Chapter 4

4 .1 Summary and Conclusion

Spatholobus parviflorus is a tropical plant belonging to Fabaceae family. SPL, the seed lectiin from *Spatholobus parviflorus,* is the first lectin purified from the genus *Spatholobus*. Purification was effectively done by ammonium sulphate fractionation and the combination of affinity chromatography using activated guargum and ion exchange chromatography (Sephadex C 50). The protein was decoupled with D-Galactose from the affinity bound guargum column. SPL is a galactose specific lectin. The present lectin is a hetero dimeric tetramer, as shown by SDS–PAGE and MALDI ToF analysis. MW of monomers are 29 kDa and 31 kDa and the tetrameric form with 120 kDa, suggesting that SPL is an oligo tetramer. These results reveals that the lectin is similar to galactose specific lectin isolated from *Dolichos biflorus (*Latha *et al*., 2006*).* The ability of lectin to agglutinate red blood cells was also investigated. Purified lectin agglutinates human erythrocytes of all blood groups, and does not show specificity towards any blood group. Preparative isoelectric foccussing showed that SPL is a basic lectin with pI 8.31. Total sugar content analysis by anthrone method provide the information that SPL contain 6.53% carbohydrate. The carbohydrate binding specificity of *S. parviflorus* lectin was probed by the hemagglutination inhibition assay. This experiment gave the idea about the relative abilities of various saccharides to inhibit lectin activities. Sugar inhibition studies showed that N-Acetyl-D-galactosamine is the best inhibitor among the sugars studied as seen in other galactose specific legume lectins, which is 16 times stronger than D-

galactose. pH stabiliy studies of *Spstholobus* lectin by using different buffers revealed that SPL is active in a pH range 2-11. The temperature stability studies gave the idea that SPL is a thermostable protein like BMA. The hemagglutination activity of SPL is not inhibited by EDTA or increased by the addition of $CaCl_2$, $MgCl_2$ and $MnCl_2$ suggesting that SPL does not require these divalent cations to exhibit its agglutination activity. Denaturation studies of SPL by using urea, GuHCl and thiourea indicate that SPL lost 50% of its hemagglutination activity at 4 M urea and GuHCl but in the case of thiourea, it was 5 M. It lost the activity completely at higher concentration of denaturants. This indicte that SPL is a globular protein and denaturate with denaturing agents like urea, GuHCl and thiourea. *Spatholobus* lectin is an antifungal lectin, against species of fungus such as *Aspergillus niger* and *Fusarium oxysporum.* A few other lectins also were reported as antifungal. Fluorascence spectra analyses at different concentraton of urea and guanidine hydrochloride showed the remarkable difference in fluorescence. It could be due to the exposing burried tryptophan groups. The "far UV" CD spectra indicate the predominant β-sheet structure of SPL and less α helical structure, a cmmon feuture of legume lectins. This was also confirmed later by crystallographic studies.

Native SPL crystal is th first lectin crystal reported from the genus *Spatholobus* and also from this laboratory. Among the different methods of crystallization used, diffraction quality crystals were obtained in hanging drop vapor diffussion method. Crystals were diffracted at 2.04Å. The data collection was done by using MAR 345 image plate. The X-ray beam with wavelength, 1.5418 Å was generated from rotating anode Cu Kα (Bruker Micro star) X-ray generator operating at 50 kv and 100 mA. Indexing, processing, scaling and merging were carried out by MOSFLM and SCALA

of CCP4 program suit. The electron density map visualization was done by a graphic software COOT. The sequence determined from electron density and sequence allignment by clustal W revealed that SPL has more than 60% sequence similarity with galactose specific lectins. SPL crystals are belonging to P1 space group and it is a hetrodimeric tetramer, formed of two canonical dimers, belongs to legume lectin family. The final model converged to R–factor 19.9 and R-free 26.4. It comprises 251 residues in α chain and 239 residues in β chain. The model has good geometry, shown by PROCHECK that 89.3% residues could be found in the most favoured region of Ramachandran plot, 10.3% residues are in the additional allowed region and none of the residues are in the generously and dissallowed region.

SPL share the structural similarity with most of the galactose specific legume lectins. The SPL contains Ca^{2+} and Mn^{2+} one each metal per molecule. Mn^{2+} is coordinated to Glu 126 OE2, Asp 128 OD2, Asp 137 OD1, His 142 and two water molecules and Ca2+ is coordinated to Asp 128 OD1 and OD2, Asn 132 OD1, Asp 137 OD2 , Tyr 130 O and two other water molecules by residues. These metal coordinating residues are conserved in legume lectins. The tetrameric association was found to be similar to the Gal/GalNac-binding tetrameric legume lectins SBA, PHA-L, UEA-II, 1LUL, 1FNY and distinctly different from FRIL and the Gal specific peanut agglutinin (PNA). The oligomerization results in the buriying of large number of residues. The surface area buried at the interface of α_1-β_1 and α_2-β_2 subunits is more than that buried at α_1-β_2 and α_2-β_1 (table 3.16), implying that α_1-β_1 and α_2-β_2 dimers formed first, before the formation of tetramer. There is a central solvent filled channel at the C terminal region in tetrameic association. SPL crystals have comparatively high solvent content

53.76 and the total number of water molecule is 693. More than 2/3rd of the solvent molecules were found in the primary solvent shell. Crystal packing of SPL along the different axes shows that the interactions between 2-fold related molecules are mainly involved at the α_1 38-42, β_1 38-42, β_1 62–66, α_1 218-221 and β_2 138-141 loops.

The three-dimensional structure of *Spatholobus* lectin provides a structure quite similar to the members of legume family lectins and lectins in general. The structure satisfactorily explains the observations based on the spectroscopic methods and biochemical analyses. The carbohydrate specificity with different sugars and the mode of carbohydrate recognition of SPL could be carried out as further studies.