

Research Article

Zinc Oxide Nanoparticle Biosynthetic Preparation Effect on One Virulence Resistance Mechanism in Clinical *Pseudomonas aeruginosa* Isolates

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Abstract

The efflux pump is a mechanism for antibiotic resistance. Plant extracts and nanoparticles (NPs) are as Suita consider as replacements for antibiotics. The current Aim was to investigate the effect of manufactured ZnO-NPs in a chemical way as compared to biosynthesized ZnO-NPs using plant leaves (Crape Myrtle *Lagerstroemia*) successfully grown in Iraq (locally known as "Coffee Rose (Al-Afranji)") to alter the current gene folding in MDR *P. aeruginosa* isolates. *P. aeruginosa* was isolated and used to check the presence or absence of the genes *mexA*, *mexB*, *oprD*, and *oprM*. Phytochemicals Present in the extract of leaves of *Lagerstroemia indica L* were screened using GC-MS, whereas standard techniques were used for the analysis of ZnO-NPs. The expression of the *MexA*, *MexB*, *OprD*, and *OprM* genes—following treatment with zinc oxide nanoparticles—was studied using the RT-PCR technique. All MDR isolates contained isolated contained the genes for *MexA*, *MexB*, *OprD* and *OprM*. The extract of *Lagerstroemia indica L*. was found to contain active chemical compounds by gas chromatography mass spectrometry (GC-MS). The size of ZnO-NPs biosynthesized via a biological method was determined to be 19 to 57 nm, whereas the sizes of those prepared via the sol-gel method ranged from 43 to 76 nm. When tested against multi-drug resistant (MDR) isolates, the MIC values were 1024, 256, and 512 micrograms/mL for the chemical preparation of ZnO-NPs, Bioengineered ZnO-NPs, and *Lagerstroemia indica L*. extract, respectively. The biologically synthesized ZnO-NPs significantly downregulated the genes *MexA*, *MexB*, *OprD*, and *OprM* in subMIC treated samples of biologically synthesized ZnO-NPs and Crape Myrtle extract compared with untreated samples.



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1. INTRODUCTION

The nanoparticle application of ZnO-NPs could benefit patients as an alternative treatment method [1]. Biological applications of ZnO nanoparticles with high biocompatibility, low toxicity, and reasonable cost. ZnO-NPs have excellent potential in biomedical applications, particularly in numerous fields, including antibiotic therapy, cancer treatment, and even the optimization of the culture medium used [2]. ZnO-NPs have been synthesized using chemical, physical, and biological techniques [3]. This biological method is effective by using natural compounds (plant extracts and microorganisms) as the encapsulation, stabilization, and reduction agents, which are substitutes for costly and harmful chemicals. However, the physical and chemical methods are costlier, labor and time consuming, and generate massive amounts of byproducts from the use of chemical agents during the reduction and precipitation processes [4]. Green biosynthesis using plants has more applications than other techniques. Plants are safer for biological systems and more readily available than biosynthesis produced by fungi and bacteria. Apart from this, the extracts of plants have a good reputation for medicinal and environmental uses as they contain compounds like terpenoids, alkaloids, phenols, tannins, and vitamins that have medicinal properties [5]. Crape Myrtle (*Lagerstroemia indica L*) agnus-castus is a plant endemic to areas of East Asia, China, and Japan that can withstand high temperatures. It is native to China and has been used as an ornamental plant due to its various colors of white, red, pink, purple, and copper [6]. The flavonoids, terpenoids, phenols, tannins, volatile oils, alkaloids, fatty acids, and other compounds have been identified in these plants [7]. The Crape Myrtle plant has a number of medicinal properties, such as antitumor activity [8] and antiviral, antibacterial, and antifungal properties [9].



The intensive activity of the compounds contained in the extract of Crape Myrtle during the green synthesis of ZnO nanoparticles with remarkable reductive properties [10] is remarkable. The study aimed to determine the effect of this treatment on the gene expression level of certain efflux pump genes in multidrug resistant (MDR) *P. aeruginosa* with the biologically synthesized ZnO nanoparticles synthesized using the extract of the leaves of *Lagerstroemia indica* L. and comparison with chemically synthesized nanoparticles of ZnO and crape myrtle alcoholic extract leaves.

Ejection pumps have three degrees of resistance. Firstly, they create a basal level of intrinsic resistance in the bacteria by gene expression. Secondly, acquired resistance is derived by second bacteria with overexpression of their efflux pumps. Third, strains under selection pressure can develop resistance, leading to the temporary overexpression of efflux pumps. Six different multidrug efflux mechanisms have been identified in *P. aeruginosa*, and the extensively studied *MexAB-OprM* is the only pump of this bacterium with a wider substrate range than any other bacterial efflux system, such as quinolones, tetracyclines, chloramphenicol, trimethoprim, beta-lactams, beta-lactamase inhibitors, macrolides, azithromycin, colistin, detergents, and dyes [11].

2. METHOD

2.1. Specimens' collection:

Ninety-five clinical samples were collected from patients suffering from skin infection, burn, and wound conditions. The samples were collected between September 1, 2025, and April 1, 2026, at Ba'aqubah Teaching Hospital, the governorate's teaching laboratories, Al-Batoul Hospital, and Al-Shifa Hospital. All samples were processed according to standard microbiological procedures, and the study received ethical approval from the Institutional Review Committee of the College of Education for Pure Sciences, University of Diyala, under reference number 223B, at the beginning of the study period.

2.2. Isolation of *P. aeruginosa* using traditional methods

Under sterile laboratory conditions, samples collected directly from individuals were cultured on various media—including MacConkey, blood agar, and Eosin Methylene Blue (EMB)—prepared according to the manufacturers' instructions and sterilized in an autoclave. The cultures were then incubated for 24 hours at 37°C to identify isolated *P. aeruginosa* colonies based on morphological characteristics, Gram staining, and specific biochemical properties.

2.3. Examination of *P. aeruginosa* with the VITEK-2 system

To verify the diagnosis, several procedures were performed. First, bacterial isolates were cultured. Following the incubation period, the turbidity of the bacterial suspensions was adjusted using VITEK densitometers (supplied by bioMérieux) to match the 0.5–0.6 values. McFarland standard. Subsequently, a single colony was selected and suspended in the solution; a Gram-negative identification card was then introduced into the bacterial suspension, and the tubes were manually loaded into the VITEK-2 system. The remaining steps were carried out in accordance with the manufacturer's instructions and the system's protocols. [12].

2.4. Identification of *P. aeruginosa* efflux pump genes

Single-plex PCR was performed on four MDR isolates selected for this study to detect the presence of efflux pump genes—specifically, *MexA*, *MexB*, *OprD*, and *OprM*—along with the *rpsL* gene, which served as a housekeeping gene. A final reaction volume of 25 µL was used, comprising 12.5 µL of "Amplicon" Master Mix (Denmark), 1.5 µL (at a concentration of 10 pmol/µL) of each primer (Bioneer, South Korea), 4 µL of DNA template, and 6.5 µL of sterile, deionized distilled water. Tables (1-2) below list the primer sequences and amplification conditions for these genes. A 1 kb DNA ladder (Roche, Germany) was used, and the reaction products were separated via agarose gel electrophoresis (2% gel containing 0.5 mg/mL ethidium bromide) at 70 V for 1.5 hours.

Table 1. The present Primer's sequence set was used in this study.

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Primers		Primer sequence (5'→3')	Product Size (bp)	Tm(°C)	Reference
MexA					
Forward	TGACCCTGAATACCGAGCTG	168	62	This study	
Reverse	GGTAGTCGGCCTCGTAGGT				
MexB					
Forward	AGGTCCAGGTGCAGAACAAG	158	62	This study	
Reverse	GTTTCGACAGGTCTTCCTTGG				
OprM					
Forward	CCATGAGCCGCCAACTGTC	180	62	[13]	
Reverse	CCTGGAACGCCGTCTGGAT				
OprD					
Forward	CTACCGCACAAACGATGAAGG	154	64	This study	
Reverse	GCCGAAGCCGATATAATCAAACG				
RpsL					
Forward	GCAAGCGCATGGTCGACAAGA	201	56	[14]	
Reverse	CGCTGTGCTCTTGCAGGTTGTGA				

Table 2. Conditions used in the PCR program for detecting the study genes.

Stages	TM(°C)	Period	Cycles number
The Initial Denaturation	94°	300s	one
The Denaturation		30s	Thirty-five
The Annealing	55°*, 57°**	30s	
The Elongation	72°	20s	
Final elongation		420s	one

Note: **MexA*, *MexB*, *OprD*, *OprM* ***rpsL*

2.5. Preparation and Characterization of *Crape Myrtle* Extract

The leaves of *Crape Myrtle* trees were bought from the “Locality” Garden in Diyala, and the botanists identified the species. To remove dust particles from the surface, the fresh crepe myrtle leaves were repeatedly washed with distilled water. The method for processing crepe myrtle leaves was modified slightly for use as described in [10]. A “Saxolite” system was used, with hexane and methanol serving as solvents, respectively. Leaf powder (100 grams) was poured into a tightly sealed funnel-shaped filter paper with inserted the “Saxolite” system. Five hundred milliliters of solvent was used, and the mixture was left at room temperature for 24 hours. Crude extract, which had darkened to a brownish color, was concentrated using a rotary evaporator in a water bath at 40 °C. The crude extract was kept in a sterile container at 4°C until use. Furthermore, an alcoholic extract of *Crape Myrtle* was prepared, which was used in the gas chromatography–mass spectrometry (GC-MS) analysis to determine the active components determine.

2.6 The Synthesis of ZnO-NPs

The main experimental steps included the biosynthesis of ZnO nanoparticles as described in [10], with some modifications, and the chemical synthesis of ZnO nanoparticles by sol-gel method [15]. *Crape Myrtle* was then extracted, and 10 g zinc nitrate ($\text{Zn}(\text{NO}_3)_2$) was added to the extract when heated under magnetic stirring at 60°C. The mixture was mixed till a white smooth paste was formed. This paste was then washed with distilled water and calcinated at 400°C for 2 hours. The outcome of this process was a white powder containing ZnO nanoparticles with the active ingredients extracted from the crepe myrtle tree (Figure 1). Apart from scanning electron microscopy (SEM), the synthesized ZnO nanoparticles were subjected to X-ray diffraction (XRD) analysis using an X-ray diffractometer to estimate their crystal structure and size estimation of the particles [16].

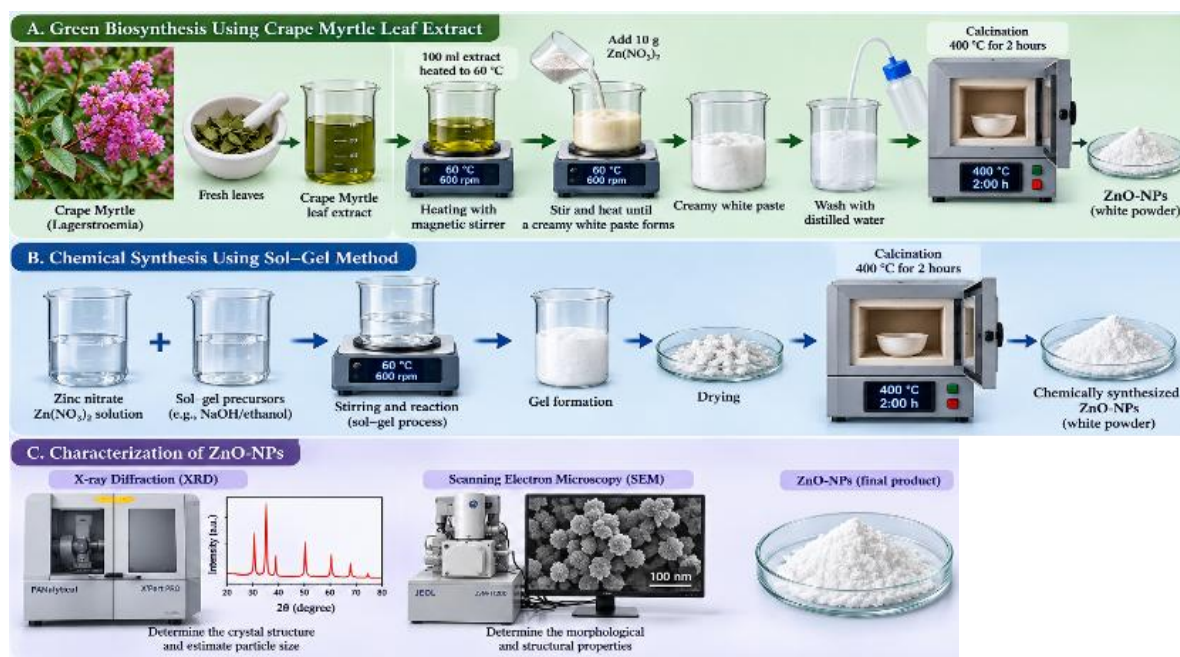


Figure 1: Preparation of zinc oxide nanoparticles: a) green synthesis; b) chemical synthesis; c) nanomaterial characterization instruments.

2.7 Antibacterial effect of ZnO-NPs and biologically synthesized ZnO-NPs using *Crape Myrtle* to determine MIC.

A stock solution with a concentration of 10,000 $\mu\text{g/mL}$ was prepared for both chemically synthesized zinc oxide (ZnO) nanoparticles and biosynthesized ZnO nanoparticles (using *Crape Myrtle* plant extract). A minimum inhibitory concentration (MIC) test was then conducted by serially adding these solutions to 96-well microtiter plates containing Mueller-Hinton broth to establish a concentration gradient (ranging from 8, 192 and 8 $\mu\text{g/mL}$). Bacterial cultures (at a concentration of 1.5×10^8 CFU/mL) were prepared and incubated overnight; subsequently, a 10 μL volume of the bacterial culture was transferred into each well using a micropipette. The plates were then incubated for 24 hours at 37°C.

Following this, 5 μ L of resazurin dye (6.75 mg/mL; Thermo Fisher, USA) was added to each well, and the plates were incubated for an additional 4 hours at 37°C. A color change from purple to pink was observed, and the MIC was defined as the lowest concentration of the compound at which no color change occurred [17].

2.8 Gene Expression Efflux Pump Gene Calculation by qRT-PCR

The change in the expression of efflux pumps was quantified by real-time quantitative polymerase chain reaction (qPCR) in four isolates of multidrug resistant (MDR) *P. aeruginosa* before and following treatment with chemically synthesized ZnONPs, biologically produced ZnONPs, and Crape Myrtle extract at sub-MIC concentrations.

2.9 RNA extraction and RT-qPCR reaction and program:

1 mL of a bacterial suspension prepared from fresh cultures of each isolate was added to 3 mL of culture broth in a tube, and the mixture was cultured under sterile conditions. After that, 1 mL of minimum inhibitory concentration (MIC) was added to each chemical. Three of these tubes were treated with ZnO-NPs, bio-ZnO-NPs, and Crape Myrtle extract, and the fourth tube was a control (untreated). Below the minimum inhibitory concentration (MIC) of each compound, the concentrations were maintained for assessing efflux pump gene expression. The Trans Zol Up Plus RNA Kit from Trans Gen Biotech, China, RNA extraction was performed according to the manufacturer's instructions, with certain modifications and procedures implemented by the researcher for samples lacking sufficient material; specifically, residual *P. aeruginosa* samples were used for extraction (to facilitate gene expression normalization). Additionally, the purity of the extracted RNA was assessed using a NanoDrop spectrophotometer (Quawell, China).

The reverse transcription reaction was performed using the GoTaq® 1-Step RT-qPCR kit, and the study genes were amplified via quantitative real-time PCR (qPCR). The assay was conducted according to the manufacturer's instructions with a total reaction volume of 20 μ L, comprising the following components: 10 μ L of 2X qPCR Master Mix; 1 μ L each of the forward and reverse primers (Table 1); 0.5 μ L each of GoScript RT Mix and MgCl₂ solution; 3 μ L of water; and 4 μ L of extracted RNA template. The primers were used in the RT-PCR thermal cycler, and the relative expression levels of the efflux pump genes were determined relative to the designated housekeeping gene, *rpsL*. (Table 3) presents the RT-qPCR protocol. The “gene folding” method was used to compare the relative expression levels of genes for both treated isolates and untreated.

Table (3). Program and conditions used in the RT-qPCR experiment

Stage	Temperature (°C)	Period	Cycle
R.T	37°	900s.	one
R.T inactivation and GoTaq® DNA Polymerase activation	95°	60s.	one
The Denaturation	95°	30s.	Forty
The Annealing	55° *	30s.	
	57° **		
The Elongation	72°	30s.	
Note. * <i>MexA</i> , <i>MexB</i> , and <i>OprM</i> ; ** <i>rpsL</i> R.T: Reverse transcriptase			

2.9 Statistical Analysis

The Statistical Analysis System, SAS (2018) program was used with the Least Significant Difference (LSD) test with Analysis of Variation, or ANOVA was employed. Gene folding calculation according to Levak 2001 [18].

3. RESULTS AND DISCUSSION

3.1 GC-MS Analysis Results

A computer-based comparison was performed between the mass spectra of these components and their counterparts in commercial spectral libraries (Wiley GC/MS), the “Mass Finder 3” and the “Baser” library of active constituents, which enabled the identification of specific components within the plant extract. Overall, the databases currently contain reference mass spectra and retention parameter data for over 3,200 authentic compounds. As shown in (Table 4) and (Figure 2).

The results revealed that the plant extract contains many bioactive substances, most notably terpenoids, fatty acids, tocopherols, and phytosterols. Phytol was the major compound (37.25%), followed by neophytadiene (7.49%), γ -sitosterol (6.10%), and squalene (5.03%). The presence of these compounds may contribute to the biological activity of the extract through reported antioxidant, anti-inflammatory, and antimicrobial activities.

Table (4): Active ingredients in the Grape Myrtle extract.

N	Compound	Chemical Classification	Molecular Formula	Retention Time (min)	Area (%)	Potential Biological Relevance
1	Neophytadiene	Diterpene / Terpene	C ₂₀ H ₃₈	22.5571	7.4851	Reported to possess potential antimicrobial and anti-inflammatory activities
2	Neophytadiene	Diterpene / Terpene	C ₂₀ H ₃₈	22.8079	0.6391	Potential biological activity; lower-quality library match than Peak 3.
3	Hexadecanoic acid, methyl ester (Methyl palmitate)	Fatty acid ester	C ₁₇ H ₃₄ O ₂	23.4200	1.3832	Fatty acid derivative with reported biological activities
4	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	C ₁₆ H ₃₂ O ₂	23.8109	1.8469	Biologically active fatty acid; reported antimicrobial and other cellular effects
5	9,12,15-Octadecatrienoic acid, methyl ester (methyl linolenate)	Polyunsaturated fatty acid ester (ω-3)	C ₁₉ H ₃₂ O ₂	25.1384	0.4191	Derivative of omega-3 polyunsaturated fatty acid
6	Phytol	Diterpene / Fatty alcohol	C ₂₀ H ₄₀ O	25.2786	37.2501	Reported antioxidant, antimicrobial, anti-inflammatory, and potential anticancer properties
7	9-Octadecenoic acid (oleic acid)	MUNFA	C ₁₈ H ₃₄ O ₂	25.4851	0.7324	Biologically important monounsaturated fatty acid
8	Squalene	Triterpene	C ₃₀ H ₅₀	30.9502	5.0297	Reported antioxidant and potential chemopreventive/anticancer properties
9	γ-Tocopherol	Tocopherol / Vitamin E	C ₂₈ H ₄₈ O ₂	32.8309	0.4640	Antioxidant activity and potential protective cellular effects
10	α-Tocopherol (Vitamin E)	Tocopherol / Vitamin E	C ₂₉ H ₅₀ O ₂	33.6348	1.1910	Strong antioxidant activity and cellular protective effects
11	Stigmasterol	Phytosterol /steroid	C ₂₉ H ₄₈ O	35.2869	1.0705	Reported anti-inflammatory, antioxidant, and potential anticancer activities
12	γ-Sitosterol (Clionasterol)	Phytosterol /steroid	C ₂₉ H ₅₀ O	36.0834	6.0953	Reported antioxidant, anti-inflammatory, and potential anticancer properties

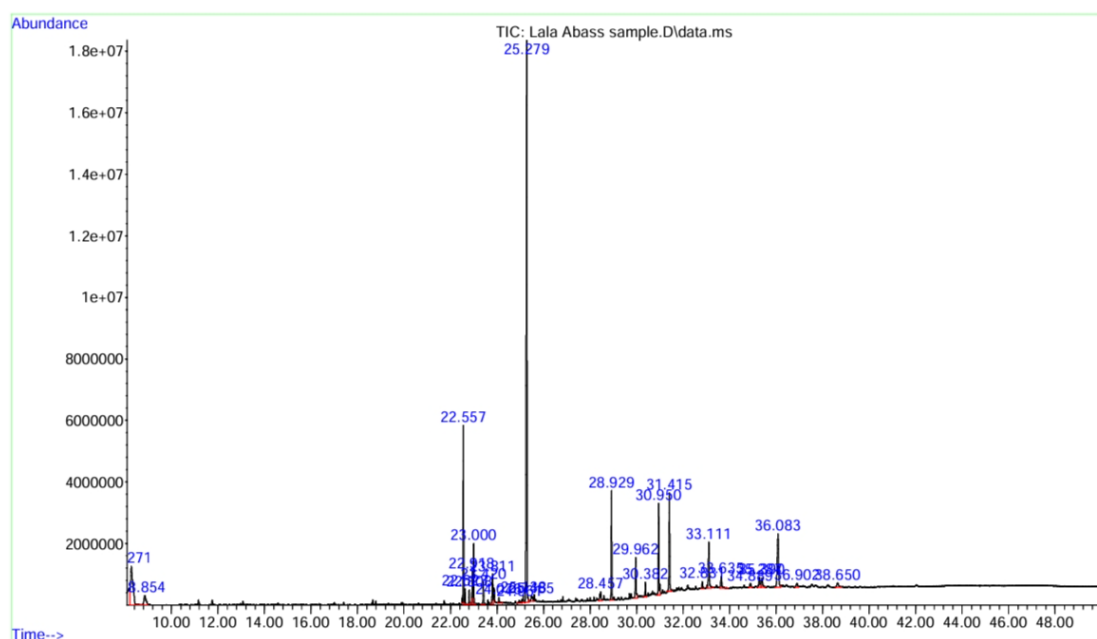


Figure 2: Gas chromatography-mass spectrometry of plant extracts

3.2 Detection of efflux pump genes in multidrug-resistant (MDR) study isolates.

Seven isolates of multidrug-resistant (MDR) bacteria were collected and confirmed as MDR by testing. The isolates, which were found to be resistant to antibiotics, were selected using the results of antibiotic susceptibility testing. Four MDR *P. aeruginosa* isolates were selected for examination of the presence of efflux pump genes (*MexA*, *MexB*, *OprD*, *OprM*) and essential gene (*rpsL*) using the conventional single-strand polymerase chain reaction (PCR) method. All (100%) of the tested isolates contained all genes.

All multidrug-resistant isolates were found to carry efflux pump genes, confirming the result of [19] that concluded the presence of efflux pump genes *mexAB-oprM* in 100% of *P. aeruginosa* isolates. Moreover, according to [19], the *MexA* and *MexB* operon genes were present in all samples, whereas the *MexAB* operon genes were located on the chromosome in *P. aeruginosa* crepe.

The results published by Abdul N. Q. in 2018, which generally agree with the present study, indicated that all confirmed isolates had the gene for *MexB* [20]. Moreover, the efflux pump is one of the significant complexes related to resistance to all major classes of antibiotics [21].

3.3 Morphological and volumetric characterization of biosynthesized zinc oxide nanoparticles.

Fresh Crape Myrtle leaves were dried and ground to synthesize zinc oxide (ZnO) particles; the resulting solutions exhibited a color shift from yellow to brown within a few hours, marking the completion of the bioreduction of zinc salts into ZnO particles. Flavonoids and phenolic compounds were found capable of converting zinc ions into ZnO particles; these phytochemicals serve as both reducing and stabilizing agents due to their non-toxic nature and antioxidant properties [22].

The findings of the current study align with a previous study [23] that observed similar color changes during the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using plant extracts. Gas chromatography-mass spectrometry (GC-MS) analysis of the leaf extract revealed the presence of numerous chemical compounds in the plant material possessing antibacterial and anti-inflammatory properties, including: phytol (C₂₀H₄₀O), phenol (C₆H₅OH), vitamin E (C₂₉H₅₀O₂), 4,5-dichloro-1,3-dioxolan-2-one (C₃H₂Cl₂O₃), squalene (C₃₀H₅₀), and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy (C₆H₈O₄). Our results align with the findings of our study [24], which demonstrated that Crape Myrtle extract contains a wide range of chemical compounds belonging to the classes of flavonoids, glycosides, triterpenoids, essential oils, and phenolic compounds. The variation in the number of identified phytochemicals may be attributed to differences in the plant's genetic composition. [25].

The X-ray diffraction (XRD) patterns of ZnO nanoparticles are shown in Figure 3. A): The XRD of ZnO peaks are located at angles ranging from (32.21, 34.90, 36.71°, 48.00°, 57.00°, 63.25°, 66.76°, 68.31°, 69.43°, 72.96°, 77.31°). These X-ray diffraction (XRD) peaks—identified as reflection planes—correspond to values ranging from (0.2460) to (0.3444). While the XRD analysis results for the samples synthesized via the biological method. B): The XRD peaks are located at angles ranging from (33.46°, 34.41°, 36.34°, 47.62°, 56.66°, 63.11°, 66.63°, 68.15°, 69.33°, 72.61°). These X-ray diffraction (XRD) peaks—identified as reflection planes—correspond to values ranging from (0.446) to (0.352). The X-ray diffraction (XRD) patterns of the bio-synthesized ZnO nanoparticles were similar to the previous results reported in [10].

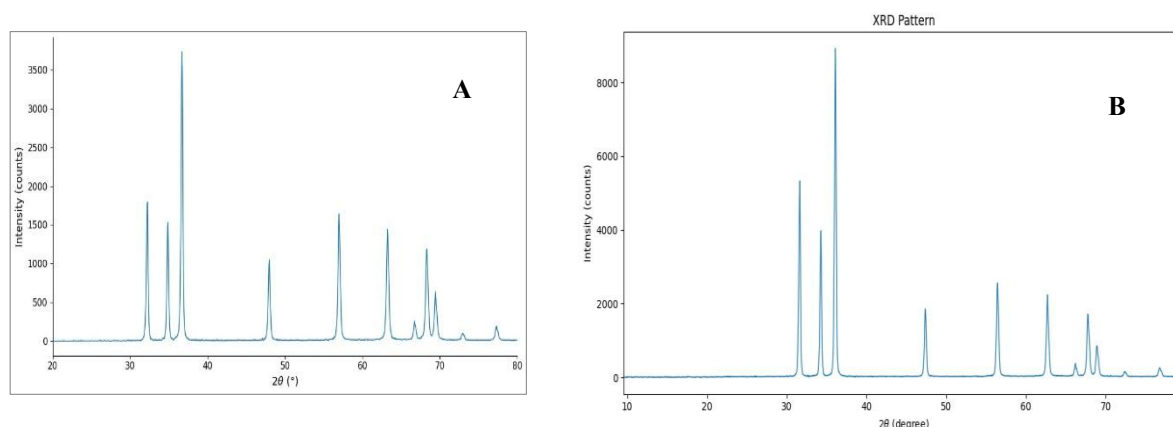


Figure 3: X-ray diffraction pattern results for the prepared ZnO nanoparticles. A) Sol-gel method, B) Biological method

The EDX analysis results shown in Figure (4A) indicate that both the chemical and biological synthesis methods for ZnO NPs yielded products composed primarily of zinc and oxygen, confirming the formation of ZnO in both cases. Oxygen was detected at a low-energy peak of 0.5 keV, while the zinc peak appeared in the 8.0–9.0 keV range—a characteristic spectral signature associated with electron transitions in zinc during EDX analysis.

The spectra of the samples show a high degree of similarity regarding peak positions, indicating that the variation in the synthesis method did not alter the fundamental elemental composition; both methods produced a material consisting mainly of zinc and oxygen. Figure (4B) also reveals the presence of carbon, which is expected given that the material was synthesized using organic plant constituents.

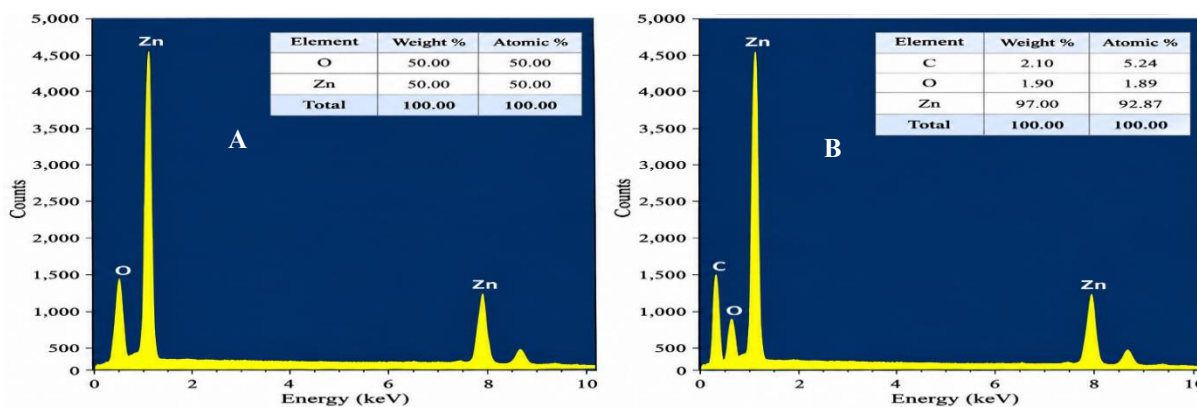


Figure 4: EDX results for (ZnO-NPs) prepared by: (A) Sol-gel method and (B) Biological method.

In (Figure 5), scanning electron microscope (SEM) images show the size and shape of the ZnO nanoparticles. (A) The particle sizes ranged from 43 to 76 nm for the sol-gel method, and the particles were spherical and compact. These results are consistent with those reported in [26], which also observed a spherical shape. In contrast, (B) the synthesis of ZnO nanoparticles using a plant extract in this study yielded particles ranging in size from 19 to 57 nm and characterized by a spherical shape.

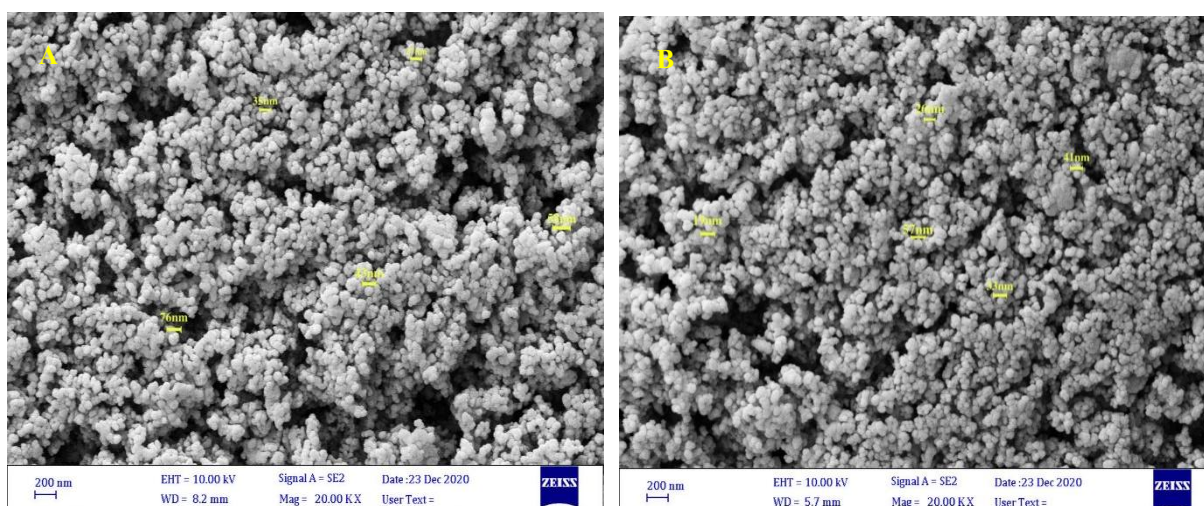


Figure 5: SEM for ZnO-NPs : (A) prepared by biological method, (B) prepared by sol-gel method.

3.4 Results of the antibacterial activity study and MIC determination

The chemically synthesized ZnO nanoparticles were found to have antibacterial activity against multidrug-resistant (MDR) *P. aeruginosa* with MIC value of 1,024 micrograms/mL. The MIC values of the biologically synthesized ZnO nanoparticles using the Crape Myrtle leaf extract were 256 micrograms/mL and 512 micrograms/mL. Some previous studies have demonstrated the antibacterial activity of ZnO nanoparticles. In a study on bio-synthesized ZnO-NP particles, the minimum inhibitory concentration (MIC) values against multidrug-resistant *P. aeruginosa* isolates were 1,600–3,200 micrograms/mL and 150 micrograms/mL with the synthesized particles, respectively [4].

The variability in reported toxicity effects of zinc oxide nanoparticles (ZnO-NP) may be due to variations in the way and scale they are produced. Research on the antibacterial effect of zinc oxide nanoparticles (ZnO-NPs) with sizes of 30–90 nm against *P. aeruginosa* revealed another mechanism by which the high negative charge on Gram-negative bacterial cell surface compared with Gram-positive bacteria could be the reason for the enhanced antibacterial activity of the ZnO-NPs. Hence, Gram-negative bacteria can support the oxidative stress mechanism in relation to the antibacterial activity of ZNPs. Comparing the antibacterial activity results, it was found that bio-synthesized ZNPs show better inhibitory effect than chemically synthesized ZNPs. There are potential adverse effects with chemical synthesis. This results in the easy penetration of bio-synthesized zinc oxide nanoparticles (Bio-ZnO-NPs) inside the bacteria cells and their attachment to the cell membrane.

The MIC of the crepe myrtle extract toward multidrug-resistant *P. aeruginosa* was 512 micrograms/mL. The crape myrtle is considered as one of the important medicinal plants which has attracted much attention and has been extensively studied by the scientists.

The result demonstrated that essential oil obtained from the Crape Myrtle tree has a positive effect on microbial cells, which is attributed to the antibacterial activity against *P. aeruginosa* (Gram-negative bacteria) [9], while the result of [10] confirmed that essential oil extracted from the Crape Myrtle tree has antibacterial activity against *P. acnes* (Gram-positive bacteria). Antibacterial activity of the alcoholic extract of Crape Myrtle leaves may be due to the presence of active phytochemicals, such as flavonoids, tannins, and phenols, or due to hydroxyl (-OH) groups in phenols, which are capable of forming hydrogen bonds with water molecules in the bacterial cell and disrupting the basic functions of the bacterial cell.

Additionally, it may be due to its inhibitory effect on enzymes, microbial adhesions, and cell membranes to block the growth of transport proteins in *P. aeruginosa*, as well as other mechanisms [27]. There are several ways to explain how zinc oxide nanoparticles (ZnO-NPs) kill bacteria. The special inhibitory mechanisms of ZnO nanoparticles could be attributed to the production of toxic ions (Zn^{2+}), which may affect the integrity of the bacterial cell wall. Within cells, nanoparticles and free metal ions interact with biochemical processes and cause changes to the structure of proteins and DNA [28]. Once the bacteria are no longer able to reproduce inside the microbial cells, cell death results in the generation of large quantities of ROS [29].

3.5 Effects of ZnO-NPs and Crape Myrtle extract on efflux pump gene expression

The total RNA was isolated from each successful isolate before and after treatment. The quantitative expression of genes was determined using the “fold change” ($2^{-\Delta\Delta Ct}$) method and the ΔCt value referred to the gene as a reference (*rpsL*). (Table 5) shows that the expression of the genes *MexA*, *MexB*, *OprD*, and *OprM* was reduced (expression ratio < 1) in isolates from the treated group compared with the untreated control group (expression ratio = 1).

Overexpression of efflux pumps, *MexA*, *MexB*, *OprD*, & *OprM*, has been shown to be linked with resistance in multi-drug resistant (MDR) *P. aeruginosa* strains, which was also supported by the experiments conducted in this research with biologically synthesized zinc oxide nanoparticles (ZnO-NPs), chemically synthesized zinc oxide nanoparticles (ZnO-NPs), and crepe myrtle extract. Furthermore, *MexAB-OprMD* seems to be very useful in the presence of antibiotics in medical applications and may play a major role in the efflux of many drugs, supporting the current results of a direct link between the expression of the genes and antibiotic resistance [30]. The results presented here suggest that the genes encoding efflux pumps are more inhibited by the synthesized biologically produced ZnO-NPs than by the chemically synthesized ZnO-NPs and Crape Myrtle extract, respectively. The gene folding levels of all the studied efflux pump genes decreased in a significant gradual line, except for *OprM*, where the expression level was decreased more by chemically synthesized ZnO-NPs than by the Crepe Myrtle extract, and *MexB*, where the expression level was decreased by both biologically synthesized ZnO-NPs and the Crepe Myrtle extract.

Considering their capacity to modify the DNA sequence and gene expression, the two treatments, ZnO nanoparticles and Crape Myrtle extract, seem to influence the gene expression of efflux genes in bacteria, causing an increase in the production of reactive oxygen species (ROS). Moreover, ribonucleic acid (RNA) is much more prone than deoxyribonucleic acid (DNA) to be damaged by ROS. Nanoparticles could increase the effectiveness of conventional antibiotic treatment by blocking multidrug-resistant (MDR) efflux pumps. The inhibitory activity of VA-C 61 extracts, on the other hand, might be due to the presence of bioactive compounds that play an important role in the process of pseudohyphae production; some of those bioactive compounds may inhibit or decrease the production of pseudohyphae.

Table 5: Compression of fold change of efflux pump genes with different parameters

Means of fold change	Material experiments (treatments)			
	Bio. Synthesis NPs	chem. Synthesis NPs	Crape Myrtle extract	Control
<i>mexA</i>	0.0006 ±0.0001 b	0.1455 ±0.05 b	0.059 ±0.01 b	1
<i>mexB</i>	0.0017 ±0.0002 b	0.1351 ±0.04 b	0.047 ±0.02 b	1
<i>oprM</i>	0.0007 ±0.0002 b	0.0500 ±0.01 b	0.002 ±0.003 b	1
<i>operD</i>	0.0008 ±0.0001	0.0500 ±0.010 b	0.128 ±0.030 b	1
Control	1.00 a	1.00 a	1.00a	-----
p-value	0.04*	0.04*	0.40*	-----

Overall, the gene fold level of all efflux genes, except *OprM*, was suppressed, with the chemically synthesized ZnO-NPs having a more pronounced effect than the Crape Myrtle extract. Untreated *P. aeruginosa* isolates (= 1) that overexpress *MexA*, *MexB*, *operD* *OprM* are thought to be linked to mechanisms that confer resistance in multidrug-resistant (MDR) *P. aeruginosa* strains. Furthermore, *MexAB-OprMD* has been shown to be very useful when the bacterium is exposed to antibiotic stress and may play an important role in the efflux of other antibiotics. This study submitted a result that emphasizes a direct correlation between antibiotic resistance and gene expression of *MexA* and *MexB*, which goes with a previous study [31]. Both the Crape Myrtle extract and the biosynthesized ZnO-NPs had the ability to change the DNA sequence and expression, which increased efflux pump genes in the bacteria. However, bioactive compounds in the Crape Myrtle extract can be responsible for the inhibitory effect of the extract [32]; some of these compounds can inhibit or limit pseudohyphal formation, adhesion, enzyme production, biofilm formation, quorum sensing, etc. Other virulence factors. Furthermore, the results showed that bio-synthesized ZnO nanoparticles along with Crape Myrtle plants had significant inhibitory activity, especially against the *MexB* gene.

In this study, the gene expression of *mexB* in *P. aeruginosa* resistant to ciprofloxacin was studied before and after treatment with the selipin nanotechnology encapsulated in nanoparticles that increased the ability of inhibiting the gene expression of efflux pumps, including *mexB* of *P. aeruginosa* [33]. Another study reported that nanoparticles had a weak inhibitory effect on the expression of *mexA* gene in the current bacterium [34]. When the efflux system is overexpressed, the accumulation of antibiotics is decreased. The efflux pumps found in *P. aeruginosa* are classified as *Mex*-type, which have very broad substrate specificity. For *P. aeruginosa* isolates, the *P. aeruginosa* genome contains the potential for efflux systems from this family, and four of them, *MexAB-OprM*, *MexCD-OprJ*, *MexEF-OprN*, and *MexXY-OprM*, are the most efficient antibiotic efflux systems [35]. Furthermore, efflux pumps can synergistically interact with other resistance mechanisms, such as multidrug resistance (MDR) [36].

This recent study on these genes is consistent with the synergy of the low-permeability outer membrane and the gene expression of several broad-spectrum multidrug efflux systems (*Mex*), the latter of which include *MexAB-OprMD* and *MexXY-OprM* that account for multidrug intrinsic resistance in *P. aeruginosa*.

The findings of the study reveal that the biosynthesized ZnO nanoparticles with Crape Myrtle leaf extract showed higher antibacterial activity than chemically synthesized ZnO nanoparticles. This is because the surface has some toxic chemical compounds that can have a negative impact on medical uses [10]. Hence, bio-synthesized ZnO nanoparticles exhibited a strong binding affinity to the cell membrane and penetrated deeply into the bacterial cell. The effective inhibitory effect of biologically synthesized ZnO nanoparticles was found to be influenced by the size of the nanoparticles and the coverage factor (alkali metals), and the surface negative charge of gram-negative bacterial cells was higher than that on the surface of bacteria gram-positive.

4. CONCLUSION

Pseudomonas aeruginosa resistance related to the *MexAB-OprMD* system. The downregulation of these genes coding for efflux pumps may diminish the ability of the antibiotic(s) to kill the cells. This finding indicated that the expression of the *MexA*, *MexB*, *OprD*, and *OprM* genes decreased more by the biologically synthesized ZnO nanoparticles than that of Crape Myrtle extract and chemically synthesized ZnO nanoparticles.

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