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Anticancer Activity of Partially Purified L-glutaminase Produced from *Pseudomonas aeruginosa* P48

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Abstract

The global need for the treatment of cancer diseases has been directed towards the use of enzymes, as these diseases have worsened in recent years for several reasons, including chemical and physical pollutants. L-glutaminase is one of the enzymes used in the treatment of tumors, as it converts the amino acid glutamine into glutamic acid, which helps inhibit the growth of cancer cells. Therefore, this study aimed to produce and purify L-glutaminase from a local isolate of *P. aeruginosa* P48 and evaluate its anticancer activity. The result of PCR with the 16SrRNA gene for identification of *P. aeruginosa* P48 showed that the *16SrRNA* gene (956bp) was presented as a single band on agarose gel as an indicator that this species was *P. aeruginosa* isolate. The L-glutaminase from *P. aeruginosa* P48 was purified utilizing ion exchange chromatography after precipitation via ammonium sulfate (0-75%), with a yield of 108.8%, a purification fold 2.189 and the specific activity was 9.5453U/mg protein. The enzyme has a molecular weight of 39 KDa. The results of the cytotoxic impact utilizing the MTT experiment indicated that partially purified L-glutaminase caused a reduction in cell viability ($p \leq 0.05$) at a dose-dependent manner on HL-60 cell lines, with a calculating IC_{50} of 143.9 µg/ml, compare with normal cell line (HdFn Cell Line) at IC_{50} of 187.3 µg/ml. After conducting an investigation of its anticancer activity, the findings were demonstrated that L-glutaminase has potential therapeutic uses in the treatment of cancer.

Keywords: L-glutaminase, *P. aeruginosa*, PCR, Anticancer activity.

النشاط المضاد للسرطان لإنزيم ل–كلوتامينيز المنقى جزئيًا والمُنتج من بكتيريا الزائفة الزنجارية P48

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الخلاصة

لقد اتجهت الحاجة العالمية لعلاج أمراض السرطان نحو استخدام الإنزيمات، حيث تفاقمت هذه الأمراض في السنوات الأخيرة لأسباب عدة منها الملوثات الكيميائية والفيزيائية. ويعد ل–كلوتامينيز أحد الإنزيمات المستخدمة في علاج الأورام، حيث يحول حامض الجلوتامين الأميني إلى حامض الجلوتاميك الذي يساعد في تثبيط نمو الخلايا السرطانية. لذلك كان الهدف من هذه الدراسة هو إنتاج وتنقية انزيم ل–كلوتامينيز من العزلة المحلية لبكتريا الزائفة الزنجارية P48، وتقييم نشاطه المضاد للسرطان. أظهرت نتيجة تفاعل البوليميراز المتسلسل مع جين *16SrRNA gene* (956bp) لتحديد بكتريا الزائفة الزنجارية P48، جين *16SrRNA gene* (956bp) كشريط مفرد على هلام الأكاروز كمؤشر على أن هذه العزلة تعود الى بكتريا الزائفة الزنجارية. تم

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تنقية ل-كلوتاميناز من بكتريا الزائفة الزنجارية P48، باستخدام كروماتوغرافيا التبادل الأيوني بعد الترسيب بكبريتات الأمونيوم (0-75٪)، مع حاصل 108.8٪، وعدد مرات تنقية حوالي 2.189 وكانت الفعالية النوعية 9.5453 وحدة/ملغرام من البروتين. ايضاً كان الوزن الجزيئي للإنزيم 39 كيلو دالتون. أشارت نتائج التأثير السام للخلايا باستخدام تجربة MTT إلى أن انزيم ل- كلوتاميناز المنقى جزئيًا قد تسبب في انخفاض حيوية الخلايا ($p \leq 0.05$) بطريقة تعتمد على الجرعة على خطوط خلايا HL-60، مع حساب IC_{50} حوالي 143.9 ميكروغرام / مل، مقارنة بخط الخلية الطبيعي (خط الخلية HdFn) عند IC_{50} بحوالي 187.3 ميكروغرام / مل. وبعد إجراء تحقيق حول نشاط الانزيم المضاد للسرطان، أظهرت النتائج ان انزيم ل-كلوتاميناز له استخدامات علاجيه محتمله في علاج السرطان.

1. Introduction

L-glutaminase (GLS) is an amidohydrolase that plays a role in the metabolism of glutamine, an amino acid that supports various bodily functions and converts it into glutamic acid. This enzyme has been produced or generated via a variety of sources, such as plants and microorganisms. The L-glutaminase has several important applications, including medical and industrial applications. Further, L-glutaminase has uses in HIV therapy and could be utilized as a cancer treatment. It also plays an indirect role in improving food flavour via increasing the L-glutamate content of intermediate ingredients [1]. L-glutaminase is considered to be a conditionally essential amino acid, although it is the most useful and abundant amino acid in the body. This is due to the fact that it becomes more difficult for the human body to synthesis sufficient amounts of L-glutaminase in response to stress or ageing, which can put a variety of cellular and bodily functions at risk [2]. As a result of their catalytic capacity to deaminate glutamine into ammonia and glutamic acid, L-glutaminase has garnered a lot of research over the course of the last 10 years. Along with the majority of other microbial enzymes, L-glutaminase produced via microorganisms is an appropriate replacement for enzymes produced via higher organisms [3]. Because the enzyme production from microorganisms is relatively rapid, inexpensive and easy [4]. According to Masisi *et al.*, the roles of L-glutaminase in various cancers have been extensively reviewed in the last few years [5]. One of the many uses for L-glutaminase is in the pharmaceutical and food sectors, among others, due to their unique capacity to stimulate the glutamines' deamidation into glutamic acids. There is hope that L-glutaminase may replace chemotherapy because of its shown capacity to drastically reduce the growth of some cancer cell lines [6]. Many different kinds of microbes, including bacteria, fungi, yeasts, and actinomycetes, have the ability to produced L-glutaminases [7]. *Pseudomonas* species can produce an impactive amount of L-glutaminase enzyme [8]. An opportunistic bacterium resistant to a variety of medications and disinfectants, *P. aeruginosa* is a species of significant medical importance that causes severe chronic and acute nosocomial infectious illnesses in patients who have had burns, catheterizations, or immunocompromised conditions [9]. *P. aeruginosa* can be known as one of the major reasons of extremely infectious diseases such as systemic pneumonia, skin inflammations, cystic fibrosis, burns, and some infections associated with septicemia, bacteremia, blood and cornea, respiratory tract, gastrointestinal, soft tissue joint and bone, and urinary tract, infectious diseases particularly in individuals with transplantation and neutropenia [10]. Some *P. aeruginosa* isolates are a kind of multi-drug resistance (MDR) bacteria that may be found in almost every setting, whether it's an animal or a plant [11]. Although some *P. aeruginosa* are pathogenic, these bacteria also produce different beneficial enzymes, including proteases, amylases, L-glutaminase etc. [12, 13]. Hence, the recent work aimed to partially purify and characterise of L-glutaminases produced from *P. aeruginosa* P48 and evaluate its anticancer activity.

2. Material and method

Identification of L-glutaminase-producing isolate

A previously isolated *P.aeruginosa* P48, after screening for its ability to produce the L-glutaminase, which was obtained from wound burn infection and identified with different examinations. After that, according to Tawfeeq *et al.,* [14], the genomic DNA was obtained from *P. aeruginosa* P48 by utilizing the HiPurA® bacterial genomic DNA purification kit. The primers used in this study for the *16SrRNA* gene (956bp) were forward `5-GGGGGATCTTCGGACCTCA-3` and reverse `5- CCTTAGAGTGCCCACCCG-3`[15]. The PCR reaction mixture consisted of 25 µl total volume, containing 12.5 µl of Master Mix, 1 µl of forward primer (10 pmol/µl), 1 µl of reverse primer, 6.5 µl of nuclease-free water, and 4 µl of genomic DNA template.Thermal cycling was carried out under the following conditions: an initial denaturation step at 95°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for 40 seconds. A final extension was performed at 72°C for 1 minute.

Extraction of L-glutaminase enzyme

After an incubation period of twenty-four hours, the enzyme from each flask was extracted via centrifuging the flasks for 10 minutes at 10,000 rpm. Then, Whatman filter paper No. 1 was utilized to filtrate the remaining liquid. It was determined that the supernatant may be used as a crude enzyme.

Determination of activity of L-glutaminase

The method described by Nadaf *et al.,*[16] was followed to determine the L-glutaminase enzyme activity. A 0.1 ml of crude enzyme sample was mixed with 2.5 ml of substrate stock solution (glutamine) and prepared in duplicate for all samples. The mixture was incubated in a water bath at 37 °C with shaking for 30 minutes. After the end of the incubation period, the reaction was stopped by adding 0.5 ml of trichloroacetic acid (TCA 5 %). The blank sample was also prepared by mixing the enzyme sample (0.1 ml) with 2.5 ml of Tris-HCl buffer (0.1 M) with a pH of 8.6 and. After the incubation period and adding TCA, 0.5 ml of the enzyme sample was added to the blank sample. After that, all tubes were centrifuged at a speed of 6000 rpm for 20 minutes, and the resulting sediment was discarded, and the clear solution was transferred to clean test tubes to determine its concentrations of release ammonia according to the direct Nesslerization method. The ammonia concentration was prepared in triplicate for all samples by mixing distilled water (4.4 ml) with the sample whose ammonia concentration (0.1 ml) was required to be detected and Nessler reagent (0.5 ml). The mixture was well mixed with a vortex for one minute. Then the absorbance was measured at the wavelength of 436 nm. For the preparation of a blank sample, 4.5 milliliters of distilled water were combined with 0.5 milliliters of Nessler reagent, and the same procedure was utilized as before. On two separate occasions, the concentration of protein and enzyme activity were measured, and the findings were reported based on the average of the two measurements. Following that, the enzymatic activity and specific activity were assessed in accordance with the following:

$$\text{Enzyme activity } \left({}^{\text{U}}/_{ml}\right) = \text{Concentration of released ammonia}/30 \times 14$$

$$\text{Concentration of released ammonia } \left(\frac{\mu g}{ml}\right) = \frac{\text{Absorbance at wavelength at 436 nm}}{\text{Slope}}$$

Where14 is a Constant and 30 is Reactive time (min.)

Protein concentration determination

According to Al-Sa'ady and Aziz [4], the Bradford method was used in order to measure the concentration of protein via utilizing a standard protein, Bovine Serum Albumin (BSA).

Specific activity determination

The specific activity of an enzyme was calculated as the following:

$$\text{Specific activity}\left(\frac{\text{U}}{\text{mg}}\text{protein}\right)=\frac{\text{Enzyme activity}\left(\frac{\text{U}}{\text{ml}}\right)}{\text{Protein concentration}\left(\frac{\text{mg}}{\text{ml}}\right)}$$

Purification of L-glutaminase

Through the use of (0-75%) ammonium sulfate precipitation and ion exchange chromatography [17], L-glutaminase was produced from a local isolate of *P. aeruginosa* P48 and then purified.

Ammonium sulfate precipitation and ion exchange separation

At a temperature of 4 degrees Celsius, solid ammonium sulfate ranging from 0-75% was progressively added into the crude enzyme (100 ml). The components were then mixed carefully for 2.45 hours. After that, the mixture was centrifuged at a speed of 10,000 revolutions per minute for twenty minutes, and the supernatant was thrown away. A buffer containing 0.2 M potassium phosphate (pH 7) was used to dissolve the precipitate, which was made up of 10 milliliters of the buffer. It was feasible to ascertain both the activity of the enzyme and the amount of protein present. According to the method suggested via [18], ion exchange chromatography (DEAE-Cellulose) was employed for L-glutaminase purification. After the phase that included the precipitation of ammonium sulfate, ten milliliters of the concentrated enzyme were transferred onto a DEAE-Cellulose column with dimensions of 23 centimeters via 1.7 centimeters. This was accomplished by moving a clean Pasteur pipette in a circular motion on the wall of the column. An equilibrating solution of Tris-HCl with a pH of 8 and a concentration of 0.2 M was used to wash the column at a flow rate of 30 millilitres per hour, with 3 millilitres being used for each fraction. This process was repeated until the optical density at 280 nm was found to be zero. The elution step was performed with a gradient concentration of sodium chloride that ranged from zero to one millimolar and was created in the same buffer. This process was done at room temperature. At a wavelength of 280 nm, the protein fractions of the washed and eluted fractions were measured. Then, the portions of the protein peaks that had enzymatic activity were collected.

Determination of L-glutaminase molecular weight

The molecular weight of L-glutaminase was determined using a Sephadex G-100 column (73×1.5 cm), which was carried out in accordance with the guidelines provided by the pharmaceutical business (Pharmacia-Sweden). The column was allowed to equilibrate overnight at a flow rate of 30 ml/hour utilizing a 0.02 M Tris-HCl buffer at a pH of 8.0. A 2 ml solution of blue dextran 2000, L-glutaminase and standard proteins (such as trypsin, BSA, aldolase, and urease) were injected separately into the column, followed collection of fractions, then tested the absorbency at 600 nm for dye and 280 nm for proteins [19]. The (ve/vo) ratio was calculated for each standard protein and for the separated fractions of partially purified L-glutaminase proteins, then standardization was done by plotting the elution volume (Ve) of each standard proteins to the void volume (Vo) of the blue dextran 2000 (Ve/Vo) versus the log value of molecular weight.

Cytotoxic impact of partially purified L-glutaminase

Following the protocol outlined in a study [20], the in vitro method was utilized to assess the potential cytotoxic impacts of L-glutaminase. Its cytotoxicity was investigated on HL-60 tumor

cells, HdFn normal cells, leukemia cells, and human dermal fibroblasts at different doses. Ascar *et al*., agreed that the MTT cytotoxic impact test should be conducted according to the manufacturer's instructions [21]. The cells were grown in 96-well microplates at a concentration of 200 µL per well, ranging from $1x10^4$ to $1x10^6$ cells /mL. After gently shaking the microplates and sealing them with sterile parafilm, they were put in an incubator set at 37 °C with 5% CO_2 for 24 hours. Wells were filled with 200 µL of a 2-fold serial dilution of the purified L-glutaminase (6.26, 12.50 ,25, 50, 100, 200, 400 µg /mL) after incubation, with the medium removed. At each control and concentration, a triplicate was performed. After 48 hours in an incubator set with 5% CO_2 at 37°C, the microplates were removed. Every well was then treated with 10 mL of MTT solution after exposure to L-glutaminase. After that, for four hours at 37°C with 5% CO_2, the microplates were put in an incubator. Subsequently, 100µL of dissolution was carefully added to each well after the medium was carefully removed, and the microplates were put in an incubator for 5 minutes. A 575 nm ELISA reader (Bio-rad, Germany) was utilized to assess optical density. For each cell line, the optical density data were evaluated statistically to estimate the required concentration to cause a 50% drop in cell viability to every cell line, through the following equation:

$$Y= D+ A-D / 1+10^{(x-logC)B}$$

A one-way ANOVA test was utilized to evaluate all of the data obtained for this investigation, and post-analysis was carried out utilizing the Duncan test, non-parametric analysis, descriptive statistics, and Mann-Whitney for independent samples. The findings were presented as mean ± SD, and $P < 0.05$ values were considered statistically significant. Graph Pad Prism version six was used to calculate statistical significance and express data as mean standard deviation (GraphPad Software Inc., La Jolla, CA).

3. Results and Discussion

Diagnosis of P. aeruginosa utilizing PCR

The current findings showed that the *16SrRNA gene* (956bp) was presented as a single band on agarose gel as an indicator that this species was *P. aeruginosa* isolates (Figure 1).

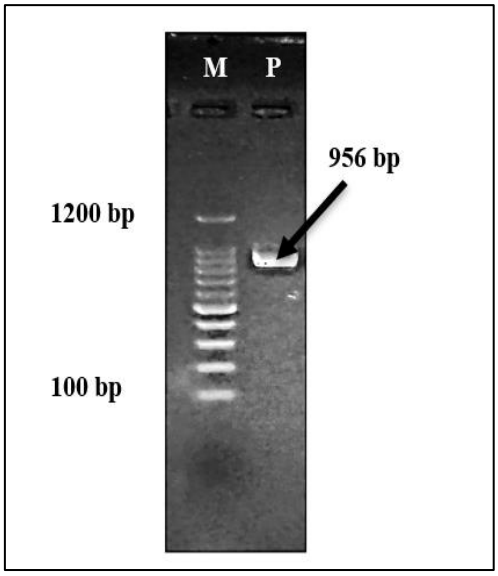

Figure 1: Agarose gel electrophoresis (1.5% agarose, 60 min at 70 volt) of amplifications of the *16SrRNA* gene (956 bp) done on agarose stained with ethidium bromide. M: DNA ladder (1200 bp); P: RCR product of 956 bp of *16SrRNA*.

Purification of L-glutaminase

Through the use of ammonium sulfate precipitation, the crude extract was refined. For the purpose of this investigation, the ammonium sulfate saturation ratios ranging from 0-75% were utilized for the L-glutaminase precipitation. After that, the second step for L-glutaminase purification after precipitation via ammonium sulfate saturation ratio (75%), the ion exchange chromatography technique (DEAE-cellulose) was utilized for this process. The findings revealed that fractions (11-21) had just one peak of enzyme activity and three protein peaks in the wash stage. L-glutaminase bears a positive charge comparable to the charge of the resin under experimental circumstances, according to the findings of the fractions with activity that were collected. Figure 2 shows an elution step that utilized gradient salt and yielded two peaks, and no L-glutaminase activity was detected. When the specific activity of this stage was 9.5453 U/mg proteins, and the fold of purification was 2.189, as shown in Table 1, the yield reached 108.8%. The reason for the increase in yield is due to the presence of inhibitors in the crude enzyme. These inhibitors were removed during ammonium sulfate precipitation.

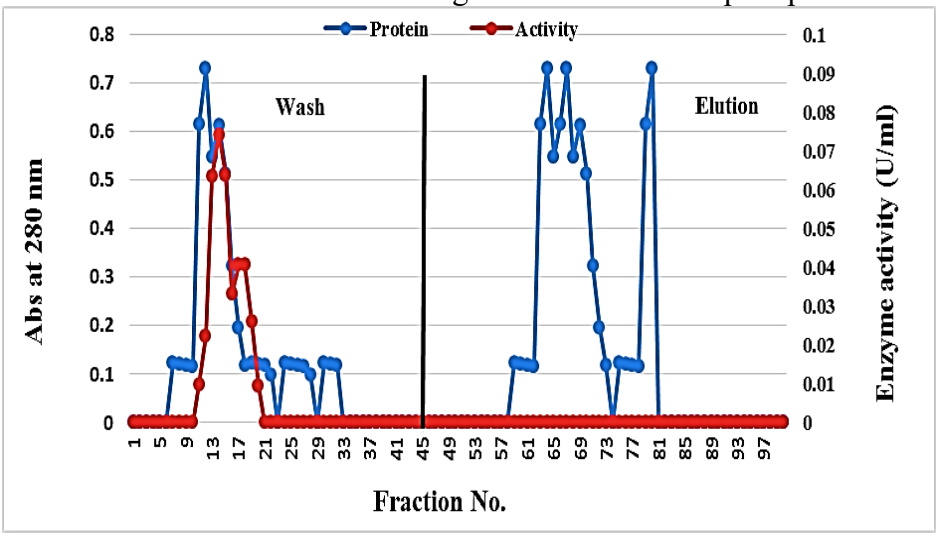

Figure 2: Purification of L-glutaminase enzyme from *P. aeruginosa* P48 utilizing ion exchange chromatography technique on a DEAE-Cellulose column of 23×1.7 cm. washed with Tris-HCl buffer (0.2 M, pH 8.0), and eluted with Tris-HCI buffer with a NaCl gradient (0.1-1 M) at 30 ml/hr as flow rate, with 3 ml for each fraction.

The charge differences-dependent separation principle is simple; the potential for reactivation for use many times, large capacity, simple handling, high power of resolution and good separation, are only a few of the numerous benefits offered via DEAE-Cellulose resin [22]. The binding of the protein to the resin depends upon the pI value of the protein and the pH of the buffer solution in which the DEAE-Cellulose is equilibrated [23]. Sephadex G-100 was utilized in a subsequent study to further purify L-glutaminase that had been previously purified utilizing the ion exchange chromatography method [24].

Table 1: The purification step of L-glutaminase from *P. aeruginosa* P48 isolate

Purification Steps	Volume (ml)	Enzyme Activity (U/ml)	Protein Concentration (mg/ml) (Mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification Fold	Yield (%)
Crude enzyme	**100**	**0.01**	**0.0024**	**4.36**	**1**	**1**	**100**
Ammonium sulfate precipitation (0-75%)	**10**	**0.12**	**0.0178**	**6.66**	**1.2**	**1.5**	**120**
Ion exchange chromatography (DEAE-Cellulose)	**24**	**0.0453**	**0.0048**	**9.5453**	**1.088**	**2.189**	**108.8**

Determination of molecular weight of L-glutaminase

An estimation of the molecular weight of L-glutaminase was made via gel filtration chromatography. Figure 3 shows that the molecular weight of L-glutaminase gained from *P. aeruginosa* was about 39000 Da, suggesting that the (Ve/Vo) ratio of this purified protein was approximately 1.81, placing it between bovine serum albumin (67,000 Da) and trypsin (23,000 Da) as standard proteins.

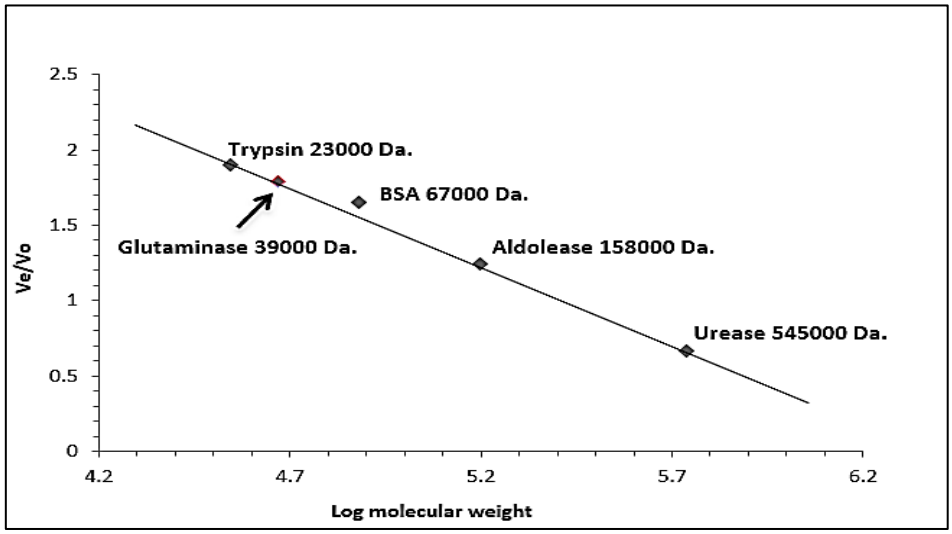

Figure 3: Establishing L-glutaminase standards according to the Ve/Vo ratio.

According to Kothapalli and MVV [25], L-glutaminase of *Streptomyces Luteogriseus* had an apparent molecular weight of 50 kDa. The molecular weight of L-glutaminase 46 kDa produced via *Streptomyces pratensis* NRC 10 [26]. Also, compared to Hassan *et al.,*[27], it was found that purified L-glutaminase has a molecular weight of 52 kDa produced from *Streptomyces sp.*

Cytotoxic impact of purified L-glutaminase (in vitro) Utilizing MTT Assay

This experiment was conducted to determine the viability of uncovered cells after examination of a range of concentrations for each constituent on the cell lines of the tumor. The percentage of treated cell viability was detected by comparing it with the normal cell line HdFn (Human dermal fibroblast, neonatal). In order to determine the cytotoxicity index of the purified L-glutaminase, HL-60 cell lines (Human promyelocytic leukemia cells) were treated with different concentrations of the enzyme for 24 hours. The results indicated that L-glutaminase affected the HL-60 cells' vitality in a concentration-dependent way. Utilizing a dose-response curve, the half-maximal inhibitory concentration (IC_{50}) for growths of cells was determined. The outcomes in Figure 4 showed that L-glutaminase gives rise to a depression in cell viability with a calculated IC_{50} of 143.9 µg/mL in a dose-based manner on HL-60 cell lines, compared with the normal cell line (HdFn cell line) with an IC_{50} of 187.3 µg/ml.

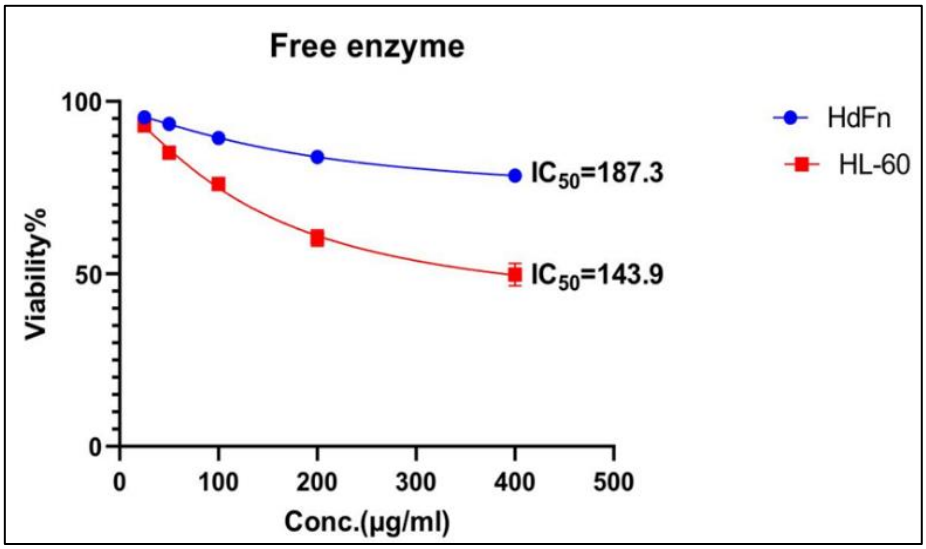

Figure 4: Dose-dependent cytotoxic effect of standard L-glutaminase on HL-60 cells and normal cell line (HdFn) after 24 hours and incubation at 37ºC.

However, statistical analysis revealed significant differences ($p \leq 0.001$) in the depression pattern between the normal cell line and the utilized concentration in HL-60 cells (Table 2 and 3). However, findings in Table 4 indicated that there were no significant discrepancies between the actual and predicted responses. However, when tested against HdFn, all of the drugs showed mild to moderate cytotoxic effects. This study reported that the impacts of *P. aeruginosa* purified L-glutaminase was observed on leukemia cancer cell lines because the enzyme L-glutaminase is responsible for converting the amino acid glutamine into glutamate. Cancer cells heavily depend on glutamine as a source of energy and building blocks for cellular structure. Therefore, this conversion can cause starvation and thus affect their growth.

Table 2: Convergence information of the effect of partially purified L-glutaminase on HL-60 cells.

Convergence Information		
PROBIT	**Number of Iterations**	**Optimal Solution Found**
	10	Yes

Table 3: Chi- square tests of the effect of partially purified L-glutaminase on HL-60 cells.

		Chi-Square	df[b]	Sig.
PROBIT	**Pearson Goodness-of-Fit Test**	21.276	5	0.001[a]

a: Confidence limits are calculated utilizing a heterogeneity factor since the significance level is smaller than.150; b: Data derived from specific instances vary from statistics based on aggregated cases.

Table 4: Cell counts and residuals of partially purified L-glutaminase effect on HL-60 cells.

No.	Conc.	No. of Subjects	Observed Responses	Expected Responses	Residual	*p*-value
1	400.000	100	49	61.326	-7.813	0.604
2	200.000	100	39	27.413	11.807	0.281
3	100.000	100	24	15.411	7.763	0.160
4	50.000	100	11	11.028	.385	0.111
5	25.000	100	5	9.111	-4.083	0.088
6	12.500	100	5	8.243	-3.451	0.081
7	6.250	100	4	8.121	-3.833	0.080

Various enzymes, from different bacterial sources have demonstrated their abilities to fight cancer cells. For example, Purified L-glutaminase isolated from *Aspergillus versicolor* Faesay4 displayed anti-cancer actions against human lung (A-549), breast (MCF-7), liver (HepG-2), and colon (HCT-116), cervical (Hela) cancer cell lines with IC_{50} 12.8, 39.61, 6.18, 11.48, and 7.25 µg/mL, respectively, and potent DPPH (2,2diphenyl-1-picrylhydrazyl) scavenging activities with IC_{50} = 50 µg/mL [28]. The *Streptomyces albogriseolus* isolate demonstrated an IC_{50} value of 101.2 µg/ml in the HePG2 cells and an IC_{50} value of 102.0 µg/ml in the cervical cancer HeLa cells utilizing the experiment of MTT, respectively [29].

The purified L-glutaminase from *Aspergillus flavus* showed an influential anti-leukemic parameter [30]. L-glutaminase was shown to have a significant function in breast cancer, according to in vitro antioxidant tests utilizing DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and anti-cancer impacts of L-glutaminase utilizing MTT assay [31]. Furthermore, A549 cells were utilized to test the anticancer impacts of both free and immobilised elastase, from *Klebsiella pneumoniae*, on lung cancer. The results showed that immobilized elastase had a stronger cytotoxic impact on A549 cells, with an IC_{50} of 142.8 µg/ml, compared to the normal cell line HdFn, which had an IC_{50} of 655.0 µg/ml [32].
With glutamine-deprivation treatment, L-glutaminase preferentially suppresses tumor development and encourages the death of cancer cells via blocking de novo protein synthesis and raising the superoxide level of oxidative stress [33]. Increasing glutamine catabolism and halting cancer development may be achieved with the use of the L-glutaminase enzyme, which is generated via halotolerant isolates. Furthermore, due to its chemical composition, saltwater may provide microbiological sources that produce enzymes with less potential for adverse impacts in therapeutic contexts[34]. Mostafa *et al*., [35] first documented *Halomonas meridiana* as a L-glutaminase producer that is utilized as a cancer agent against the colon.
According to the present study, the enzyme L-glutaminase produced from local isolate *p. aerogenosa* P48 had the antitumor impact. This indicates that the enzyme L-glutaminase has the ability to break down cancer cells.

4. Conclusions

The L-glutaminase enzyme produced by *P. aeruginosa* P48 was successfully partially purified using ammonium sulfate precipitation and ion exchange chromatography (DEAE-Cellulose), both of which proved to be effective. After conducting an investigation of its anticancer activity, positive findings were discovered against various cancer cell lines (HdFn and HL-60), suggesting that L-glutaminase may have potential therapeutic uses in cancer treatment. It would be beneficial to do more studies in order to investigate the whole scope of its impactfulness and safety profiles for clinical use.

Conflict of Interest

There is no possible bias on the part of the writers.

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