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Selective Cytotoxic and Antibiofilm Activities of *Pseudomonas Aeruginosa* Exotoxin A Against A549 Lung Cancer Cells

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Abstract

Background: Cancer is still one of the global leading causes of death, and there are currently therapies that have limited efficacy because they don't act on every type of cancer. This has triggered extensive curiosity about novel bioactive chemicals, such as bacterial toxins, including Exotoxin A from *P. aeruginosa*, as potential targets of anticancer treatment.

Methodology: A total of 356 clinical samples were collected and tested for *P. aeruginosa* with common biochemical methods, the VITEK II system, and PCR targeting 16S rRNA and exoA genes. Exotoxin A concentrations were measured by ELISA, and the toxin was partially purified by ammonium sulfate precipitation and ion exchange with gel filtration chromatography. A549 lung cancer cells and normal human fibroblast (NHF) cells were compared with purified toxin cytotoxicity using the MTT assay. Minimum inhibitory concentration (MIC) was measured and toxin biofilm inhibition activity was calculated.

Results: Of all samples investigated, 124 (34.8%) were *P. aeruginosa*. Among the isolates, a great resistance to antibiotics was found including ciprofloxacin. Molecular analysis confirmed *that 16S rRNA* and *ExoA* genes were present in all isolates (100%). Exotoxin A concentration varied from 1634.13 to 4793.79 pg/mL, and levels were higher in isolates that came from burns. The purified toxin had a molecular weight of about 65.8 kDa. Functional experiments demonstrated that A549 cells exhibited a potent dose-dependent cytotoxic effect while NHF cells were less affected, implying that the substance was selectively lethal. No remarkable antibacterial activity was detectable using MIC test, but there was varying antibiofilm activity for all the strains of bacteria.

Conclusion: Exotoxin A has shown to exhibit selective and major cytotoxic activity against lung cancers, and may be a candidate for targeted anti-cancer therapy. The limited antibacterial activity and the variable antibiofilm activity corroborate the selectivity of this product for eukaryotic cells and its applicability for future therapy should be better investigated.

الفعالية السُمّية الخلوية الانتقائية والمضادة للأغشية الحيوية للسم الخارجي أ المستخلص من الزائفة الزنجارية ضد خلايا سرطان الرئةA549

نبأ عبد الجبار احمد ولقاء مجيد عزيز

الخلاصة

المقدمة: لا يزال السرطان أحد الأسباب الرئيسية للوفاة على مستوى العالم، وهناك حاليًا علاجات ذات فعالية محدودة لأنها لا تؤثر على جميع أنواع السرطان. وقد أثار هذا فضولًا واسعًا حول المواد الكيميائية النشطة بيولوجيًا الجديدة، مثل السموم البكتيرية، بما في ذلك السم الخارجي أ من بكتيريا الزائفة الزنجارية، كأهداف محتملة لعلاج السرطان.

العينات وطرق العمل:

تم جمع 356 عينة سريرية وفحصها للكشف عن بكتيريا الزائفة الزنجارية باستخدام الطرق الكيميائية الحيوية الشائعة، ونظام VITEK II، وتقنية تفاعل البوليميراز المتسلسل (PCR) التي تستهدف جينات 16S rRNA وexoA. وقد تم قياس تركيزات السم الخارجي أ باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA)، وتم تنقية السم جزئيًا عن طريق الترسيب بكبريتات الأمونيوم والتبادل الأيوني باستخدام كروماتوغرافيا الترشيح الهلامي. وقد تمت مقارنة سمية السم المنقى مع خلايا سرطان الرئة A549 وخلايا الأرومة الليفية البشرية الطبيعية باستخدام اختبار MTT. وتم قياس الحد الأدنى للتركيز المثبط (MIC) وحساب نشاط تثبيط الغشاء الحيوي للسم.

النتائج:

من بين جميع العينات التي تم فحصها، كانت 124 عينة (34.8%) من نوع الزائفة الزنجارية. ولوحظت مقاومة عالية للمضادات الحيوية، بما في ذلك السيبروفلوكساسين، بين العزلات. وأكد التحليل الجزيئي وجود جينات 16SrRNA وexoA في جميع العزلات (100%). وتراوح تركيز السم الخارجي أ من 1634.13 إلى 4793.79 بيكوغرام/مل، وكانت المستويات أعلى في العزلات المأخوذة من الحروق. وبلغ الوزن الجزيئي للسم المنقى حوالي 65.8 كيلو دالتون. وأظهرت التجارب الوظيفية أن خلايا A549 أظهرت تأثيرًا سامًا قويًا يعتمد على الجرعة، بينما كانت خلايا NHF أقل تأثرًا، مما يشير إلى أن المادة قاتلة بشكل انتقائي. ولم يُرصد أي نشاط مضاد للبكتيريا ملحوظ باستخدام اختبار MIC، ولكن لوحظ تباين في النشاط المضاد للأغشية الحيوية لجميع سلالات البكتيريا.

الاستنتاج:

أظهرت السموم الخارجية من النوع أ نشاطًا سامًا للخلايا انتقائيًا وكبيرًا ضد سرطانات الرئة، وقد تكون مرشحة للعلاج الموجه للسرطان. ويؤكد النشاط المضاد للبكتيريا المحدود والنشاط المضاد للأغشية الحيوية المتفاوت انتقائية هذا المنتج للخلايا حقيقية النواة، وينبغي دراسة إمكانية استخدامه في العلاج المستقبلي بشكل أفضل.

1. Introduction

Pseudomonas aeruginosa is a type of cardiac Gram-negative rod-shaped dynamic opportunistic organism that occurs in a variety of ecological situations(Riedel *et al.*, 2019). It is a highly uncommon nosocomial pathogen, especially in immunocompromised patients, and is responsible for severe cases of severe and sometimes fatal cases of P. The pathogenic activity of *P. aeruginosa* is primarily due to its incredible plasticity, large array of virulence factors, and various regulatory networks that have allowed *P. aeruginosa* to survive to tell a story, persist in host tissues, and evade immune surveillance. The virulence of aeruginosa is determined by various factors that alter its adhesion, invasion, and persistence in host tissues(Wood *et al.*, 2023) . They all include secreted enzymes, pigments, toxins, and the ability to form biofilms. The ability of microbial pathogens to form biofilms is an essential component of chronic infections, and biofilms are key vendors that improve survival (as protection of cells from antimicrobial responses and host responses) and further induce prevention and expanded colonization. aeruginosa. aeruginosa well-known exhibits extreme resistance to more than one antibiotic, including carbapenems, which additionally complicate treatment and promote persistent infection (Pang *et al.*, 2019)(Meletis *et al.*, 2012). Among these important determinants of virulence, bacterial toxins are particularly relevant because of their direct effect on the host cell function(Riedel *et al.*, 2019). Such toxins have recently attracted significant attention as pathogenic agents, as well as potentially therapeutic targets, particularly in cancer therapies, due to their selective cytotoxic activities. Several studies have reported that bacterial toxins, such as lipopolysaccharides (LPS), exhibit cytotoxic effects against cancer cell lines including HeLa and MCF-7, reducing cell viability in a concentration-dependent manner(Moutab, Abdul-Rahman Ibrahim and Aziz, 2019; Mutaab Alqaissy and LM, 2021). Exotoxin A (PE) belongs to the most potent and best-known virulence factors from *P. aeruginosa*. It is a single-chain protein toxin that enters the extracellular environment under certain conditions, for example, limitation of iron and environmental stress(West, 2000). Exotoxin A has a good affinity for mammalian cells for efficient internalization and the subsequent disruption of cellular activities thereof. Exotoxin A cytotoxic mechanism includes the inhibition of protein biosynthesis mediated by ADP-ribosylation of elongation factor-2 (EF-2) by nicotinamide adenine dinucleotide (NAD+)(West, 2000). EF-2 inactivation due to this manipulation stops polypeptide elongation, impedes protein synthesis, and causes apoptosis and cell death. In turn because of its exact workings, Exotoxin A exhibits robust cytotoxic effects, especially in the presence of rapidly dividing cells. Cancer is still one of the common causes of death worldwide, and there is an urgent requirement for better and more selective therapeutic strategies. As we know, Exotoxin A is an excellent candidate for cancer therapy because Exotoxin A is an effective and selective tumor growth inhibitor. As well, it has been found to induce apoptosis by its inclusion into developed engineered immunotoxins, thus giving rise to a potential therapeutic target for selective anticancer activities against tumor cells(Almami, 2025). The cell line A549, from human lung carcinoma, is available to us as an in vitro model for the measurement of cytotoxicity and therapeutic efficacy based on in vitro model evaluation. It has demonstrated a robust platform for assessing toxin-induced tissue damage and the selectivity of cytotoxic agents for cancer cells(Huang *et al.*, 2024). Though Exotoxin A has a clearly established cytotoxic effect in its body, it has not been extensively reported about its antibiofilm effect as well as selective toxicity against cancer cells. Accordingly, the aims of this study is to isolate and purify Exotoxin A form bacterial samples of *P. aeruginosa* using standard biochemical methods, to evaluate its antibiofilm activity in biofilm-forming bacterial strains, and to investigate its cytotoxic characteristics in A549 cancer cell line and in normal cells by means of a MTT assay to investigate the concept of dual-functional biological therapeutic effect of Exotoxin A.

2. Materials and Methods

2.1. Sample Collection

A total of 356 anonymized clinical samples from patients with UTIs, ear, sputum, burns, and wounds were collected at Al Ramadi Teaching Hospital between July and December 2025.

2.2. Isolation and Identification of *Pseudomonas aeruginosa*

Samples from various sources like urinary tract infections (UTI), wounds, burns, and otitis media infections from patients attending hospitals who had been infected with these infections were collected. Conventional biochemical and morphological assays were performed to determine bacterial isolates. These organisms were grown on MacConkey, Blood, and Cetrimide agar (HiMedia). Morphological characteristics, dimensions and organization were analyzed using an optical microscope. Confirmation of the putative isolates was carried out using the VITEK® 2 Compact system (bioMérieux, France).

2.3. Antimicrobial Susceptibility Tests

Antibiotic susceptibility was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the guidelines set by CLSI (2025)(James *et al.*, 2025).The drugs examined included meropenem (10 µg), imipenem (10 µg), ciprofloxacin (10 µg), levofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), piperacillin/tazobac (10 µg) , and amikacin (30 µg). The results were categorized as sensitive, intermediate, or resistant based on CLSI breakpoints.

2.4. Detection of *16SrRNA* Gene and *exoA* Gene

The 1*6SrRNA* gene and *exoA* gene were identified using PCR and the sequence shown in Table1 was used. And the genes were detected according to the program shown in Table2. Amplified DNA fragments were examined by utilizing electrophoresis in agarose gel (2%). Gels have been stained with ethidium bromide and were photographed by using gel documentation system with UV light.

Table1: Primer Sequences and Expected PCR Product Sizes For *16srrna* and *exoA* Genes

Gene		Sequence of forward and reverse Primer (5′- 3′)	TM (˚C)	Product bp	Manufacturing company (Origin)
16SrRNA	F	GGTGGTTCAGCAAGTTGGAT	59	583	Microgene (Korea)
	R	CACCGGCAGTCTCCTTAGAG			
exoA	F	CTGGAGCGCAACTATCCCAC	59	191	Microgene (Korea)
	R	CCGAAGACGATGCTTTGCG			

Table2: The Program for *16SrRNA* and *exo A* Gene Amplifications.

Steps	Temperature (°C)	Time	Cycles
Initial Denaturation Activation	94	5 min	1
Denaturation	95	30 sec	30
Annealing	59	30 sec	
Extension	72	30 sec	
Final extension	72	5 min	1

2.5. Exotoxin A Detection

The level of Exotoxin A was determined using a sandwich enzyme-linked immunosorbent assay (ELISA). The concentration of Exotoxin A in the samples was measured using a specific human *P. aeruginosa* Exotoxin A (PEA) ELISA kit (Sun long Biotech Co. Ltd; catalog number SL1497Hu).

2.6.Exotoxin A Partial Purification

The procedure employed in the present study was conducted in accordance with that described by (Whitaker and Bernhard, 1972).Ammonium sulfate ($(NH_4)_2SO_4$) was added at varying saturation percentages (20, 30, 40, 50, 60, 70, 80, and 90%) to precipitate the toxin, and the resulting precipitate was separated by centrifugation at 10,000 rpm for 20 minutes.

2.7.Exotoxin A Concentration

Following centrifugation of the bacterial suspension, the resulting supernatant was concentrated from 1 L to 10 mL via sucrose dialysis within a dialysis bag.

2.8.Ion-Exchange Chromatography for Partial Purification of Exotoxin A

The purification proceeded according to ion-exchange chromatography methods previously discussed (Jawad and Rasheed, 2022). A DEAE-cellulose column (20 × 3 cm) was equilibrated with 0.01 M Tris-HCl buffer (pH 8.0). Toxin A was added to the column, and unbound proteins were removed by washing with the same buffer. Bound proteins were eluted using a linear NaCl gradient. Fractions were collected, monitored at 280 nm, and the concentration of exotoxin A was measured using ELISA.

2.9. Purification of Toxin by Gel Filtration Chromatography

The purification was accomplished by standard gel filtration chromatography. Sephacryl S-200 column (1.5 × 35 cm) was equilibrated with 0.2 M Tris-HCl buffer (pH 8.0). Toxin A (previously purified using ion-exchange chromatography) was added to the column. Elution was performed in the same buffer at constant flow rate, and fractions were collected and monitored at 280 nm. The concentration of exotoxin A in the fractions was measured by ELISA.

2.10. SDS - Polyacrylamide Gel Electrophoresis

Procedure for separating proteins is done along these lines and SDS-PAGE procedures carried out for low molecular weight protein. Protein specimen was ready and denatured prior to loading. Electrophoresis was applied at constant voltage, and protein bands, including Exotoxin A, were visualized after adding Coomassie Brilliant Blue to it.(Schägger, 2006).

2.11. Minimum Inhibitory Concentration (MIC)

The MIC of Exotoxin A was determined by a resazurin-based turbidimetric assay following the broth microdilution method (Teh *et al.*, 2017). Serial twofold dilutions of Exotoxin A were prepared in Mueller–Hinton broth in 96-well plates. To the wells, bacterial suspension adjusted to 0.5 McFarland standard was added and then incubated for 18–24 h at 37 °C. Resazurin solution was added, and plates were further incubated. A color change from blue to pink, where bacterial growth was detected, and MIC was determined to be the lowest concentration that prevented this color change.

2.12. Anti-biofilm Activity

The activity of Exotoxin A against biofilm was determined in clinical isolates of *S. aureus*, *E. coli*, and *K. pneumoniae* identified using the VITEK system and confirmed as biofilm producers. This assay was carried out via the microtiter plate method as described previously with minor changes to the procedure(Zamil, 2025). Bacterial suspension was incubated with Exotoxin A in Mueller–Hinton broth supplemented with glucose in 96-well plates. After incubation, non-adherent cells were removed from wells and washed and dried. The biofilm formed was stained with crystal violet, and the bound dye was solubilized by ethanol. Absorbance was determined with a microplate reader at 580 nm and biofilm inhibition was calculated with the standard formula.

2.13. Cell Culture Conditions

Cell lines were grown in Minimum Essential Medium (MEM; US Biological, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Capricorn Scientific, Germany). 100 IU/mL penicillin and 100 µg/mL streptomycin were added. Cells

were kept in a humidified incubator at 37 °C and 5% CO_2. Exponentially growing cells were employed for all the experiments(Easty *et al.*, 1981).

2.14. Cytotoxicity Assay

Cells were seeded at 1×10^4 cells per well, followed by incubation 24–72 h and incubation to yield a single layer of monolayer. Different concentrations of Exotoxin A were used to incubate the cells for 72 h, then added MTT solution (2 mg/mL) and added DMSO to dissolve the formazan crystals. These experiments were carried out on wells containing 96-well plates. Microplate reader measurement was conducted at 492 nm(Freshney, 2015). Cytotoxicity was determined according to the classic formulation:

$$Cytotoxicity\ (\%) = \frac{\text{OD\_control} - \text{OD\_sample}}{\text{OD\_control} \times 100} \quad (1)$$

2.15. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Two-way ANOVA was used to examine differences between isolates in terms of resistance intensity and biofilm formation capacity. Tukey's multiple comparisons test was also applied to assess the toxin's effect on normal and cancer cells. Results are expressed as mean ± standard deviation, and values were considered statistically significant at a $p \leq 0.001$ level.

2.16. Ethical Committee Approval

According to the ethical approval letter no. AS-EC/0014 dated January 13, 2026, the Research Ethics Committee of Fallujah University - College of Science- Biotechnology department granted ethical approval for this research. The committee reviewed the research concept and reviewed the sub-material and authorization for the trial as its donation, where participant consent was formed. The use of clinical samples was changed to anonymous. According to the study report, between July and December 2025, Ramadi Teaching Hospital took 356 anonymous medical samples from patients suffering from burns, wounds, ear infections, sputum and urinary tract infections Researchers promised to use the simplest of the samples for non-information, in public research. And now to not include any of the contributors to the research group in choice often in-patient care. In addition, the ethics committee authorized the researchers to replace any changes to the protocol, and any unforeseen circumstances that might arise during the course of the observations.

3. Results

3.1.Isolation and Identification

Of these 356 clinical samples, 124 isolates (34.8%) were designated as *P. aeruginosa*, as shown in Fig.1.

3.2. Antibiotic Susceptibility

Antibiotic Susceptibility The isolates exhibited remarkable resistance to ciprofloxacin, meropenem, and levofloxacin, as shown in Fig.2.

3.3. Detection of *16SrRNA* Gene and *exoA* Gene

All isolates showed significant amplification of *16S rRNA* gene confirming the identification of *P. aeruginosa*. In addition, the *exoA* gene was detected in 100% of the isolates, as shown in Figs.3 and Fig.4.

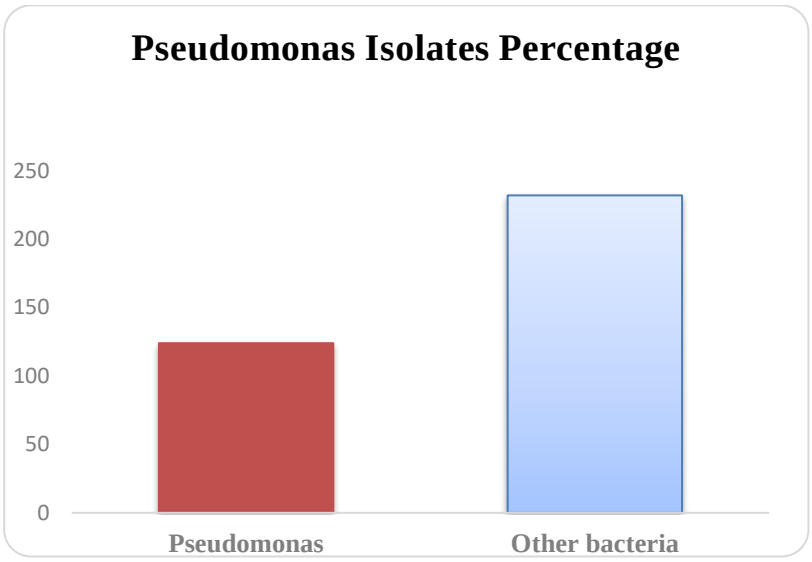

Figure1: The Percentage of *P. aeruginosa* in Different Clinical Samples.

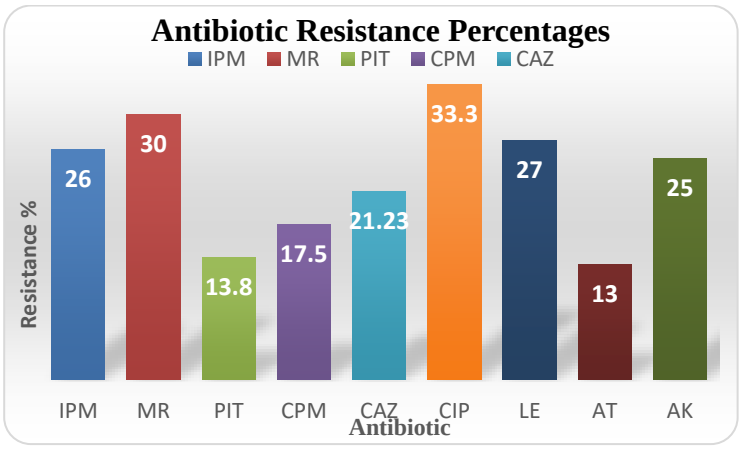

Figure2: The Distribution of Antibiotics Resistant by *p. aeruginosa* Using Disc Diffusion Method

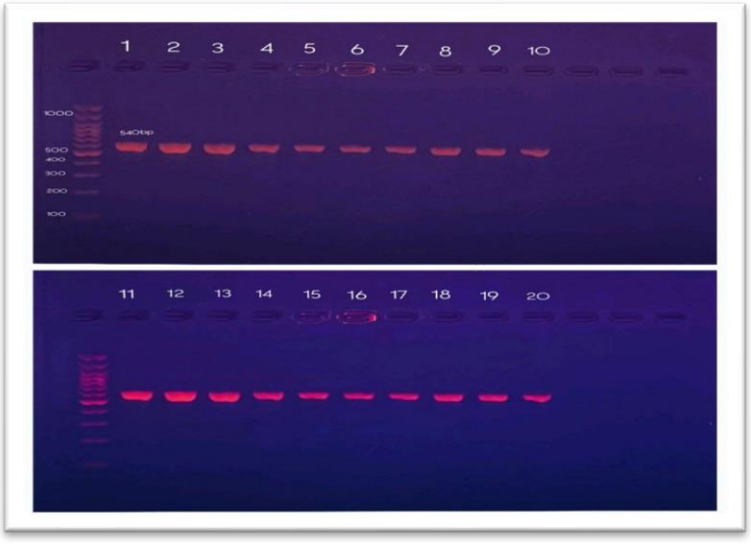

Figure3: Gel Electrophoresis Showing PCR Amplification of 16srRNA Gene of *p. aeruginosa* 540bp

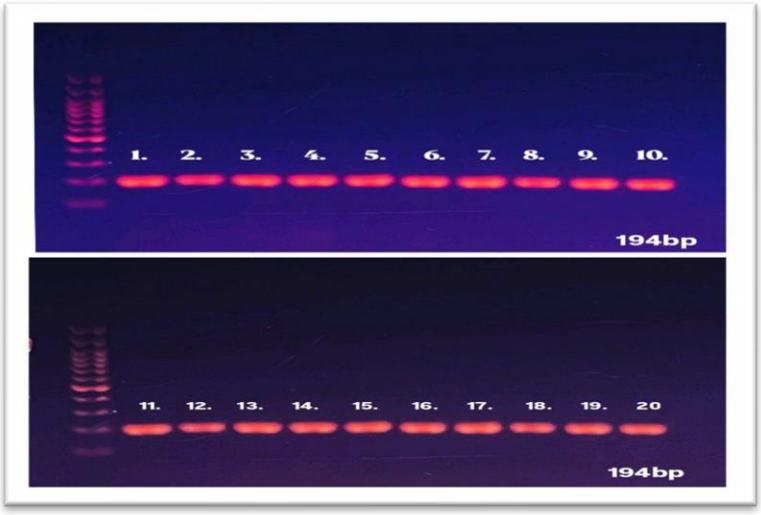

Figure4: Gel electrophoresis Showing PCR Amplification of *exoA* Gene of *p. aeruginosa* 194bp.

3.4. Exotoxin A Concentration

Exotoxin A concentrations varied from 1634.13 to 4793.79 pg/mL, with peak values detected in burn isolates, as shown Fig.5.

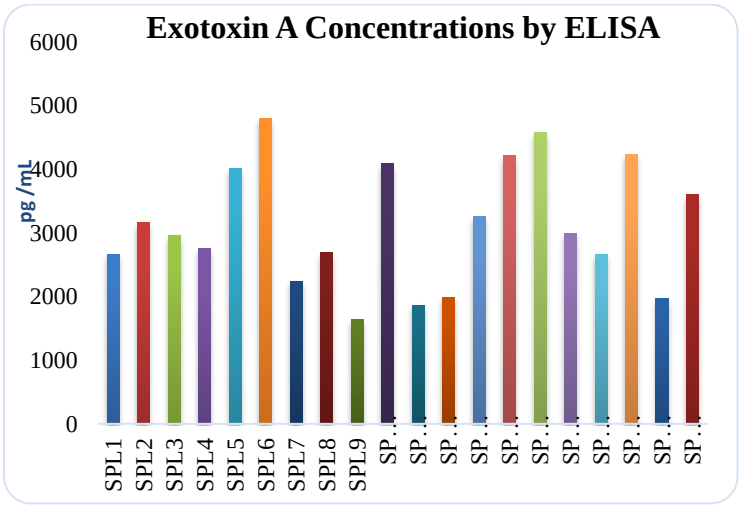

Figure5: Exotoxin A Concentrations in Clinical Isolates Measured by ELISA.

3.5. Purification and Molecular Characterization of Exotoxin A

Ion-exchange chromatography found two protein peaks around the elution, as shown in Fig.6. Gel filtration chromatography gave a single, symmetrical peak, according to Fig.7. SDS-PAGE showed a single protein band which had a molecular weight of around 65.8 kDa.

3.6. MIC Assay

No MIC value for Exotoxin A was found in the concentration range tested against the bacterial isolates examined.

3.7. Anti-biofilm Activity

Exotoxin A exhibited varying effects in biofilm production among the bacterial isolates tested. In some isolates, the emergence of biofilm was diminished, while in others increased biofilm forming appeared as determined from Fig.8.

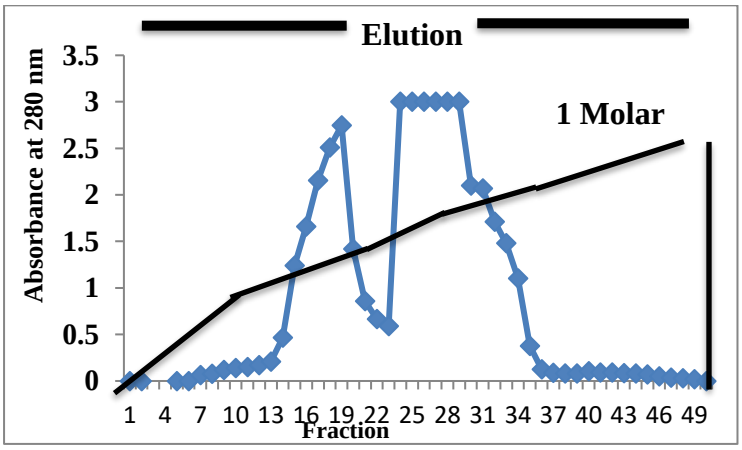

Figure6: Purification of Exotoxin A from *p. aeruginosa* by Ion Exchange Chromatography.

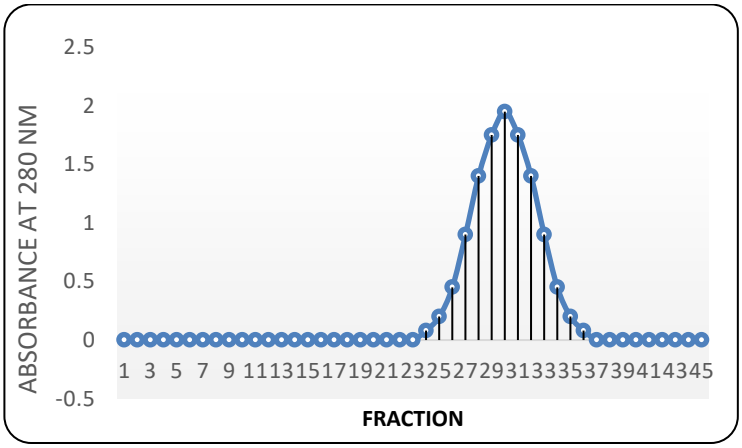

Figure7: Gel filtrations Chromatography of Toxin A.

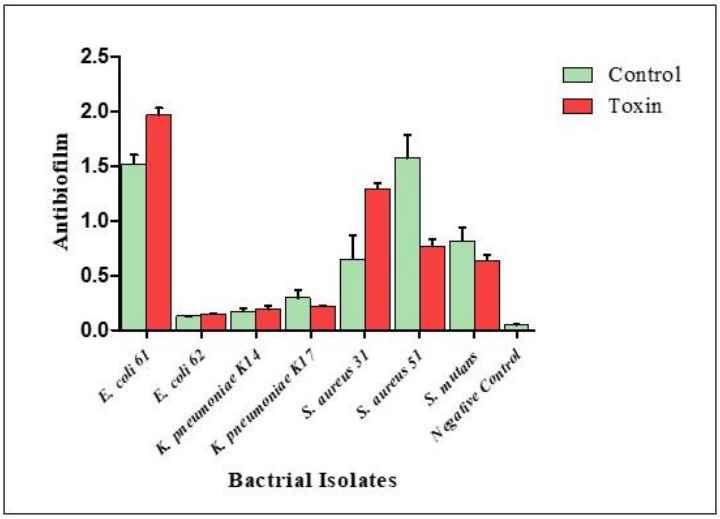

Figure8: Differential Effects of Exotoxin A on Biofilm Formation Across Bacterial Strains.

3.8. Cytotoxic and Selective Effects of Exotoxin A on A549 and NHF Cell Lines

Exotoxin A showed dose-dependent activity on A549 lung cancer cells based on their inhibitory curve depicted in Fig.9. Its greatest activity (1000 µg/mL) in decreasing cell viability was statistically significant against lower concentrations and

the control ($P < 0.05$). The lower concentrations (500, 250, 125, 62.5, and 31.25 µg/mL) exhibited decreasing cytotoxic effects, confirming a concentration-dependent response. The lowest concentration was 62.5 µg/mL, which exerted a statistically significant cytotoxic effect, while 31.25 µg/mL did not differ from the control group.

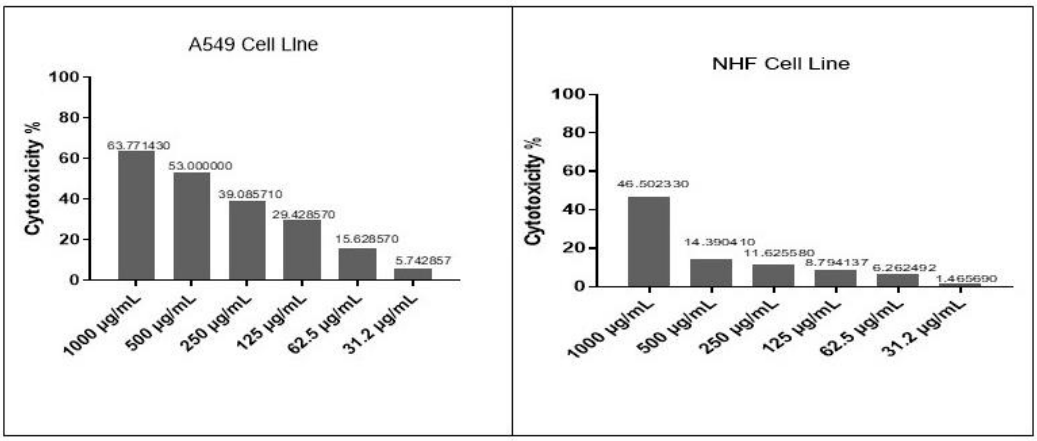

Figure9: Inhibitory Curve Showing the Effect of Exotoxin A on Cell Viability
The X-Axis Represents the Concentration of The Test Compound (In µg), And the Y-Axis *Represents* the Percentage of Cell Death (Cytotoxicity).

The IC_{50} score of Exotoxin A for A549 cells was around 151.3 µg/mL Fig.10. while the IC_{50} value against normal human fibroblast (NHF) cells was approximately 509.1 µg/mL, as shown in Fig.10.Afterward examining these results under a microscope, it was concluded that treated A549 cells had distinct apoptotic characteristics, including cell shrinkage, rounding, and nonadherence, compared to untreated cells, as shown in Fig.11. Exotoxin A induced dose-related cytotoxicity in A549 and NHF cell lines. A549 cells were more sensitive to the toxin, and the optical density value decreased markedly with increasing concentrations of the toxin (particularly at 1000 and 500 µg/mL). The sensitivity of NHF cells was lower, with less pronounced optical density changes at lower toxin concentrations. Statistical analysis determined that there were significant differences at higher concentrations, and lower concentrations had non-significant effects (ns), as illustrated in Fig.12.

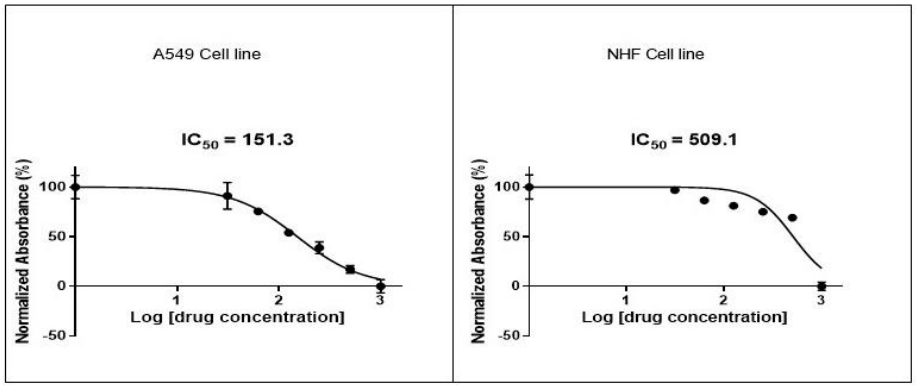

Figure10: Dose-response curve used to determine the IC50 value of Exotoxin A on cancer cells. The x-axis represents the concentration of the test compound (in µg), and the y-axis represents the percentage of cell inhibition. The IC50 value, represented by the Log of drug concentration of the test compound X that inhibits 50% of cell viability.

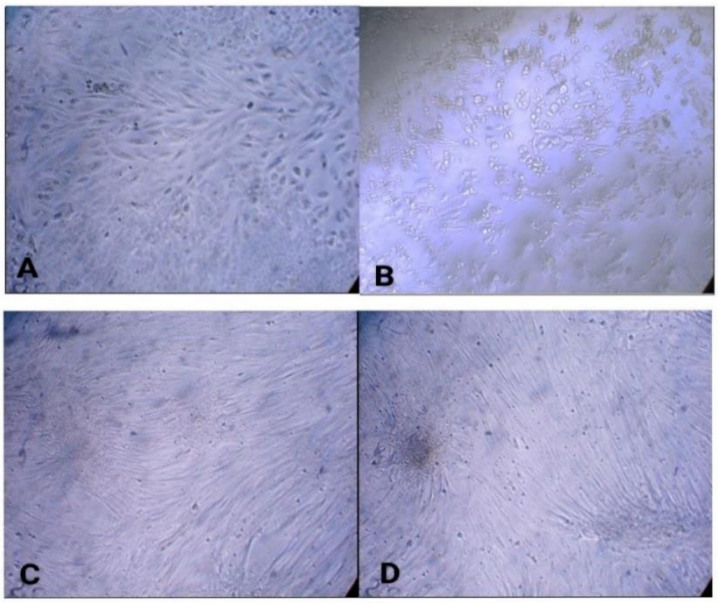

Figure11: Morphological changes in A549 and NHF Cells Before and After Treatment with Exotoxin A. A: A549 cells (un traeted), B: A549 cells (traeted), C: NHF: cells (un traeted), D: NHF cells (traeted).of the test compound X that inhibits 50% of cell viability.

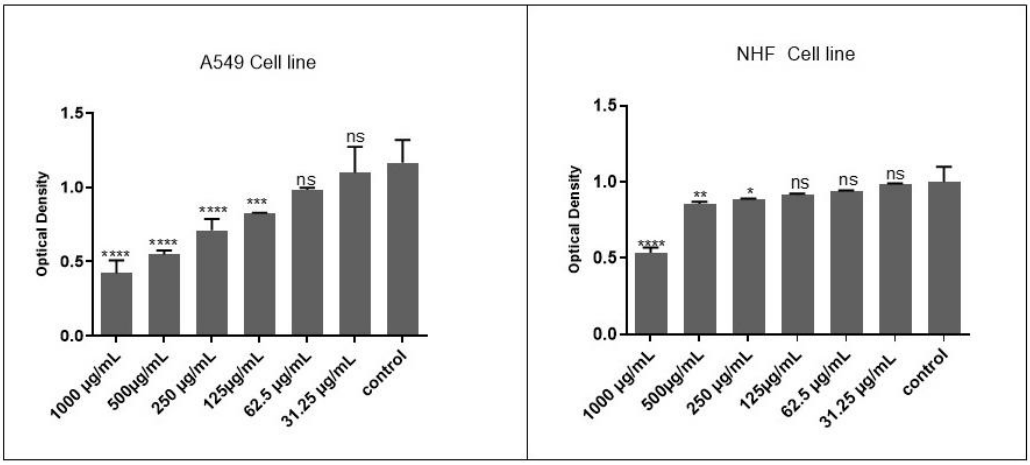

Figure12: Optical Density Reading Curve

4. Discussion

The prevalence of *Pseudomonas aeruginosa* in this study was relatively high compared with previous reports. (Yaseen and Hassan, 2025) reported similar prevalence rates, while lower prevalence rates were documented in other studies(Asamenew *et al.*, 2023)(Maharjan, 2022). Variations among studies may be associated with differences in sample types, hospital environments, patient populations, and infection sources, particularly in burn and wound infections where *P. aeruginosa* is highly prevalent (Vidaillac and Chotirmall, 2021). The high resistance observed against ciprofloxacin, meropenem, and

levofloxacin may be associated with several resistance mechanisms including efflux pump overexpression, porin loss, β-lactamase production, and target-site mutations, which are frequently reported among multidrug-resistant *P. aeruginosa* strains (Elmosallamy *et al.*, 2024)Zhu *et al.*, 2025). The detection of the *16S rRNA* gene in all isolates verified the molecular identification of *P. aeruginosa*, confirming previous molecular investigations on clinical isolates(Saeed, Mayea and Shalal, 2023) . Also, the *exoA* gene was found in all isolates (100%), which shows the broad distribution of this virulence determinant in clinical strains (Ghazaei, 2022; SALMAN, 2015). The high concentrations of Exotoxin A in burn isolates might be a consequence of the heightened pathogenicity often associated with burn-associated *P. aeruginosa* infections. (El-Shaer *et al.*, 2016) reported similar findings. Other mechanisms regulating exotoxin production in clinical isolates include quorum sensing systems and environmental stress conditions (Schaber *et al.*, 2004). Ion-exchange chromatography also showed two separate protein peaks during elution, indicating that Exotoxin A was partially purified, and the gel filtration chromatography indicated that there was a single peak representing one symmetrical peak, this led to a higher purified protein. SDS-PAGE shows that there is one protein band at ~65.8 kDa which results in calculation of the predicted molecular weight in Exotoxin A (Lory, Strom and Johnson, 1988) was measured to give the predicted molecular weight as well. The effectiveness of the stepwise purification is confirmed by these findings. Given the absence of a detectable MIC value, it is unlikely that Exotoxin A is serving primarily as a traditional antibacterial agent. Earlier reports indicated that Exotoxin A mainly exerts antagonistic effects at eukaryotic cells on its inhibition of protein synthesis (exposing a non-antibacterial capability)(Zhang *et al.*, 2008). This finding is consistent with that of the selective biological activity observed in the present study. The antibiofilm assay yielded different responses for bacterial isolates. It is possible that incomplete disruption of cellular metabolic activity or quorum sensing pathways may be the cause of diminished biofilm production in some isolates. Whereas increased biofilm production, seen in some isolates, may point towards stress-induced or hormetic responses triggered by toxin exposure (Hashmi *et al.*, 2014). Other inconsistencies in the antibiofilm responses have been reported in previously evaluated studies quantifying bacterial metabolites and toxins used against biofilm-forming isolates (Zhang *et al.*, 2008). The current study showed that A549 lung cancer cells exposed to exotoxin A exhibited dose-dependent cytotoxicity, for which increasing doses resulted in increased reduction in cell viability. Cancer activity was highest at 1000 µg/mL and decreases at lower concentrations followed by decreasing effects, providing the mechanism of action at a concentration-dependent scale. The cytotoxic effect of Exotoxin A probably depends on its strong inhibition of protein synthesis via ADP-ribosylation of elongation factor-2 (EF-2), eventually killing cells. A549 cells respond to toxin more than NHF normal cells, a mechanism that could potentially be linked to high metabolic activity and proliferation of cancer cells. The data are consistent with previous studies done in a concentration-dependent manner by (Moutab, Abdul-Rahman Ibrahim and Aziz, 2019; Mutaab Alqaissy and LM, 2021) confirming the cytotoxic effect of bacterial products on cancer cell lines of HeLa and MCF-7 cells. Similarly, the lesser cytotoxicity of NHF cells in this current study may also exhibit a selective impact towards malignant cells. The IC_{50} of A549 cells is significantly lower than the value for NHF cells, indicating selective cytotoxicity toward malignant cells. This selective response confirms previous studies indicating Exotoxin A's potential as a targeted anticancer agent, particularly when included in the context of engineered immunotoxins (Almami, 2025). Morphological study revealed typical apoptotic changes for A549 cells: cell shrinkage, rounding, and detachment from the culture surface. From the previous work demonstrating that Exotoxin A induced apoptosis via inhibition of protein synthesis by ADP-ribosylation of elongation factor-2 (EF-2) (Zhang *et al.*, 2008;Huang *et al.*, 2024) , these findings are consistent. The fact that NHF cells have lower sensitivity towards Exotoxin A at lower concentrations of toxin also confirms Exotoxin A's selective action against cancer cells. While cytotoxic effects were detected at higher concentrations, as observed through optical density readings, normal cells were still relatively less

affected than A549 cells. These results indicate that Exotoxin A may have the potential to preferentially attack malignant cells while exerting reduced toxicity toward normal tissues. The present study demonstrated that Exotoxin A shows its potential for selective anticancer activity against A549 lung cancer cells with comparatively lower activity against normal fibroblast cells. Such findings support Exotoxin A as a selective therapeutic candidate for cancer treatment.

Conclusion

The study demonstrated that medical isolates of *Pseudomonas aeruginosa* produced Exotoxin A, which was partially purified and was detected by an ~65.8 kDa protein band that was consistent with the expected molecular weight. Indeed, Exotoxin A demonstrated no consistent antibacterial activity suggesting that its major effect tends towards eukaryotic cell rather than bacterial inhibition. Cytotoxicity assays demonstrated dose-dependent toxic effect for A549 lung cancer cells (IC50 = 151.3 µg/mL), and for normal NHF cells IC50 = 509.1 µg/mL (indicated selective toxicity against cancer cells). Anti-biofilm activity varied among bacterial isolates, showing inconsistent effects under different conditions. These results point out the importance of Exotoxin A as a selective anticancer, rather than a classical antibacterial approach. To do so, the purification accuracy should be increased, identification of protein using sophisticated molecular technologies, mechanisms of selectivity should also be explored, safety and efficacy of the sample used in larger in vitro as well as in vivo models, should also be investigated.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding this study.

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