

## Extraction, Characterization and Partial Purification of L-Asparaginase from the Leaves of *Arachis Hypogaea* L.

Niranjana. J, Kandasamy Arun Gandhi\*, D. Sunmathi and P. Nanthavanan

Research Department of Biotechnology, Dr. N.G.P Arts and Science  
College (Autonomous), Coimbatore, Tamilnadu, India.

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L-asparaginase has been a promising therapeutic agent in the treatment of acute lymphoblastic leukemia and lymphoma. In recent times, due to the side effects of commercially available bacterial L-asparaginase and its unavoidable importance, plants are being explored as the source of L-asparaginase. The enzyme L-asparaginase was partially purified from *Arachis hypogaea* L. The crude enzyme extract was subjected to different purification steps including ammonium sulphate precipitation, dialysis followed by separation on Sephadex G-100 gel filtration (size exclusion chromatography) to obtain partially pure form of L - asparaginase. The enzyme was partially purified to 118 folds and contained specific activity of 4686.86 U/mg with 9.85% yield. SDS-PAGE electrophoresis of the partially purified enzyme revealed that it was a single protein with molecular weight of 70 kDa. The study on physicochemical properties showed that L - asparaginase from *Arachis hypogaea* L. was potassium-dependent in nature, where its optimum pH of enzyme activity was found to be 8.0 and temperature as 40°/50°C with reaction time of 15 - 20 minutes. Also it was observed that the L-asparaginase activity increased with the presence of metal ions such as Na<sup>+</sup>, Mg<sup>++</sup>, making it an enzyme dependent on metal ions for its reaction. In addition to this, it was revealed that the enzyme was partially inhibited in presence of certain chelators. The specificity of L-asparaginase obtained from *Arachis hypogaea* L. with lack of urease activity and minimal glutaminase activity along with less cytotoxicity on human blood indicated it as an efficient chemotherapeutic agent that could be investigated further in future studies.

**Keywords:** *Arachis Hyogaea* L; Cytotoxicity; Gel Filtration; Groundnut Plant; L-Asparaginase; Sephadex G-100; Size Exclusion Chromatography.

L-asparaginase (EC 3.5.1.1) is an enzyme belonging to "Ntn-hydrolases" family, catalyzes the hydrolysis of L-asparagine producing L-aspartic acid and ammonia<sup>1,2</sup>. The inability of the leukemic cells to synthesize L-asparagine due to the absence of L-asparagine synthetase has made the enzyme L- asparaginase as a key therapeutic agent in the treatment of acute lymphoblastic leukemia (ALL)

and lymphoma. As the leukemic cells requires high amount of L-asparagine, they rely on the exogenous sources for their proliferation and survival. Injection of L-asparaginase results in depletion of L-asparagine in blood, which will lead to starvation of tumor cells selectively whereas normal cells are protected from L-asparagine starvation since they are capable of synthesizing this amino acid

\*Corresponding author E-mail: arungandhicbe@gmail.com



which is essential for both protein synthesis and cell survival<sup>3</sup>.

There are two formulations of L-asparaginase used clinically for ALL treatment, one from *Erwinia chrysanthemi* and other from *Escherichia coli*. However, its long term use was known to result in normal cell toxicity, rapid clearance of enzyme in blood stream and patient's hypersensitivity<sup>4</sup>. The cell toxicity indicated that the L-asparaginase's toxicity is due to enzyme glutaminase activity and bacterial endotoxins in enzyme preparations. Therefore in attempt of minimizing various side-effects, plants are explored for the production of L-asparaginase and are known to be less toxic compared to bacterial L-asparaginase<sup>5</sup>. In plants, L-asparagine is known to be nitrogen compound and may accumulate under stress conditions<sup>6</sup>. There are two types of plant L-asparaginase such as potassium- dependent and potassium- independent asparaginase. Although these enzymes have high relative sequence similarity, potassium independency of L-asparaginase is not well understood. This study is done in order to partially purify and characterize L-asparaginase from the leaves of *Arachis hypogaea* L. (groundnut plant) as a plant source.

## MATERIALS AND METHODS

### Plant material

The plant was collected from the agricultural field at Puliampatti, Erode district. It was authenticated as *Arachis hypogaea* L. by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (No.: BSI/SRC/5/23/2018/Tech/2324).

### Crude enzyme extract

The crude enzyme extract was prepared by homogenizing 1 gram of shade dried leaves of *Arachis hypogaea* L. in 0.1M Phosphate buffer with pH 7.5 at 3°C containing  $\beta$ -mercaptoethanol and 1mM EDTA. The process was carried out in chilled condition. The homogenate was centrifuged at 12,000 rpm for 30 minutes at 3°C. The supernatant was designated as crude enzyme extract.

### Partial purification of L-asparaginase Ammonium sulphate precipitation

The extracted crude enzyme was precipitated by adding ammonium sulphate salt

with continuous stirring using magnetic stirrer providing 70% saturation. The precipitated enzyme was refrigerated for an hour and centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellet obtained was re-suspended in 0.02M phosphate buffer (pH 6.9) and subjected to dialysis with phosphate buffered saline of pH 7.4. After series of dialysis, the sample was centrifuged at 13,000 rpm for 20 minutes at 4°C. The supernatant was collected and was subjected to next step of purification.

### Sephadex G-100 (Gel filtration)

The concentrated enzyme preparation was added on the top of Sephadex G-100 column equilibrated with 100mM Phosphate buffer (pH 7.0) and eluted with the same buffer with the flow rate of 0.4ml/minute. The fraction showing high L-asparaginase activity was selected and the homogeneity was checked by SDS- PAGE.

### Asparaginase activity Assay

The activity of L-asparaginase was measured by using Nessler's reagent<sup>7</sup>. The L-asparaginase catalyzes L-asparagine to L-aspartic acid and ammonia and later react with Nessler's reagent to produce pale yellow to orange color product. The reaction mixture consisted of 0.5 ml of 40 mM L-asparagine, 0.5 ml of 50 mM phosphate buffer (pH 8.0) and 0.1 ml of enzyme sample. The tubes were incubated for 15 minutes at 37°C. The reaction was stopped by adding 0.5 ml of 20% Trichloroacetic acid (TCA). The blank was prepared without the enzyme sample. The reaction mixture was centrifuged at 8000 rpm for 5 minutes. To 1 ml of supernatant, 3 ml of distilled water and 1 ml of Nessler's reagent was added. The mixture was incubated for 20 minutes at room temperature and the absorbance was measured at 570 nm using Colorimeter (ELICO). The ammonia released in the reaction was determined based on the standard curve obtained with ammonium sulphate, where 1  $\mu$ mole of ammonium sulphate is equal to 2  $\mu$ moles of ammonia liberated. One unit of L-asparaginase activity is defined as the amount of the enzyme that liberates 1  $\mu$ mole of ammonia per minute at room temperature.

### Protein Determination

Protein was quantified by using Nanophotometer (Implen N 60) – Protein UV (A260/ 280nm).

## Characterization of L-asparaginase

### Potassium dependency of L-asparaginase

To determine the nature of L-asparaginase from *Arachis hypogaea* L., the enzyme preparation was pre-incubated with potassium chloride of different concentration (20 mM, 40 mM, 60 mM, 80 mM and 100 mM) for 15 minutes prior to the addition of substrate. The enzyme activity was determined under assay condition and relative activity (ratio of enzyme activity in presence of potassium chloride and absence of potassium chloride) was calculated. The activity which was assayed on absence of L-asparagine was taken as 100%.

### Effect of buffer pH

The effect of buffer pH on L-asparaginase activity was studied by incubating the enzyme preparation in the 50 mM phosphate buffer of various pH ranges (5.0, 6.0, 7.0, 8.0 and 9.0) with 40 mM L-asparagine for 15 minutes at room temperature. Then enzyme activity was determined and relative activity (ratio of enzyme activity of reaction mixture at different pH and enzyme activity of control at pH 8.0) was calculated. The activity which was assayed at pH 8.0 was taken as 100%.

### Effect of reaction time

The effect of reaction time on L-asparaginase activity was studied by incubating the reaction mixture for different period of time (10, 15, 20, 25 and 30 minutes) at room temperature. Then enzyme activity was determined and relative activity (ratio of enzyme activity of reaction mixture at different reaction time and enzyme activity of control with specific reaction time). The activity which was assayed at 10 minutes reaction time was taken as 100%.

### Effect of temperature

Optimum temperature for the L-asparaginase activity was determined by incubating the reaction mixture at different temperatures (20, 37, 40, 50, 60 °C). Then the enzyme activity was determined and relative activity (ratio of enzyme activity of reaction mixture at different temperature and enzyme activity of control at specific temperature) was calculated. The activity which was assayed at room temperature was taken as 100%.

### Effect of substrate concentration

The effect of substrate concentration

on L-asparaginase activity was determined by incubating the enzyme with different concentration (20 mM, 40 mM, 60 mM, 80 mM and 100 mM) of L-asparagine for 15 minutes. Then the enzyme activity was determined and relative activity (ratio of enzyme activity at different substrate concentration and enzyme activity with 40 mM substrate concentration) was calculated. The activity which was assayed at 40 mM substrate concentration was taken as 100%.

### Substrate specificity

To determine the specificity of L-asparaginase, the enzyme reaction mixture was made with 40 mM of each L-glutamine, L-glutamic acid, L-aspartic acid, DL-ornithine and urea as these are analogs of L-asparagine. The enzyme activity was determined under assay conditions and relative activity (ratio of enzyme activity in presence of different analogs as substrate and L-asparagine as substrate) was calculated. The activity which was assayed on presence of L-asparagine was taken as 100%.

### Effect of metal ions

The effect of different metal ions on L-asparaginase activity was determined by pre-incubating enzyme alone with 100 mM metal ions such as Na<sup>+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup> for 15 minutes prior to adding the substrate. Then enzyme activity was determined and relative activity (ratio of enzyme activity in presence and absence of metal ions) was calculated. The activity which was assayed on absence of metal ions was taken as 100%.

### Effect of chelators and surfactant

The effect of different chelators and surfactant on L-asparaginase activity was determined by pre-incubating enzyme alone with 10 mM chelators such as EDTA, SDS, urea, β-mercaptoethanol and tween-20 for 15 minutes prior to adding the substrate. Then enzyme activity was determined and relative activity (ratio of enzyme activity in presence and absence of chelator) was calculated. The activity which was assayed on absence of metal ions was taken as 100%.

### Cytotoxicity study

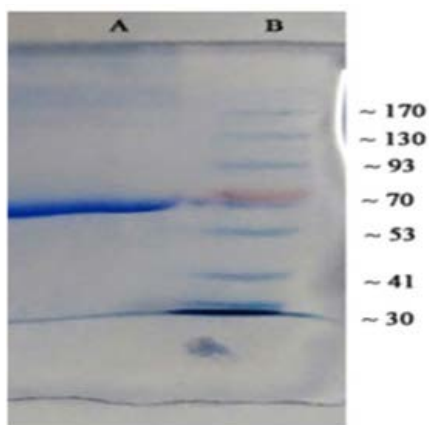
#### *in-vitro* hemolytic activity test

The cytotoxicity of the partially purified L-asparaginase was studied based on the lysis of RBCs. 5 ml of blood was collected in a polystyrene tube containing EDTA (anticoagulant). The blood

sample was centrifuged at 800 rpm for 3 minutes. The supernatant containing plasma and buffy coat phase was discarded while the pellet/ cell suspension was washed with phosphate buffered saline (PBS) and centrifuged at 800 rpm for 3 minutes (this process was repeated thrice). 0.5 ml of cell suspension and 0.5 ml of partially purified enzyme was added in PBS. The mixture was incubated for 30 minutes at 37°C and was centrifuged at 1500 rpm for 10 minutes. The supernatant was added to round bottom 96 well microtitre plates and checked for its absorbance value (at 540 nm) using microplate reader ELISA Reader (BIORAD). The PBS containing blood sample acted as negative control whereas distilled water containing blood sample served as positive control. The hemolytic activity (%) was calculated.

## RESULTS AND DISCUSSION

The crude enzyme extract obtained from *Arachis hypogaea* L. was partially purified



**Fig. A.** Molecular Weight Determination (SDS-PAGE). A- Partially Purified Asparaginase From Leaves of *Arachis Hypogaea* L. Showing 70 Kda, B- Protein Marker

by a series of purification steps that included salt precipitation dialysis and size exclusion chromatography (gel filtration), where the purification fold increased after each purification step. The enzyme assay and protein determination was carried out after each step of purification. The dialyzed ammonium sulphate precipitate added to Sephadex G- 100 gel filtration column was eluted with 100 mM phosphate buffer (pH 7.0) at the flow rate of 0.4 ml/minute and the fraction showing highest total enzyme activity of 1392 U was selected for further characterization. The results of purification of L-asparaginase are summarized in Table 1. The partially purified L-asparaginase was subjected to SDS-PAGE and the molecular weight was found to be 70 kDa (Figure A).

In general, plant L-asparaginase is commenced into two categories such as potassium dependent and potassium non-dependent L-asparaginase. Investigation was made to determine the nature of partially purified L-asparaginase from *Arachis hypogaea* L. by incubating it in different concentrations of potassium chloride ranging from 20 mM to 100 mM concentration (Table 2, Figure 1).

The enzyme activity was gradually increased from 403 U/ml to 504.5 U/ml showing that presence of potassium increased the L-asparaginase activity when compared to the control (enzyme in absence of potassium chloride) with minimum enzymatic activity of 403.6 U/ml (relative activity, 100%). Similarly, stimulation of L-asparaginase activity on addition of potassium was reported in *Phaseolus multiflorus*, *Zea mays* and *Lupinus arboreus* as well as potassium independency was reported in *Lupinus angustifolius*, where dependent form was found to be less stable<sup>8</sup>.

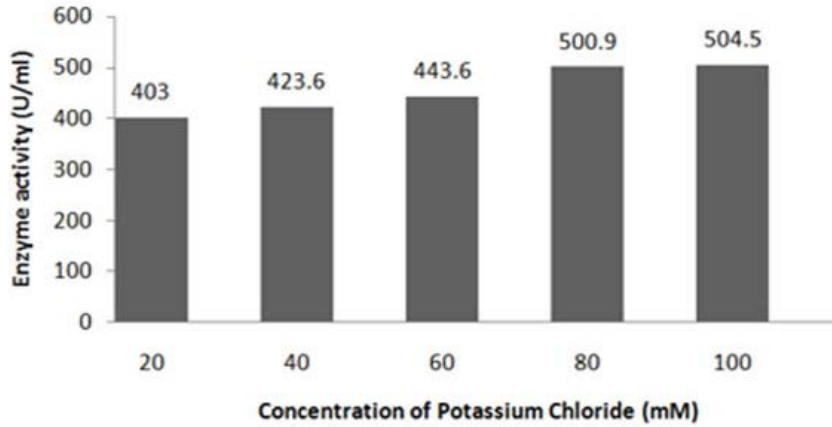
The L- asparaginase was active over a broad range of pH where it exhibited pH optimum at 8.0 (Table 3, Figure 2). The enzyme activity

**Table 1.** Purification of L-asparaginase from leaves of *Arachis hypogaea* L.

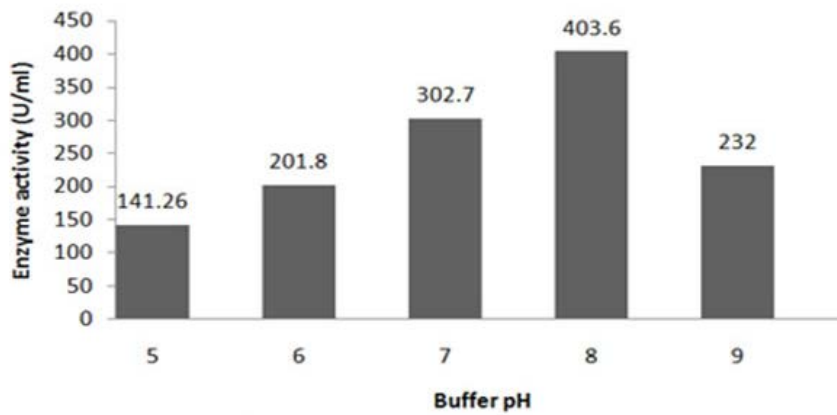
Purification steps	Collected volume of sample(ml)	Total Enzyme activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	14	14126	358.05	39.45	-	100
Post-dialysis	14	5650.4	84.21	67.09	1.7	40
Sephadex G-100filtration	3	1392	0.297	4686.86	118.80	9.85

increased gradually from pH 5.0 till pH 8 with the maximum enzyme activity 403.6 IU/ml (relative activity, 100%) However, there was decrease in

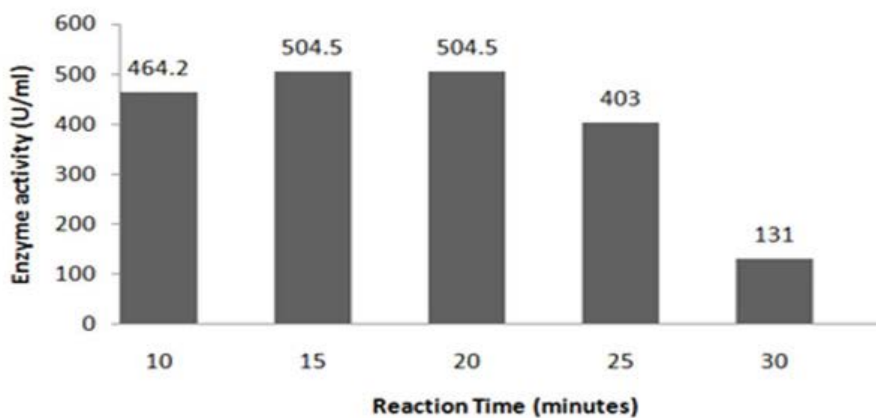
the activity as the pH of buffer increased to 9.0 providing enzyme activity as 232 IU/ml (relative activity, 57.48%). The enzymes residual activity



**Fig. 1.** Potassium Dependency of L-Asparaginase Obtained from *Arachis Hypogaea* L



**Fig. 2.** Effect of Buffer Ph on Activity of L-Asparaginase Obtained from *Arachis Hypogaea* L



**Fig. 3.** Effect of Reaction Time (Minutes) on Activity Of L-Asparaginase Obtained from *Arachis Hypogaea* L

was higher at pH 9.0 while compared to relative activity at pH 5.0 with enzyme activity of 141.26 IU/ml. Thus, it is known that L- asparaginase from *Arachis hypogaea* L. can lose its activity at minimum and maximum pH range of buffer, being partially stable at pH 7.0 with enzyme activity 302.7 IU/ml (relative activity, 75%) and completely stable at pH 8.0 (relative activity, 100%).

Similarly, highest enzyme activity of 399.5 IU/ml at pH 8.0 was reported in *Pisum sativum*<sup>9</sup>, *Spinacia oleracea*<sup>7</sup> and in *Phaseolus*

*vulgaris* seed<sup>10</sup>. The optimum pH was found to be comparative with L-asparaginase extracted from various microorganisms<sup>11-12</sup>.

The effect of reaction time on the enzyme-substrate complex is necessary to determine the efficiency of an enzyme over a period of time. The L-asparaginase activity increased over the time of incubation up to 25 minutes (Table 4, Figure 3) and found to decrease as the time of incubation extended. The enzyme activity was maximum (504.516 U/ml; relative activity, 125%) at time period of 15 minutes and 20 minutes of incubation when compared to enzyme activity 464.2 U/ml; relative activity, 100% at 10 minutes. The enzyme

**Table 2.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. at different concentration of potassium chloride

Concentration of Potassium chloride (mM)	Relative activity (%)
Control 20	10099.8
40	104
60	109
80	124
100	125

**Table 5.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. at different temperature ( °C )

Temperature (°C)	Relative activity (%)
20	32.4
37	100
40	125
50	125
60	74.9

**Table 6.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. at varying substrate (L-asparagine) concentrations

Substrate concentration (mM)	Relative activity (%)
20	75
40	100
60	107.5
80	110
100	125

**Table 3.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. at different buffer pH

Buffer pH	Relative activity (%)
5	34.9
6	50
7	74.82
8	100
9	57.48

**Table 4.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. at varying reaction time (minutes)

Reaction time (minutes)	Relative activity (%)
10	100
15	125
20	125
25	99.8
30	32.4

**Table 7.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. in relation with substrate specificity

Substrate	Relative activity (%)
L-asparagine	100
L-glutamine	17
L-glutamic acid	-
DL-ornithine	-
L-aspartic acid	-
Urea	-

activity was slightly decreased with incubation time of 25 minutes providing activity of 403.6U/ml, where the relative activity (99.8%) was higher when compared to the relative activity (32.45%) at reaction time of 30 minutes showing that the activity of L-asparaginase from *Arachis hypogaea* L. decrease with increase in reaction time. Similar result was reported in *Spinacia oleracea*, showing increase in enzyme activity at reaction time of 15 minutes<sup>7</sup>, whereas L-asparaginase from *Pisum sativum* was reported to have highest enzyme activity at 30 minutes<sup>9</sup>. However, L-asparaginase activity of *Arachis hypogaea* L. had minimum enzyme activity (131 IU/ml) at the reaction time of 30 minutes.

The study of effect of temperature on enzyme activity was made in order to know the stability of enzyme (Table 5, Figure 4). The L-asparaginase activity was found to be minimum of 131 U/ml at 20°C (relative activity, 32.4%) where as highest enzyme activity of 504.5 U/ml

was noted at 40°C and 50°C (relative activity, 125%). As the temperature was increased to 60°C, the enzyme activity was reduced to 302.6 U/ml (relative activity, 74.9%) showing 40°C/50°C as optimum temperature of L-asparaginase from *Arachis hypogaea* L. Similarly, L-asparaginase from *Solanum nigrum*, *Spinacia oleracea* and *Phaseolus vulgaris* seeds were found to be highest at 40°C and partially minimum at 50°C<sup>13-14</sup>.

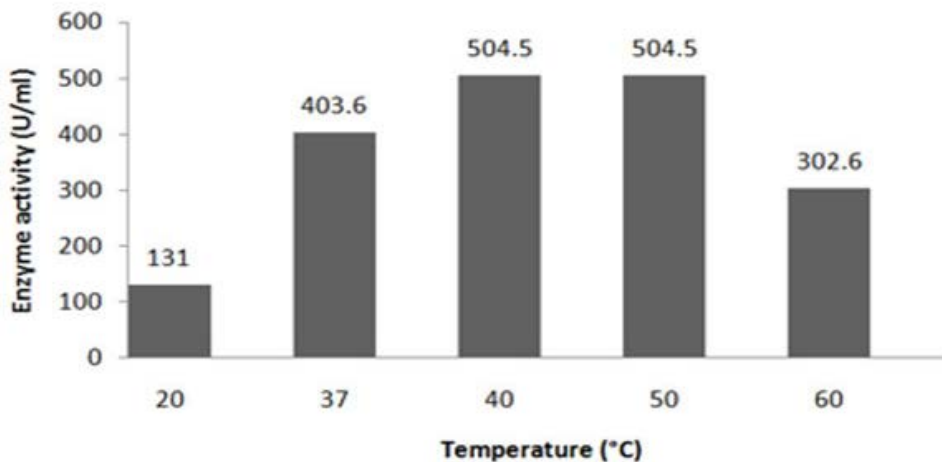
When L-asparaginase was incubated with different substrate concentration (Table 6, Figure 5), the maximum L-asparaginase activity of 504.5 U/ml (relative activity, 125%) was noted with 100 mM substrate concentration and minimum activity of 70.6 U/ml (relative activity, 17.5%) with 20 mM substrate concentration. The enzyme activity was found to be relative amongst three different concentrations (40 mM, 60 mM and 80 mM) with relative activity of 100%, 107.5 % and 110% respectively. This indicates the gradual increase of enzyme activity of L-asparaginase from

**Table 8.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. in presence of metal ions

Metal ions	Relative activity (%)
Mg <sup>2+</sup>	115
Na <sup>+</sup>	107
Cu <sup>2+</sup>	82
Hg <sup>2+</sup>	62
Zn <sup>2+</sup>	-

**Table 9.** Relative activity (%) of L-asparaginase from *Arachis hypogaea* L. in the presence of chelators and surfactant

Chelators/Surfactant	Relative activity (%)
EDTA	75
â- mercaptoethanol	75
Urea	100
SDS	17
Tween-20	115



**Fig. 4.** Effect of Temperature (°C) on Activity of L-Asparaginase Obtained from Arachis Hypogaea L

*Arachis hypogaea* L. with increase in substrate concentration. However, it was reported that L-asparaginase activity of *Pisum sativum* was higher (332.7 U/ml) with 200 mM concentration and partial with 100 mM concentration of substrate exhibiting, enzyme activity of 140 U/ml<sup>[9]</sup>.

The substrate specificity of an enzyme act as an important characteristic to determine the efficiency of it as a drug. When L-asparaginase was incubated with L-asparagine and its analogs such as L-glutamine, L-glutamic acid, DL-ornithine, L-aspartic acid and urea at concentration of 40mM, respectively (Table 7, Figure 6). The result showed that partially purified L-asparaginase from *Arachis hypogaea* L. had high specificity towards L-asparagine with enzyme activity of 403.6 U/ml

(relative activity, 100%). However, 17% activity against L-glutamine showing enzyme activity of 70.6 U/ml was observed. No other substrate was found to interact with enzyme. Specifically, no interaction with urea indicates that L-asparaginase from *Arachis hypogaea* L. lacks urease activity. Similarly, substrate specificity analysis carried out with L-asparaginase of *Phaseolous vulgaris* showed activity against different analogs with lesser than 2% of relative activity<sup>[10]</sup>, whereas L-asparaginase from microbe had no specificity against the analogs expect L-glutamine (relative activity lesser than 10%)<sup>15</sup>.

Metal ions are known to play an important role in maintaining the active configuration of enzyme at elevated temperature protecting it

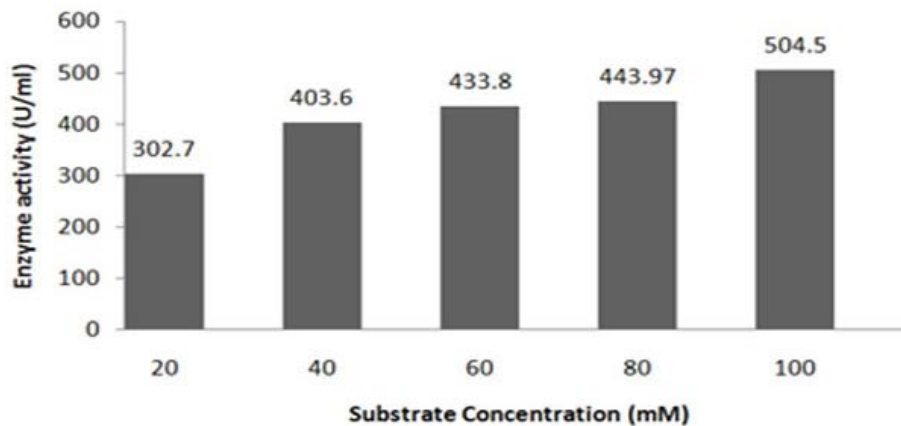


Fig. 5. Effect of Substrate Concentration (Mm) on Activity of L-Asparaginase Obtained from Arachis Hypogaca L

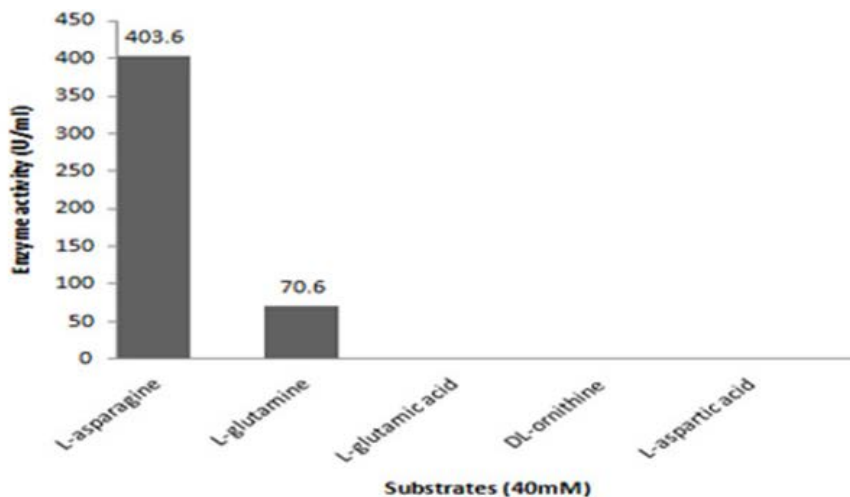


Fig. 6. Substrate (40mm) Specificity of L-Asparaginase obtained From Arachis Hypogaca L



from thermal denaturation<sup>16</sup>. The effect of various metal ions was determined by incubating partially purified L-asparaginase with metal ions (100 mM) 15 minutes prior to the addition of substrate (Table 8, Figure 7). L- asparaginase activity was not affected by the metal ions such as  $Mg^{2+}$ ,  $Cu^{2+}$ ,  $Hg^{2+}$  and  $Na^{+}$ . It was evident that the enzyme activity increased on presence of  $Mg^{2+}$  and  $Na^{+}$  as enhancers showing relative activity of 115% (464 U/ml) and 107% (433.8 U/ml) respectively when compared to enzyme activity of 403.6 U/ml in absence of metal ions (relative activity, 100%). It suggests that these metal ions can act as co-factors, which can help to increase the enzymatic reaction. However, heavy metal ions like  $Hg^{2+}$ ,  $Cu^{2+}$  partially inhibited the L-asparaginase activity with relative activity of 62% and 82% respectively.

The presence of  $Zn^{2+}$  completely inhibited the L-asparaginase activity from *Arachis hypogaea* L. The studies made on L-asparaginase from *Pectobacterium carotovorum* showed the similar partial inhibition of enzyme activity on presence of  $Hg^{2+}$  and  $Cu^{2+}$ <sup>12</sup> whereas in *Solanum nigrum*,  $Na^{+}$  enhanced the enzyme activity but  $Hg^{2+}$  acted as a complete inhibitor<sup>13</sup>.

When different chelators (10 mM) were incubated in the reaction mixture (Table 9, Figure 8), it was found that EDTA and  $\beta$ -mercaptoethanol partially inhibited the enzyme resulting in the enzyme activity of 302.6 IU/ml and 302.7 IU/ml respectively with 25 % of inhibitory action. The result was similar to the study, where EDTA partially inhibited the L-asparaginase activity from *Phaseolus vulgaris*<sup>10</sup>. SDS consisted of maximum

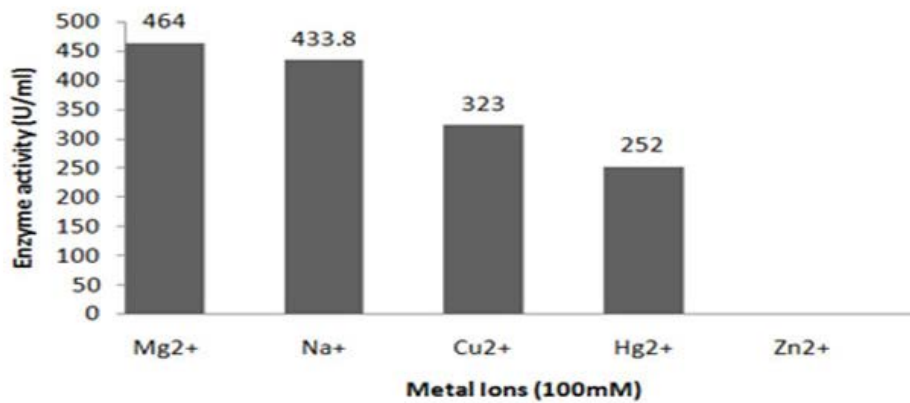


Fig. 7. Effect of Metal Ions (100mm) on Activity of L-Asparaginase from Arachis Hypogaea L

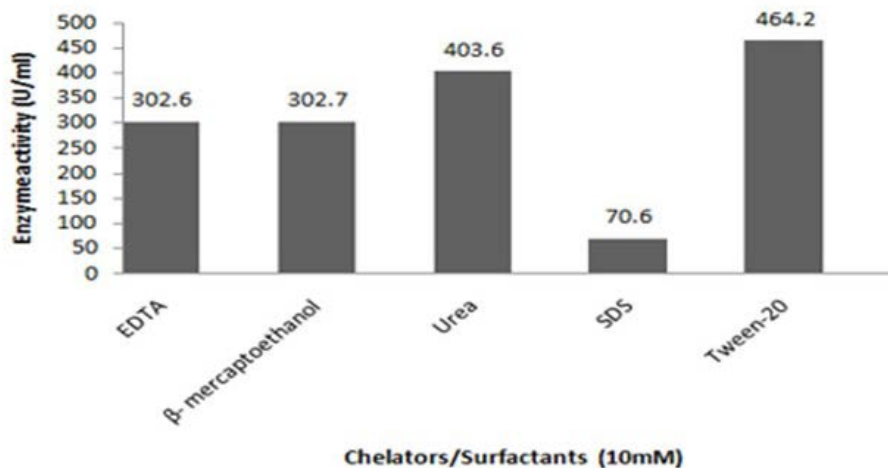


Fig. 8. Effect of Chelators/Surfactants (10mm) on Activity of L-Asparaginase Obtained from Arachis Hypogaea L

inhibitory action (83%) on L-asparaginase from *Arachis hypogaea* L. providing enzymatic activity of 70.6U/ml whereas urea had no effect on L-asparaginase activity. However, results of certain studies showed urea as a strongest inhibitor<sup>13</sup>. Tween 20 was found to enhance the L-asparaginase activity (464.2 U/ml) with relative activity of 115%, where it suggests that surfactants appears to increase quick and direct contact of enzymes with substrate site. Similar result was reported in *Streptomyces brollosae* NEAE-115<sup>16</sup>.

Cytotoxicity of L-asparaginase from *Arachis hypogaea* L. was studied *in-vitro* viz., hemolytic activity assay. The cell suspension was incubated with 100µl of enzyme fraction with protein content of 99µg along with positive and negative control. On obtaining absorbance value (at 540 nm) and calculating the percentage of hemolytic activity, the test sample containing L-asparaginase provided 80% cell viability. This indicated that partially purified L-asparaginase from *Arachis hypogaea* L. is less toxic to healthy blood cells and can be administrated as drug with further purification. Similar result has been reported in L-asparaginase from *Bacillus lichenformis*<sup>15</sup>.

### CONCLUSION

This study clearly revealed that leaf extract of *Arachis hypogaea* L. is a potent source of L- asparaginase with the molecular weight of 70 kDa. The enzyme contained similar physiochemical properties of L- asparaginase produced earlier from other sources. The study on various environmental conditions such as pH, temperature showed that L- asparaginase from *Arachis hypogaea* L. can be stable at wide range of pH , showing highest enzyme activity at pH 8 and at temperature 40°C as well as 50°C. The excellent characteristic of this enzyme such as catalytic activity over range of pH, temperature and high substrate specificity makes it highly valuable to be exploited as a potent anti-cancer agent. As the partially purified L- asparaginase from *Arachis hypogaea* L. is in urease- free form and with minimal glutaminase activity, it can reduce the side effects during course of anti-cancer therapy. Moreover, it's less toxicity against the anticoagulated blood supports its efficiency as a therapeutic agent and it can further

investigated for its capacity as a potential candidate for treating acute lymphoblastic leukemia and lymphoma in future studies.

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