International Journal of Applied Research

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(7): 628-631
www.allresearchjournal.com
Received: 15-04-2015
Accepted: 17-05-2015

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Isolation, Purification & Characterization of L-Asparaginase from dry seeds of *Pisum sativum* and *Vigna radiata*

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Abstract
L-Asparaginase is an extracellular enzyme having potential therapeutic application. The present work describes the isolation, extraction, purification, identification, characterization, protein estimation of powder of dry seeds of *Pisum sativum* and *Vigna radiata*.
Seeds of *Pisum sativum* and *Vigna radiata* were extracted for the production of –asparaginase. L-asparaginase was assayed according to Nesselerization method based on the conversion of L-asparagine to ammonia and L-aspartate.
Crude enzyme was partially purified by using dialysis technique. Maximum concentration of enzyme was found in *Pisum sativum* as compared to *Vigna radiata*. The purified enzyme was characterized for effect of temperature, pH, enzyme concentration & substrate concentration.

Keywords: *L-Asparagine, Pisum sativum, Vigna radiata* and *L-asparaginase.*

1. Introduction
L-asparaginase (L-asparagine amid hydrolase, E.C.3.5.1.1) was introduced in the therapeutics to treat acute lymphoblastic leukemia. However, L-asparaginase is of special significance because of the fact that tumour cells are deficient in L-asparaginase synthetase activity, which restricts their ability to synthesize the normally non-essential amino acid L-asparagine, required for the growth and survival of cancer cells. Therefore, tumour cells are dependent on exogenous supply of L-asparagine from body fluids. Administration of L-asparaginase within the body does not affect the functioning of normal cells because they possess an inherent property to synthesize L-asparagine for their own requirements, but reduces its concentration in the plasma pool. Thus induces a state of fatal starvation in the susceptible tumour cells. L-asparaginase is an important therapeutic enzyme and is produced by bacteria (Upadhyay *et al.,* 2012) [11].
The plant asparaginase has been less studied (Borek and Jaskoliski, 2001). In plants, L-asparagine is the major nitrogen storage and transport compound (Sieciechowicz *et al.*, 1988). In many legumes, asparaginases liberate ammonia from asparagines that is necessary for protein synthesis. There are two groups of such proteins, called potassium-dependent and potassium-independent asparaginases. Both enzymes have significant levels of sequence similarity (Lough *et al*., 1992b).
The medical utilization of L-asparaginase from the reported sources suffer the limitations of eliciting immunological responses leading to hypersensitivity in the long-term usage, allergic reactions, anaphylaxis and instance of spontaneous resistance of the tumor cells. So the present investigation was undertaken with an aim to search for some excellent L-asparaginase producer and study optimization criteria for the production of cost effective, eco-friendly and a potent L-asparaginase in near future.

2. Materials and Methods
Sample Collection
Seeds of *Pisum sativum* and *Vigna radiata* were collected during August 2014 from the local market of Akola. Healthy looked seed were washed three to four times with distilled water to remove surface dust and other foreign particles, and then stored in clean dry container at 4 °C.

Extraction of Asparaginase
10 gm of seed powder were mixed with three volume of 0.05 M. Potassium phosphate buffer, pH 8.00 containing 1.5 M sodium chloride, 1 mM PMSF, 1 mM EDTA, and 10% (W/V) glycerol, then centrifuged at 10,000 RPM for 20min. supernatant was used as crude enzyme.

Enzyme Assay
Mashbum and Wrist on (1963) determined the rate of hydrolysis of asparagine by measuring released ammonia; one unit releases one micromole of ammonia per minute at 37 °C and pH 8.6 under specific condition.
Asparaginase was assayed according to Nesserlization method based on the conversation of L-Aspargine to ammonia and L-Asparatate (Ren *et al*., 2010).

Standard Graph
In to a series of test tube 1 ml of each 0.1 µm, 0.2 µm, 0.3 µm, 0.4 µm, 0.5 µm, 0.6 µm, 0.7 µm, 0.8 µm, 0.9 µm and 1.0 µm of ammonium sulphate were added from working solution. Then 2.5 ml of 1.5 M TCA was added. To this 1 ml of 1 N NaOH was added. It was followed by 0.2ml EDTA (0.1M) to each tube to overcome the encountered turbidity. After 2 min 0.5 ml of Nessler's Reagent was added. The OD was measured after 5 min. at 425 nm. Blank was adjusted by distilled water. A standard curve was constructed by taking ammonium sulphate (µm/ml) on x axis and optical density on y axis.

Dialysis method
The samples were then dialysed by using dialysis membrane having pore 3.2u

Protein Estimation, Enzyme Assay and Optimization Studies of partially purified Enzyme
Partially purified samples were subjected to protein estimation was by Folin-Lowry's method. The L-asparaginase assay was determined by measuring released ammonia. The standard graph of ammonium sulphate was plotted. With the help of this graph L-asparaginase was assayed from partial purified sample by taking OD at 425 nm (Kushwaha *et al*., 2012) [6].
Further the L-asparaginase was characterized to check the effect of pH, temperature, enzyme concentration and substrate concentration by maintaining respective pH, respective temperature, substrate and enzyme concentration (Kushwaha *et al*., 2012) [6].

3. Result and Discussion
Protein Estimation
Sample I i.e. *Pisum sativum* found to have highest protein content of 0.89 mg/ml. as compared to sample no. II i.e. *Vigna radiata* 0.76 mg/ml protein found.

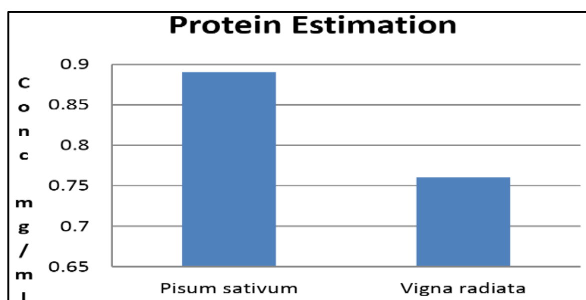

Fig 1:

Enzyme Assay
Amount of ammonia released from the purified sample of seeds was calculated by plotting standard graph of ammonium sulphate. The concentration in unknown sample was calculated i.e. Sample I (*Pisum sativum*) showed 20 u/mg while Sample II (*Vigna radiata*) showed 0.17 u/mg activity.

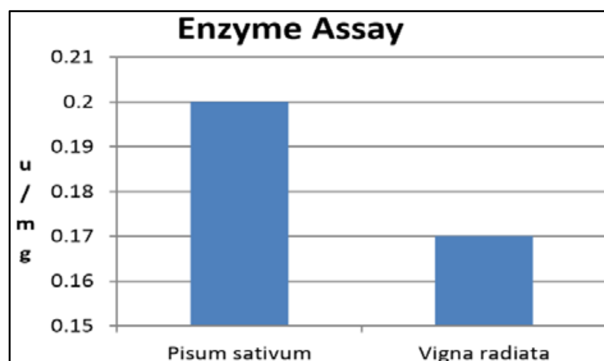

Fig 2:

The specific activity of unknown sample was calculated by following formula.

$$U/mg = \frac{\text{Micromole of ammonia released}}{\text{10 min X mg enzyme in Reaction}}$$

Specific activity of Sample I (Pisum sativum) was found to be 0.0061u/mg & Specific activity of Sample II (Vigna radiata) was found to be 0.0053 u/mg.

Characterization of Enzyme
The crude and purified enzyme was characterized for the effect of pH, temperature, enzyme concentration and substrate concentration and result were noted as follows.

Effect of pH on L-Asparaginase activity
It was observed that enzyme shows the maximum activity at pH 7. At acidic pH 5 enzyme activity was low and at alkaline pH 9 and 11 less activities were obtained as compared to pH 7.

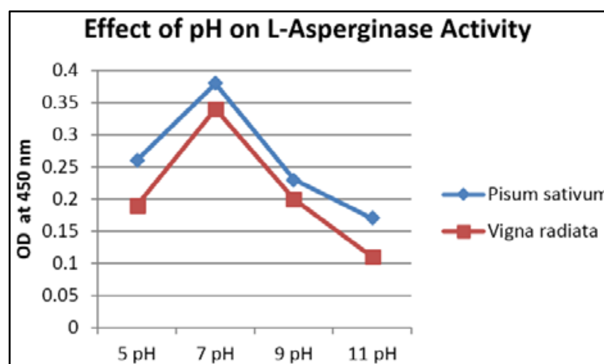

Fig 3:

Effect of temperature on L-Asparaginase activity
Effect of temperature on L-Asparaginase activity was studied. It was observed that at 37 °C maximum enzyme activity was observed for enzyme from sample. At low temperature, enzyme activities were lowered and at higher

temperature it shows less activity as compared to 37 °C. When there was again increase in the temperature upto 100 °C enzyme activities was found zero. Sample No. 2 was found to exhibit high enzyme activity than sample No. 1 at 37 °C which is about 0.38 um/mg and sample No. 1 was found to be 0.31 ug/mg at temperature 37 °C.

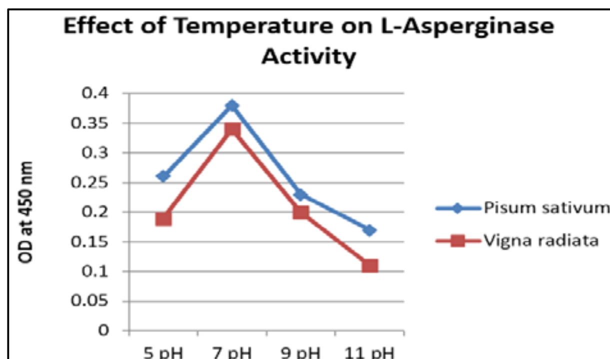

Fig 4:

Effect of enzyme concentration on L-Asparaginase activity

Effect of enzyme concentration on the enzyme activity was studied. It was observed that a maximum enzyme activity was present at 0.4 mmolar concentration

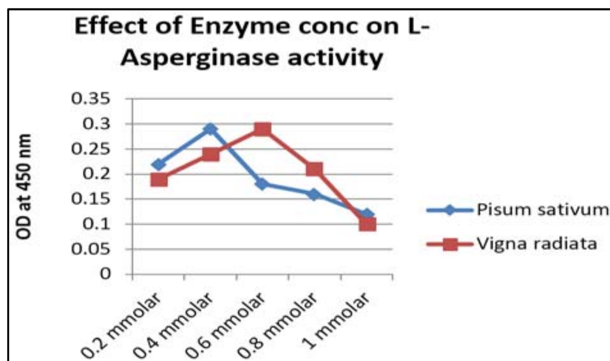

Fig 5:

Effect of Substrate concentration on L-Asparaginase activity

Effect of substrate concentration on the enzyme activity was studied. It was observed that as the concentration of substrate increases, the enzyme activity also increases. Highest activity was found on 0.6 molar concentration of substrate thereafter that enzyme activity decreases.

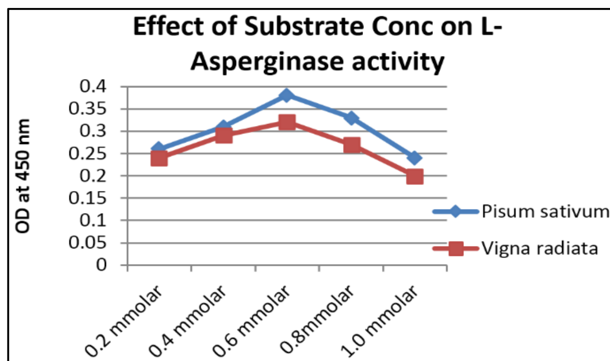

Fig 6:

4. Discussion

L-asparaginase in the treatment of leukemia and other lymphoproliferative disorders has extensively studied, for these reasons L-asparaginase has established itself to be an indispensable component (Umesh *et al*., 2007) [10]. So keeping this in mind present work was carried out to have more and maximum L-Asparaginase production.

In the present investigation the enzyme L-Asparaginase was isolated from the seeds of plants. L-Asparaginase was extracted from dry seed powder of the plants by homogenization method (Chang and Farden, 1981). The L-Asparaginase present in the extract was confirmed by Asparaginase Assay

Both the samples I and II showed good concentration of L-Asparaginase, *Pisum sativum* showed higher concentration than *Vigna radiata*. The crude sample was purified by using dialysis technique. The purified enzyme was further characterized for the effect of temperature, pH, Substrate concentration, and enzyme concentration.

The enzyme from both samples showed maximum activity at 37 °C temperature. The enzyme activity was found to be increased at pH 7, below and above this value it was less. While increase in substrate and enzyme concentrations showed that enzyme activity also increases but upto certain limit after that it declines. The maximum emzyme activity was observed at enzyme concentration of 0.6 µ /mg and substrate concentration of 0.6 µ/mg.

Several studies reported the optimum temperature 37 °C and pH 7 for the maximum L-Asparaginase activity (Kushwaha *et al*., 2012) [6].

5. Conclusion

The following conclusions have been drawn on the basis of present piece of work.

1) Most of studies of L-Asperginase are from bacteria but plants also found to be good source for the isolation of L-asparaginase.
2) The L-Asparaginase containing plant was found belong to Leguminous family
3) *Pisum sativum* show more L-Asparaginase activity then *Vigna radia*
4) The purified enzyme will be better therapeutic agent than bacterial Asparaginase since it has many side effects.

6. References

1. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology. New age International (P) Ltd., Publishers, New Delhi. 3rd Edition, 2003.
2. Audipudi Amritha, Pallavi R, Naga Ratna Supriya G. Characterization of L- Asparginase producing bacteria from Mangrove soil, International Journal of Chem Tech Research 2013; 5(1):109-112.
3. El-Bessoumy AA, Mohamed S, Jehan M. Production, Isolation, and Purification of LAsparaginase from *Pseudomonas Aeruginosa* 50071 Using Solid-state Fermentation. Journal of Biochemistry and Molecular Biology 2004; 37(4):387-393.
4. Eliana Chagas P, Laelaslov Sodek. Purification and properties of Asparaginase from test of immature seeds of pea, 2012.
5. Kamble KD, Bidave PR, Muley VY, kamble LH, Bhadange DG, Musaddiq M. Characterization of L-asparginase producing bacteria from water, farm and

saline Soil. Bioscience Discovery 2012; 3(1):116-119.
6. Kushwaha Akhilesh, Faiyaz Ahmed, Jayanand, Pushpendra Singh. Production and purification of L-Asparginase from bacterial source. International journal of universal pharmacy and life sciences 2012; 2(2):39-62.
7. Neelam Verma, Mandeep Kataria, Kuldeep Kumar, Jyoti Saini. Comparative study of L-Asparaginase from different cytotypes of Withania somnifera. Dunai and Its purification.
8. Paglla Udaya, Rao CSVR, satish Babu Rajulapati. Studies on L-Asparginase production by using Staphylococcus Capitis, Journal of Chemical, Biological And Physical Sciences 2013; 3(1):201-219.
9. Savitri, Neeta Asthma, Wamik Azim. Microbial L-asparginase: A potent Antitumour Enzyme. Indian Journal of Biochemistry 2002; 2:184-194.
10. Umesh K, Shamsher S, Wamik A. Pharmacological and clinical evaluation of Lasparaginase in the treatment of Leukemia, Critrev oncol hematol 2007; 61:208-221.
11. Upadhay Ramraj, Akanksha Saxena, Naveen Kango. Screening and production of tumour inhibitory L-asparginase by bacteria isolated from soil. Asian Journal of Pharmaceutical and Clinical Reasearch 2012; 5(3):135-137.
12. Vijay Nimkande, Dipali Barate. Studies on L-Asparaginase producing bacteria from soil sample of Akola District, 2014.