Many enzymes are known for their therapeutic actions. L-asparaginase from bacteria is known for its anticancerous property and is used in treatment of Acute Lymphoblastic Leukemia (ALL). However, one of the major limitations of the bacterial drug is the contamination of bacterial endotoxin which causes side effects. Therefore different plant species of Solanaceae and Fabaceae family were screened for L-asparaginase as there were reports on presence of this enzyme in plants.

34 plant species were screened using Nessler’s method. Out of the 34 plant species screened, Capsicum annum, Pisum sativum, Lycopersicum lycopersicum, Datura innoxia, Withania somnifera, Vigna sps and Tamarindus indica showed high L-asparaginase activity. Therefore these plant species were further subjected for protein estimation. Out of all, Withania somnifera showed highest specific activity. Therefore, further work was carried out only on Withania somnifera.

The protein from W. somnifera was purified using ammonium salt precipitation, acetone precipitation, sephadex G-75 gel filtration and Ion exchange chromatography. The peak fractions obtained on the basis of activity assay during each step of purification were monitored using Nessler’s method for enzyme activity and estimation of protein using U.V absorption and PAGE.

The purification of protein was confirmed by a single band obtained in SDS PAGE, Native PAGE and 2-D Gel electrophoresis. The molecular weight of the enzyme was determined using medium sized standard molecular weight markers in PAGE. The molecular weight was found to be 72 KDa in
native PAGE. The enzyme is a dimer with each subunit having a molecular weight of approximately 36 KD.

Characterization of the enzyme was done using various biochemical methods as well as biophysical methods. The molecular weight of the enzyme was found to be 72 KD and pI value of the protein was found to be between 5.0 to 5.5 on IPG strip (03 - 10). The enzyme showed optimum activity at pH 7.2 and it was found to be highly stable between pH 7.0 to pH 7.5 using sodium acetate, phosphate and sodium borate buffers of different pH range within 3.0 to 10.0 in an activity assay. L-asparaginase enzyme activity assay was also performed using varying temperatures for 4° C to 70° C by keeping the pH optimum. 37° C was found to be the optimum temperature for the enzyme and it was stable up to 50° C. The Km value was determined by Lineweaver-Burk double-reciprocal plot (LB plot) using different substrate concentrations ranging from 1mM to 5 mM. The kinetic parameter values such as Km was 6.1 x 10^{-2} mM; kcat was 178.57 sec^{-1}; kcat/Km: was 2927.37 sec^{-1}/mM; Vmax was 714.28 µmoles/min/mg.

The antitumor activity assay showed that a remarkable suppression of lymphocyte cell culture was observed at 2 IU/tube of L-asparaginase from Withania somnifera. The results of the cytotoxicity of L-asparaginase to human leukemia cells showed that the enzyme induced a slight decrease in cell viability between 0 IU (control) and 0.5 IU, followed by a gradual decrease between 1 and 5 IU.

Total RNA isolated by commercial Kit (Genei) method, gave a good sharp band for RNA. The total RNA isolated was used for the cDNA preparation of L-
asparaginase gene. Reverse transcriptase method was used to obtain the cDNA from total RNA. The sequence was submitted to NCBI gene bank (FJ645259).

The gene was successfully optimized for E.coli expression system. To obtain the required expression product, prSET-A vector was chosen and the fusion product was named pr/WsA. Cell-free extract of the E. coli BL21 (DE3) showed high asparaginase activity with a specific activity of 17.3 IU/mg protein. The His - Tag fusion protein was purified through Nickel CL-Agarose column and 55.8 IU/mg was obtained as a final product. The K_m values for the recombinant l-asparaginase with l-asparagine and l-glutamine were determined as 0.075 and 4.5 mM, respectively. The purified enzyme showed very low glutaminase activity, which is about 2% of l-asparaginase activity. The activity was significantly lower than that exhibited by the E. coli and E. chrysanthemi enzymes.

In structure characterization, the amino acid sequence was analyzed using the block web server. “SMART” tool was used for determining the domain region of the sequence from Withania somnifera. The identification of conserved sequence was done through “Motif Scan”. “PRED TMBB” tool was used to analyze the amino acid position where there was in or out position.

“Compute pI/Mw” tool from ExPASy server was used to analyze the pI value and molecular weight of the sequence. For calculating the hydrophobicity “ProtScale” tool was used while “Statistical Analysis of Protein Sequences” (SAPS) was used to evaluate content of amino acid in sequence and its individual charge distribution.
Summary

For characterization of the secondary structure “SOPMA” web tool was used for prediction of secondary structure and “TMpred” was used for characterization of secondary structure. The protein structure was predicted by homology modeling method using software like “spdbv” and server like “swiss model” and “CPH model 2.0”. The computed model was checked for its stability on the basis of energy and the value for the energy minimization was -27455.36 KJ/mol. The ΔG° showed negative value which indicates that the structure symmetry was spontaneous and highly stable in nature.

ProSA tool was used for validation of the structure on basis of energy and “WHATIF” server was used for analyzing structure scale and symmetry, atom coordination, nomenclature, geometric, accessibility, bumping, 3-D database, B-factor and hydrogen bond and structure analysis and an over Z-score of -0.075 the structure was obtained.

The homology model of the W. somnifera L-asparaginase obtained was superposed with the structure of Human Taspase1 (PDB code: 2a8J) using “Topmatch” web tool. Active sites on the protein structure were evaluated through “spdbv” software. The protein was highly specific for the ligand L-asparagine. “Argus lab” was used for docking and to find out the best ligand pose on the structure. The best ligand pose had an energy value of -4.35269 kcal/mol. The amino acid residues of the active site were almost same as those reported in other sources.