Original Research Article

ISOLATION, KINETICS AND *IN VITRO* ANTI-LEUKEMIC ACTIVITY OF *CAPSICUM ANNUM* L-GLUTAMINASE

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ABSTRACT

This study is aimed at exploring the potential of developing naturally occurring L-glutaminase isolated from green chilies as anti-leukemic drug which selectively targets cancer cell metabolism unlike other non-selective chemotherapeutics. This was done by determination of its specific activity and optimum condition for L-glutaminase activity followed by partial purification and *in vitro* cytotoxicity assay. Activity of L-glutaminase was measured in the aqueous extract of fruits by Nesslerization method after homogenization in liquid nitrogen and extraction with 0.05 M sodium borate buffer (pH 8.5). Results showed that L-glutaminase activity per milligram of total protein was 18.7 U/mg. Optimum conditions for the catalytic activity of crude L-glutaminase extracted from chili fruits were studied. Results showed maximum activity of L-glutaminase was achieved when the enzyme was incubated with 250 mM of l-glutamine at 37°C for 30 minutes in the presence of phosphate buffered saline at pH 7.2. The maximum velocity (Vmax) and affinity constant (Km) of L-gutaminase were 14.1 mM and 90.2 mM respectively. Crude L-glutaminase was purified by salting out using seven different concentrations of ammonium sulfate ranging from 20% to 80% saturation. Specific activity of purified L-glutaminase was increased from 18.7 to 98.5 U/mg at when salted out in 50% ammonium sulfate solution. This result indicated the high efficiency of ammonium sulfate precipitation as purification technique. Assessment of anti-leukemic property was done by comparing the cytotixicity of L-glutaminase with doxorubicin and combination of them against THP-1 cell line by MTT assay. Results showed significant anti-cancer activity with inhibition percent of 72.75 % in case of combination of the enzyme with doxorubicin when compared to the enzyme alone (60.28%). These findings concluded that L-glutaminase of green chilies could be developed as anti-leukemic agent owing to its high content in chilies, ease of purification and signicant cytotoxicity against human monocytic leukemia cells.

Keywords: Chilies, *in vitro* anti-leukemic activity, L-glutaminase, *Capsicum annum*

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Running title: Kinetics and anti-leukemic activity of *Capsicum annum* L-glutaminase.

INTRODUCTION

One area of L-glutamine research which has recently gathered much attention is the effect of L-glutamine on cancer therapy regimens. Numerous clinical studies have been conducted utilizing L-glutamine in conjunction with radiation and chemotherapy, with promising results [1].

In addition to L-glutamine being the principal metabolic fuel for the rapidly proliferating cells of the intestines and immune system, L-glutamine is also the main fuel for most rapidly growing tumors, which have high L-glutaminase activity, similar to small intestine enterocytes. Tumor growth can deplete skeletal muscle L-

glutamine and glutathione, providing less fuel for enterocytes and creating a catabolic, cachectic state. It is suggested the tumor can become a "L-glutamine trap," further enhancing systemic glutamine loss [2]. Pathological cancer cell growth relies on maintenance of proliferative signaling pathways with increased autonomy relative to non-malignant cells. Several lines of evidence argue that L-glutamine reinforces activity of these pathways. In some cancer cells, excess L-glutamine is exported in exchange for leucine and other essential amino acids. This exchange facilitates activation of the serine/threonine kinase mTOR (mammalian target of rapamycin complex), a major positive regulator of cell growth [3].

Loss of the epithelial cell-cell adhesion molecule E-cadherin is a component of the epithelial-mesenchymal transition, and is sufficient to induce migration, invasion, and tumor progression [4]. Addiction to L-glutamine may oppose this process because glutamine favors stabilization of tight junctions in some cells [5]. Furthermore, the selection of breast cancer cells with the ability to grow without L-glutamine yielded highly adaptable subpopulations with enhanced mesenchymal marker expression and improved capacity for anchorage-independent growth, therapeutic resistance, and metastasis *in vivo* [6]. It is unknown whether this result reflects a primary role for L-glutamine in suppressing these markers of aggressiveness in breast cancer, or whether prolonged glutamine deprivation selects for cells with enhanced fitness across a number of phenotypes.

Unlike normal cells, leukemic cells does not depend on L-glutamine synthetase, they directly depend on the exogenous supply of L-glutamine from the blood for their growth and survival. Therefore, blood L-glutamine serves as a metabolic precursor for the nucleotide and protein synthesis of tumor cells .Consequently, L-glutaminase causes selective death to L-glutamine dependent tumor cells by blocking the energy route for their proliferation [7].

Microbial L-glutaminase has gained a prime focus since the discovery of its anti-cancer properties. Imada *et al*. (1973) had tested the amidase activities of thousands of microbes [8]. This was followed by the isolation and purification of L-glutaminases [9,10,11] and their structure determinations [12,13] with the ultimate aim of developing these enzymes as a therapeutic agents.

Since the discovery of the first L-glutaminase in *Escherichia coli* [9], investigators have been looking continuously for serologically new structures of L-glutaminase in attempt to surpass the immune inactivation of the drug which additionally support that the research in plant type L-glutaminase is worth doing.

In this study we aim to investigate natural products potential for anti-leukemic activity. It is an attempt of drug discovery through biotechnical oriented approach by using naturally produced L-glutaminase of green chilies (*Capsicum annum*). This attempt is targeting the exploitation of the country's rich resources of the chilies plant.

L-glutaminase (EC 3.5.1.2) is an amidohydrolase that catalyses the hydrolytic conversion of its substrate L-glutamine to L-glutamic acid and ammonia. It can be developed as a biopharmaceutical for cancer therapy as it facilitates high l-glutamine catabolism, consequently deprives cancerous cells of a vital metabolite; L- glutamine needed for energy and growth. This in fact, results in a selective starvation and apoptosis of L-glutamine addictive tumor cells because unlike normal cells they lack L-glutamine synthetase which replenishes L-glutamine to normal cell [14].

The development of L-glutaminase as an anti-cancer for leukemia namely acute lymphoblastic leukemia (ALL) would be extremely beneficial due to the fact that the disease most commonly occur in children. This group of patients is greatly vulnerable to the deadly side effects associated with the non-selective chemotherapeutic agents. On the other hand, none of these cytotoxic effects would be present when

considering a biomolecule like L-glutaminase for therapy. This is attributed to its inability to enter the cells and only negatively affect the metabolism of cancer cells by breaking down L-glutamine extracellularly [15].

MATERIALS AND METHODS

Extraction of L-glutaminase

Extraction of L-glutaminase from 250 g of local *capsicum* fruits was achieved according to Bano and Sivaramakrishnan (1981) and Abdulla *et al.* (2012) by homogenization with liquid nitrogen. Forty five grams of frozen homgenate was extracted with two volumes of 0.05 M borate buffer, pH 8.5, and then centrifuged at 10000 rpm for 20 minutes. Supernatant was regarded as crude enzyme [16,17]. The crude extract was divided into two halves, one half was used for kinetic studies followed by lyophilization and The other half was subjected to ammonium sulfate precipitation of L-glutaminase then lyophilized and stored at 4 ^{o}C.

Enzyme assay

L-glutaminase activity was determined by adding 100 µl of crude enzyme to 400 µl of 25 mM L-glutamine (dissolved in extraction buffer), mixed gently and incubated at 37^{o}C for 30 minutes. After incubation, 500 µl of 1 N sulfuric acid was added to the reaction mixture to stop the reaction, then the mixture was centrifuged at 8000 rpm for 2 minutes to remove the precipitated proteins, and the ammonia concentration was determined in clear supernatant by the direct Nesslerization method.

Nesslerization was done by mixing 190 µl of deionized water with 10 µl of supernatent and 25 µl of Nessler reagent in microtiter plate. The mixture was shaken well, and absorbance was measured at 400 nm in 1 to 3 minutes. Concentration of ammonia was then determined from Ammonium Chloride standard graph. Total protein was measured by Biuret method. Enzyme and substrate blanks were included in all measurements [11]. One L-glutaminase unit (U) is defined as the amount of enzyme which liberates 1 µmole of ammonia per minute under experimental conditions. L-glutaminase activity was calculated according to the following equation [8]:

Activity (u/ml) = Concentration of ammonia***/***time of reaction

Specific activity (u/mg) = Activity (u/ml)***/***protein (mg/ml)

Determination of optimum conditions for crude L-glutaminase activity

Effect of different factors on crude L-glutaminase activity was studied according to Elshafei *et al*. (2014) [11]. These factors include substrate concentration, pH of reaction medium, reaction temperature.

Partial purification of L-glutaminase by ammonium sulfate precipitation

The specific enzyme activity of the crude extract were assayed and subsequently subjected to ammonium sulfate precipitation. In 7 separate tubes ammonium sulfate was added an gently mixed at 4 ^{o}C to 1 ml of crude extract that gave final concentrations of 20%.30%, 40%, 50%, 60%, 70%, and 80% w/v saturation.

The precipitate obtained after centrifugation at 10000 rpm at 4 ^{o}C for 15 minutes was washed 2 times with deionized water and dissolved in a suitable volume of phosphate buffered saline (pH 7.2), then enzyme activity and total protein were determined after each saturation. The optimum salting out concentration of the enzyme was established on the basis of enzyme activity [18].

L-glutaminase preparation for bioassay

Lyophilized powder of both crude and partially purified L-glutaminase were carefully weighted (0.1 g) of the crude and partially purified enzyme was dissolved in 1 ml of PBS followed by measurement of activity which was 80 U/ml and 104 U/ml for crude and partially purified L-glutaminase respectively.

5.1 Cell Culture

Human monocytic leukemia cells (THP-1) propagated in L15 cell culture media supplied by 10 % calf fetal serum and L-glutamine and sterilized by microfiltration through 0.2 micron filter. THP-1 cell were cultivated in 50 Cm^3 cell culture flasks and incubated in 5% CO_2 incubator and till appropriate cell density was reached**.**

Microculture tetrazolium (MTT) assay

This colorimetric assay is based on the capacity of Mitochondaria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] into an insoluble purple formazan product which is measured spectrophotometrically. The reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

The THP-1 cell culture was grown to in L 15 media, then the cell count was adjusted to1×10^4-10^5 cells/ ml using medium L 15 containing 10% fetal bovine serum (FBS). To each well of 96 well microtitre plates, 80 µl of diluted cell suspension was added followed by 100 µl medium. Then, 20 µl of crude L-glutaminase, partially purified L-glutaminase, 40 µl doxorubicin (5mg/ml) and combination of doxorubicin (5mg/ml) and both crude L-glutaminase and partially purified L-glutaminase. A control was set by adding 20 µl of PBS After 72 hours, cells were centrifuged at low speed to attach it to the bottom of the microtiter plate then, the supernatant was flicked off and 100 µl of fresh complete media was added to all wells after that three 2 fold serial dilution of the samples in growth medium was prepared to test the effect of three deferent concentration of each sample then incubated at 37ºC in 5 % CO_2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, 50µl of MTT dye was added to each well which was prepared with concentration of 5mg/ml in PBS. The plates were gently shaken and wrapped in aluminum foil and incubated for 4 hours at 37ºC in 5% CO_2 incubator. The supernatant was removed, 100 µl of Dimethyl sulfoxide (DMSO) was added, and the plates were gently shaken to solublize the formed formazan. The absorbance was measured using multiskan spectrophotometer at a wavelength of 570 nm. The percentage growth inhibition was calculated using the formula below:

$$\textbf{\% cell inhibition} = 100-\{(Ac-At)/Ac\} \times 100$$

Where, At= Absorbance value of test compound; Ac=Absorbance value of control

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RESULTS

Detection of L-glutaminase in *Capsicum annum* L. fruits

Extract of chilies yielded a significant amounts of the enzyme. Results in table (1) showed total and specific L-glutaminase activities.

Table (1): L-glutaminase activity and specific activity in the extract of fruits of *Capsicum annum* L.

Chili fruits weight (g)	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)	Total activity (U)
40	70	172.1	9.18	18.7	12047

Optimum conditions for L-glutaminase activity

Optimum conditions for L-glutaminase activity were studied after extraction of the enzyme from plant fruits. These conditions include the optimum substrate concentration, pH of the reaction mixture, temperature of the reaction, and the enzyme concentration. Changing in any of these parameters may affect the enzyme activity.

2.1 Effect of buffer pH

Effect of pH on the activity of L-glutaminase activity produced by *Capsicum annum* L. was studied. Results presented in table (2) and illustrated in figure (1) showed that maximum L-glutaminase activity was obtained when pH of the reaction mixture was adjusted to 7.2, at this value; the enzyme activity was 6.55 U/mg.

Table (2): Effect of pH on L-glutaminase activity

pH	Activity (U/mg)
5	0.347
6	0.57
7.2	6.55
8	3.49
9	2.74
10.6	1.82
12	1.45

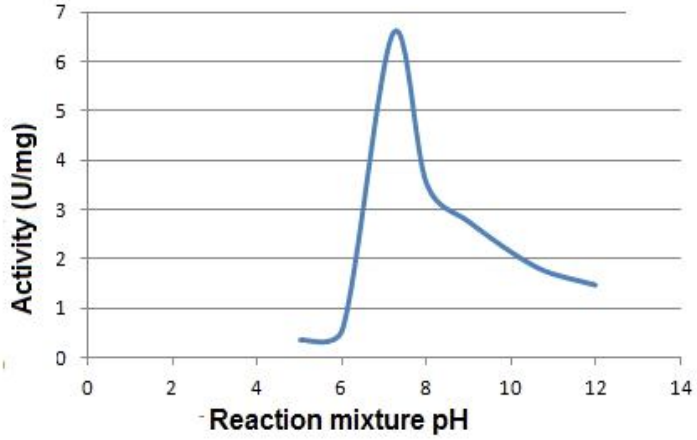

Figure (1): Effect of buffer pH on the activity of L-glutaminase of *Capsicum annum*.

2.2 Effect of temperature

In order to determine the optimum reaction temperature for the activity of L-glutaminase extracted from fruits of *C. annum* L., different temperatures (15, 30, 37, 50, and 60°C) were used for this purpose. It was chosen with regard to the climate surrounding the plant.

Results presented in table (3) and illustrated in figure (2) showed that the maximum activity of L-glutaminase was obtained when the temperature of the reaction mixture was 37°C. At this temperature, enzyme activity was increased to 0.35 U/mg.

Table (3): Effect of temperature on L-glutaminase activity

Incubation temperature (^{0}C)	Activity (U/mg)
15	0.268
30	0.287
37	0.351
50	0.281
60	0.129

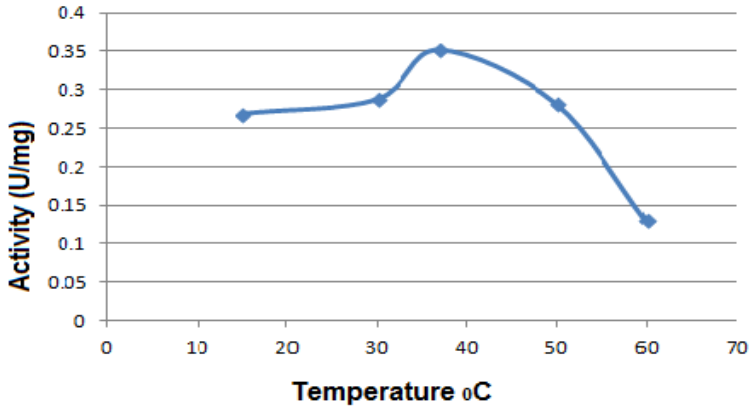

Figure (2): Effect of temperature on the activity of L-glutaminase extracted from fruits of *Capsicum annum* L.

2.3 Effects of substrate concentration

In order to determine the enzyme kinetics of L-glutaminase, seven concentrations (10, 25, 50, 100, 150, 200, and 250 mM) of enzyme substrate (L-glutamine) were used for this purpose.

Results mentioned in table (4) and illustrated in figure (3) showed that the activity of L-glutaminase increased gradually with the increase in L-glutamine concentration. Maximum activity of L-glutaminase was obtained when the substrate concentration was 250 mM, at this concentration; L-glutaminase activity was 8.9 U/mg. This concentration (250 mM) of L-glutamine was regarded as saturable for L-glutaminase active site, and was used in determining the effect of enzyme concentration on activity.

According to results mentioned in figure (4), the Km value for the green chilies L-glutaminase was 90.2 mM and Vmax was 14.1 mM/min.

Table (4): L-glutaminase kinetics

L-glutaminase concentration	Activity (U/mg)
0.029	479.18
0.059	272.19
0.11	149.66
0.23	74.87
0.47	35.19
0.95	21.4
1.9	11
3.8	6.57
7.61	4.93

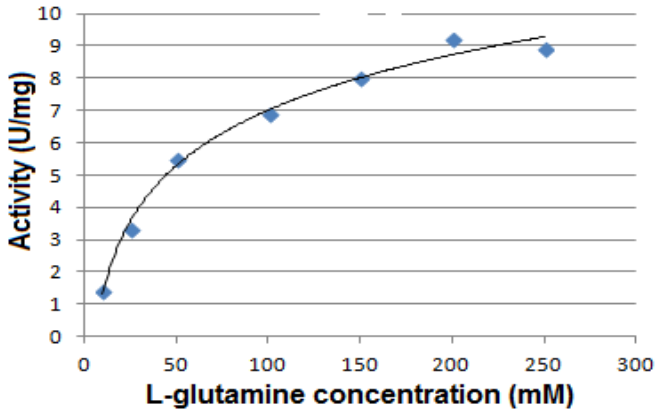

Figure (3): Effects of substrate (L-glutamine) concentration on the activity of L-glutaminase extracted from fruits of *Capsicum annum* L. incubated at 37°C for 30 minutes, pH=7.2.

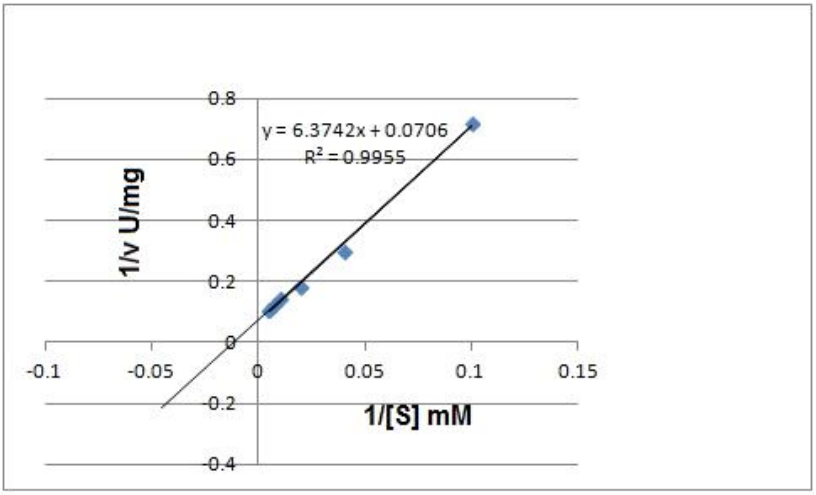

Figure (4): A Lineweaver-Burk plot was used to determine Km and Vmax of L-glutaminase from the fruits of *Capsicum annum* L.

These kinetic constants were calculated from the graph equation as follows:

1/V= Km/ Vmax and 1/[s] = 1/Vmax, where 1/Vmax = intercept, Km/Vmax = slope, V = reaction velocity and [S] = substrate concentration. Substituting these constants in the graph equation yielded the value of Vmax = 14.1 mM/min and Km = 90.2 mM.

Partial purification of L-glutaminase by ammonium sulfate precipitation

The crude enzyme extract was subjected to ammonium sulfate precipitation with 7 different saturations. Then the total enzyme activity and total protein in each of these concentrations were determined.

Partial purification of L-glutaminase produced by *Capsicum annum* L. using ammonium sulfate precipitation showed that the best fraction was obtained when 50% ammonium sulfate was used in comparison with the crude enzyme and other ammonium sulfate concentrations (figure 5). It gave the maximum values of total

activity, specific activity and yield of the L-glutaminase enzyme which reached (92.7 U/ml, 98.5 U/mg and 53.8%) respectively. The purification fold of the purified enzyme was 5.2 when 50% ammonium sulfate was used.

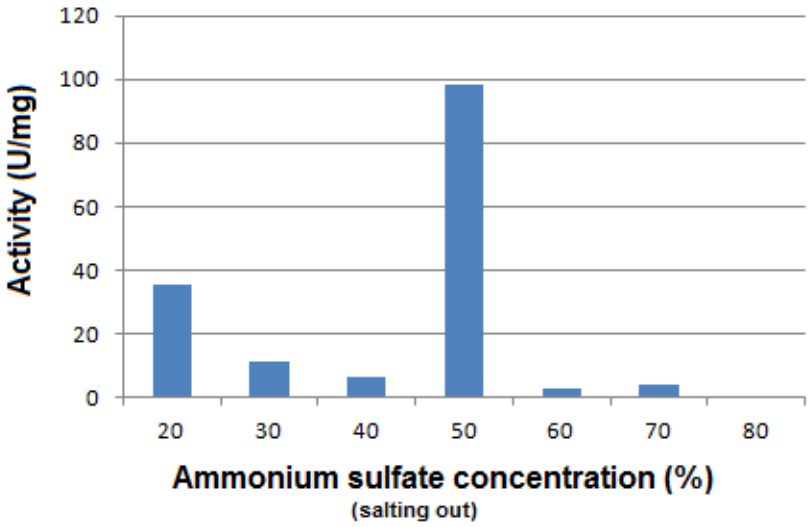

Figure (5): Ammonium sulfate fractionation of L-glutaminase

The anticancer effect of crude enzyme against (THP-1) cell line by MTT assay

The viability of human monocytic leukemia cells (THP-1) treated with crude chilies extract and doxorubicin each on its own is tested by the Microculture tetrazolium assay (MTT). Three different enzyme activities 80, 40 and 20 U/ml were gave 57.03%, 56.3%, and 53.9 respectively as presented in table (5).

Table (5): MTT assay of crude L-glutaminase against THP-1 cell line

Treatment	Activity/ concentration	Abs Mean ± SD	Inhibition (%)	*IC50
L-glutaminase Crude (U/ml)	80	0.1656 ± 0.012	57.037	3.949
	40	0.0007 ±0.1683	56.335	
	20	0.007 ±0.1777	53.906	
Doxorubicin (mg/ml)	5	0.01 ±0.1183	69.293	
Control (PBS)	-	0.0083±0.385547	0	

***IC 50:** is the concentration that decrease the cell viability by 50%.

The anti-cancer effect of semi purified enzyme against (THP-1) by MTT assay

The viability of human monocytic leukemia cells (THP-1) treated with semi purified chilies extract and doxorubicin each on its own is tested by the Microculture tetrazolium assay (MTT). Three different enzyme activities 104, 52 and 21 U/ml gave 60.2 %, 54.9% and 51.5 % respectively as presented in table (6).

Table (6): MTT assay of Semi-purified L-glutaminase against THP-1 cell line

Treatment	Activity/ concentration	Abs Mean ± SD	Inhibition (%)	*IC50
L-glutaminase Semi-purified (U/ml)	104	0.1531± 0.012	60.282	21.549
	52	0.005 ±0.1736	54.965	
	21	0.001 ±0.1866	51.593	
Doxorubicin (mg/ml)	5	0.01 ±0.1183	69.293	
Control (PBS)	-	0.0083±0.385547	0	

The anticancer effect of Combination (crude + Doxorubicin) against THP-1 cell line by MTT assay

Three different enzyme activities were selected by serial dilution of the chilies crude extract and combined with three different concentrations of doxorubicin then the Inhibition percentage of the combination against THP-1 cell was measured by MTT assay. .the results are presented in table (7).

Table (7): MTT assay of combination (Crude + Doxorubicin) against THP-1 cell line

Treatment	Activity/ concentration	Abs Mean ± SD	Inhibition (%)	IC50
L-glutaminase Crude (U/ml)+Doxorubicin (mg/ml)	80+5	0.1090± 0.006	71.716	13.524
	2.5+40	0.01 ±0.1424	63.048	
	1.25+20	0.006 ±0.1715	55.494	
Doxorubicin (mg/ml)	5	0.01 ±0.1183	69.293	
Control (PBS)	-	0.0083±0.385547	0	

The anticancer effect of Combination (Semi purified + Doxorubicin) against THP-1 cell line by MTT assay

The viability of human monocytic leukemia cells (THP-1) treated with semi purified chilies extract and doxorubicin in combination on its own is tested by the Microculture tetrazolium assay (MTT). Three different enzyme activities were selected by serial dilution of the semi-purified chilies L-glutaminase extract. The results are presented in table (8).

Table (8): MTT assay of combination (Semi-purified+ Doxorubicin) against THP-1 cell line

Treatment	Activity/ concentration	Abs Mean ±SD	Inhibition (%)	IC50
L-glutaminase Semi-purified (U/ml)+Doxorubicin (mg/ml)	104+5	0.1050±0.005	72.757	15.988
	2.5+52	0.003±0.1347	65.059	
	1.25+21	0.024±0.1688	56.199	
Doxorubicin (mg/ml)	5	0.01±0.1183	69.293	
Control (PBS)	-	0.0083±0.385547	0	

DISCUSSION

Homogenization of plant material in liquid nitrogen facilitated the breakup of cells prior to extraction without heat denaturation of L-glutaminase. In addition, using 0.05 M sodium borate buffer (pH 8.5) for extraction of L-glutaminase from green chilies yielded an activity of 18.7 U/mg of the enzyme. This extraction buffer could release large amounts of enzyme molecules from the cells lysate which agreed with the reported maximum L-glutaminase activity and solubility of 5.3 U/mg/ml at pH 9 [11].

Optimum pH for L-glutaminase activity extracted from green chili fruits in this study was found to be 7.2. In general, this agreed with most plant enzymes which showed maximum activity at or near neutral pH [19].

On the other hand most of the reported microbial L-glutaminases function optimally at alkaline pH with the exception of *Escherichia coli* (pH=7) [18]. These include bacterial L-glutaminase of *Rhizobium etli* (pH=8.3-8.5) and *Bacillus pasteurii* (pH=9) [20, 21], and fungal L-glutaminase of *Penicillium politans* (pH=7.5-8.5) and *Penicillium brevicompactum* (pH=8.5) [22, 11]. Optimum activity of green chilies L-glutaminase at neutral pH strongly support the idea of developing it as cancer therapeutic because of its maximum activity in the pH of the blood and even cell culture media for *in vitro* testing.

Activity of *C annum* L. L-glutaminase has been found to be maximum when incubated at 37 ^{0}C. This was agreed the reported maximum L-glutaminase activity from most microorganisms at 37°C [8]. However, it was found that L-glutaminase B of *Escherichia coli* functioned optimally at 25 ^{0}C which may discourage its utilization as a drug in human beings due to its less diminished activity at body temperature (37 ^{0}C). Other microbial glutaminases has enhanced activity at high temperatures include L-glutaminase of the bacterium *Rhizobium etli* had an optimal enzyme activity at 45 ^{0}C [20], while the fungal L-glutaminase of *Penicillium politans* and *Penicillium brevicompactum* had its optimum activity at even higher temperatures of 60 ^{0}C and 50 ^{0}C respectively [22, 11].

Optimum activity of green chilies L-glutaminase (0.35 U/mg) at the body temperature 37 ^{0}C may make it easier for *in vitro* and *in vivo* testing of the biological activity. In addition, it returned 80% of its activity (0.28 U/mg) at 50 ^{0}C. This may enhance its shelf life stability substantially in comparison with other protein therapeutics.

L-glutamine concentration required to saturate the active site of L-glutaminase of *C annum* was 250 mM and affinity constant Km of L-glutaminase was 90.2 mM. This was considerably larger than the Km values for most of the L-glutaminases from other microbial sources, which were of the order of 0.01 mM. The enzyme from green chilies has thus a lesser affinity for the substrate L-glutamine.

It has been reported that most microbial L-glutaminases had low Km values with the exception of the fugus *Penicillium politans* (Km=7500 mM) [22]. These include bacterial L-glutaminase of *Escherichia coli* (Km=4.5 mM), *Rhizobium etli* (Km=1.5 mM) and *Bacillus pasteurii* (Km=9.5 mM) [20, 21], and fungal L-glutaminase of *Penicillium brevicompactum* (Km=1.66 mM) [22, 11].

The enzyme from green chilies might be an 'asparaginase-glutaminase' enzyme. A number of such enzymes with both glutaminase and asparaginase activities were purified from filamentous fungi [23]. Another possibility for the high Km value of the enzyme is that it could be a γ-Glutamyltranspeptidase (GGT) that catalyzes the hydrolysis of γ-glutamyl compounds and transfer of their γ-glutamyl moieties to amino acids and peptides. GGT has been reported in *Bacillus subtilis* by Kijima and Suzuki (2007) and is able to catalyze the hydrolysis of glutamine to glutamic acid [24].

L-glutaminase salted out by 50% ammonium sulfate saturation had increased the specific activity of the crude extract from 18.7 U/mg to 98.5 U/mg which proved the high efficiency of salting out technique for purification. These findings were totally different from that reported by Abdallah et *al.* who found that 80% ammonium sulfate was best salting out concentration which increased the specific activity of L-glutaminase of *Streptomyces avermiyilis* from 4.9 to 9.7 U/mg [16]. Green chilies L-glutaminase was purified by 5 folds using ammonium sulfate precipitation while microbial L-glutaminases were not much purified by this technique. The bacterial L-glutaminases of *Escherichia coli*, *Bacillus pasteurii* and *Rhizobium etli* were purified by 1, 2 and 3 folds by ammonium sulfate salting out respectively [22, 11].

The growth inhibition percent of both crude and partially purified L-glutaminase (57.03%, 60.28%) against human monocytic leukemia cells were slightly different in comparison with crude L-glutaminase of *Penicillium brevicompactum* tested against Hep-G2 [Human hepatocellular carcinoma cell line], MCF-7 [Breast cancer cell line], HCT-116 [Colon cell line] and A549 [Human lung Carcinoma] with inhibition % 65.3%, 41%, 34.2% and 33% respectively [11].

The effect of combination almost equal to the effect of doxorubicin alone this may be due to the rapid onset of action of doxorubicin in comparison with L-glutaminase which takes a long time to initiate the apoptotic process through L-glutamine starvation of THP-1 cells.

Inhibition of THP-1 cells growth by combination of L-glutaminase and doxorubicin (72.75%) when doxorubicin (69.29) was used alone. This suggested the substitution of doxorubicin (indiscriminating chemotherapeutic agent) with L-glutaminase of green chilies to eliminate its lethal side effect or reduce its use in the treatment of acute lymphoblastic leukemia in children which considered the most vulnerable group of patient affect by the cytotoxicity.

L-glutaminase of chilies might be an alternative natural and relatively cheap source of the enzyme which fends off the hazards and fastidious culture requirement of microbial L-glutaminases.

Results showed that the inhibition % of THP-1 cell growth by partially purified L-glutaminase (60.2%) was more than that obtained by crude L-glutaminase (57.03%) which reflected the advantage offered by purification on enhancing the anti-cancer activity may be due to eliminating any interfering substances in the crude extract that might affect the cells viability.

CONCLUSIONS

It was concluded that L-glutaminase isolated from fresh green fruits of *C. annum* L. proved to be approptiate source for L-glutaminase that functioned optimally in the presence of L-glutamine substrate at a concentration of 250 mM in phosphate buffer (pH 7.2) at 37°C. L-glutaminase extracted from fruits of *C. annum* L. can be purified partially using ammonium sulfate salting out technique with minimal enzyme losses at 50 % saturation. *C. annum* L. L-glutaminase proved to have anti-cancer activity against THP-1 cell line close to that of doxorubicin and without major increase in cytotoxicity when used in combination.

RECOMMENDATIONS

Purify L-glutaminase of green chilies to a high level of purity and test efficacy as well as cytotoxicity of purified L-glutaminasein different tumor cell lines. Furthermore, investigate other plant sources for producing L-glutaminase particulary other members of the Solanaceae family taking into consideration economic value and commercial volume of these sources.

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