International Journal of Design & Nature and Ecodynamics
Vol. 20, No. 10, October, 2025, pp. 2255-2263
Journal homepage: http://iieta.org/journals/ijdne

Inhibitory Effect of *Euphorbia tirucalli*-Mediated Selenium Nanoparticles on Biofilm Formation in *Pseudomonas aeruginosa* Harboring *gacA* and *pslA* Genes

Fatima Hussein Jabbar*, Souad Khalil Ibrahim

Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad 10053, Iraq

Corresponding Author Email: fatima.Hasan2202@ihcoedu.uobaghdad.edu.iq

https://doi.org/10.18280/ijdne.201005

Received: 19 August 2025
Revised: 14 October 2025
Accepted: 24 October 2025
Available online: 31 October 2025

Keywords:
Pseudomonas aeruginosa, biofilm inhibition, Euphorbia tirucalli, selenium nanoparticles (SeNPs), sub-MIC, antibiofilm agents, gene expression (gacA, pslA)

ABSTRACT

Pseudomonas aeruginosa, a bacterium known to cause wound and burn infections, appears in the form of a biofilm associated with chronic infections and antibiotic resistance. This study investigated the inhibitory impact of extract from *Euphorbia tirucalli* (ETE) and selenium nanoparticles (SeNPs) that were made from ETE on the biofilm formation of *P. aeruginosa* isolates that have the *gacA* and *pslA* genes, in addition to examining the impact on gene expression. Biosynthesis of selenium nanoparticles was achieved using an "all-green" methodology using the alcoholic extract of *E. tirucalli* as a reducing and stabilizing agent. Characterization of the nanoparticles was achieved through UV–Visible spectroscopy, Fourier transform-infrared spectral analysis, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, Atomic Force Microscopy, X-Ray Diffraction analysis, and zeta potential. The antibacterial and antibiofilm effects were conducted using microdilution and microtiter plate methods, respectively. Gene expression of *gacA* and *pslA* was studied by Quantitative Real-Time PCR. ETE and Et.SeNPs significantly inhibited biofilm formation at sub-MIC levels, with Et.SeNPs demonstrated a greater inhibitory effect ($p = 0.001$). A minimal inhibitory effect on *pslA* expression was noted, but *gacA* expression was unchanged ($p = 0.80$), and the trend for *pslA* expression was non-significant ($p = 0.25$). The results point to the notion that the antibiofilm effects of Et.SeNPs might have mechanisms that do not rely solely on direct downregulation of these genes, indicating inhibition through other pathways. Notably, green synthesized Et.SeNPs provide potential utility as adjunctive agents in the treatment of biofilm-associated infections from *P. aeruginosa*.

1. INTRODUCTION

The field of biomedical and pharmaceutical research has greatly benefited from the general advancement of nanomaterials due to their unique physicochemical properties (e.g., high surface-area-to-volume ratio and tunable reactivity). Among the different kinds of metallic nanoparticles, selenium nanoparticles (SeNPs) are a particular class of material that has garnered attention as selenium is an essential trace element associated with antioxidant defense and immune modulator; the nanoscale form of selenium has, in addition, exhibited greater bioactivity and lower toxicity than bulk selenium [1, 2]. Green or biological synthesis of SeNPs can be achieved through the use of microorganisms and plant extracts, which is an environmentally friendly and biocompatible approach without toxic chemicals, and enables better control of nanoparticle morphology and stability at the nanoscale [3].

Plant extracts act as natural reducing and stabilizing agents in nanoparticle preparation and provide bioactive phytochemicals such as flavonoids, phenolics, terpenoids, and alkaloids [4, 5]. The biomolecules have a dual role: They mediate the synthesis of the SeNPs by reducing elemental selenium to SeNPs and impart inherent antimicrobial and antibiofilm properties. Recent research indicates that SeNPs made from plant materials possess a potent ability to inhibit biofilm formation by bacteria, such as Staphylococcus aureus, Escherichia coli, and *Pseudomonas aeruginosa* [6, 7]. Screening of SeNPs created from *Euphorbia tirucalli* or related plant species (*E. retusa*) has shown good antibacterial, antioxidant, and antibiofilm efficacy by disrupting cell membranes and disrupting quorum-sensing signals [8-10]. There remains limited data, however, on the antibiofilm properties of *E. tirucalli*-mediated SeNPs against *P. aeruginosa* biofilms [11].

Pseudomonas aeruginosa is an opportunistic pathogen that is frequently correlated with chronic wounds and burn infection types [12]. Its ability to develop biofilms contributes to the persistence of infection and multidrug resistance. The biofilm development of *P. aeruginosa* is orchestrated by complicated signals, especially quorum-sensing (QS) systems and many genetic determinants. The *gacA*/*gacS* two-component regulatory system is especially important in the regulation of virulence factor production, such as pyocyanin, lipase (LipA), and lipolytic enzyme (LipS), and synthesis of extracellular polysaccharides encoded by the pel and psl

operons [13, 14]. The *gacA* regulator targets transcription of small RNAs (RsmY and RsmZ), which are involved in biofilm-related genes, establishing it as a target for antibiofilm approaches [15].

Due to the occurrence of multidrug-resistant *P. aeruginosa* and the inability of standard antibiotics to damage biofilms, the need for alternative therapeutic strategies is warranted. Plant-derived selenium nanoparticles are promising for therapeutic purposes, due to their two beneficial properties, which include antimicrobial and antibiofilm properties, in addition to their low cytotoxicity and sustainable biosynthesis [16, 17].

Thus, this study aimed to assess the suppressive capacity of *Euphorbia tirucalli* extract (ETE) and selenium nanoparticles (Et.SeNPs) fabricated from plant-based extracts on the capacity of *Pseudomonas aeruginosa* isolates that contained the *gacA* and *pslA* genes to form biofilms. The *gacA* and *pslA* genes were selected as they are key genes that regulate virulence and biofilm formation in *P. aeruginosa* isolates. This study emphasizes green-synthesized SeNPs as potential antibiofilm agents and contributes to monitoring the molecular gene expression in *P. aeruginosa* to better understand potential mechanisms other than conventional antibiotics through characterizing these nanoparticles.

2. MATERIALS AND METHODS

2.1 Bacterial isolates and cultural identification

2.1.1 Ethical approval and sample collection

The Scientific Committee of the College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Iraq, approved this research (Approval No.: EC: -65, 29/12/2024). All work with clinical samples complied with institutional and national ethical guidelines in accordance with the 1964 Helsinki Declaration. Written informed consent for use of the samples was obtained from all patients and/or their legal representatives before samples were taken. A total of 117 wound and burn samples were obtained from patients admitted to the Ghazi Al-Hariri hospital, the Specialized Burn Hospital, Al-Kindi Educational Hospital, the Al-Kadhimain City Hospital, and Baquba Educational Hospital from November 6 and December 25, 2024. Samples were taken aseptically and transported under sterile conditions to the microbiology laboratory for culture and analysis.

2.1.2 Bacterial isolation and identification

The samples were incubated on Cetrimide agar (Hi-Media) and incubated at 37°C for 24 h. Identification of colonies exhibiting characteristic pigmentation, morphology, and biochemical reactions was carried out for colonies suspected to be *Pseudomonas aeruginosa. P. aeruginosa* identification by the VITEK-2 automated system (BioMérieux) confirmed 45 isolates as *P. aeruginosa*, of which 10 were sent for further molecular investigation.

2.2 DNA and RNA extraction

Genomic DNA and total RNA were extracted according to manufacturer protocols using ABIOpure (USA) and TRIzol™ reagent (Invitrogen, USA). The purity and concentration of DNA and RNA were assessed using a Quantus Fluorometer as well as spectrophotometric readings of A260/280 ratios ranging from 1.8 to 2.0, confirming acceptable purity for PCR and qPCR analysis. All samples were stored at -20°C until analysis.

2.3 Primer design and PCR conditions

Primers for the *gacA*, *pslA*, and *fbp* genes were chosen from previously published works [14, 17]. PCR amplification was performed in 25 µl reactions, containing 1 µl of each primer (10 pmol), 10 µl of Go Taq Green Master Mix (Promega, USA), 1 µl DNA template, and nuclease-free water. Cycling conditions were as previously specified [14]: Denaturation at 95°C for 5 min, followed by 35 cycles through denaturation (95°C, 30s), annealing (55–63°C depending on target), and extension (72°C, 30s), ending with final extension at 72°C for 5 min.

2.4 Preparation of *Euphorbia tirucalli* extract and green synthesis of selenium nanoparticles

Healthy *E. tirucalli* specimens were gathered from Diyala, Iraq, and analyzed under the direction of Dr. Israa Kareem Nasrallah (University of Baghdad).

The latex extract was prepared with a Soxhlet extraction apparatus employing 80% methanol: 20% distilled water at 80°C for 8 h. The subsequent solution was allowed to evaporate under reduced pressure at 45°C, and the dried extract was stored at 4°C [18].

In terms of green method synthesis, 10 g of *E. tirucalli* extract was added to 90 ml of 2 mM sodium selenite (Na_2SeO_3) and ultrasonicated for 30 min in order to mix it consistently. It was placed in the dark for 3 h to facilitate a homogeneous orange-red suspension [19].

2.5 Characterization of Et.SeNPs

The characterization of selenium nanoparticles prepared with *E. tirucalli* extract (Et.SeNPs) was performed using an UV–Vis spectrophotometer (SHIMADZU, Japan), a FTIR spectrometer (Shimadzu, Japan), SEM and EDX (Bruker, USA), AFM (Aa3000, Angstrom), XRD (Bruker, USA), and a zeta potential analyzer (Thermo Scientific, USA).

These analyses confirmed morphology, elemental composition, crystalline nature, and nanoparticle stability.

2.6 Gas Chromatography–Mass Spectrometry (GC–MS) analysis

The extracts of *Euphorbia tirucalli* were analyzed with GC-MS (Shimadzu QP2010 Ultra, Japan) with the use of an Rtx-5MS capillary column. Helium was the carrier gas set to 1.0 ml/min. The oven temperature was programmed from 60°C to 280°C at a rate of 10°C/min. The mass spectra were acquired in the electron impact mode (70 eV, with m/z 40–500). The identification of superb compounds was made based on comparison with NIST and Wiley libraries, and the relative abundance was calculated from the peak areas.

2.7 Determination of MIC and sub-MIC

MIC and sub-MIC values for *E. tirucalli* extract (ETE) and Et.SeNPs were determined using the microdilution method in 96-well plates as described by O'Toole [20]. Biofilm inhibition was assessed by the microtiter plate assay, with

crystal violet staining and absorbance measurement at 560 nm.

2.8 Quantitative Real-Time PCR (qRT-PCR)

For gene expression analysis, RNA templates were reverse transcribed into cDNA using the RevertAid First Strand cDNA Kit (Thermo Scientific). qRT-PCR was performed with SYBR Green chemistry on a QuantStudio 3 (Applied Biosystems).

Two internal reference genes, 16S rRNA and *fbp* (fructose-bisphosphate aldolase), were chosen for normalization as we observed stable expression of these genes in *P. aeruginosa* during various stress conditions. Relative expression of *gacA* and *pslA* was calculated based on the method of $2^{-\Delta\Delta CT}$ [21].

Normalization was validated by comparison of Ct values (SD < 0.2) of the 16S rRNA and *fbp* reference genes, confirming they would be appropriate for expression analysis.

2.9 Statistical analysis

All experiments were conducted in triplicate. Results were expressed as mean ± SD. Differences between control and treated groups were analyzed using t-tests, with $p \leq 0.05$ considered significant and $p \leq 0.001$ highly significant [22].

3. RESULTS AND DISCUSSION

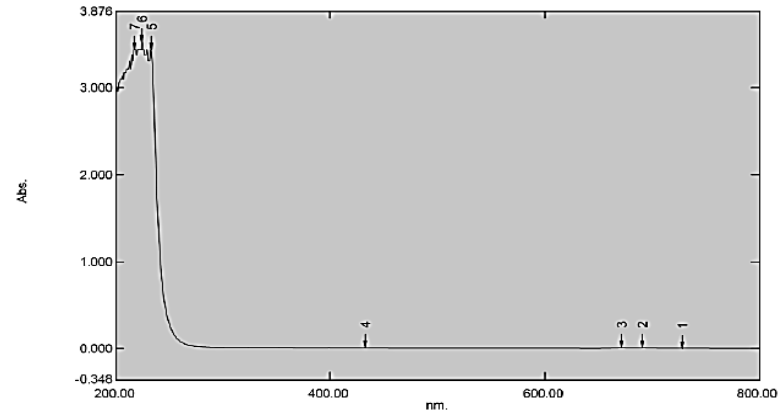
3.1 Bacterial isolates and cultural identification

A total of 117 samples were collected, and only 75 (64.10%) showed growth. Using the Vitek-2 System, 45 (38.46%) of the isolates were cultured on Cetrimide agar, and some showed growth, producing colonies that were yellow-green, blue-green, or colorless.

3.2 Characterization of *E. tirucalli*-mediated selenium nanoparticles

The successful biosynthesis of selenium nanoparticles (Et.SeNPs) was visually confirmed by observing the color change of the reaction mixture changed from clear to orange-red (indicating the reduction of selenite ions, $Se^{4+} \rightarrow Se^{0}$).

UV–Vis spectroscopy exhibited strong absorption peaks at 209.5 and 219 nm (Figure 1), confirming the typical optical properties of SeNPs [23]. FTIR analysis indicated the presence of characteristic absorption bands of –OH, C=O, and C–H groups (Figure 2), revealing that phenolics and flavonoids from plants play a role as both reducing and stabilizing agents [24].

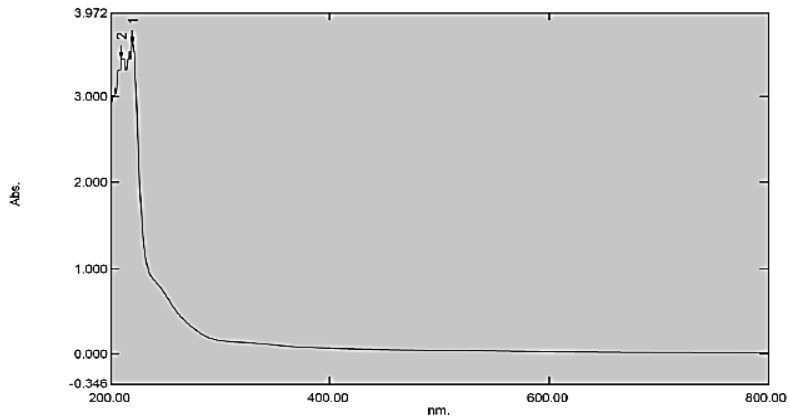


Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

Sample Preparation Properties
Weight:
Volume:

No.	P/V	Wavelength	Abs.	Description
1	↑	729.00	0.007	
2	↑	691.50	0.009	
3	↑	671.50	0.009	
4	↑	432.50	0.009	
5	↑	232.50	3.436	
6	↑	224.00	3.524	
7	↑	216.50	3.436	

(A)



Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

No.	P/V	Wavelength	Abs.	Description
1	↑	219.00	3.612	
2	↑	209.50	3.436	

(B)

Figure 1. UV–Vis absorption spectrum of the compound showing characteristic peaks at different wavelengths of (A) Alcoholic *E. tirucalli* extract ETE, (B) Selenium nanoparticles mediated with alcoholic *E. tirucalli* extract Et.SeNPs

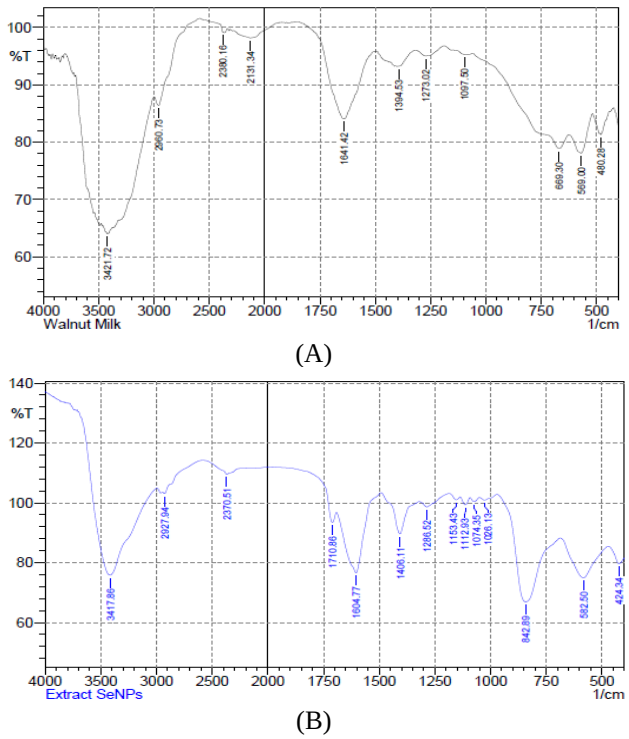


Figure 2. FTIR spectra showing functional groups present in (A) *E. tirucalli* milk extract and (B) Biosynthesized selenium nanoparticles (SeNPs)

SEM imaging demonstrated spherical nanoparticles ranging from 32–42 nm (Figure 3), while EDX spectra confirmed the elemental composition with selenium as the dominant component (46.5%), followed by oxygen and carbon residues (Figure 4).

In addition to the SEM data, a Dynamic Light Scattering (DLS) analysis was carried out. The DLS indicated an average hydrodynamic diameter of 58.4 nm and a polydispersity index (PDI) of 0.31, which indicates moderate uniformity in size (Figure 5). The zeta potential (+53.6 mV) demonstrated excellent colloidal stability [25]. Therefore, these findings together affirm that the Et.SeNPs from *E. tirucalli* extract are nanosized, stable, and bioactivity-rich with potential capping compounds (Figure 6).

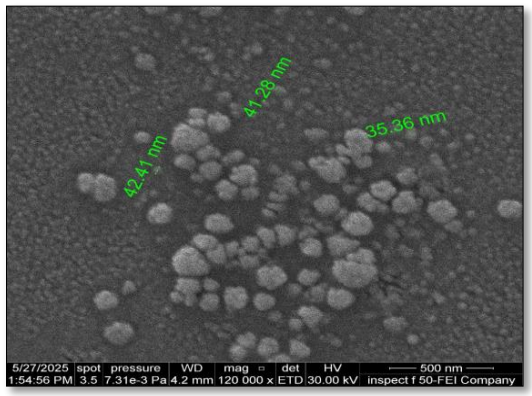
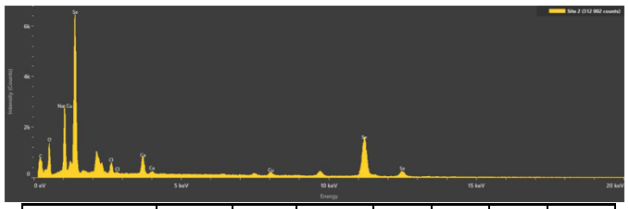


Figure 3. SEM image of biosynthesized selenium nanoparticles (SeNPs) showing particle sizes in the range of 32–42 nm



Element	Se	C	O	Na	Cl	Ca	Cu
Weight %	46.5	13	20	13	2.7	3.5	1.4

Figure 4. EDX spectrum confirming the elemental composition of *E. tirucalli* mediated selenium nanoparticles (Et.SeNPs), showing strong Se signals

The results of (XRD) showed that during the peaks θ2, there were selenium particles at (21.78 and 29.7), while the peaks (25.02 and 31.23) correspond to the extract of alcoholic *E. tirucalli* (Figure 7).

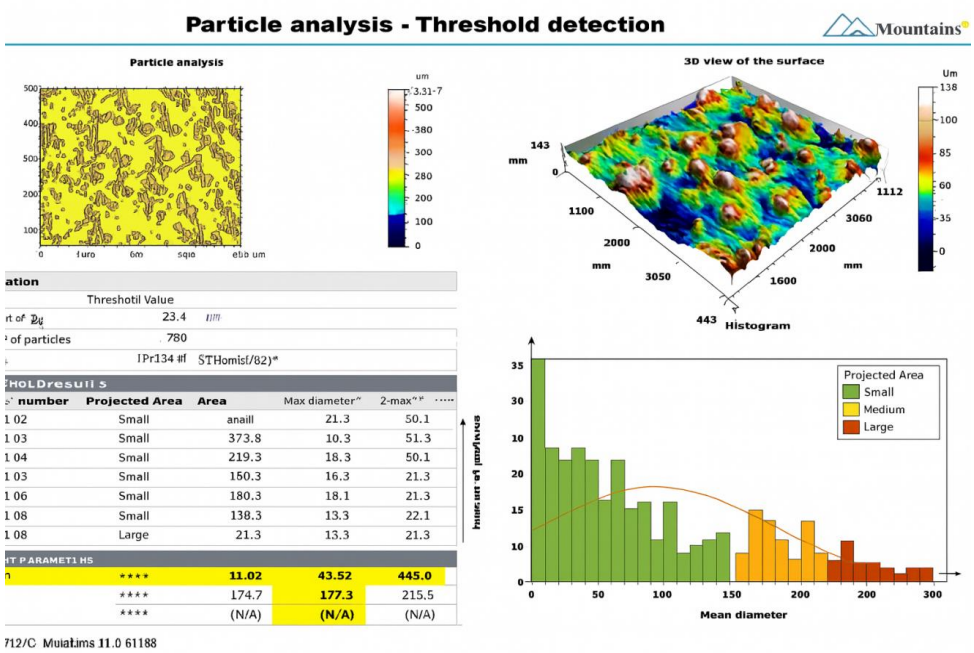


Figure 5. AFM particle size and surface analysis of *E. tirucalli* mediated selenium nanoparticles (Et.SeNPs) showing mean diameter distribution

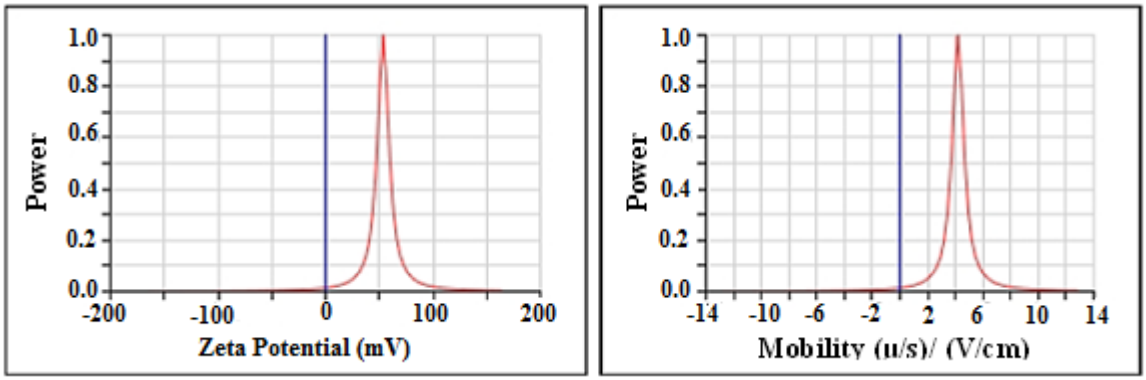


Figure 6. Zeta potential and mobility analysis of *E. tirucalli* mediated selenium nanoparticles (Et.SeNPs) showing high stability (+53.69 mV)

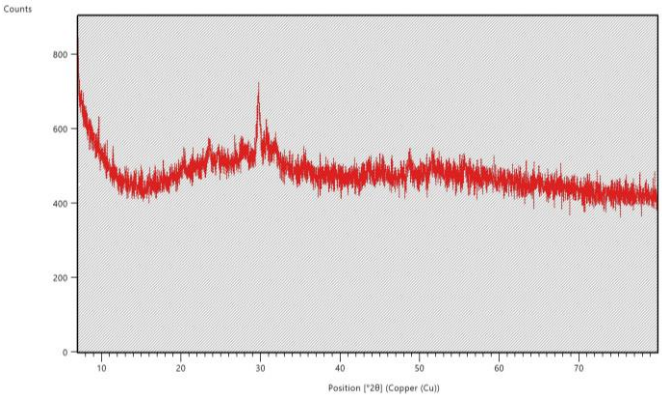
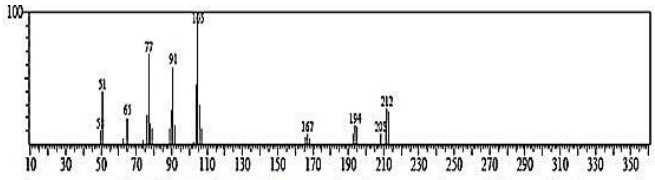


Figure 7. X-ray diffraction (XRD) pattern of *E. tirucalli* mediated selenium nanoparticles (Et.SeNPs) showing characteristic crystalline peaks



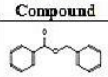
Chemical Formula	Shape of chemical Compound	Compound Name Chemical	Weight	Area	Retention Time (RT)	Peak
$C_{14}H_{12}O_2$		Benzoic acid, Phenylmethyl ester	212	%100	15.417	28

Figure 8. GC-MS analysis of bioactive compounds identified from the alcoholic extract of ETE

3.3 Gas Chromatography–Mass Spectrometry (GC–MS) analysis

The GC–MS profile of *Euphorbia tirucalli* extract demonstrated the presence of various bioactive phytochemical compounds, such as terpenoids, diterpenes, triterpenoids, esters, and phenolic derivatives (Figure 8). These classes of compounds are well documented for their antimicrobial and antioxidant effects [22]. Terpenoids and phenolic compounds, in particular, are known to act as natural reducing and stabilizing agents in the biosynthesis of metallic nanoparticles, reducing selenite ions ($Se^{4+} \rightarrow Se^{0}$), and stabilizing nanoparticles as the capping agent in the nanostructure [2]. The presence of these compounds in *E. tirucalli* extract may, therefore, account for the successful synthesis of Et.SeNPs and their noted stability, represented by the zeta potential analysis. Additionally, many of these same phytochemicals, such as β-amyrin, phytol, and squalene, have been noted to provide both antibacterial and antibiofilm activities that may work with selenium to produce a synergistic effect to inhibit *P. aeruginosa* bacteria with Et.SeNPs [26].

3.4 Minimum inhibitory concentration (MIC) and sub-MIC determination

Both *E. tirucalli* extract (ETE) and Et.SeNPs exhibited inhibitory effects against *P. aeruginosa*. The MIC and sub-MIC values were determined using the 96-well microtiter plate assay, where blue wells indicated no growth, MIC, and pink wells indicated minimal bacterial survival (sub-MIC). Representative images of the colorimetric assay have been appended to visually show the performance on the MIC and sub-MIC endpoints. The MIC of ETE was determined to be 3.75 mg/ml, and the sub-MIC was 1.88 mg/ml. The MIC of Et.SeNPs were determined to be 83.3 µg/ml and sub-MIC 41.65 µg/ml, confirming the nanoparticle formulation had significantly improved antibacterial activity.

3.5 Inhibition of biofilm formation

Quantitative assessment using the microtiter plate method (MTP) demonstrated that both ETE and Et.SeNPs had significant inhibition of biofilm formation ($p \leq 0.05$), with Et.SeNPs showed a stronger effect ($p \leq 0.001$). On average, the percentage inhibition ranged from 48–87% for Et.SeNPs and 16–76% for ETE (Table 1).

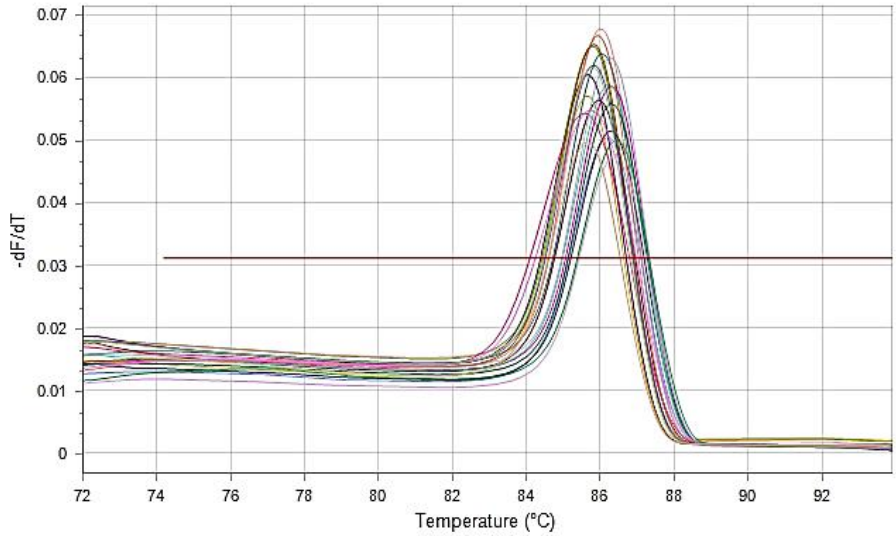
The differences in inhibition observed between isolates may be linked to several variables, such as variation in biofilm matrix composition, where strains with increased extracellular polymeric substances (EPS) thickness are more resistant [27]. Differences in nanoparticle interaction also exist, and variations in the charge on the surface of cells and outer membrane permeability will influence SeNP adherence. Genetic diversity between isolated strains may also play a role in virulence gene expression and quorum-sensing regulation [28]. Variability in metabolic activity may also affect the isolates' response to sub-MIC concentrations.

This variation is consistent with previous reports that some strains of *P. aeruginosa* can demonstrate differences in susceptibility to SeNPs based on their EPS composition, quorum-sensing systems, and tolerance to oxidative stress [29, 30].

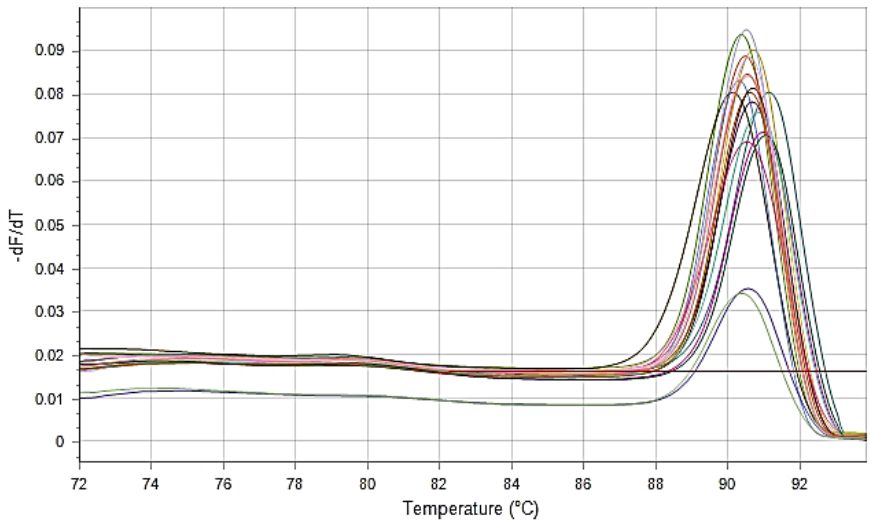
Table 1. Effect of sub-MIC concentrations of *E. tirucalli* extract (ETE) and selenium nanoparticles (Et.SeNPs) on biofilm formation of *P. aeruginosa* isolates

Sub-MIC of ETE (gm/ml)	The Average Biofilm of Each Isolate Before Treatment	The Average Biofilm Formation of Each Isolate After Treatment ETE	Inhibition Rate (%)	p-Value
1.88	0.66	0.50	24%	
1.88	0.75	0.54	28%	
1.88	1.22	0.47	61%	
1.88	0.92	0.47	49%	0.0397*
1.88	2.28	0.53	76%	
1.88	0.81	0.29	64%	
1.88	0.68	0.57	16%	
Sub-MIC of Et.SeNPs (gm/ml)	**The Average Biofilm of Each Isolate Before Treatment**	**The Average Biofilm Formation of Each Isolate After Treatment Et.SeNPs**	**Inhibition Rate (%)**	**p-Value**
41.65	0.66	0.34	48%	
41.65	0.75	0.32	57%	
41.65	1.22	0.34	%72	
41.65	0.92	0.30	%67	*0.0127
41.65	2.28	0.28	%87	
20.83	0.81	0.20	%75	
20.83	0.68	0.21	%69	
P-value#		0.001*		

*#t-test *Significant differences at $p \leq 0.05$*



(A)



(B)

Figure 9. Real-time PCR of (A) *gacA*, (B) *pslA*

3.6 Gene expression analysis of *gacA* and *pslA*

According to qRT-PCR analysis, there was no significant difference in *gacA* ($p = 0.80$) or *pslA* ($p = 0.25$) expression between treatment with ETE and Et.SeNPs and the untreated control (Figure 9). Although *pslA* expression decreased slightly (mean fold change = 0.88 ± 0.22), it was not statistically significant.

This minor reduction may have resulted from the underpowered sample size ($n = 7$ isolates) and the fact that we were only using sub-MIC concentrations, which may not have been high enough to provide access to detectable transcriptional downregulation.

Regardless, the consistent inhibitory effectiveness on biofilm formation with no significant transcriptional change suggests that the antibiofilm mechanism of Et.SeNPs likely operate through post-transcriptional or physicochemical interactions, including: inhibition of EPS production (or stability), production of reactive oxygen species (ROS) that damage the cellular membrane, and/or inhibition of Quorum Sensing signaling molecules (e.g., N-acyl homoserine lactones) [31].

Furthermore, these ideas correspond with previous studies that demonstrated SeNPs could limit biofilm production without direct inhibition of gene transcription by disrupting either intercellular communication or biofilm matrix degradation [1, 32].

3.7 Comparative sensitivity among isolates

In particular, *P. aeruginosa* isolates showed sensitivity to Et.SeNPs that were dependent on strain, with inhibition rates between 48% and 87%.

This variability may be related to either differences in cell envelope structure, specifically the density of lipopolysaccharides and porins, differences in antioxidant defense mechanisms, which could neutralize oxidative stress induced by nanoparticle exposure, and the presence or absence of biofilm-associated genes, particularly *gacA* and *pslA* genes known to regulate polysaccharide biosynthesis and adherence properties [33].

Clearly, clarifying nanoparticle-bacteria interactions is complex; additional transcriptomic and proteomic studies are needed to better understand the molecular mechanisms responsible for resistance and sensitivity of *P. aeruginosa* to SeNPs.

4. CONCLUSIONS

In conclusion, *Euphorbia tirucalli* extract (ETE) and SeNPs (*Euphorbia tirucalli* extract (ETE)-synthesized selenium nanoparticles) inhibited biofilm formation in *Pseudomonas aeruginosa*. The SeNPs showed greater antibiofilm activity compared to the crude extract. Gene expression analysis suggests that this antibiofilm activity may not be established through direct gene regulation, but possibly through alternate pathways, such as quorum-sensing disruption or impairment of extracellular polymeric substance (EPS) stability and interactions. Based on the strong antibiofilm efficacy, stability, and biosafety, the green-synthesized SeNPs are promising candidates as antimicrobial adjuvants for application in wound dressings, burn therapies, and medical device coatings. Further molecular and in vivo studies are suggested to substantiate our findings and understand the underlying mechanisms.

ACKNOWLEDGMENT

The authors thank the Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Iraq, for their help in completing the current research.

REFERENCES

[1] Mikhailova, E.O. (2023). Selenium nanoparticles: Green synthesis and biomedical application. Molecules, 28(24): 8125. https://doi.org/10.3390/molecules28248125

[2] Salman, M.F., Al-Mudallal, N.H.A.L., Ahmed, M.E. (2025). Cytotoxic effect of biogenic selenium nanoparticles using bacteriocin of Acinetobacter baumannii isolated from burns and wound infections. Iraqi Journal of Science, 66(4): 1535-1549. https://doi.org/10.24996/ijs.2025.66.4.13

[3] Al-Kurdy, M.J., Khudair, K.K., Al-Kinani, L.H. (2020). Synthesis and characterization of black currant selenium nanoparticles (Part I). The Iraqi Journal of Veterinary Medicine, 44(2): 25-34. https://doi.org/10.30539/ijvm.v44i2.974

[4] Wali, A.T., Alqayim, M.A.J. (2019). Biosynthesis, characterization and bioactivity of selenium nanoparticles synthesized by propolis. The Iraqi Journal of Veterinary Medicine, 43(1): 197-209. https://doi.org/10.30539/iraqijvm.v43i1.490

[5] Zambonino, M.C., Quizhpe, E.M., Mouheb, L., Rahman, A., Agathos, S.N., Dahoumane, S.A. (2023). Biogenic selenium nanoparticles in biomedical sciences: Properties, current trends, novel opportunities and emerging challenges in theranostic nanomedicine. Nanomaterials, 13(3): 424. https://doi.org/10.3390/nano13030424

[6] Edo, G.I., Mafe, A.N., Ali, A.B.M., Akpoghelie, P.O., et al. (2025). Green biosynthesis of nanoparticles using plant extracts: Mechanisms, advances, challenges, and applications. BioNanoScience, 15: 267. https://doi.org/10.1007/s12668-025-01883-w

[7] Rasha, M., Neihaya, H.Z. (2025). Effect of bio-synthesize selenium nanoparticles from *E. coli* bacteria on biofilm of pathogenic microbes. Iraqi Journal of Biotechnology, 24(SI): 146-158. https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/788/572.

[8] Hameed, H.Q., Hasan, A.A., Abdullah, R.M. (2019). Effect of *Olea europaea* L. extraction and TiO_2 nanoparticles against *Pseudomonas aeruginosa*. Indian Journal of Public Health Research & Development, 10(6): 1218-1223. https://doi.org/10.5958/0976-5506.2019.01459.1

[9] Binckley, S., Zahra, F. (2025). Euphorbia tirucalli toxicity. In StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK574526/.

[10] Abduljabbara, B.T., El-Zayat, M.M., El-Halawany, E.S.F., El-Amier, Y.A. (2024). Selenium nanoparticles from Euphorbia retusa extract and its biological applications: Antioxidant and antimicrobial activities.

Egyptian Journal of Chemistry, 67(2): 463-472. https://doi.org/10.21608/ejchem.2023.214819.8069

[11] Nagime, P.V., Pandey, V.K., Rajpal, C., Jayeoye, T.J., Kumar, A., Chidrawar, V.R., Singh, S. (2025). Biogenic selenium nanoparticles: A comprehensive update on the multifaceted application, stability, biocompatibility, risk, and opportunity. Zeitschrift für Naturforschung C, 80(11-12): 627-655. https://doi.org/10.1515/znc-2024-0176

[12] Salih, S., Khalil, S. (2019). The inhibitory effect of partially purified lipopolysaccharide extracted from *Pseudomonas aeruginosa* bacteria on Candida glabrata yeast. Biochemistry and Cell Archives, 19(2): 4207-4209. http://www.connectjournals.com/file_html_pdf/3051002H_4207A.pdf.

[13] Lafta, I.J., Sadeq, Z. (2024). *Pseudomonas aeruginosa* is an effective indicator for screening quorum sensing inhibition by plant extracts. The Iraqi Journal of Veterinary Medicine, 48(1): 54-62. https://doi.org/10.30539/nzsc0y49

[14] Anupama, R., Mukherjee, A., Babu, S. (2018). Gene-centric metegenome analysis reveals diversity of *Pseudomonas aeruginosa* biofilm gene orthologs in fresh water ecosystem. Genomics, 110(2): 89-97. https://doi.org/10.1016/j.ygeno.2017.08.010

[15] Fahmy, N.F., Abdel-Kareem, M.M., Ahmed, H.A., Helmy, M.Z., Mahmoud, E.A.R. (2025). Evaluation of the antibacterial and antibiofilm effect of mycosynthesized silver and selenium nanoparticles and their synergistic effect with antibiotics on nosocomial bacteria. Microbial Cell Factories, 24: 6. https://doi.org/10.1186/s12934-024-02604-w

[16] More, P.R., Pandit, S., De Filippis, A., Franci, G., Mijakovic, I., Galdiero, M. (2023). Silver nanoparticles: Bactericidal and mechanistic approach against drug-resistant pathogens. Microorganisms, 11(2): 369. https://doi.org/10.3390/microorganisms11020369

[17] Anderson, G.G., Yahr, T.L., Lovewell, R.R., O'Toole, G.A. (2010). The *Pseudomonas aeruginosa* magnesium transporter MgtE inhibits transcription of the type III secretion system. Infection and Immunity, 78(3): 1239-1249. https://doi.org/10.1128/iai.00865-09

[18] Salem, S.S., Shaban, Badawy, M.S.E.M., Al-Askar, A.A., Arishi, A.A., Elkady, F.M., Hashem, A.H. (2022). Green biosynthesis of selenium nanoparticles using orange peel waste: Characterization, antibacterial and antibiofilm activities against multidrug-resistant bacteria. Life, 12(6): 893. https://doi.org/10.3390/life12060893

[19] Ohikhena, F.U., Wintola, O.A., Afolayan, A.J. (2017). Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal, 2017(1): 9658598. https://doi.org/10.1155/2017/9658598

[20] O'Toole, G.A. (2011). Microtiter dish biofilm formation assay. Journal of Visualized Experiments, 47: e2437. https://doi.org/10.3791/2437

[21] Kim, H.Y. (2013). Statistical notes for clinical researchers: Assessment of normal distribution (2) using skewness and kurtosis. Restorative Dentistry & Endodontics, 38(1): 52-54. https://doi.org/10.5395/rde.2013.38.1.52

[22] Díaz-Pérez, S.P., Solis, C.S., López-Bucio, J.S., Valdez Alarcon, J.J., Villegas, J., Reyes-De la Cruz, H., Campos-Garcia, J. (2023). Pathogenesis in *Pseudomonas aeruginosa* PAO1 biofilm-associated is dependent on the pyoverdine and pyocyanin siderophores by quorum sensing modulation. Microbial Ecology, 86(1): 727-741. https://doi.org/10.1007/s00248-022-02095-5

[23] Yang, R., Li, Q., Zhou, W., Yu, S., Liu, J. (2022). Speciation analysis of selenium nanoparticles and inorganic selenium species by dual-cloud point extraction and ICP-MS determination. Analytical Chemistry, 94(47): 16328-16336. https://doi.org/10.1021/acs.analchem.2c03018.s001

[24] Ibrahim, S.S.S., Ansari, Y.N., Puri, A.V., Patil, V.V., Gaikwad, S.S., Haroon, R.A. (2024). Recent progress in the green synthesis, characterization, and applications of selenium nanoparticles. BIO Integration, 5(32). https://doi.org/10.15212/bioi-2024-0063

[25] Alqaraleh, S.Y., Al-Zereini, W.A., Mwafi, N.R., Jaffal, S.M., Al-Qtaitat, A.I. (2024). The green synthesis of selenium nanoparticles: A comprehensive review on methodology, characterization and biomedical applications. Research Journal of Pharmacy and Technology, 17(8): 4054-4062. https://doi.org/10.52711/0974-360x.2024.00629

[26] Abd Rashed, A., Jamilan, M.A., Abdul Rahman, S., Amin Nordin, F.D., Mohd Nawi, M.N. (2024). The therapeutic potential of agarwood as an antimicrobial and anti-inflammatory agent: A scoping review. Antibiotics, 13(11): 1074. https://doi.org/10.3390/antibiotics13111074

[27] Ibrahim, S.K. (2020). Study the effect of *Linum usitatissimum* (flax seeds) and *Eucalyptus rostrata* (Eucalyptus) leaves extracts on *Pseudomonas aeruginosa* growth in vivo and *in vitro*. Biochemical and Cell Archives. https://repository.uobaghdad.edu.iq/articles/rRYQMooBVTCNdQwCMZH8?page=2945.

[28] Mohammed, H.A., Zgair, A.K. (2022). Detection of quorum sensing genes of *Pseudomonas aeruginosa* isolated from different areas in Iraq. Iraqi Journal of Science, 63(11): 4665-4673. https://doi.org/10.24996/ijs.2022.63.11.5

[29] Puri, A., Mohite, P., Ansari, Y., Mukerjee, N., Alharbi, H.M., Upaganlawar, A., Thorat, N. (2024). Plant-derived selenium nanoparticles: Investigating unique morphologies, enhancing therapeutic uses, and leading the way in tailored medical treatments. Materials Advances, 5(9): 3602-3628. https://doi.org/10.1039/d3ma01126g

[30] Sans-Serramitjana, E., Gallardo-Benavente, C., Melo, F., Pérez-Donoso, J.M., Rumpel, C., Barra, P.J., Durán, P., Mora, M.D.L.L. (2023). A comparative study of the synthesis and characterization of biogenic selenium nanoparticles by two contrasting endophytic selenobacteria. Microorganisms, 11(6): 1600. https://doi.org/10.3390/microorganisms11061600

[31] Elkady, F.M., Badr, B.M., Saied, E., Hashem, A.H., Abdel-Maksoud, M.A., Fatima, S., Malik, A., Aufy, M., Hussein, A., Abdulrahman, M., Hashem, H.R. (2025). Green biosynthesis of bimetallic copper oxide-selenium nanoparticles using leaf extract of *Lagenaria siceraria*: antibacterial, anti-virulence activities against multidrug-

resistant *Pseudomonas aeruginosa*. International Journal of Nanomedicine, 2025: 4705-4727. https://doi.org/10.2147/ijn.s497494
[32] Habeeb, Z.A., Jameel, S.K., Ahmed, M.E. (2025). Synthesis, characterization, and cytotoxic evaluation of selenium nanoparticles. Biomedical and Pharmacology Journal, 18(1). https://doi.org/10.13005/bpj/3139
[33] Touati, A., Ibrahim, N.A., Tighilt, L., Idres, T. (2025). Anti-QS strategies against *Pseudomonas aeruginosa* infections. Microorganisms, 13(8): 1838. https://doi.org/10.3390/microorganisms13081838