# CHAPTER 6

# SUMMARY


**6. SUMMARY:**

Lectins are proteins or glycoproteins which can reversibly bind to specific sugar moiety present in glycoconjugates. They are ubiquitous in nature and found in all organisms ranging from viruses, bacteria, plants, and animals. They play significant roles at the cellular and molecular level for many biological recognition processes including carbohydrates, and proteins. Lectins are also responsible for mediating the attachment and binding of bacteria, viruses, and fungi to their intended targets or host cells. Accumulating literature reports suggest that Legumes are the most studied and rich source of lectins. A large number of legume lectins have been isolated from various sources and are well characterized for their specificity and biological activities. Because of their diverse roles and applications, there is an expanding emphasis on the isolation, purification, and characterization of lectins from different sources.

In the present study, a lectin was isolated and purified from the seeds of a leguminous plant *Meizotropis buteiformis* collected from in and around Chini-inkhol Village, Kangpokpi District, Manipur, India. The main aim of this study is to purify and characterize the lectin for its biochemical and physical properties. The lectin was assayed by the hemagglutination assay method of Devi *et al.*, 2009 involving a standard serial dilution procedure using 2% suspension (v/v)

of rabbit RBC. Lectin activity was taken as hemagglutination titre, the reciprocal of the highest dilution of lectin sample at which complete hemagglutination occurred and it was expressed in terms of hemagglutination unit (HAU), that is the minimum amount of lectin required for complete agglutination under the standard assay condition. Protein was estimated following the method of Lowry *et al.*, 1951 using crystalline Bovine serum albumin as standard. Carbohydrate specificity of the lectin activity was determined by hemagglutination inhibition assay taking various carbohydrates and glycoproteins following a slight modification of the method of Sawhney *et al.*, 1996.

The lectin was purified from the crude lectin preparation by a procedure involving successive steps of ammonium sulphate fractionation, and affinity chromatography. The crude lectin preparation was found to have a typical specific activity of 729.6HAU/mg protein. To undergo further purification steps, the crude lectin preparation was subjected to 50-80% ammonium sulphate fractionation, and its corresponding specific activity was found to be 2402.8 HAU/mg with 3.29 purification fold and 70.59% recovery. Among the sugars and sugar derivatives tested for preliminary sugar specificity of lectin, disaccharide Lactose was found to be the most potent inhibitory sugar followed by a monosaccharide D-Galactose. So, the partially purified ammonium sulphate fraction was subjected after dialysis to affinity chromatography of lactose-sepharose 4B column. Then the bound lectin was eluted using 10mM Lactose in PBS solution. The affinity eluted lection fractions were pooled and found to have a specific activity of 360658.8HAU/mg and a percentage recovery of 35.22% with 494.32 fold purification when compared to the crude preparation. The electrophoretic homogeneity of the affinity eluted lectin was determined by acidic native PAGE (pH 4.3) which showed a single band under native conditions.

Following the successful purification, the lectin was characterized with respect to certain physicochemical properties. The native molecular weight of the purified lectin was determined by gel filtration using Sephadex G-75 column

and found to be 75kDa. This native molecular weight of the lectin compares well with many of the leguminous plant lectins reported in the literature. The subunits molecular weights of the lectin, determined by SDS-PAGE both in reduced and non-reduced condition coupled with silver staining, were found to be 36kDa and 38kDa which are designated as subunit A and B respectively. Combining the result of native molecular weight determination with that of the subunit molecular weight determination by SDS-PAGE, it is inferred that the purified lectin is a heterodimeric protein made up of two non-identical subunits (subunit A and B) linked non-covalently without a disulfide bond. These subunit structures are also in agreement with many of the earlier reports on the subunit structure of leguminous lectins. Like other leguminous lectins, the purified lectin in the present investigation is strongly inhibited by disaccharide Lactose followed by monosaccharide D-Galactose. Though many other leguminous lectins required divalent metal ions for their activity, the activity of the lectin purified from *M. buteiformis* seeds does not show any effect either in the presence or absence of divalent metal ions ($Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Mn^{2+}$, and $Sn^{2}$) and also does not require the given divalent metal ions. Similar results of non-requirement of the metal ions by other lectins are also reported in previous works and compared well with the purified lectin.

The pH optima of the lectin activity were found to be relatively broad ranging from 6.0 to 8.0 which compares well with the pH optima of other leguminous lectins. The lectin was completely stable from pH 6.5 to 7.5 which is in conformity with those lectins from the leguminous family. Full activity of the lectin was observed at the assay temperature from $30^0$C to $50^0$C. A decrease in lectin activity was observed below $30^0$C and above $50^0$C as well. No activity was observed at $80^0$C and above. With respect to thermal stability, the purified lectin subjected to 10mins incubation in PBS exhibited complete stability from $0^0$C to $50^0$C with a $\mathbf{t_{1/2}}$ value of $61^0$C. These observed effects of assay temperature and thermal stability are comparable to or in conformity with literature reports on other leguminous lectins. Moreover, the M. buteiformis

lectin does not show blood group specificity as it agglutinates well with all RBC belonging to human blood group A, B, AB, and O, with the O group being 16 times more specific than either A or B group. However, the observed hemagglutination activity of the lectin towards human blood groups A, B, or AB, and O was 32 and 2 times lesser respectively than that of observed lectin activity towards rabbit RBC. These results are comparable with those of many other lectins reported in the literature. The purified M. buteiformis lectin is a glycoprotein in nature and the total neutral carbohydrate content of the lectin was found to be 11.4% (w/w) of the total weight of the lectin. These results compare well with other reported leguminous lectins in the literature. The purified lectin does not show any antibacterial activity against the tested three bacteria namely *E-coli, Bacillus subtillis, Micrococcus luteus.* In contrast, some other leguminous lectins reported were found to show anti-microbial properties against many bacteria.

Based on the results of peptide mass fingerprinting using Liquid chromatography tandem mass spectrometry (LC-MS/MS) and subsequent analysis by Mascot Sequence matching software (Matrix Science), no match was found with any of the known proteins in the database. However, from the protein view report for the matched protein, it was found that M. buteiformis lectin showed only upto 50% protein sequence coverage against β-chain of Butea monosperma lectin (**ANNEXURE I**). From this result, it was concluded that the lectin, purified from the seeds of *Meizotropis buteiformis* is a novel lectin which is not reported and characterized earlier. For the future work plan, more fundamental and functional characteristics of this lectin including three-dimensional structure, binding site structure, carbohydrate interaction kinetics, mitogenicity, cytotoxicity, *etc.* are yet to be determined. Research works may also be directed towards a better understanding of this newly purified lectin with respect to its physicochemical and functional characteristics.