

## ISOLATION AND PURIFICATION OF ANTICANCER PROTEIN EXOTOXIN A FROM *Pseudomonas aeruginosa*

<sup>1</sup>L. Q. Jawad  
Researcher

<sup>2</sup>H. A. R. R. Rasheed  
Lecturer

<sup>1</sup>AL-Esraa University College, Baghdad, IRAQ

<sup>2</sup>Dept. Biotech. Sci. Col., University of Baghdad, Baghdad Iraq

layla\_k\_j@outlook.com

hala.aljendeel@gmail.com

### ABSTRACT

Toxins produced from bacteria constitute promising antitumor agents in treating different cancer types. Exotoxin A from *Pseudomonas aeruginosa* is the highly toxic virulence component that bind to specific cell receptors. This study aimed to purify Exotoxin A from clinically isolated *Pseudomonas aeruginosa*. A total of 150 bacterial isolates were taken from clinical samples (burn and wounds) and were screened on selective media and identified as *P. aeruginosa* using biochemical analysis and molecular test by PCR amplification technique utilizing specific primer 16srRNA. Exotoxin A produced from *P. aeruginosa* were screened and purified by two steps that include gel filtration and ion-exchange chromatography. In this study, 68 isolates were characterized as *P. aeruginosa*, furthermore, real-time PCR proved that these isolates revealed 100% specificity, sensitivity and had positive amplified bands with a size of 249bp. The concentration of Exotoxin A extracted from *P. aeruginosa* using Trypticase soy broth was 18 Mg/ml. The percent of Purification and recovery for Exotoxin A was 21.4 %, 40% respectively. Clinical Isolates of *Pseudomonas aeruginosa* have the potential to produce Exotoxin A that is responsible for pathogenicity.

**Keywords:** bacterial isolates, biochemical analysis, PCR, toxins, ion exchange, gel filtration column.

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عزل وتنقية بروتين مضاد للسرطان Exotoxin A من بكتريا *Pseudomonas aeruginosa*

حلا عبد الكريم رشيد  
مدرس

ليلى قيس جواد  
باحث

قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

المستخلص

تشكل السموم الناتجة من البكتريا عاملاً مضاداً للأورام وواعداً في علاج أنواع السرطان المختلفة. Exotoxin A الناتج من *pseudomonas aeruginosa* هو من المكونات الفوعة شديدة السمية التي ترتبط بمستقبلات خلوية معينة. تهدف هذه الدراسة على تنقية الـ Exotoxin A المعزول من العينات السريرية لبكتريا *pseudomonas aeruginosa*. وجد انه من مجموع 150 عذلة بكتيريا تم عزلها من عينات سريرية (جروح وحروق) تم فحصها على وسط انتقائي وتحديدتها على انها *p. aeruginosa* باستخدام التحليل الكيميائي الحيوي والاختبار الجزيئي بتقنية التضخيم PCR باستخدام برايمر محدد 16srRNA، تم تأكيد تشخيص التحليل الكيميائي الحيوي باستخدام جهاز VITEK-2 Compact system. تم غرلة عزلات *P. aeruginosa* المنتجة للـ Exotoxin A وتنقية الـ Exotoxin A على مرحلتين الأولى تتضمن الترشيح الهلامي والثانية التبادل الايوني الكروماتوغرافي. في هذه الدراسة تم تصنيف 68 عذلة على انها *p. aeruginosa*، بالإضافة الى ذلك اثبت تشخيص PCR انها 100% *P. aeruginosa* حيث تظهر بنطاقات مكبرة موجبة بحجم 249 نقطة أساس. كان تركيز Exotoxin A المستخلص من *P. aeruginosa* باستخدام وسط 18 Trypticase soy broth 18 مل/مل كانت نسبه التنقية والاسترداد للـ Exotoxin A 21.4% و 40% على التوالي. العزلات السريرية من *Pseudomonas aeruginosa* لديها القدرة على انتاج Exotoxin A المسؤول عن الامراضية.

الكلمات المفتاحية: العزلات البكتيرية، التحليل الكيميائي الحيوي، تقنية تضخيم PCR، السموم، التبادل الايوني واعمدة الترشيح الهلامي.

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## INTRODUCTION

*Pseudomonas aeruginosa* is a common bacteria that can infect immunocompromised people and cause nosocomial infections in hospitals (27). This gram-negative bacterium has the rod form is bacilli non-sporulation motile by polar flagellum aerobic (3). And an opportunistic pathogen that causes different types of infections (Example skin, eyes, ears, respiratory tract, urinary tract, sepsis originating from the stomach, softness Infections of the skin, of the bone, and the joints) (4), is a bacterial pathogen taking responsibility for a large part of the burning units and surgery wards in hospitals (23). It is also the main source of chronic lung infections that lead to the deaths of cystic fibrosis patients (22). *Pseudomonas aeruginosa* Generation of virulence factors such as alkaline protease, Elastase, hemolysin, and pyocyanin that could cause harm to Tissue like a stream of blood (20). This bacterium was found to be one of the causative agents of bacteria causing bacteremia in the patient's tissue or organ transplant, causing approximately 28% of bacteremia at all (2). A very toxic virulence component produced naturally by *P. aeruginosa* is exotoxin A (PE), This toxin was first detected by Liu, A strain that is deficient in protease production was considered a good producer of toxin compared with the strain that was actively producing protease (9,29). Exotoxin A is a heat-labile single polypeptide, consisted of 613 amino acid series with a molecular weight of 66 kilodalton (25,18), Exotoxin A which inhibits protein biosynthesis (27). These types of toxin enhance the transference of ADP ribose from NAD<sup>+</sup> (nicotinamide adenine dinucleotide) to diphthamide, which leads to suppress the synthesis of protein and consequently lead to eukaryotic cells death (18), which kills the cells through inhibition of protein synthesis, formed by *Pseudomonas aeruginosa*. The main PE domains elucidated by their crystallographic structure contain the Ia domain (1-252 amino acids), which combines for the receptor of the R2 macroglobulin sited in cells of the animal. Domain II (253-364 amino acids), Contains a proteolytic position placed from 279 to 280 amino acids for furin cleavage, and a translocating a sequence of the

protein (amino acids 280-313) besides Domain III (amino acids 400-613), containing the enzyme ADP-ribosylation in amino acids (400-602), Replacing last one 5 PE amino acids, REDLK, endoplasmic KDEL retention sequence reticulum (ER) boosts cytotoxicity, the standard model which explains how exotoxin A produced from *Pseudomonas aeruginosa* killing cells by several steps: The exotoxin binds with their receptors, The protease furin cause cleavage in amino acids 279 and 280 and is affected into endosomes then unfolds at low pH. The bond of disulfide is reduced between Cys265 and Cys287, and the toxin's carboxylic terminus combines to the receptor of KDEL and is moved from the trans-Golgi To ER where the sequences of domain II encourage cytoplasmic protein translocation, possibly over pre-existing ER holes, the catalytic elongation factor2 of toxin ADP-ribosylates in the cytosol, (EF2)-EF2 Obstructs protein synthesis and participate in the programmed death of the cell (15). It was shown that the introduction by microinjection of just a few PE molecules into a cell's cytoplasm is enough to destroy the cell (15). PE-based recombinant toxins have been produced to kill cancer cells, containing different binding domains. With just a few hundred locations per cell, these immunotoxins can destroy cells (11), even though barely a small proportion of the molecules binding toxins in the cytoplasm ultimately enters (14). if the toxin is administered ineffectively to the cytosol, the targeted cell may avoid intoxication. Therefore, it is of clinical significance to elucidate and refine the measures required for PE to cellular intoxication (15). Is exotoxin A (PE) generated in vitro by most *P. aeruginosa* clinical isolates yet occurs to be generated in humans through infections with *P. aeruginosa*. Toxin in its natural state loses the complete enzymatic function. Maximum activity of ribosylation of ADP is explained once toxin is denaturized, AND after *Pseudomonas* proteases cleave it to yield 37-kDa fragment active in the enzyme (21). the *Pseudomonas aeruginosa* toxin is secreted and is altered widely through cell selection to yield and transmit an enzymatic fragment (37-kDa) to the cytosol, the first phase in the intoxication

method includes binding PE toxin to definite receptors after that endocytosis activated by the receptor(7,28) Powerful emission of toxins shows a significant role in the disease-producing pathogenesis microorganisms, As these factors may function on goals that are far from the colonization site (10). Excretion means the movement of a polypeptide from the site of synthesis. For cytoplasmic and outer membranes, this latter lacks a proton motive power and energy source. While an outer layer contains prions, which allow the hydrophilic solutes to pass freely the pore size is very small to enable the free distribution of large proteins on periplasm (29). This research was performed on extraction of exotoxin A and purification it from clinical isolates of *Pseudomonas aeruginosa* in Iraq.

## MATERIALS AND METHODS

### Collection of samples

During this study, 150 swabs were collected from clinical samples (burn and wounds) from different hospitals: Teaching Hospital of Baghdad Medical city, Al-Yarmouk hospital. Al-Kindi hospital, and another private laboratory for the period Between October 2019 to January 2020, these samples were incubated at 4 °C during transportation. The bacterial isolates were isolated by culturing in Brain heart infusion broth and incubated at 37 °C for 24h, then inoculated using the loopful on selective media (MacConkey agar) with incubation at 37 °C for 24h.

### Bacterial Identification

All samples identified by morphology, biochemical test, VITEK-2 System, and Molecular identification

#### Morphological identification

The bacteria have been examined after their morphological properties Defined by Cetrinide agar

Depending on colonies shape, color, texture, and edges. The capability of these colonies to produce pigment as pyocyanin and pyoverdine were examined (17).

### Microscope examination

The developing colonies were examined applying a light microscope used for their slide staining along with gram-stain, shape, cell arrangement, spore formation, and the existence of capsule (5).

### Biochemical test

The biochemical examinations for the colonies were obtained and as follows: MacConky agar, Simmon`s citrate, Catalase test, Oxidase test, to check the biochemical examination, the Vitek-2 compact device used growth-based technology to classify the bacteria in clinical samples as an automated microbiology analyzer device (12).

### Cetrinide Agar (Production of pyocyanin pigment)

The bacteria`s capability to produce pyocyanin pigment on cetrinide agar were identified, the isolated colonies were then incubated at 37°C for 24hrs, the presence of green color indicates positive results (12).

### Growth at 42 °C

The bacterial opportunity to grow at 42°C was examined, it was sub-cultured on the plate of nutrient agar then incubated for 24 hrs. The positive result at the temperature of 42°C indicated the presence of growth (12).

### Molecular identification

The molecular methods were used to characterize and identify the isolates, PCR amplification technique was used to analyze phylogenetic tree of specific 16srRNA gene in pathogenic strain of *Pseudomonas aeruginosa*.

**Table 1. Oligonucleotide primer sequences used for PCR amplification of 16S Rrna**

	Primer sequence	Product size
16s(f) Forward	5'CGG ACC TCA CGC TAT CAG AT3'	249bp
16s(r) Reverse	5'CTG CCC TTC CTC CCA ACT TA3'	

PCR was run in a programmable thermocycler having an initial delay at 95 °C for 5 min and final delay at 72 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30sec., annealing at 58 °C for 1min followed by extension at 72 °C for 30 sec. PCR amplified product was resolved in a 2% agarose gel by electrophoresis and analyzed using gel documentation system. The reaction setup for PCR is illustrated in Table 2.

**Table 2. PCR amplification reaction**

Step	Temperature	Time	Number of cycle
Initial denaturation	95 °C	5 min.	1 cycle
denaturation	94 °C	30 sec.	30 cycle
Annealing	58 °C	1min.	
Extension	72 °C	30sec.	
Final extension	72 °C	5min.	1 cycle

### Screening the *Pseudomonas aeruginosa* isolates to produce protease enzyme

#### Protease test

The isolates of *Pseudomonas aeruginosa* were screened in the agar of skim milk for their ability in producing protease enzyme (12)

#### Culture conditions

The cultures were grown at 32°C for 20 to 24 hrs. in a shaker incubator at 250 rpm. To get extreme aeration, 50 ml of the culture was prepared in 300 ml flask. To adjust the cell density, 1ml of culture was taken to prepare appropriate dilution with normal saline using a reader at 620nm, the inoculum was prepared with initial absorbance 0.5. The growth curve was estimated as OD. per unit time. Spectrophotometers measure the fraction of light that is absorbed by a solution and report that as absorbance units. The absorption reads after every two hours during the 24hrs then draws a growth curve (8).

#### Production of exotoxin A

*Pseudomonas aeruginosa* was grown in Tryptic soy broth to produce exotoxin A. the production media were prepared by melting 30g of powder in 100 ml distilled water. then, the media were dialyzed overnight at 4°C against 0.01M tris- OH buffer using a magnetic stirrer. The dialysate that goes out of the dialysis bag were completed to the volume of 1000ml with distilled water and transferred to 1L flask medium was sterilized using autoclave for 15 minutes at 121°C, monosodium glutamate (0.05M) and glycerol (1%) were filter sterilized using 0.45Mm filter and were added to the dialyzed medium. 50ml of the medium was distributed in every 20 sterile flasks. Then 0.5ml of inoculum was added to the flasks and incubated using a shaker incubator (150 rpm) for 18h at 32°C. in

the last part, the bacterial suspensions were centrifugated for 20 min at 10,000xg.

Exotoxin A concentration was measured using a specific sandwich ELISA kit (Sun long Biotech Co. Ltd; catalog number SL1497Hu)

#### Exotoxin A Concentration

Directly, the supernatant from centrifuged bacterial suspension was concentrated with sucrose from 1L to 10 ml using a dialysis bag.

#### Exotoxin A Purification by Gel Filtration Chromatography with Sephadex G-150

Sephadex G-150 gel column 0.5×30 cm was formulated as suggested by means of the company of Pharmacia Fine Chemicals. The 0.01 M of Tris -OH buffer (pH 8) was used to wash and equilibrate the column; 10 milliliters of concentrated crude extract were applied. Using the same equilibration buffer, the elution was accomplished a rate of flow around (3 ml/fraction) and absorption of all fractions was measured at 280 nm by using the UV spectrophotometer. The peaks were gathered and checked for Exotoxin A and protein concentrations.

#### Toxin purification using Ion exchange chromatography

Ion exchange chromatography was made as stated by Whitaker and Bernard in 1972. A CMC-column 0.5×30cm was adjusted and washed several times with 0.01 M Tris-OH pH8. 6 milliliters of the concentrated toxin purified by the Sephadex G-150 column was added to the column. In wash steps, the samples were collected at a flow rate of 3 ml/fraction. The bound proteins were eluted using NaCl in a linear gradient from 0.5 to 1 M with 0.01 M Tris-OH buffer pH8. All the obtained samples were measured by UV spectrophotometer at 280 nm, then tested for exotoxin A and protein concentrations.

#### Determination of Protein concentration

According to the Bradford method, the concentration of proteins was determined (11). A typical bovine serum albumin curve was performed by using a stock solution containing different concentrations of BSA (20,40,60,80,100 mg/ml). Then 2.5ml of Coomassie G-250 and 400 µl of tris- HCL were mixed and left at room temperature for 2 min, the blank composed of 500 µl from the buffer of Tris-HCL and 2.5 ml from reagent of Bradford, then absorbance for test and blank

was measured at 595 nm. A standard curve between the concentrations and absorbance of BSA was plotted. The concentration of protein in toxin samples was measured by mixing 100µl of a sample containing toxin dissolved in the buffer of Tris-HCL exposed to same earlier addition, then their absorbance was documented at 595nm.

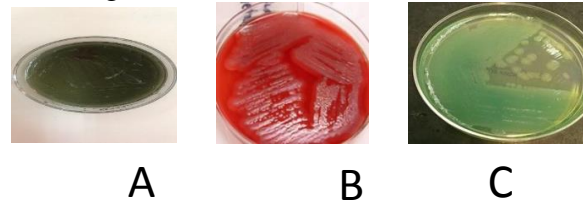
## RESULTS AND DISCUSSION

**Isolation and identificatio of Pseudomonas aeruginosa:** Findings showed that amongst 150 samples obtained from various clinical sites (burns and wounds) of patients in Teaching Hospital of Baghdad Medical city, Al-Yarmouk hospital. Al-Kindi hospital, and other private laboratories, Between October 2019 to January 2020 infection, only 68 isolates suggest typical morphological features, biochemical test, and molecular identification belonged to *P. aeruginosa*, whereas the remaining 82 isolates related to different infective bacteria.

### Identify infective isolates

Detection of *Pseudomonas aeruginosa* relay on bacterial isolation and identification of bacteria in a laboratory, which is Identified according to its morphology with Gram stain, and straight appearance or a little curved, Non-sporulating, mobile, gram-negative rod with aerobic growth. Characterization of bacterial colonies using gram staining methods where the bacterial sample was found as gram-negative with rod form, non-spore-forming, straight or little curved, that is agreed with Kenneth, (13). The characteristics of morphology were performed upon the colonial shape and forming colorless colonies with 1-3 mm in diameter after cultivation on MacConkey Agar because of non-lactose fermentation. In brain heart infusion agar *P. aeruginosa* exhibited mucoid with green color characteristics. In general, clinical samples produce a smooth colony, which has a big, smooth fried egg form with smooth rims and an elevated appearance. The presence of green color on cetrimide agar identify the bacteria's capability to produce pyocyanin pigment which indicates a positive result for *P. aeruginosa*. Cultivation on Cetrimide agar (N-acetyl-NNN-trimethyl-ammonium-bromide) is used to prevent bacterial growth except *Pseudomonas aeruginosa*. Its acts as a

quaternary structure cationic ammonium cleanser that is responsible for releasing phosphor and nitrogen from bacterial strain except for *P. aeruginosa* (24). Additionally, the capability of *pseudomonas aeruginosa* to make pigment was detected through cultivation on cetrimide agar for pyocyanin production (water-soluble, phenazine, blue-green, non-fluorescent pigment) which is activated in the presence of potassium sulfate and magnesium chloride in the broth (13).



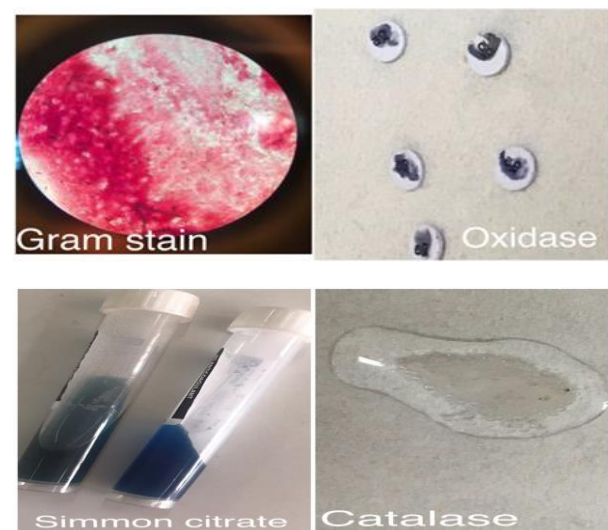
**Figure 1. culturing of Pseudomonas aeruginosa in A/Brain heart infusion agar B/ MacConkey Agar C/ cetrimide agar**

### Biochemical tests

Bacterial isolate based on analyses of morphological and, microscopic characteristics. Was subjected to several biochemical tests, which showed that only 68 of the 150 isolates were suspected of belonging to *Pseudomonas aeruginosa spp.*

**Table 3. Biochemical test used in *p. aeruginosa* identification**

Biochemical test	<i>P. aeruginosa</i>
Oxidase	+
Catalase	+
Simmons Citrate	+
Gram stain	-



**Figure 2. Biochemical test used in *p. aeruginosa* identification**



To confirm the biochemical examination, the Vitek-2 compact device used growth-based technology to classify the bacteria in clinical samples as an automated microbiology analyzer device.

bioMérieux Customer: Microbiology Chart Report Printed Sep 1, 2020 21:33 CDT

Patient Name: Location: Patient ID: Physician: Lab ID: 03 Isolate Number: 2

Organism Quantity: Selected Organism : *Pseudomonas aeruginosa*

Source: Collected:

Comments:

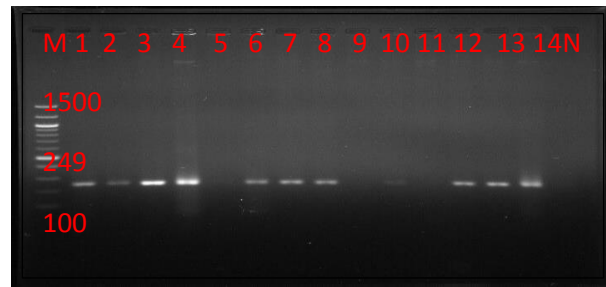
Identification Information	Analysis Time: 4.82 hours	Status: Final
Selected Organism	99% Probability <i>Pseudomonas aeruginosa</i> Bionumber: 0003053003500000	
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	βCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	αGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	βMAL	-	19	βMAN	-	20	βMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	-	32	αSOR	-
33	SAC	-	34	αTAG	-	35	αTRE	-	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	βHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

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**Figure 3. Vitek II test for *p. aeruginosa***

**Molecular identification** Total Genomic DNA was extracted from 150 isolates of *pseudomonas spp.* to perform a molecular identification and detection of *pseudomonas aeruginosa*. Genetic identification using 16SrRNA was submitted using specific primers as shown in (Table 1) and PCR amplification techniques as revealed in (table 2), the results revealed that all 68 isolates (100 %) had positive amplified bands with a size of 249bp.



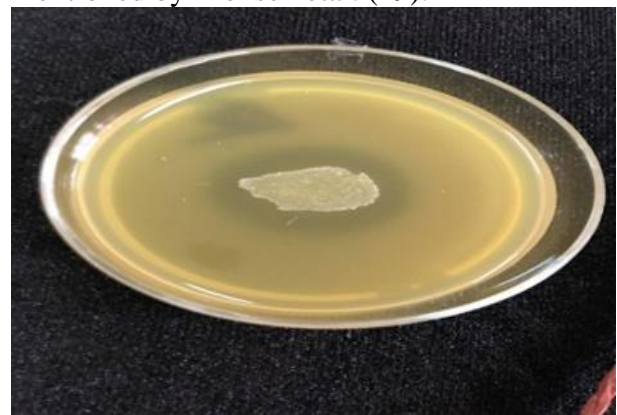
**Figure 4. Gel electrophoresis showing PCR amplification of 16srRNA Gene of *p. aeruginosa* 249bp**

#### Growth at 42 °C

Colonies cultured at 42°C for 24 hours on nutrient agar, showed the ability to grow up at this temperature, and the positive result was indicated by the presence of suggested growth.

#### Protease generating enzyme

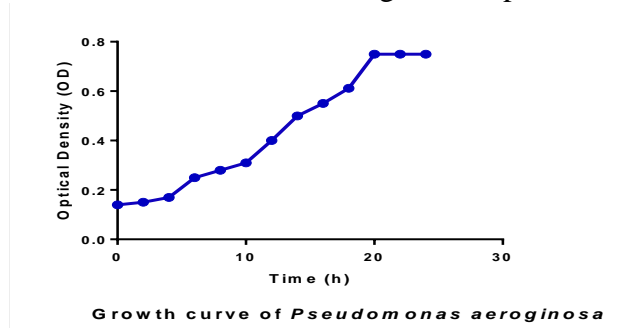
From the total of 68 bacterial isolates that belonged to *P. aeruginosa spp.*, only 54 strains (79.41%) provide positive results for the production of protease besides 14 isolates (20.59%) revealed negative test for protease after cultured in skim milk agar, our result disagrees with Alane et al. (1), who confirmed that the ratio of isolated *P. aeruginosa* that is positive to protease test and exotoxin A were more than 95% (1). The negative protease strains were considered toxin A producers because the positive strain of *pseudomonas aeruginosa* destroys the exotoxin by protease enzyme, it is compatible with Shahanra et al. (26), Who confirmed that Exotoxin A were purified from the supernatant of non-proteolytic strains of *P. aeruginosa*, PA 103, by a number of physicochemical processes (26). Furthermore, numbers of evidence indicate that in *P. aeruginosa* culture, the toxin is deactivated via proteolytic enzymes as mentioned by Mohsen et al. (19).



**Figure 5. *Pseudomonas aeruginosa* positive to Protease test**

### Culture conditions

For optimum cell production of Exotoxin A, the cells should be at the log-phase growth. The end of Log-phase growth of *Pseudomonas aeruginosa* were achieved following 18 hours of incubation in Tryptic soy broth with inoculum 0.5 at 37°C, shaking in 150 rpm.



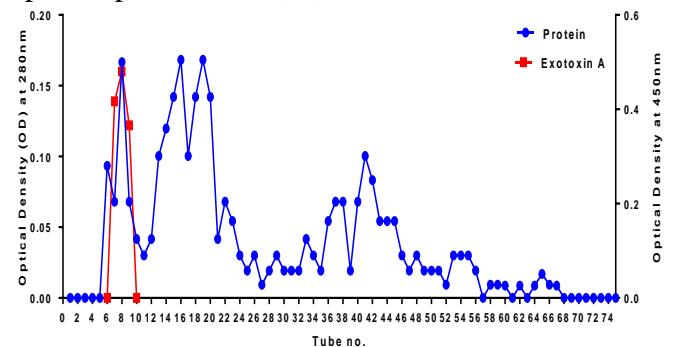
**Figure 6. Growth curve of *Pseudomonas aeruginosa***

**Exotoxin A Production:** The exotoxin A production by *P. aeruginosa* was achieved using a dialyzable part of Trypticase soy broth (TSBD), supplemented with 1% glycerol and 0.05M monosodium glutamate. The sandwich ELISA technique was applied to determine exotoxin A concentration in supernatants of cultures. Standard curve was used to calculate the total concentration of exotoxin A by plotting the absorbance values against different concentrations of purified exotoxin A that added to wells (0.15ng to 1.8ng/ well).

### Purification of toxin by Gel Filtration Chromatography with Sephadex G-150

Exotoxin A purification were done using column chromatography methods. The crude of Exotoxin A was concentrated with sucrose and applied to Sephadex G-150. The elution curves achieved in these methods are illustrated in figure 7, which revealed several protein peaks with one peak contained the exotoxin A, the percent of purification and recovery of exotoxin A were 7.18 % and 70%, respectively that illustrated in (table 4). Chromatographic purification using Sephadex column G-150 revealed that one toxin peak and several protein peaks appeared following elution of the exotoxin A using 0.01 M of Tris -OH buffer (pH 8). Fractions that contained exotoxin A were collected, combined for applying to immunological assay to measure the concentration of exotoxin A and total protein concentration. The result showed a significant loss of exotoxin A, that is

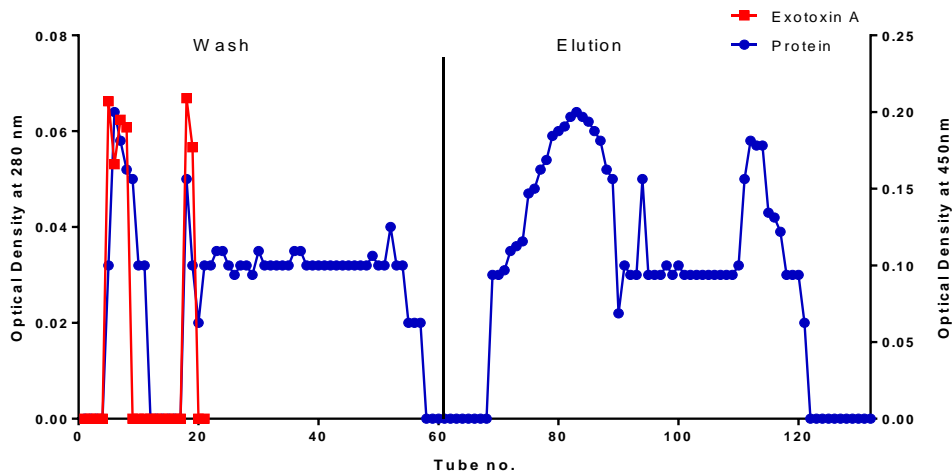
compatible with Liu *et al.* (16) who reported the same loss in toxin during gel filtration with a partial purification (16).



**Figure 7. Exotoxin A purification using chromatographic gel filtration (Sephadex G-150)**

### Ion exchange chromatography Purification of PE:

The second step in the purification of exotoxin A was Ion exchange method, after purifies exotoxin A using gel filtration. fractions that contain the exotoxin A were collected and applied to *Carboxymethyl cellulose column (CMC)* equilibrated with 0.01M Tris- OH, then Sodium chloride (NaCl) was added with gradient concentration from 0.5 to 1 M with a buffer of 0.01 M Tris-OH pH8.0. A volume of 9 ml of exotoxin A was loaded to the CMC column and then eluted using the buffer supplemented with NaCl gradients. The results are shown in figure (8) revealed the presence of two peaks of protein and two peaks of exotoxin A in the wash step. In the Elution step, only three peaks of protein were indicated with no toxin activity. The percent of purification and recovery of exotoxin A in the first peak was 21.4 % and 40%, respectively. While the second peak exhibited 17.74 purity% and 18.3 recovery% that illustrated in (table 4). Ion exchange chromatography with CMC, this step produced further purification for exotoxin A, the result revealed two protein peaks and two Toxin peaks through the washing step of the CMC column due to the negatively charged toxin which is confirmed by Masuyer.(18) who describes the structure of exotoxin A which have a unique structure containing a metal-binding site with a cleft that negatively charged and play important role in the interaction among interdomain that eventually effect on toxicity (18). The purified exotoxin A for two peak exhibits a total increase in purity of about 21.4 and 17.74, receptively .



**Figure 8. Exotoxin A purification using Ion exchange chromatography (CMC) column with dimension 0.5x30 cm and the Flow rate was 30 ml/hrs**

**Table 4. The purification steps of exotoxin A from *Pseudomonas aeruginosa***

Purification Stage	Volume (ml)	Protein (Mg/ml)	Total Protein (Mg)	Exotoxine conc. (Mg/ml)	Total Exotoxine conc.(Mg)	Recovery%	Purity%
Supernatant	10	370	3700	18	180	100	4.87
Sephadix G-150	9	195	1755	14	126	70	7.18
cmc first peak	12	28	336	6	72	40	21.4
cmc second peak	6	31	186	5.5	33	18.3	17.74

## CONCLUSION

In conclusion, the bacterial isolates of *P. aeruginosa* were isolated from burn and wound infection. These isolate especially the strains that are not producing protease enzyme, showed the capability to secrete a considerable amount of Exotoxin A in the production medium. While the protease producing strain of *P. aeruginosa* showed no or decreased amount of Exotoxin A. the purification protocol of exotoxin A, used in this paper, was accomplished with the recovery of around 40% and purity about 21.4%, which is assumed as an acceptable ratio to measure the cytotoxic effect of exotoxin A on cancer cell lines which is now in progress.

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