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Study of the Effect of Green Nanocomposite from *Salvia officinalis* Plant on pslA Gene in Ciprofloxacin-resistant *Pseudomonas aeruginosa* Bacteria

Baidaa Jamel Nama [a*]
and Kiaser Abdulsajjad Mohmmed Hussain [a]

[a] Department of Biology, College of Education for Pure Science, University of Kerbala, Iraq.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The current study aimed to get ready aqueous extract and nanocomposite of the Salvia officinalis plant using the green synthesis method, and to identify silver nanoparticles, an AFM microscope was used, the antibiotic was loaded onto the nanocomposite, and the genetic expression of the psla gene was studied in the Pseudomonas aeruginosa bacteria. The study groups included five groups, the first group was treated with the antibiotic. Ciprofloxacin, the second group was treated with the aqueous extract of the Salvia officinalis plant, the third group was treated with the nanocomposite,

**Corresponding author: Email: baidaa.j@s.uokerbala.edu.iq;*

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the fourth group was treated with the extract loaded with the antibiotic, and the fifth group was treated with the nanocomposite loaded with the antibiotic. The results showed that change in color from yellow to dark brown Indicates the presence of nanoparticles and that the average size average granular size of the extract loaded with the antibiotic is 132.2nm, while the average granular size of the free extract is 85.49nm, and the average granular size of the nanocomposite loaded with the antibiotic is 92.25nm, while the average granular size of the free nanocomposite is 35.42nm, which indicates the success of the process of loading the antibiotic ciprofloxacin onto the nanocomposites, and down-regulation occurred in Gene expression of the psla gene in P. aeruginosa. In the fourth treatment, the aqueous extract of Salvia officinalis is loaded with the antibiotic by (0.8643±0.0095 fold change) and in the fifth treatment, the nanocomposite is loaded with the antibiotic by (0.4357±0.0088 fold change) when the concentration is greater than the MIC.

Keywords: Green synthesis; nanotechnology; Salvia officinalis; pslA gene; Pseudomonas aeruginosa.

1. INTRODUCTION

The issue of antibiotic resistance is escalating globally, and this leads to an increase in the rate of disease, death, and healthcare costs. The spread of resistance relies on multiple variables, such as the misuse of antibiotics, the low level of hygiene in society, safe food, antibiotic accumulation, and Insufficient infection control implementation in clinics and hospitals. The use of antibiotics in the animal and food industries and their presence in the environment, and multidrug-resistant bacteria have become a threat to patients who necessitate medical devices, including blood catheters and ventilators [1-4] Bacteria quickly develop new resistance mechanisms that provide defence against the impacts of antibiotics due to mutations that occur in some bacterial cells that make them resistant to the effects of antibiotics. After that, this advantage is transmitted to the next offspring, which is a generation with complete resistance to the antibiotic [5]. It has been determined Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa have all been characterized as pathogens exhibiting exceptionally elevated levels of antibiotic resistance, yielding a declining range of treatments accessible to these organisms [6]. Pseudomonas aeruginosa, a Gram-negative bacterium, falls under the Pseudomonadaceae family, which is known for its resistance to multiple antibiotics. Pseudomonas aeruginosa, a bacterium, has the greatest death rate across all causes of bloodstream infections, accounting for up to 60% of deaths in burn patients. This malignant infection is often not discovered until A positive blood culture for PA means that the patient is already suffering from bacteraemia. A rapid diagnostic test capable of detecting PA at an early stage when It still exists. In minimal quantities within the burn wound could serve as an early indicator since it will enable earlier and more efficient therapeutic intervention [7]. Virulence factors (virulence) or virulence genes. Among these genes is the psla gene, which is related to the process of biofilm development in the bacterium Pseudomonas aeruginosa. Gene expression of the psla gene is responsible for the phenotype of biofilm formation by the bacterium, Pseudomonas aeruginosa [8]. Among them is the process of biofilm formation, which are groups of microorganisms adhered to a surface and covered by an exopolysaccharide matrix membrane. As they move from plankton to a surface-attached community in response to some specific environmental signals, phenotypic characteristics develop in such bacteria. Bacterial biofilm formation is a common characteristic that enables enhanced survival in challenging environments, such as those with limited nutrients or exposure to antimicrobial agents [9,2]. Foot ulcer infections can also be attributed to bacterial biofilms that form on the surface and secrete extracellular polymeric substances (EPS) that serve as a protective barrier. Regarding the immune defences of the host and the use of antibiotics [10], it is resistant to many antibiotics, including ciprofloxacin, which is a drug belonging to the fluoroquinolone antibiotic group. It has proven its effectiveness in treating various bacterial infections since 1987, and has been listed as an essential drug by the World Health Organization [11]. Ciprofloxacin is a broad-spectrum antibiotic and is the second-generation fluoroquinolone. It can be used in the therapeutic management of a broad spectrum of bacterial infections. (both positive and negative) and toxins. It is used to hinder or inhibit certain infections that may occur due to gonorrhoea bacteria, pneumonia, and toxins or infections of the skin, bones, joints, stomach area, and prostate. It works to

discourage serious injuries that occur in the long term [12].

To confront the problem of bacterial resistance to multiple drugs, scientists have invented nano-delivery systems that have demonstrated high efficiency in overcoming many anatomical and functional barriers to delivering the drug to the target site. Thus, they have been able to produce highly effective drugs with few side effects [13]. Green nanotechnology is a field that focuses on Making products that are environmentally friendly, safer for all living organisms, less expensive and more stable than other manufacturing methods such as bacteria, fungi, yeasts and viruses or using different plant parts such as leaves, stems, fruits, peels, etc. The "green synthesis" of nanoparticles has garnered significant interest in the use of metal oxides, such as silver oxides (Ag-NO_3), due to their optical, chemical, and electrical properties [14] It has been found that nanomaterials face the problem of increasing resistance to bacteria because of their distinct characteristics, the dimensions of which fall within the nanoscale of (1-100), like increased stability, solubility, ease of production, and biocompatibility. The small size of nanoparticles relative to their surface area is one of the most important properties. These materials have been distinguished [15] and many studies have proven that plants are considered a safe source for the pharmaceutical industry and a preventive source for many diseases, due to the medicinal effectiveness of plant compounds that carry antioxidant and antimicrobial properties [16]. Among the medicinal plants is the sage plant, which belongs to the Lamiaceae family, and is often known as the “salvation plant,” derived from the Latin word “salvarem,” which means “preservation or treatment.” It has been used for a variety of purposes, including reducing perspiration, treating sore throats (used as a gargle), regulating menstrual cycles, fighting infections, improving lipid status and liver function, improving appetite and digestion, and enhancing mental ability [17].

2. MATERIALS AND METHODS

2.1 Isolation and Diagnosis of Bacteria

100 samples from both sexes, with ages ranging from (1 day to 70) years, were collected from burn and surgical patients and paediatric patients for patients hospitalized. The number of burn isolates was 35 isolates, surgical isolates were 52 isolates, and children were 13 isolates. The isolates were transferred in swab form and placed in a sterile carrier medium. From hospitals in the Holy Governorate of Karbala, Al-Hussein General Hospital and Children's Teaching Hospital, all isolates were grown on MacConkey agar and Blood agar medium and incubated for 24 hours at 37 ℃ and the bacteria were diagnosed using the VITEK-2 Compact System device.

2.2 Preparation of the Aqueous Extract and Nanocomposite of *Salvia officinalis*

15 g of dried *salvia* powder was weighed and 300 ml of boiled distilled water was added to it and left for 30 minutes at room temperature. It was filtered using pieces of gauze and then filtered twice using filter papers (Whatman No. 1). The filtrate was then placed in a centrifuge and dried. The filtrate was placed in an oven at a temperature of (45°C) to obtain a dry extract in powder form. The powder was placed in a sealed, opaque tube and stored until used. Green Ag-NPs were manufactured by bioabsorption of Ag+ in a clean solution of Salvia officinalis extract. By taking 1 mM of silver nitrate, the plant extract (1.5 gm of ready-made dry plant extract into 100 ml of non-ionic distilled water) was dropped onto the alkaline $AgNO_3$ solution with a ratio of (80% silver nitrate solution: 20% plant extract) and was done. Mixing for 30 minutes at 45-55°C. Then the mixture is monitored for 3 hours. Observed the colour of the mixture changing from yellow to dark brown, indicating the formation of Ag-NPs [18].

2.3 Diagnosis of Nanoparticles

AFM This technique is used to determine various characteristics like the particles' shape, size, surface area, fractal dimensions, and pores. In addition, the orientation, interference, and nanoparticle dispersion can be determined using these techniques. Calculating the height and size of nanoparticles using AFM technology is an advantage of AFM compared to traditional microscopes, including TEM and SEM [19]. All the prepared compounds are within nanoscale limits.

2.4 Preparation of MIC Concentrations of Antibiotic CIP, Extract Sao, and Nanocomposite Sao /NPs

The MIC concentration of the antibiotic ciprofloxacin is equal to 4µg/ml from Vietek, the

MIC concentration of the Sao extract equals 8 µg/ml and the MIC concentration of the nanocomposite equals 8 µg/ml [20] To prepare the MIC concentration of the extract loaded with the antibiotic and the nanocomposite loaded with the antibiotic, half a ml of the antibiotic concentration was combined with half a ml of the extract or compound nano concentration.

2.5 Molecular Detection of Biofilm Formation from *Pseudomonas aeruginosa*

The minipreps DNA kit was used for DNA extraction and purification based on the manufacturer's instructions Wizard Minipreps DNA kit (Promega/USA), A pair of primers was used for the amplification of a fragment that covers the entire Psla genes (Forward primer: 5'-CACTGGACGTCTACTCCGACGATAT-3 and Reverse primer: 5'-GTTTCTTGATCT TGTGCAGGGTGTC-3'), and 16s RNA (Forward primer: 5-CAGCTCGTGTCGTGAGATGT -3 and Reverse primer : 5- CGTAAGGGCCA TGATGACTT-3), A single reaction mixture contained 2-5 µl DNA extract, 25 µl Master mix (reaction buffer, 1 µl; nucleotide mixture dNTP, 10 mM; and Taq DNA polymerase, 0.5µl), 2µl of 10 pmol/µl of forward primers specific and, 2µl of 10 pmol/µl of reverse primers specific in a total volume of 25 µl. The amplification of Psla was run given these circumstances: 5 minutes at 94˚C, followed by 30 cycles of 1 minute at 94˚C, 1 minute at 60˚C, and 1 minute at 72˚C, and finally, 10 minutes at 72˚C. The resulting PCR products were run in 1.5% agarose gel electrophoresis.

2.6 Relative Gene Expression of the pslA Gene under the Influence of Nanoparticles

After distributing the bacterial cells and exposing them to treatments that weaken the process of biofilm formation, five distinct groups were formed. The first group was just antibiotic ciprofloxacin, which was considered a control group due to natural resistance to the antibiotic ciprofloxacin. The second group was the aqueous extract of *Salvia officinalis*, and the third group was the nanocomposite group. The fourth group represented the extract group loaded with the antibiotic, and the fifth group was the nanocomposite group loaded with the antibiotic.

300 µl of bacterial cells inoculated on the wells of a microtiter plate containing 50 µl of treatments, after 18 hours of incubation, 350 µl of bacterial mixture was taken in new 1.5 microcentrifuge tubes for RNA extraction, The bacterial cells were first centrifugated at 13000 rcf for 3 minutes, The cell pellet was then resuspended in an RNase-free lysozyme reaction solution and incubated at 37°C for 10 minutes to facilitate cell lysis. This step was followed by the use of the easy-spin™ Total RNA Extraction Kit/ Korea, by the manufacturer's protocol, to complete the RNA extraction process.

At a wavelength of 260 and 280 nm (A260 and A280, respectively), the RNA samples' absorbance was measured. An estimate of the extracted RNA's purity can be obtained using the 260/280 nm absorbance ratio (A260/A280) in a (NANO spectrometer / Bioneer, Korea).

Using the AddScript Reverse Transcriptase Kit Bioneer/Korea by the manufacturer's protocol, the total RNA that was extracted was converted into complementary DNA (cDNA). The reaction was set up in a 20 µL volume, with the RNA template and reagents gently mixed and added to the GoTaq® qPCR (promega/USA). The reaction was then run on a thermal cycler using the program specified by the manufacturer. The synthesized cDNA was either immediately used for qRT-PCR.

The qRT-PCR experiment using the GoTaq® qPCR mix of the Promega/ USA via Setting up two reactions for each pair of primers, using the cDNA for Psla and the housekeeping 16 sRNA gene as a control. The suitable volume of the reaction mixture was carried to each well of a PCR plate and done on ice to get 20 µl of the final reaction volume and the target genes' cycle threshold (Ct) values were derived by deducting the Ct of the housekeeping 16 sRNA gene from the Ct of the target genes: PslaΔCt = Ct Psla - Ct 16 sRNA, the fold change in gene expression for all Pseudomonas clinical isolates (all five groups) was calculated as: Fold change = $2^{-\Delta\Delta ct}$ [21].

3. RESULTS AND DISCUSSION

3.1 Isolation and Diagnosis of Bacteria

The outcomes of the current investigation showed the role of the VITEK2 device in identifying bacterial isolates. It is considered a faster and more accurate method than traditional methods. It also demonstrates the speed of detection and the efficiency of diagnosing samples away from contamination. The percentage of bacteria was as in Fig. 1.

klebsiella pneumonia24% ‹pseudomonas aeruginosa 21 % ‹E. coli 13%coagulase negative staphylococcus 8 ‹ %staphylococcus aureus7% ‹Enterobacter aerogene s 5% ‹Staphylococcus Haemolytic us 4% shigella4% ‹Acinetobacterbaumannii complex 4% ‹Enterococcus Avium% 2‹ Enterococcus 4%‹ Enterobacter cloacae complex1%‹Serratia 1%‹Enterococcus gallinarum2%.

3.2 Preparation of the Aqueous Extract and Nanocomposite of *Salvia officinalis*

The development of silver nanoparticles was monitored according to the alter in color, as the colour of the reaction mixture began to change from yellow to dark brown after three hours (Fig. 2), which represents the silver nanoparticle formation, resulting from the particle reduction of Ag+ metal ions. Nanosilver Ag via active molecules present in S. officinalis extract such as violin compounds and organic acids [22].

3.3 Diagnosis of Nanoparticles

In Fig. 3 the results of the current study showed atomic force microscope images. The coefficient of roughness of the external surface of the salvia nanocomposite particles was 3,698 nm, while the coefficient of surface roughness of the aqueous extract of the salvia plant was 6,732 nm, where the difference before and after converting the aqueous extract of the salvia plant to the nanocomposite was 3,034 nm. Which indicates the success of the process of preparing the nanocomposite from the extract. The root mean square of the salvia nanocomposite is 4,534 nm and the root mean square of the salvia extract is 9,461. The difference in the root mean square is 4,927 nm, which indicates that the nanocomposite falls within the limits of the nanoscale [23] the surface area of the salvia nanocomposite was 0.7983, and the surface area of the aqueous extract of the salvia plant was 0.6174 nanometers. This is an important indication that the surface is a good receptor for the particles to be loaded, and the texture direction for the salvia nanocomposite was 0.002567 and for the aqueous extract of the salvia plant was 180.0. While the maximum height of the peak for the salvia nanocomposite is 16.39 and for the aqueous extract of the salvia plant is 44.98, and the maximum height of the hole for the salvia nanocomposite is 12.45 and for the aqueous extract of the salvia plant is 70.10.

The results of the current study showed in Fig. 4 that the average granular size of the salvia nanocomposite SaoNPs was 35.42 nm, after the average granular size of the Salvia extract SaoNPs was recorded as 85.49 nm, and the difference between the average sizes was 50.07 nm, as shown in Fig. 4.

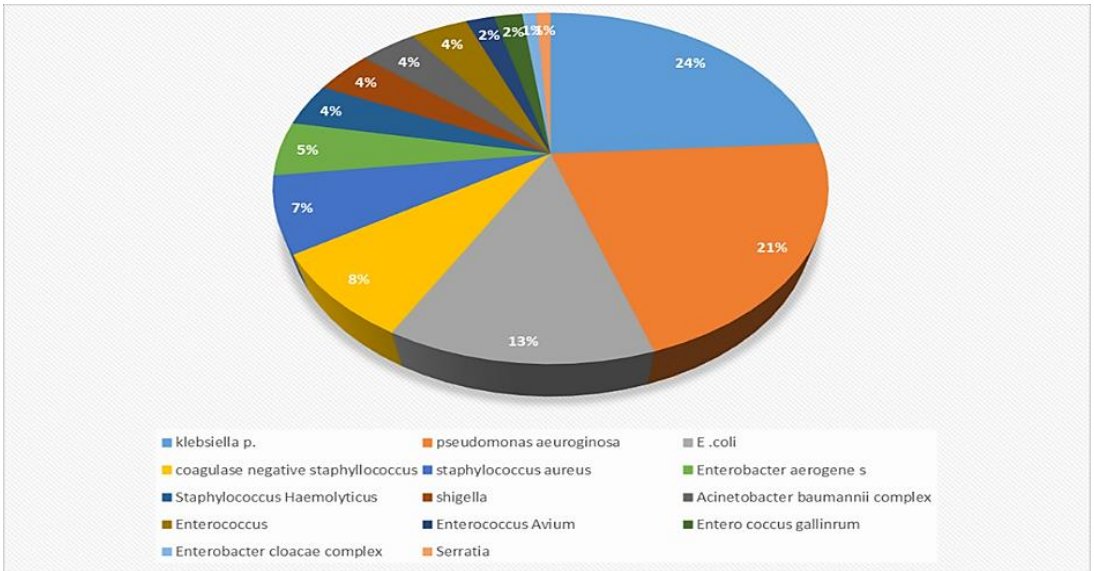

Fig. 1. shows the percentages of bacterial isolates

Fig. 2. Change of colour of the aqueous extract of Salvia officinalis from yellow to dark brown (A) solution of the aqueous extract of the Salvia plant. (B) solution of silver nitrate AgNO3. (C) Aqueous extract of the plant Salvia officinalis after reacting with a 1 mM AgNO3 solution after three hours

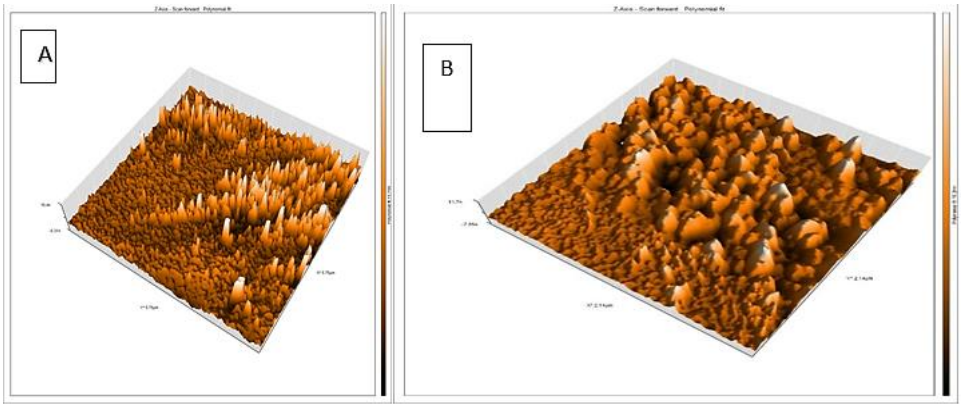

Fig. 3. Atomic force microscope images (A)3D image of the *salvia officinalis* nanocomposite (B) 3D image of the aqueous extract of *Salvia officinalis*

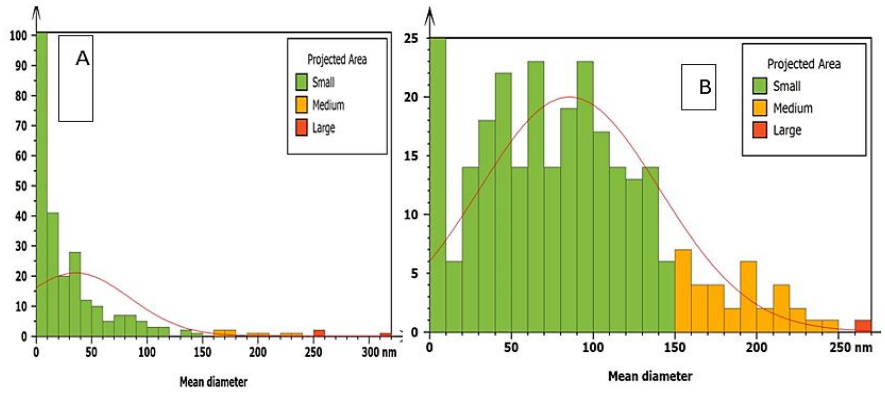

Fig. 4. Distribution of different proportions of particle sizes (A) *Salvia officinalis* nanocomposite (B) *Salvia officinalis* aqueous extract

In Fig. 5 The study's findings revealed that the nanocomposite loaded with the drug ciprofloxacin had a roughness coefficient of 15.20 nanometers, while the roughness coefficient of the extract loaded with the drug ciprofloxacin was 18.58 nanometers. This is necessary to ensure that the drug is loaded onto the nanocomposite after converting the salvia into a nanocomposite. The root mean square ratio of the nanocomposite loaded with ciprofloxacin was 18.94, while that of the extract loaded with ciprofloxacin was 25.91. The

difference in root mean squared was 6.97 nm, and the surface area of the nanocomposite loaded with ciprofloxacin was 3.962%, while the surface area of the extract loaded with the drug was 1.251%. The results showed that the texture direction of the nanocomposite loaded with the drug was 3.490, and the texture direction of the extract loaded with the drug ciprofloxacin was 180.0, while the maximum height of the peak in the nanocomposite loaded with the drug ciprofloxacin was 47.24 nm and for the extract loaded with the drug was 62.04. The results showed that the maximum height of the crater in the nanocomposite loaded with the drug ciprofloxacin was 3.962. As for the extract containing the drug ciprofloxacin, 1.251.(3-6).

3.4 Polymerase Chain Reaction for Psla Gene Detection

In this study, total DNA was extracted from all clinical isolates of P. aeruginosa, and the findings revealed that all clinical isolates contained Psla genes, which are associated with the quorum sensing (QS) phenomenon in P. aeruginosa. Fig. 7 The Psla gene encodes the Psla protein via PCR product size 1116 bp, which is an important component of the quorum sensing (QS) system in P. aeruginosa. Psla protein acts as a QS signal synthase, which is accountable for the generation of the autoinducer molecule N-3-oxo-dodecanoyl-L-homoserinelactone (3O-C12-HSL) [24].

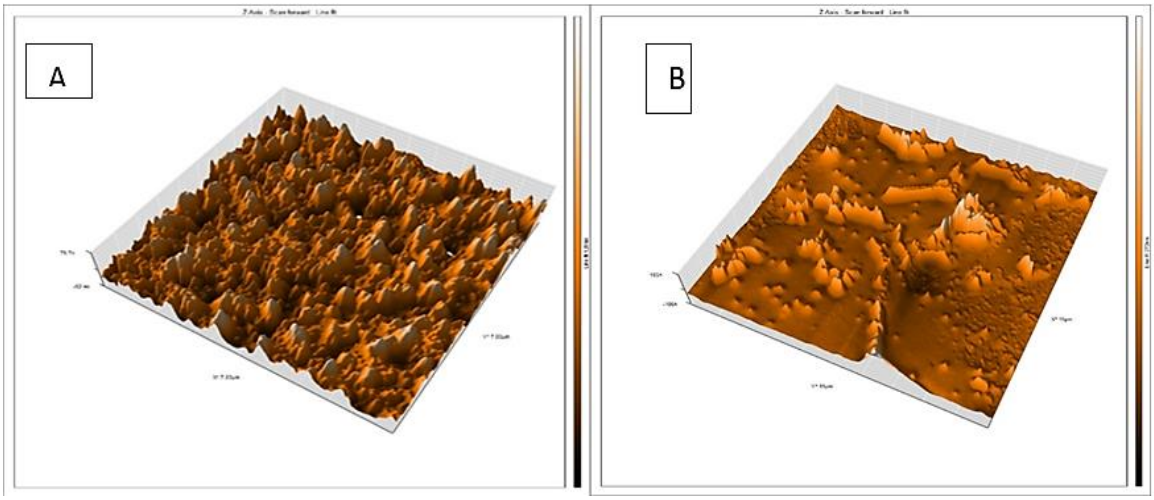

Fig. 5. Atomic force microscopy images (A) 3D image of the Salvia nanocomposite loaded with CIP drug (B) 3D image of a salvia extract loaded with a drug

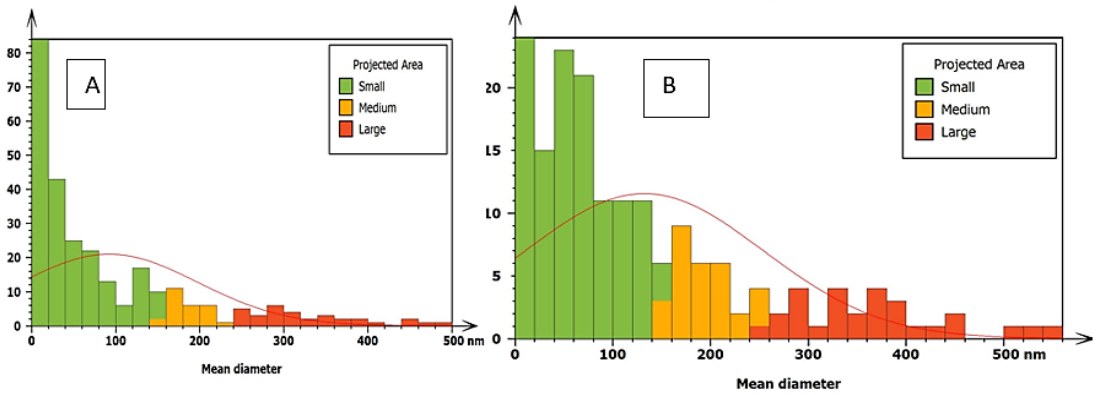

Fig. 6. Distribution of different percentages of particle sizes (A) Salvia nanocomposite loaded with CIP drug (B) Salvia aqueous extract loaded with CIP drug

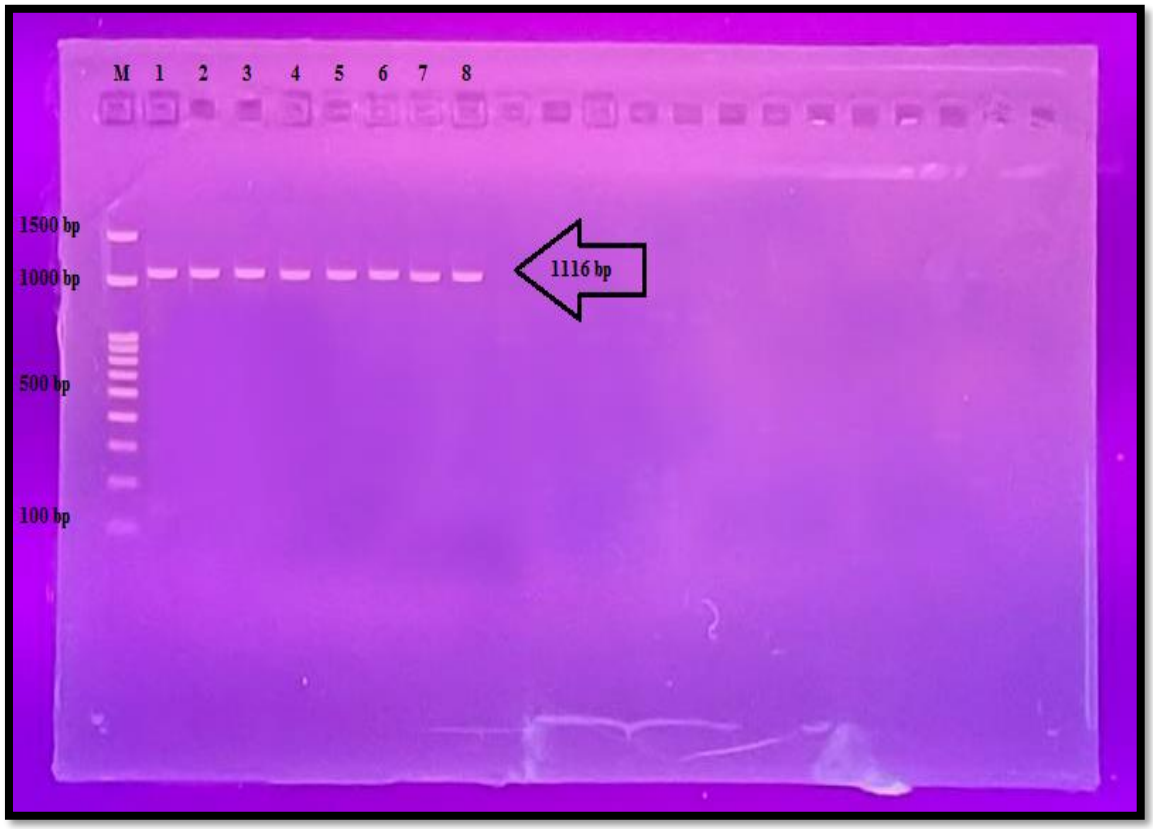

Fig. 7. agarose gele elctrophoresis for Psla gene in psudomas areogenoza isolates; M: DNA marker; 1-8 positive PCR product 1116 bp for Psla gene detection in psedomnas areogenoza

The outcomes of the current investigation illustrated that all P. aeruginosa isolates examined in our study exhibited the capacity to generate biofilms, and these biofilm-forming isolates also showed high levels of antibiotic resistance (100%). Our study is consistent with [25], who found that all *P. aeruginosa* isolates possess an antibiotic resistance pattern and can distribute the pslA gene responsible for biofilm production in extracts acquired from wastewater in a burn center in Gilan, Iran. The outcomes of the current investigation were consistent with the work conducted by [26] from Tehran, Iran, which showed a very rapid biofilm formation rate, as more than 96% of P. aeruginosa isolates gathered from patients Burns are capable of forming biofilms.

It is also not consistent with the results reported in the study conducted by [27] from Tabriz, Iran, where 79% (79%) of their clinical P. aeruginosa strains were identified as biofilm producers.

3.5 Gene Expression of the pslA Gene Using the Relative Quantification

Method of quantitative measurement of the housekeeping gene: Quantitative transcription real-time PCR (RT-qPCR) was carried out to measure relative quantification (gene expression analysis) of 16sRNA gene expression levels of Pseudomonas aeruginosa normalized by 16sRNA gene expression.

The RT-qPCR quantification technique in the real-time PCR system was based on threshold cycle numbers (CT) values to detect the amplification of target genes. The critical value of the control group that was considered treatment ranged (CT = 30), while the other four groups, which include the free nanocomposite group and the free extract group have critical value ranging from (31) and (32), respectively. As for the remainder, the nanocomposite group with the antibiotic and salvia extract with the antibiotic groups, have critical value recorded as (CT = 30 and CT = 33), respectively Fig. 8.

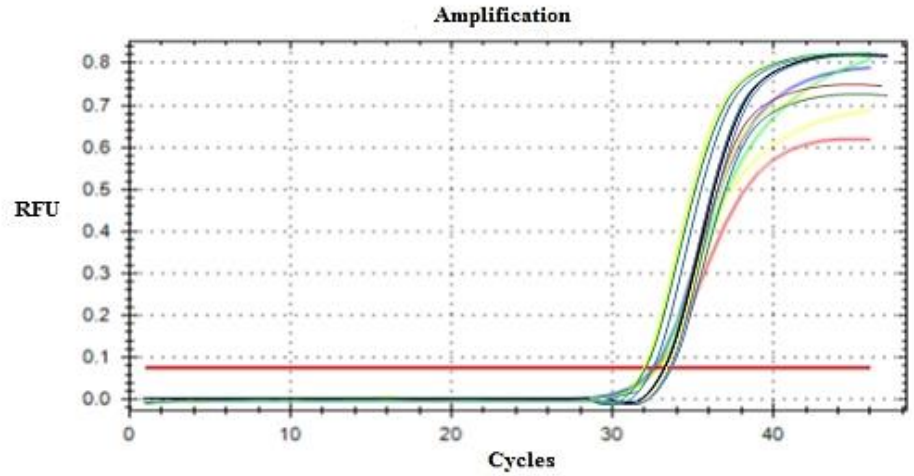

Fig. 8. gene expression amplification curve for the real-time polymerase chain reaction (Real time-PCR) technique for the 16sRNA gene of the Pseudomonas aeruginosa bacteria, where the line curves show: the zero line curve: the CIP antibody group, the black line curve: the free extract group, the red line curve: the compound group Free nanoparticles, blue line curve: extract + antibiotic group. Green line curve: nanocomposite + antibiotic group

Relative expression of psla gene: The levels of gene expression for the pslA gene showed clear significant differences in the levels of gene expression in the isolate groups of the treatments and the control group, as the gene expression results showed a clear decrease (down regulation) in the fourth groups, T4, and the fifth groups, T5, So, it was different with the first group, T1, the second group, T2, and the third group , T3, on the other hand, gene expression results were recorded as 3.994, 4.1256, 5.0172, 0.8643, and 0.4357, respectively, compared with the gene expression of the control group, which is equivalent to 1 (fold change) according to the Livak method and as shown in Fig. 9.

The psla gene expression results from Pseudomonas aeruginosa biotechnology in the fourth treatment (T4), the aqueous extract loaded with the antibiotic at a concentration of (4 + 8 µg /ml), showed a downregulation of 0.8643±0.0095 (fold change) and the fifth treatment, T5, the nanocomposite loaded with the antibiotic at a concentration of (4 +8 µg /ml) decreased by 0.4357±0.0088 (fold change) as contrasted with the control sample in which the level of psla gene expression was up-regulated by 3.994±0.098 (fold change). On the other hand, the gene expression of the psla gene in the second groups, T2, aqueous extract of the Salvia officinalis plant have 4.1256 ± 0.0724 (fold change) and the third groups, T3 nanocomposite, gene expression was 5.0172 ± 0.0751 (fold change). This level indicates the presence of significant differences in expression of genes between the groups and compared to the control group. The Graphs (3-9) shows the relative gene expression for the psla gene.

The outcomes of gene expression for the psla gene of the Pseudomonas aeruginosa bacteria showed that the fourth treatment, T4, the aqueous extract of the Salvia officinalis plant, loaded with the antibiotics, and the fifth treatment, T5, the nanocomposite, loaded with the antibiotics, at a concentration of C1>MIC, negatively affects (reducing the RNA concentration) the level of gene expression, and thus affects the formation of the biofilm of the Pseudomonas aeruginosa bacteria, while the first treatment, T1, ciprofloxacin, the second groups, T2, the free aqueous extract, and the third groups, T3, the free nanocomposite, showed a clear effect on the level of gene expression of the Psla gene (an increase in RNA concentration), and this indicates the activity and effectiveness of this gene in activating and sustaining the membrane biosynthesis and also plays an important role in Qurum sensing in bacteria.

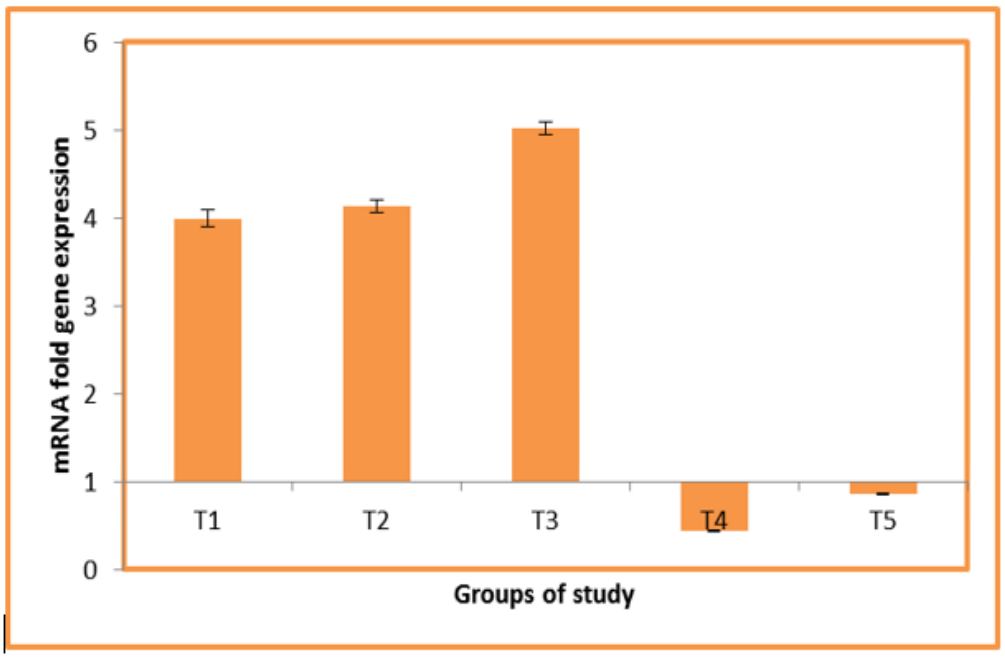

Fig. 9. Shows the gene expression of the Psla gene in Pseudomonas aeruginosa bacteria

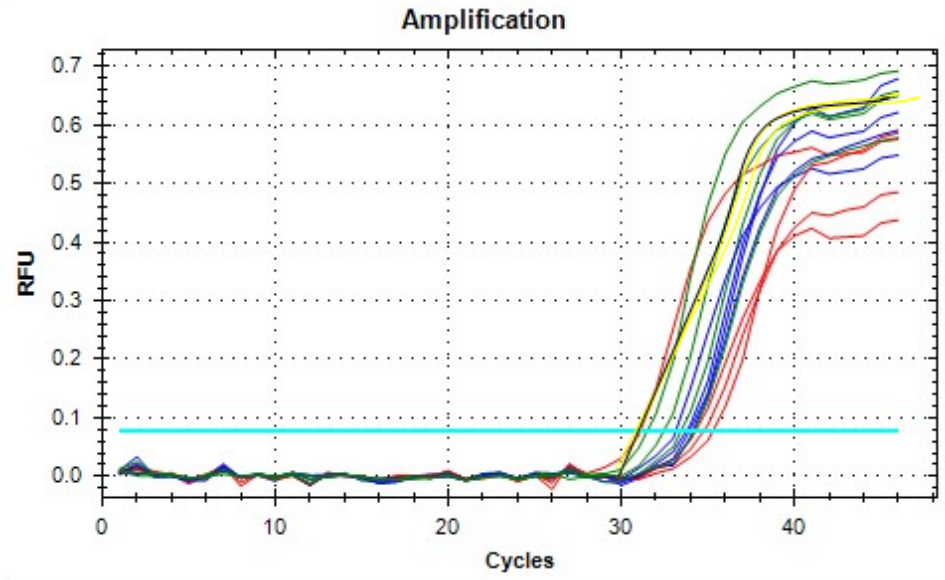

Fig. 10. Gene expression amplification curve for the real-time polymerase chain reaction (Real time-PCR) technique for the psla gene of the Pseudomonas aeruginosa bacteria, where the curves show: green curve: nanocomposite group, yellow curve: free extract group, blue curve: antibiotic group The black curve: the nano group + the antibiotic. The red curve: the extract group + the antibiotic

The current study and gene expression results also indicated that raising the concentration resulted in a drop in the gene expression of the Psla gene of Pseudomonas aeruginosa bacteria for both the fourth groups, T4, and the fifth groups, T5. This study agreed with [28], and this may be due to the effect of the separated silver nanoparticles. Or combined with antibiotics, which leads to total suppression of biofilms, noticed within 24 hours, in addition to the good compatibility of mixing nanoparticles with antibiotics [29] Silver nanoparticles can inhibit the

formation of biofilms by preventing the production of EPS, which is an adhesive material necessary to bind cells and hold them together. AgNPs, once attached to the cell membrane, can reach and build up in the bacterial cytoplasm, and these accumulated particles lead to the inactivation of enzymes through coagulation with compounds, they contain sulfur and phosphorus, including those found in proteins, which are accountable for the creation of EPS, which leads to the inhibition of biofilms [30] A bigger surface area makes small nanoparticles better at inhibiting biofilms and thus provide a larger surface area, to oxidize and release Ag+, and their small size permits them to reach colonies inside biofilms and adhere to the cell membrane, and disrupting its permeability and respiration, and because biofilms are defined by the existence of nutrient-transporting water channels or pores in the matrix, and large nanoparticles cannot penetrate these channels [31] AgNPs and the ions associated with these particles exhibit antimicrobial properties through structural damage. and components of biofilms and blocking the metabolism of bacteria through various actions, AgNPs interact with biofilms, penetrate them, migrate inward, and interact with the main components of biofilms including proteins, nucleic acids, lipids, and van der Waals, ionic interactions, polysaccharides via Hydrophobicity, electrostatics, and hydrogen bonding [32].

4. CONCLUSIONS

The combination of silver nanoparticles and Salvia officinalis /or antibiotics Ciprofloxacin demonstrated the ability to inhibit the expression of the pslA gene involved in biofilm formation in Pseudomonas aeruginosa, this suggests that the silver nanoparticles, possibly in conjunction with bioactive compounds present in the plant extract or antibiotics, have the potential to disrupt or prevent the formation of biofilms by *Pseudomonas aeruginosa*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVED

The ethical approved and informed consent procedures was taken from ethical review boards and committee college of education for pure science university of kerbala.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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