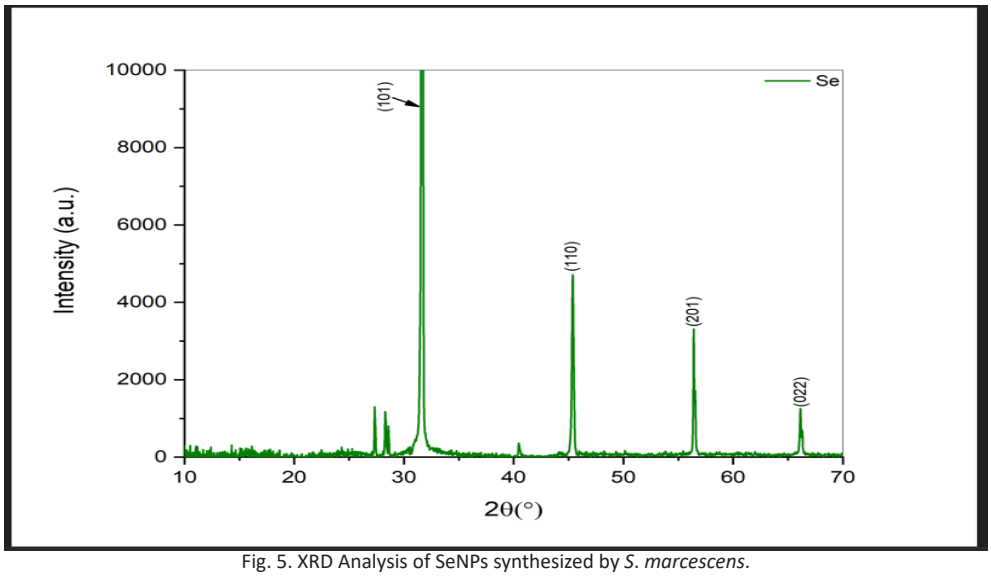


Fig. 5. XRD Analysis of SeNPs synthesized by *S. marcescens*.

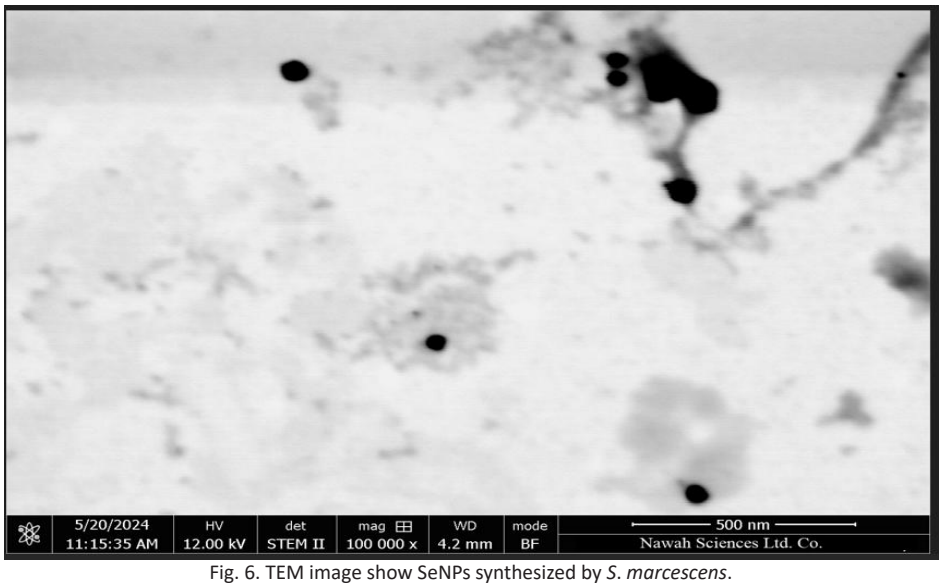


Fig. 6. TEM image show SeNPs synthesized by *S. marcescens*.

file No. 01-085-0565. The average particle size was calculated at 67.9 nm using the Debye-Schärer equation.

The form and size of SeNPs were determined using TEM. Fig. 6, selenium particles with a measuring ruler of around 500 nm are evenly distributed and mostly spherical in shape. Fig. 7 show the size distribution of TEM for SeNPS synthesized by *S.marcescens*.

The Fe-SEM images of the freshly created SeNPs has been shown in Fig. 8. The surface morphology of SeNPs showed that they were spherical in shape and evenly dispersed.Fig. 9 showed size distribution of SeNPs.

The presence of an elemental selenium was confirmed during SeNPs by EDS analysis. It can be said that the peaks seen in Fig. 10 is due to the chemicals present in the bacterial extracts, and the presence of signals such as oxygen, carbon, silicon, chloride, etc.may have arisen from organic

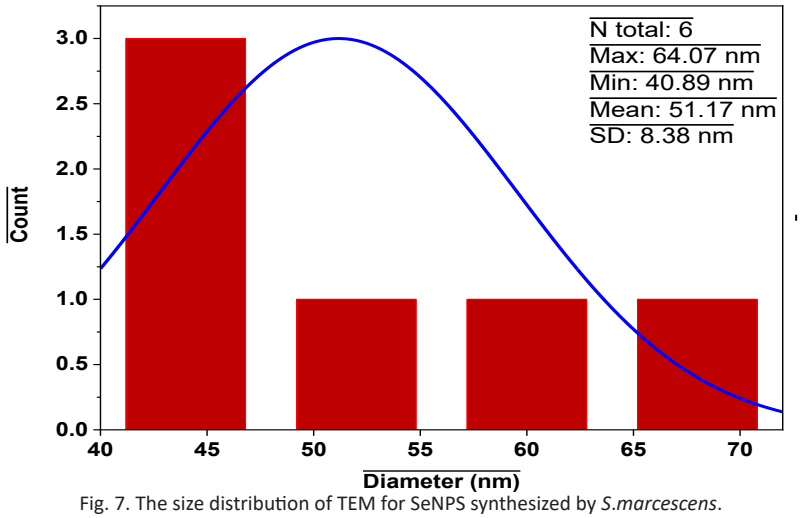


Fig. 7. The size distribution of TEM for SeNPS synthesized by *S.marcescens*.

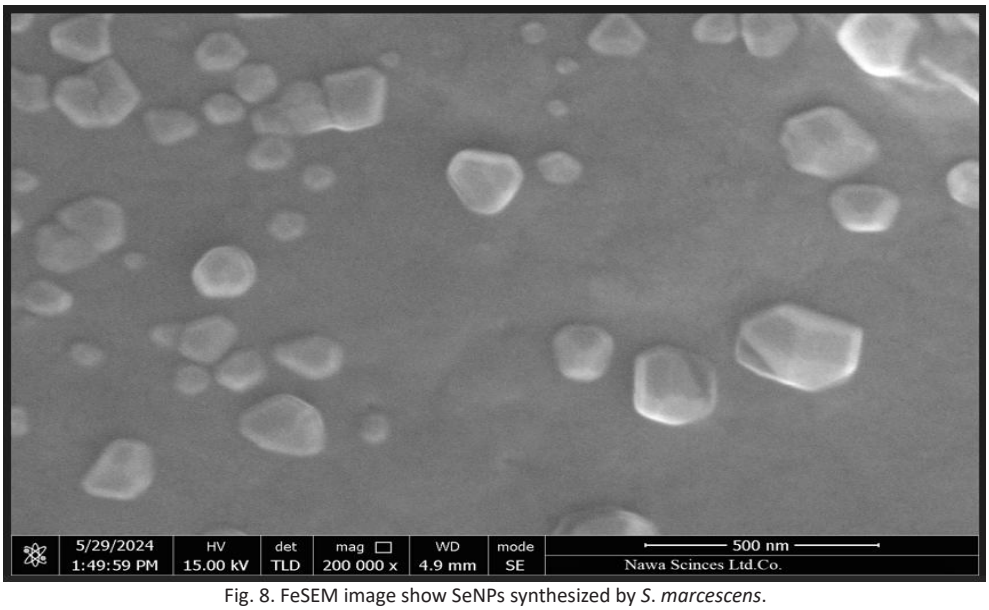


Fig. 8. FeSEM image show SeNPs synthesized by *S. marcescens*.

biomolecules or phenolic compounds on the surface of the nanoparticles[12] . Fig. 11 show EDX-Mapping images of SeNPs which show a map of the distribution of atoms for each element of the nanoparticles, where it showed that the atoms are distributed homo-geneously in the map of SeNPs.

zeta value of SeNPs synthesized from (*S. marcescens*), it was (-19.8mv) Fig. 12.The more negative/positive charge on the surface of the nanoparticles is important for long-term stability, as it prevents the particles from agglomerating in the medium [27].

Antibacterial Activity

Diseases are the leading cause of death in the world, so recent interest has been given to finding ways to eliminate these diseases, including the use of nanoparticles as antimicrobials due to their antimicrobial properties. In this study, SeNPs synthesized from pathogenic bacteria filtrates *S.marcescens* were used as antibacterial agents, where the anti-bacterial activity of SeNPs was tested on Gram-positive *S.aureus* and Gram-negative *E.coli* bacteria, and the antibiotic gentamicin was used as a positive control.SeNPs showed significant activity against both types of

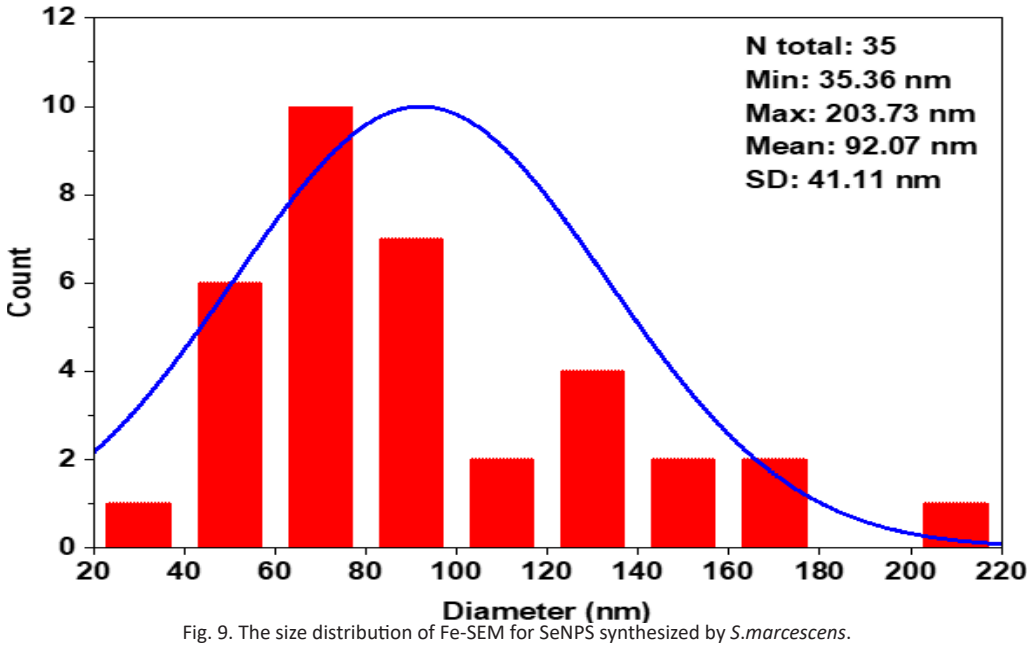
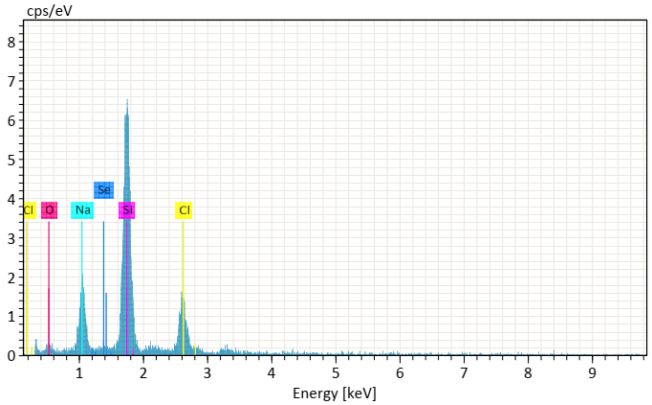


Fig. 9. The size distribution of Fe-SEM for SeNPS synthesized by *S.marcescens*.



Element	At. No.	Mass Norm. [%]	Atom [%]
Oxygen	8	8.33	13.82
Sodium	11	21.34	24.64
Silicon	14	46.04	43.52
Chlorine	17	23.88	17.88
Selenium	34	0.40	0.14
		100.00	**100.00**

Fig. 10. EDX spectra of SeNPs synthesized by *S. marcescens*.

positive and negative bacterial isolates, as Fig. 14B shows the inhibition zone of SeNPs on bacteria *E.coli* and it was 20mm while Fig. 14A shows the inhibition zone on bacteria *S.aureus* and it was 24mm i.e. a significant result compared to the antibiotic gentamicin (20mm) Fig. 13. Generally, SeNPs have size and concentration dependent effects against different microorganisms [28].

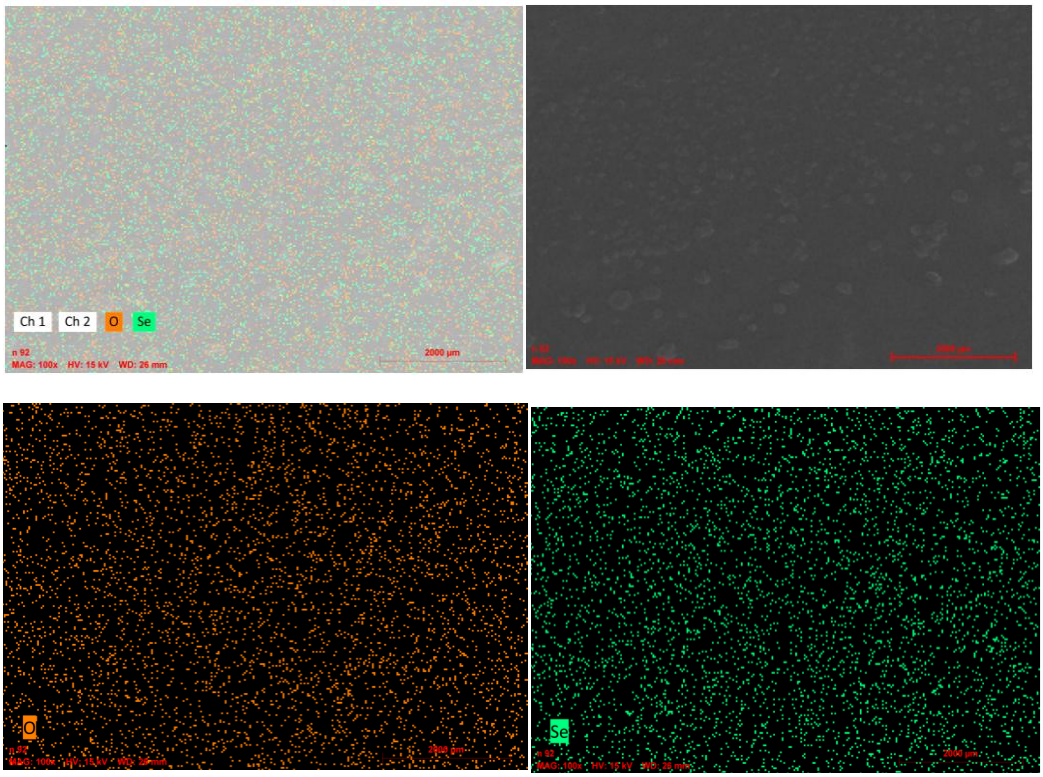


Fig. 11. EDX-Mapping images of SeNPs synthesized by *S. marcescens*.

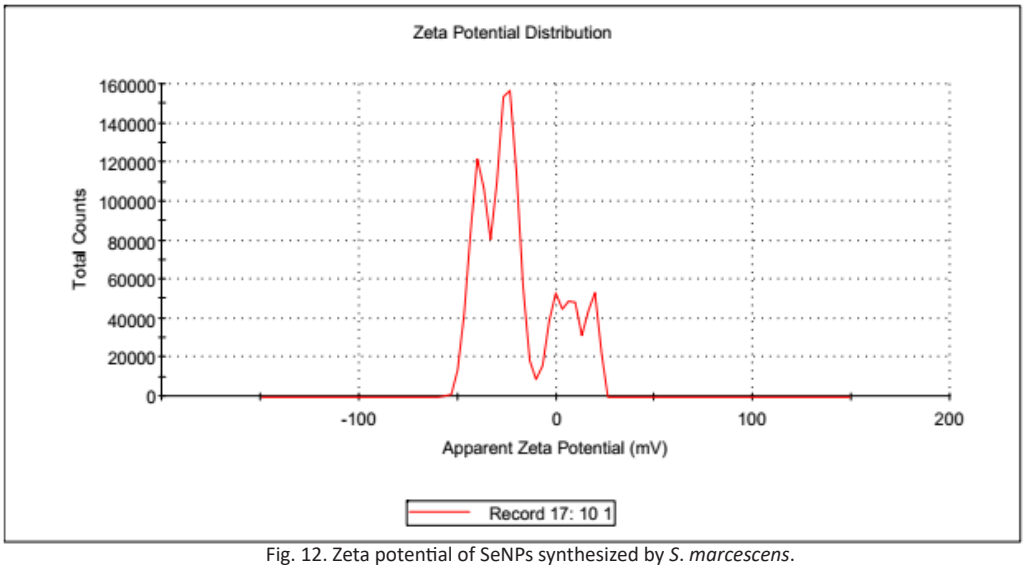


Fig. 12. Zeta potential of SeNPs synthesized by *S. marcescens*.

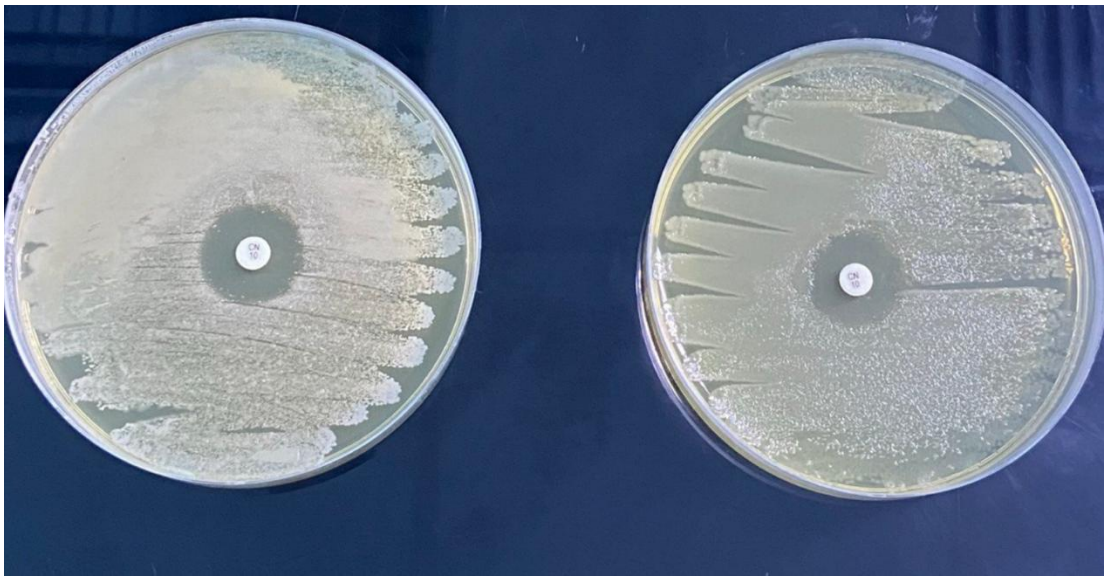


Fig. 13. Inhibition zone of gentamicin on A) *S.aureus* B) *E. coli*.

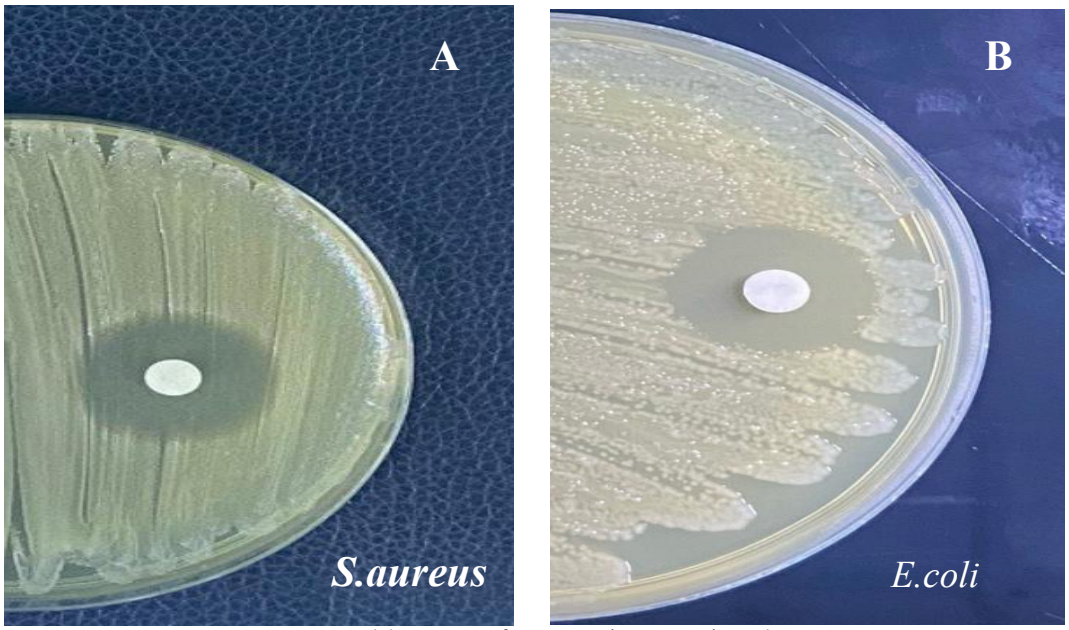


Fig. 14. Inhibition zone of SeNPs on A) *S.aureus* B) *E. coli*.

Further, selenium and tellurium NPs exhibit anti-microbial activity and destruction of bio-film formation.

CONCLUSION

The present study gives a positive result about the use of SeNPs as effective alternative antibacterials, which were biosynthesis using pathogenic bacteria, which were identified and determined by DNA extraction. After biosynthesis and obtaining nanoparticles were characterized by various tests, Uv-visible, FTIR, XRD,Zeta potential. The size and shape of the particles were determined by Fe-SEM,TEM, finaly EDX-mapping

analysis.these results,which indicated that SeNPs can be biosynthesis from some pathogenic bacteria under ideal conditions. After that, the biological activity of the biosynthesis nanoparticles was tested on two types of pathogenic bacteria to find out if they have inhibitory ability, and indeed they gave a high probability of inhibition.

CONFLICT OF INTEREST

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

REFERENCES

1. Silva GA. Introduction to nanotechnology and its applications to medicine. Surg Neurol. 2004;61(3):216-220.
2. Mateo D, Morales P, Ávalos A, Haza AI. Comparative cytotoxicity evaluation of different size gold nanoparticles in human dermal fibroblasts. J Exp Nanosci. 2015;10(18):1401-1417.
3. Horikoshi S, Serpone N. Introduction to Nanoparticles. Microwaves in Nanoparticle Synthesis: Wiley; 2013. p. 1-24.
4. Verma S, Hoffmann FW, Kumar M, Huang Z, Roe K, Nguyen-Wu E, et al. Selenoprotein K Knockout Mice Exhibit Deficient Calcium Flux in Immune Cells and Impaired Immune Responses. The Journal of Immunology. 2011;186(4):2127-2137.
5. Li Y, Li X, Zheng W, Fan C, Zhang Y, Chen T. Functionalized selenium nanoparticles with nephroprotective activity, the important roles of ROS-mediated signaling pathways. Journal of Materials Chemistry B. 2013;1(46):6365.
6. Khiralla GM, El-Deeb BA. Antimicrobial and antibiofilm effects of selenium nanoparticles on some foodborne pathogens. LWT - Food Science and Technology. 2015;63(2):1001-1007.
7. Shedbalkar U, Singh R, Wadhwani S, Gaidhani S, Chopade BA. Microbial synthesis of gold nanoparticles: Current status and future prospects. Advances in Colloid and Interface Science. 2014;209:40-48.
8. Webster TJ, Tran. Selenium nanoparticles inhibit Staphylococcus aureus growth. International Journal of Nanomedicine. 2011:1553.
9. Wang Q, Webster TJ. Nanostructured selenium for preventing biofilm formation on polycarbonate medical devices. Journal of Biomedical Materials Research Part A. 2012;100A(12):3205-3210.
10. Peiris MK, Gunasekara CP, Jayaweera PM, Arachchi NDH, Fernando N. Biosynthesized silver nanoparticles: are they effective antimicrobials? Mem Inst Oswaldo Cruz. 2017;112(8):537-543.
11. Ahmed MH, El-Hashash MA, Marzouk MI, El-Naggar AM. Synthesis and antitumor activity of some nitrogen heterocycles bearing pyrimidine moiety. J Heterocycl Chem. 2020;57(9):3412-3427.
12. Sayed R, Saad H. Gold Nanoparticles: Green Synthesis, Characterization and Biological Activities. Egyptian Journal of Chemistry. 2021.
13. Dewan TM, Hateet RR. Biosynthesis of Silver Nanoparticles from Microbacterium sp. for Determination of Antibacterial and Antitumor. Iraqi Journal of Industrial Research. 2023;10(2):105-116.
14. Merlino CP. Bartolomeo Bizio's Letter to the Most Eminent Priest, Angelo Bellani, Concerning the Phenomenon of the Red-Colored Polenta. J Bacteriol. 1924;9(6):527-543.
15. Ai B, Hushi T. Japanese Journal of Health and Human Ecology. 2006;72(1):3-14.
16. Mishra A, Sundaravadivel P, Tripathi SK, Jha RK, Badrukhiya J, Basak N, et al. Variations in macrophage migration inhibitory factor gene are not associated with visceral leishmaniasis in India. Journal of Infection and Public Health. 2019;12(3):380-387.
17. Ibrahim IAJ, Kareem TA, Azeez YM, Falhi HK. Phylogenetic tree analysis based on the 16S sequence alignment for Klebsiella spp. isolated from different sources. Iraqi Journal of Science. 2019:2618-2628.
18. Kim YJ, Singh P, Yang D-C, Singh H, Wang C, Farh ME-A, et al. Biosynthesis, characterization, and antimicrobial applications of silver nanoparticles. International Journal of Nanomedicine. 2015:2567.
19. Valentina Y. Optimisation of Reactant Concentration in Biosynthesis of Silver Nanoparticles using Pathogenic Bacteria Isolated from Clinical Sources and their Characterisation: A Recent Study. Innovations in Microbiology and Biotechnology Vol. 3: Book Publisher International (a part of SCIENCEDOMAIN International); 2022. p. 45-55.
20. Qais FA, Shafiq A, Khan HM, Husain FM, Khan RA, Alenazi B, et al. Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya koenigii (L.) against Multidrug-Resistant Pathogens. Bioinorg Chem Appl. 2019;2019:1-11.
21. Kotakadi VS, Gaddam SA, Venkata SK, Sarma PVGK, Sai Gopal DVR. Biofabrication and spectral characterization of silver nanoparticles and their cytotoxic studies on human CD34 +ve stem cells. 3 Biotech. 2016;6(2).
22. S.S P, Rudayni HA, Bepari A, Niazi SK, Nayaka S. Green synthesis of Silver nanoparticles using Streptomyces hirsutus strain SNPGA-8 and their characterization, antimicrobial activity, and anticancer activity against human lung carcinoma cell line A549. Saudi J Biol Sci. 2022;29(1):228-238.
23. Zhang X-F, Liu Z-G, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int J Mol Sci. 2016;17(9):1534.
24. R N, S M, S JP, P P. Green synthesis and characterization of bioinspired silver, gold and platinum nanoparticles and evaluation of their synergistic antibacterial activity after combining with different classes of antibiotics. Materials Science and Engineering: C. 2019;96:693-707.
25. Sapkota A, Thapa A, Budhathoki A, Sainju M, Shrestha P, Aryal S. Isolation, Characterization, and Screening of Antimicrobial-Producing Actinomycetes from Soil Samples. Int J Microbiol. 2020;2020:1-7.
26. Srivastava N, Mukhopadhyay M. Biosynthesis and structural characterization of selenium nanoparticles mediated by Zooglea ramigera. Powder Technol. 2013;244:26-29.
27. Nano-therapeutic efficacy of green synthesized gold nanoparticles (gAuNPs) and its antibacterial efficacy. Indian Journal of Biochemistry and Biophysics. 2022.
28. Subash-Babu P, Al-Saran N, M. Alshammari G, Naif Al-Harbi L, Hussain Alhussain M, Shamlan G, et al. Evaluation of Biosafety, Antiobesity, and Endothelial Cells Proliferation Potential of Basil Seed Extract Loaded Organic Solid Lipid Nanoparticle. Front Pharmacol. 2021;12.