



# Antibacterial Effect of Manganese Nanoparticles Loaded on Prodigiosin against Pathogenic *Pseudomonas aeruginosa*

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**Abstract:** *Pseudomonas aeruginosa* are opportunistic pathogens that can cause infections in the lungs, skin, and eyes of people with cystic fibrosis (CF), HIV/AIDS, and burns and abrasions. In this study, the biosynthesis method of manganese nanoparticles (MnO NPs) was performed using pigment of *Serratia marcescens*, called Prodigiosin, which is utilized as a stabilizing and reducing agent. The amid of study was to investigate the antimicrobial activity of manganese nanoparticles on clinical isolate of *P. aeruginosa*. The results, one hundred and eighty samples were collected from burn and wound infections of different patients with different ages and sexes, from Kadhimiya Hospital, Karkh General Hospital and Yarmouk Hospital during the period from September 2022 to December 2022, whereas all these samples were subjected to different examinations in order to isolate *P. aeruginosa*. The influences of varied concentrations (25, 50, 100 and 200 µg/ml) of MnO NPs on bacteria *P. aeruginosa* were demonstrated. The antibacterial action was showed to be immediately reliant on the concentration of MnO NPs. The maximum inhibition zones around *P. aeruginosa* isolate were 28 mm at concentration 200 µg/ml of MnO NPs, while the minimum regions of inhibition were set at 25 µg/ml MnO NPs concentrations, were 10 mm. In conclusion, manganese nanoparticles that loaded on prodigiosin showed effective antibacterial activity against *P. aeruginosa*.

**Keywords:** MnO NPs, prodigiosin, *Serratia marcescens*, *Pseudomonas aeruginosa*.

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## Introduction

*P. aeruginosa* is motile, aerobic, non-spore forming, rod-shaped and gram-negative bacteria, belong to the Pseudomonadaceae family which includes the *Pseudomonas* genus and *P. aeruginosa* is one species of this genus (1,2,3). *P. aeruginosa* are ubiquitous microorganisms found in both natural and human-made environments, including those involving animals and plants (4). Pseudomonads are opportunistic pathogens that can cause infections in the lungs, skin, and eyes of people with cystic fibrosis (CF),

HIV/AIDS, and burns and abrasions (5). *Serratia marcescens* is gram-negative rod-shaped bacillus belongs to the family Enterobacteriaceae (6). Prodigiosin (PG), is one of alkaloid secondary metabolites released from numerous microorganisms including *Nocardia* spp., *Streptomyces lividans*, *Vibrio gazogenes*, *Pseudomonas magneslorubra*, *S. rubidaea*, *Serratia marcescens*, *Pseudoalteromonas rubra*, etc. The chemical structure of this pigment includes three pyrrole rings. This natural pigment lives up to its imposing moniker by displaying a wide

range of useful properties, including those that are immunosuppressive, antifungal, antimalarial, anticancer, antibacterial, etc (7).

Particles with a mean diameter of less than or equal to 100 nm and a high surface-to-volume ratio are known as nanoparticles (NPs) (8,9). The visual features, catalytic activity, and antimicrobial capabilities of nanoparticles have garnered a lot of attention in recent years. There is a greater possibility of their use in fields like medicine, communications, and electronics because of all their special qualities. Nanoparticles, endowed with these characteristics, have proven useful in a wide variety of industries (10). Manganese considered as a high-performance metal in numerous applications such as catalysis, photoelectronics, electrochemistry, electronics, purification, water treatment, biosensors, biomedicine, and medicine etc. (11).

For these reasons, manganese nanoparticles are a promising new antibacterial treatment option. Consequently, the goal of the recent work is to determine whether or not manganese nanoparticles synthesized with Prodigiosin from *Serratia marcescens* can inhibit the growth of *P. aeruginosa*.

#### **Materials and methods**

##### **Collection, isolation and identification of bacteria**

A total of one hundred-eighty samples were obtained from burn and wound infections of different patients with different ages and sexes, from Kadhimiya Hospital, Karkh General Hospital and Yarmouk Hospital during the period from September 2022 to December 2022. All samples were subjected to various examinations, including microscopic examination (gram staining), cultural characteristics

(MacConkey agar and Cetrimide agar) and biochemical tests (catalase, oxidase, indole, Simmons citrate and urease tests) in order to isolate and identify isolates of *P. aeruginosa* (12,13,14).

In addition, a ready isolate of *Serratia marcescens* were obtained after confirming its species using VITEK-2 system.

##### **Extraction and purification of prodigiosin**

After 48 hours of incubation at 28 degrees Celsius, the cell-free broth culture of *Serratia marcescens* was used to harvest the prodigiosin. The culture medium was placed in centrifuge for 15 minutes at 8000 rpm to remove any debris. The collected supernatant was then systematically removed, and methanol (250ml) was added to the collected cells before being well mixed for three hours at temperature of room. After combining methanol with culture medium, the resulting mixture was placed in centrifuge at 8000 rpm for twenty minutes, after which the supernatant was obtained and filtered through a fine mesh filter (0.2  $\mu\text{m}$ , millipore filter). Hence, a rotary evaporator set at 70 °C was utilized for concentration of the methanol filtrate, and two as much chloroform was added to the pigment extraction mixture. The methanol and chloroform were thoroughly combined in a reparatory funnel, and then the organic chloroform phase was separated and left to dry at 45 °C. When finished, the pigment was added to methanol for dissolving and stored in an opaque bottle in the refrigerator (15).

##### **Syntheses of manganese oxide nanoparticles (MnO<sub>2</sub> NPs)**

Manganese oxide nanoparticles (MnO<sub>2</sub> NPs) were synthesized via the

biological synthesis approach using manganese (II) sulphate monohydrate ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 98.0%) (16). In a typical procedure, 5 gm of manganese (II) sulphate monohydrate was added to deionized distilled water (DDW) for dissolving utilizing the technique of sonication for thirty minutes (solution A). Furthermore, 10  $\mu\text{g/ml}$  of prodigiosin was dissolved to create solution (B). The two solutions (A and B) were then fully combined in an ultra-sonication bath for half an hour at a temperature of 50 °C and a pH of 7.0 before being stored in the dark for 12 hours. Then, the mixture was placed in centrifuge and rinsed with DDW multiple times. The resulting weight residuals were then left to dry at 60 °C and stored in the darkness until needed(17).

#### **Characterization of Manganese oxide nanoparticles ( $\text{MnO}_2$ NPs)**

Different techniques were utilized in order to characterize  $\text{MnO}_2$  NPs, including ultra-violet visible light (UV-Vis) and fourier transforms infrared (FTIR) (17).

#### **Antibiotic susceptibility test**

Ten antibiotics were utilized in order to estimate the multi-drug resistance isolate of *P. aeruginosa*. These antibiotics (symbol,  $\mu\text{g}$ ) as follows: Tobramycin (TOB, 10  $\mu\text{g}$ ), Piperacillin-tazobactam (PIT, 100/10  $\mu\text{g}$ ), Meropenem (MEM, 10  $\mu\text{g}$ ), Azithromycin (AT, 30  $\mu\text{g}$ ), Ceftazidime (CAZ, 30  $\mu\text{g}$ ), Piperacillin (PRL, 100  $\mu\text{g}$ ), Ofloxacin (OF, 5  $\mu\text{g}$ ), Levofloxacin (LE, 5  $\mu\text{g}$ ), Gentamicin (CN, 10  $\mu\text{g}$ ) and Imipenem (IPM, 10  $\mu\text{g}$ ) according to CLSI (18), whereas the results were represented as resistance, intermediate and sensitive.

#### **Antibacterial test**

The agar well diffusion technique was used to determine the MIC of biologically generated Mn NPs for their antibacterial properties against the selected isolate (19). In this case, 25 ml of sterile medium made from Müller Hinton agar was poured into pre-cleaned Petri dishes and let to set overnight in a lab. The agar medium with the grown test species was expanded using the sterile cotton swab method. As a result, solutions of varying concentrations of Mn (25, 50, 100, 200  $\mu\text{g/ml}$ ) were introduced into the previously drilled wells. At 37 degrees Celsius, the obtained plates were incubated for a period of 24 hours. After that, we determined the size of the no-go area surrounding each of the prepared wells (17).

#### **Results and discussion**

##### **Isolation and identification of bacterial samples**

A total of 180 samples were subjected to various examinations. Firstly, cells of *P. aeruginosa* were given negative gram reaction and appeared as single bacterial cell or arranged in small pairs, rods. The cultural characteristics of *P. aeruginosa* were determined on MacConkey agar and Cetrimide agar. On MacConkey, colonies of this bacterium were appeared as pale shape because of *P. aeruginosa* was non-lactose fermented bacterium, while greenish-yellow color colonies of this bacterium were appeared on cetrimide agar medium, due to the ability of *P. aeruginosa* to survive with the toxic cetrimide material (12-14), as shown in (Figure 1). In total, only 60 isolates were identified as *P. aeruginosa*.

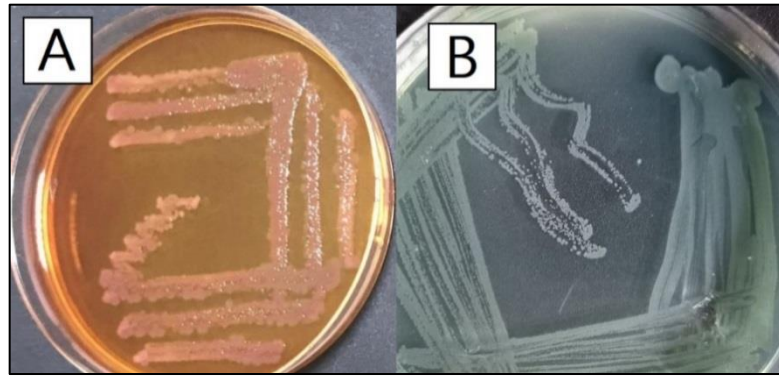


Figure (1): Colonies of *P. aeruginosa* on selective media: A) MacConkey agar, B) Cetrimide agar.

Several biochemical tests were performed to confirm that the isolates were *P. aeruginosa*. The results of all

isolates were investigated as in (Table 1)(12).

Table (1): The biochemical tests of *Pseudomonas aeruginosa* isolates

Test	Result
Oxidase	+
Catalase	+
Indole test	-
Methyl red	-
Vogues-Proskauer	-
Simmons Citrate test	+

Furthermore, the ready isolate of *Serratia marcescens* that utilized for production of prodigiosin after

performing VITEK-2 system, as shown in (Figure 2).

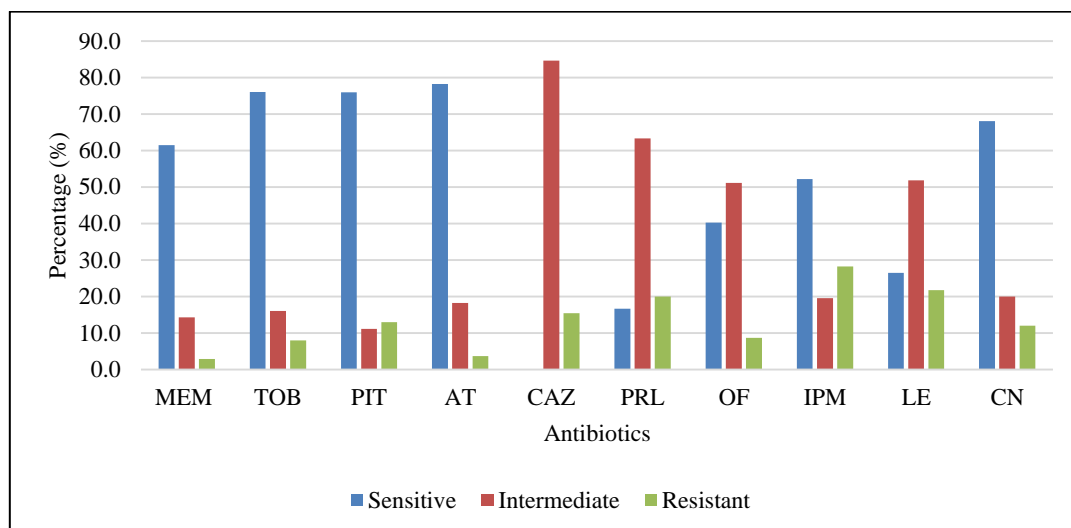
Organism Quantity:		Selected Organism : <i>Serratia marcescens</i>		Source:		Collected:											
Comments:																	
<b>Identification Information</b>		<b>Analysis Time:</b> 3.97 hours		<b>Status:</b> Final													
<b>Selected Organism</b>		99% Probability		<i>Serratia marcescens</i>													
<b>ID Analysis Messages</b>		Bionumber:		6125711455006210													
<b>Biochemical Details</b>																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	+
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure (2): Identification of *Serratia marcescens* isolate using VITEK 2 system

**Antibiotic susceptibility test**

Ten antibiotic discs were utilized for estimate the multi-drug resistant *P. aeruginosa* isolate in order to use this isolate for further steps. The results were showed that majority of *Pseudomonas aeruginosa* isolates were sensitive to antibiotics, including Meropenem, Tobramycin, Piperacillin-

tazobactam, Azithromycin and Gentamicin with 82%, 76%, 82%, 86% and 68%, respectively, as shown in (Figure 3). In addition, the majority of isolates were show intermediate resistance to Ofloxacin, Piperacillin and Levofloxacin with 74.6%, 60.3% and 86.3%, respectively (18).



**Figure (3): Antibiotic susceptibility test of *Pseudomonas aeruginosa***

The growth and spread of multidrug-resistant (MDR) strains of *P. aeruginosa* have lately become health problem for many reasons. To begin with, *P. aeruginosa* is a leading cause of death from infection, especially in hospitals and among those with impaired immune systems. Second, it can be selectively favored and disseminate antimicrobial resistance in vivo to an extraordinary degree. Third,

the rapid and widespread dissemination of "high-risk" *P. aeruginosa* clones is a hazard to global public health that must be investigated and addressed with haste and resolve (20). The multi-drug resistant isolate of *P. aeruginosa* were selected for further experiments, after performing VITEK2-system, which ensure that this isolate was *P. aeruginosa*, as shown in the following (Figure 4).

bioMérieux Customer:		Microbiology Chart Report		Printed February 10, 2023 8:22:27 AM AST	
Patient Name: Noor.		Patient ID: Clinical		Physician:	
Location:		Isolate Number: 1			
Lab ID: 142					
Organism Quantity:		Selected Organism: <i>Pseudomonas aeruginosa</i>		Source:	
Comments:				Collected:	
Identification Information		Analysis Time: 7.98 hours		Status: Final	
Selected Organism		Bionumber: 0003051103500352		Pseudomonas aeruginosa	
ID Analysis Messages					
Biochemical Details					
2	APPA (-)	3	ADO -	4	PyrA -
5	1ARL -	7	dCEL -	9	BGAL -
10	H2S -	11	BNAG -	12	AGLTp -
13	dGLU +	14	GGT +	15	OFF -
17	BGLU -	18	dMAL -	19	dMAN -
20	dMNE +	21	BXYL -	22	BAlap +
23	ProA +	26	LIP -	27	PLE -
29	TyrA +	31	URE -	32	dSOR -
33	SAC -	34	dTAG -	35	dTRE -
36	CIT +	37	MNT +	39	SKG -
40	ILATk +	41	AGLU -	42	SUCT +
43	NAGA -	44	AGAL -	45	PHOS -
46	GlyA -	47	ODC -	48	LDC -
53	1HISa +	56	CMT +	57	BGUR -
58	0129R +	59	GGAA -	61	IMLTa +
62	ELLM -	64	ILATa +		

**Figure (4): Identification of *Pseudomonas aeruginosa* using VITEK 2 system.**

**Production of prodigiosin pigment**

*Serratia marcescens* were incubated for 12 hours before their prodigiosin synthesis was initiated.

Changes in medium hue, observed predominantly during the stationary phase, may be attributable to prodigiosin accumulation (21).

## Characterization of MnO nanoparticles Ultra-violet visible light (UV-Vis) investigation

The optical characteristics of the biosynthesized MnO<sub>2</sub> NPs were studied

utilizing UV-Vis spectroscopy method. As demonstrated in (Figure 5), the attained MnO<sub>2</sub> NPs showed a pronounced UV absorption at around 440 nm (17).

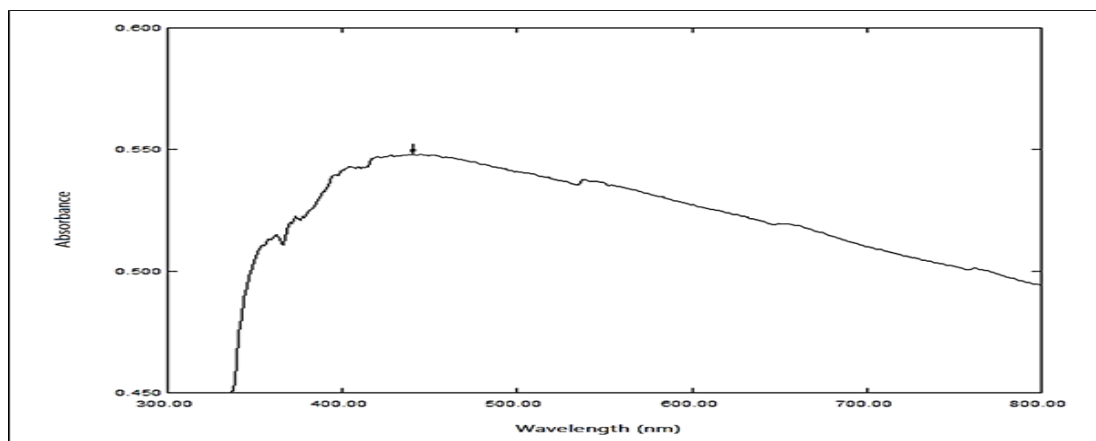


Figure (5): Spectrum of UV-Vis of the MnO NPs.

## Fourier transforms infrared (FTIR) spectroscopy analysis

(Figure 6) displays the FT-IR data for the MnO<sub>2</sub> NPs produced via biosynthesis. It is common to see a succession of absorption peaks between 400 and 4000 cm<sup>-1</sup>, which correspond to hydroxyl and carboxylate groups in the substance. In particular, the C-

Harmonics stretching mode is responsible for a broad frequency range about 3433.05 cm<sup>-1</sup>. The N-O (Nitrocompounds) stretching vibration is responsible for the majority of the other peaks around 1560.30 cm<sup>-1</sup>. In addition, the C-C (in-ring) aromatics stretching mode is responsible for the peak observed at 1537.73 cm<sup>-1</sup> (17).

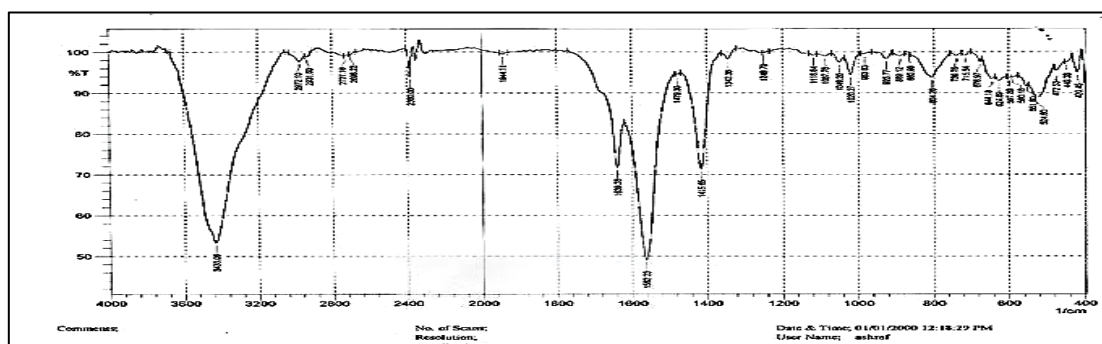


Figure (6): FTIR spectrum of the biosynthesized MnO.

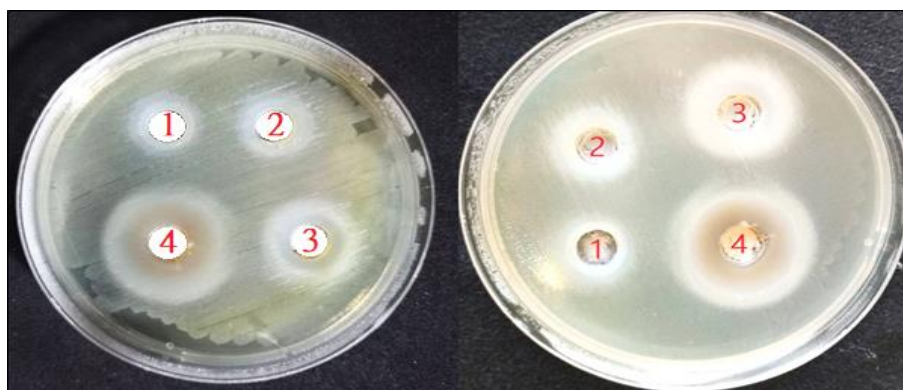
## Inhibitory activity of nanoparticles

The antibacterial activity of the biosynthesized MnO<sub>2</sub> NPs at various concentrations (25, 50, 100, 200) mg/ml is depicted in Figure 6. The antibacterial activity was found to be directly dependent upon the MnO<sub>2</sub> NPs

concentration. It was found that increasing the concentration of MnO NPs increased their antibacterial action. The maximum inhibition zones around *P. aeruginosa* isolate were 28 mm at concentration 200 mg/ml of MnO<sub>2</sub> NPs, whereas the minimum inhibition, zones

were located at 25 mg/ml MnO<sub>2</sub> NPs concentrations, were 10 mm. It is clear to be noticed that the MnO<sub>2</sub> NPs antibacterial activity is immediately dependent on the utilized concentrations. The MnO<sub>2</sub> NPs exhibited strong antibacterial activity against pathogenic bacteria,

including *P. aeruginosa* (22,23). In addition, this antimicrobial activity may attribute to prodigiosin which also exhibited bactericidal and bacteriostatic activities against several microorganisms, including *P. aeruginosa* (24) (Figure 7).



**Figure (7): Antibacterial activities of the biosynthesized MnO<sub>2</sub> NPs against *P. aeruginosa* at concentrations of: 1) 25 µg/ml, 2) 50 µg/ml, 3) 100 µg/ml and 4) 200 µg/ml.**

## Conclusion

Manganese nanoparticles that loaded on prodigiosin showed effective antibacterial activity against *P. aeruginosa*. It is clear to be noticed that the MnO<sub>2</sub> NPs antibacterial activity is immediately dependent on the utilized concentrations.

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