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PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A GLUCOSE/ MANNOSE SPECIFIC LECTIN FROM HYACINTH BEAN (LABLAB PURPUREUS (L.) SWEET) USING CON A SEPHAROSE 4B AFFINITY CHROMATOGRAHY

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Abstract

Lectin from Hyacinth Bean (*Lablab purpureus* (L.) Sweet) was isolated by ammonium sulphate precipitation and purified by Con A Sepharose 4B affinity chromatography. This lectin, named LPL, agglutinated human, rat and chicken erythrocytes. The sugar specific studies were performed and among the several sugars tested, d-mannose and d-glucose inhibited agglutination of the lectin. The temperature stability of LPL was up to 60°C.The activity of LPL was completely lost at lower pH. LPL when subjected to SDS-PAGE, a single band, which when compared with marker proteins, corresponds to a molecular weight of 25 kDa approximately. The activity of LPL was reduced by demetallisation with EDTA and retained by the addition of Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, and Zn^{2+} which shows the metal dependency of the lectin. Denaturing agents like Urea and Thiourea decreases the activity on LPL higher concentrations

Keywords: *Lablab purpureus* (L.) Sweet), Lectins, Con A Sepharose 4B, SDS-PAGE, Denaturing agents

Introduction

In the early years of lectin research, legume seed extracts displayed different haemagglutination properties when assayed with erythrocytes from different animal erythrocytes (Landstein & Raubischek 1908). Leguminosae family was a rich repository of many structurally and medicinally active lectins, which are multivalent, cell agglutinating proteins with exquisite sugar binding properties (Etzler, 1986; Rudiger, 1988; Sharon and Lis, 1990; Irlanda *et al*., 2017). Many legume lectins such as *Canavalia ensiformis* (Roh and Lee., 2002), *Cicer reticulatum L.* (Gupta *et al*., 2017), *Cicer reticulatum L.*(Gautham *et al*., 2017), *Lathyrus sativus* (Sawhney *et al*.,1996) *Psophocarpus tetragonolobus L.* (Pueppke., 1979) *Glycine max* (Bondar et al., 2016), *Arachis hypogaea* (Chacko and Appukuttan.,2000) , *Pisum sativum* (Rashmi and Iyyaswami,2019; Trowbridge.,1974) *Maackia amurensis* (Imberty *et al*., 2000) *Phaseolus vulgaris* (Cummings *et al*., 1982; Kaneda *et al*., 2002) have been reported. These lectins displays a variety of biological properties including antimicrobial (Charungchitrak *et al*., 2010), insecticidal (Wang *et al*., 2017) and antitumor activities (Lacerda *et al*., 2017).The specificity of legume lectins for some typical animal glycans, has led to the suggestion that legume lectins play a role in plant defense against

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insects and/or predator animals (Chrispeels *et al*., 1991).

Lablab purpureus (L.) Sweet) is a annual or short-lived perennial vines belongs to the family Leguminosae which is a native to Africa and it is cultivated throughout the tropics for food (Maass *et al*., 2010; Sheahan *et al*., 2012). The fruit is a legume pod variable in shape, size, and color. It is usually several centimeters long and bright purple to pale green. The plant was reported to shown anti-inflammatory, antioxidant, cytotoxic potential, antifungal, antihyperglycemic and antinociceptive potential (Habib *et al*., 2012; Fakhoury *et al*., 2001; Ahmed *et al*., 2015; Rahman and Akhter, 2018). Hemagglutinating activity in seeds of Hyacinth bean was first reported by Boyd and Reguera (1949) and then by Tobiska (1959). Lectin from *Lablab purpureus* has been isolated and characterized by several groups, though there are many studies about Hyacinth bean has been published, the variations of biochemical and biophysical properties differs with varieties of *Lablab purpureus* used and/or the different climates in which they were grown(Guran et al., 1983; Mo et al., 1990). In the present paper, we describe purification of lectin from the seeds of *Lablab purpureus* and its biological characterization.

Materials and Methods

Chemicals

Con A Sepharose 4B column were purchased from GE Healthcare Bio-sciences, (Sweden).Acryl amide, Bis-acrylamide, ammonium per sulfate, β-mercaptoethanol, TEMED, Glycine, and sodium dodecyl sulfate were from Sigma (St. Louis, MO, USA).Sugars, PEG and α-D-methylmannoside were purchased from Himedia (Mumbai). Ammonium sulfate, Tris hydrochloride, EDTA, Sodium chloride, Sodium acetate, Sodium hydroxide, Hcl, Methanol, Glycerol, Glacial acetic acid was the analysis grade commercially available. Low molecular weight protein markers are purchased from BR BIOCHEM Life sciences (New Delhi).

Collection of Plant Material

Lablab purpureus (L.) Sweet) used in the study were purchased from Krishipura, Pangappara, Trivandrum, Kerala, India. Voucher specimens (KUBH 10251) were deposited on Department of Botany, University of Kerala.

Preparation of Crude Extract and Ammonium Sulphate Precipitation

Hyacinth Beans were homogenized using an electric blender with 20 mM Tris-Hcl buffer pH 7.4 (1:10) ratio and the extract was filtered through a double-layered cheese cloth and centrifuged. Protein was precipitated from this supernatant with 0 – 80 % saturation of ammonium sulphate was added in small fractions with constant stirring on an ice bath. The resultant sample was centrifuged (8000 × g, 30 min, 4 °C) in a cooling centrifuge (Eppendorf; 5804R,Germany) and the protein obtained was then resuspended in Tris-Hcl buffer pH 7.4 and dialysed (Sigma; 25×16mm) in the same buffer for 3 days after several buffer changes to remove ammonium sulphate. The fraction after dialysis was loaded on to Con A Sepharose4B affinity column for further purification.

Purification of Lectin Using Con A Sepharose4B affinity chromatography

Purification of lectin from resuspended pellet was done using Con A Sepharose 4B affinity

chromatography column (1.6×60 cm) .The column used consists of 5 ml of Concanavalin A coupled to Sepharose 4B by the cyanogen bromide method. Dialysed precipitate was loaded on the column using Tris-Hcl, pH 7.4, as the binding buffer and washed with the same solution and fractions (10ml) were collected. Flow rate was adjusted to 1 ml/min. The column was washed with ten column volume of binding buffer to remove any unbound/weakly bound proteins and the presence of protein in each fraction was monitored at 280nm with spectrophotometer (Jasco V630, Germany). When the absorbance was below>0.05; bound proteins were eluted by 0.1M α-D-methylmannoside and dialysed with Tris-Hcl and finally against distilled water. The high specific fractions were pooled, dialysed and checked haemagglutination activity (Charungchitrak *et al*., 2011).

SDS-PAGE and Coomassie Staining

10% Sodium-dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (1970). The molecular mass of the protein was determined using low molecular weight markers (BR BIOCHEM Life sciences ,New Delhi).The concentrated protein fractions eluted in different pH buffers were subjected sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS -PAGE). The protein bands in the gel after electrophoresis were visualized by Coomassie staining (Blum *et al*., 1987).

Hemagglutinating assay

Haemagglutination assays were carried out on according to procedure described by Debray (1981). Human blood samples were taken from healthy blood donors of Department of Biochemistry. Rat erythrocytes were taken from animal house of Dept of biochemistry, University of Kerala .The erythrocytes were washed thrice in Tris-Hcl by centrifugation at 1,000rpm. Finally 10 % R.B.C suspensions were prepared and used for determination of haemagglutination activity, which was done in round bottomed micro titre plates (Tarsons products, Mumbai). Haemagglutination activity was visualized by eye after 1 hour by examining cells under a light microscope (Alwana *et al*., 1998).

Protein Estimation

Lowry's method (1951) was used in determining the protein content of the crude extract and the fraction obtained from gel fraction with Bovine serum albumin as standard.

Carbohydrate biding specificity

For the inhibition test, 100µl of serial two fold dilution of the various sugars or glycoproteins are first added to each well of 96-well micro titre plate. An equal volume of LPL was added to each well, and this was gently shake and incubated for 1 hour at room temperature. Finally 100µl of human ABO erythrocyte suspension was added. After 1 hour the haemagglutination inhibition titre was scored visually (Vazquez *et al*., 1997).

Effects of temperature and pH on hemagglutinating activity

Effects of temperature and pH on hemagglutinating activity were evaluated according to Shet and Madaiah *et al*., 1987. LPL (1mL) was incubated for 20min at 30-100^{0}C and cooled down for 10min and haemagglutination were performed. To find out effect of pH in the haemagglutination assay the following buffer solutions were used. Glycine-Hcl pH 2.8, Acetate

pH 4.3, Tris-Hcl pH 8.5, Glycine –NaOH pH 10.5 buffers were used.

Effects of metal ions

To evaluate the effect of divalent metal ions was previously dialyzed against 5 mM EDTA (24 h at 4 °C) followed by150 mM NaCl (6 h at 4 °C) to eliminate EDTA. Subsequently, the haemagglutination of dialyzed was evaluated in the presence of 100 mM $CaCl_2$, $MgCl_2$ $MnCl_2$, $BaCl_2$, $FeSO_4$ and $ZnSo_4$ (Devi *et al*., 2011).

Effect of denaturing agents

To determine the effect of denaturing agents ie, Guanidine hydrochloride and Thiourea on lectin activity, wide range of concentrations from (0.5-5M) was prepared The 50 µL of each solution was incubated with 50 µL of lectin solution (1mg/ml) in a microtitre plate at 37°C for 1 hour and the haemagglutination activity was checked for untreated and treated samples (Kaur *et al*., 2005).

Results and Discussion

Lectins were abundantly present in legumes, they accumulate in the protein bodies to well characterized storage proteins (Sultan *et al.*, 2004).Lectin was isolated by precipitation with solid ammonium sulfate (0 - 80%) followed by dialysis against the same buffer for 2 days with regular buffer changes. Presence of lectin after salting out and dialysis was confirmed by haemagglutination and then subjected to Con A Sepharose 4B affinity chromatography. The eluted portion with 0.1M α-D-methylmannoside the active fractions (No.14-16) were pooled and dialysed against Tris-Hcl with several changes for 78h. This lectin was named LPL and used for further studies. The elution profile of is shown in the figure: 1. Protein content in the sample was estimated to 47.39 mg/ml (Table 1). LPL eluted as a single peak which showed haemagglutinating activity as shown in figure 2 and then subjected to SDS -PAGE and then compared with low molecular weight markers showed a band with an apparent molecular mass of 25 KDa (Fig-2) which demonstrated that the purification strategies were effective. LPL agglutinates with human ABO, rat and chicken erythrocytes tested (Table 2). Though several sugars were used for the inhibition study the binding property, glucose and mannose was found to be potent inhibitors of LPL. The result of inhibition of haemagglutination by LPL was summarized in Table-3. This finding was supported by previous studies of lectin from earlier workers on *Lablab purpureus* (Saha *et al*., 2014; Leopoldo *et al*., 1994; Fakhoury *et al*., 2001). From fig-3, it is clear that the activity of LPL was stable upto 60^0C and decreases at higher temperature and completely lost at the temperature of 90^0C. In temperature stability studies, LPL showed remarkable stability and tolerance towards high temperature as the uniform activity was maintained till 60 °C and completely loses at 90 °C.The thermostability of lectin can differ in accordance with the association degree of protein structure on hydrogen bonds (Vogt *et al*., 1997), existence of glycosylation (Öberg *et al*., 2011) as well as complexity of its tertiary structure (Carvalho *et al*., 2015). It was found that the LPL exhibited maximal specific activities between pH (6 - 7) respectively, and the stability loses at lower pH (2.4 - 4.3) and decreases at higher pH (8-10.8) (fig-4) against the whole buffers tested. The pH stability loses in lower conditions, even though, higher pH showed a negative impact. Lectin from *A. tortuosam* showed a loss of haemagglutination activity both in the highly acidic (less than 2)

and basic (10 and above) pH conditions (Dhuna *et al*., 2005). The activities of LPL were completely lost in the presence of EDTA, and the addition of divalent ions Ca^{2+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Fe^{2+} and Zn^{2+} regains the activity of lectin considerably **(**Fig-5). Metal ion analysis revealed that LPL requires metal ions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Fe^{2+}, Zn^{2+}) as it shows complete deactivation with EDTA. Metal ion specificity is a general physicochemical property observed in most legume lectins, indicating they are essential for haemagglutination activity (Moreira *et al*., 2006). The reducing agents Guanidine hydrochloride, urea and thiourea inhibits the activity of LPL with increasing concentrations **(**Fig-6). Among the denaturing agents urea and thiourea are the potent inhibitors of LPL in higher concentrations. The high concentration of denaturing agents was supposed to allow water molecules to disrupt the hydrophobic interactions in the interior of lectin that support its native conformations (Singh and Saxena, 2013). Addition of various denaturing agents have been found to affect tertiary structure and henceforth haemagglutination activity of lectins (Islam and khan., 2012, Kabir *et al*., 2012, Ynalvez *et al*., 2012).However there are not any literature available on the metal ion specificities and denaturing properties of LPL to our knowledge.

Conclusion

In the present study 25 KDa lectin was isolated and purified from *Lablab purpureus* (LPL) by Con A Sepharose 4B affinity chromatography column which is mannose/glucose specific.LPL were found to be stable at pH between (7-8) and temperature upto 60^0C.LPL were also metal dependent and its activity was decreased by increased concentrations of urea and thiourea. The seeds of Lablab purpureus which is used as food stuff and it contains lectin protein which has exquisite biophysical and biochemical properties. In the future the protein isolate could be used for blood typing, bacterial typing and may have the potential to play role as biotechnological tools. Hence further work can be continued for exploring its medicinal value as well as its other therapeutic uses.

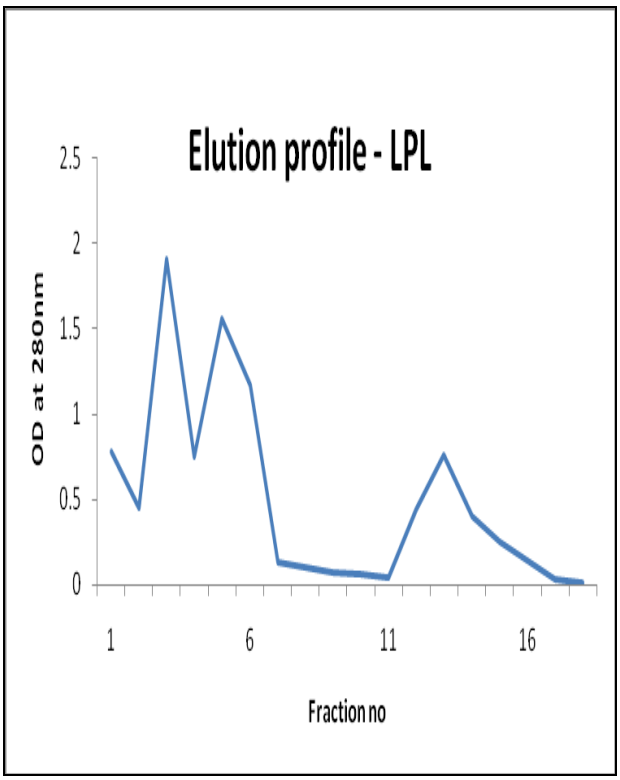

Figure 1. Purification of LPL. Affinity chromatography of LPL on Con A Sepharose 4B column. Elution with Tris-Hcl buffer followed by 0.1M α-D-methylmannoside

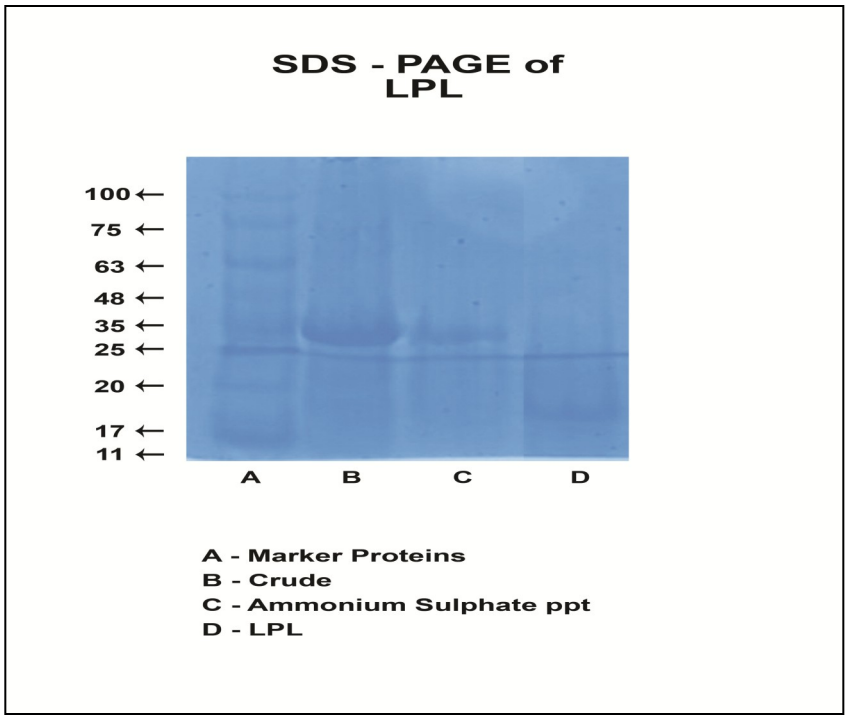

Figure 2. SDS-PAGE

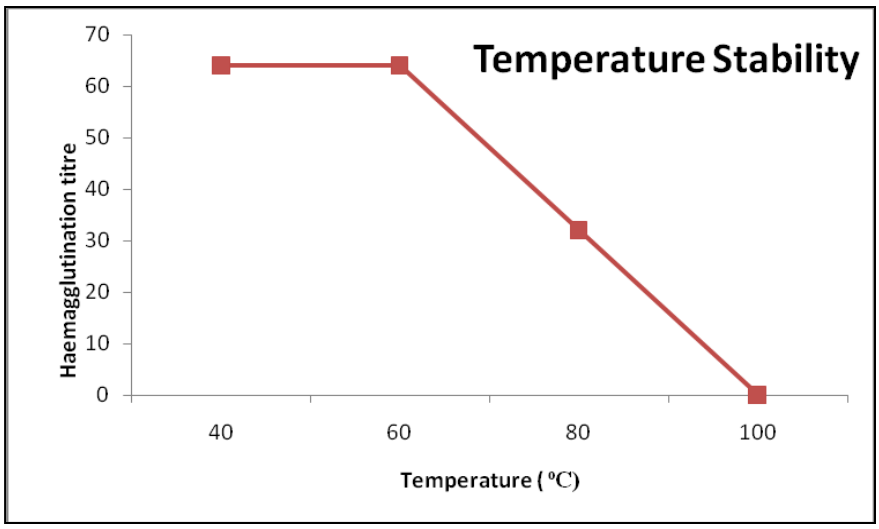

Figure 3. Effect of temperature on the hemagglutinating activity of LPL

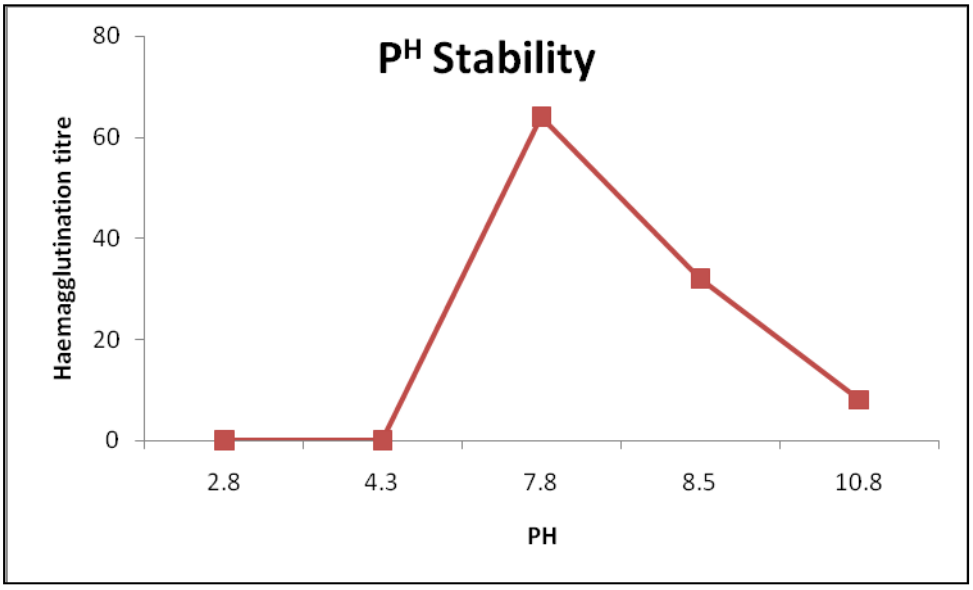

Figure 4: Effect of pH on the hemagglutinating activity of LPL

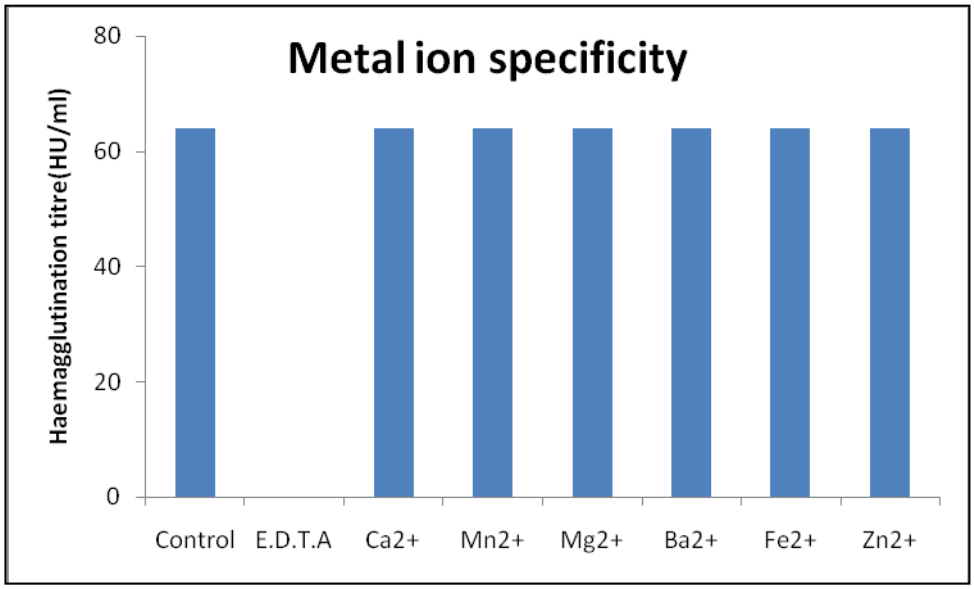

Figure 5. Effect of Metal ions on the hemagglutinating activity of LPL. Hemagglutinating ac tivity (HA) of EDTA-treated LPL after the addition of Metal ions (100Mm). Details are given under materials and methods

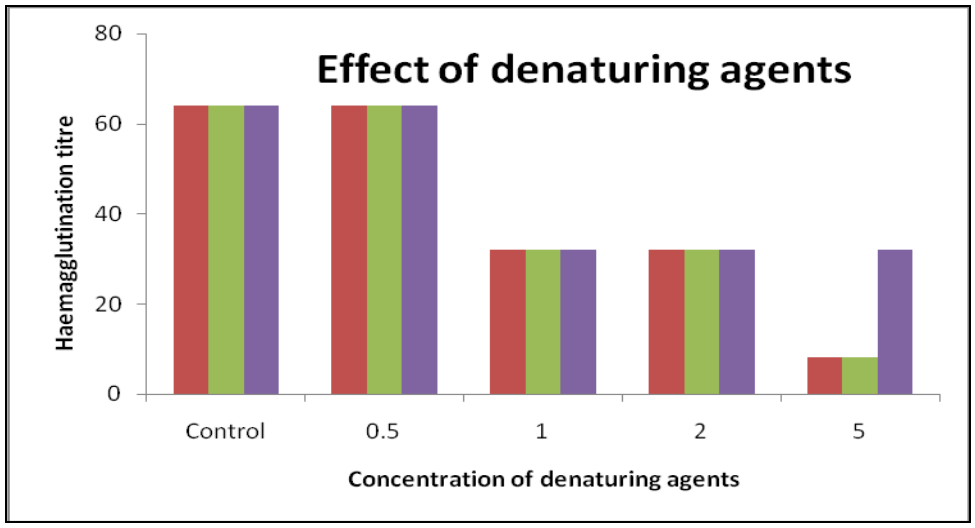

Figure 6. Effect of denaturing agents on the hemagglutinating activity of LPL

Table.1 Summary of purification of LPL

Sample	**Protein (mg/mL)**	**HAU**	**Specific activity**	**Yield of protein (%)**
Crude extract (100ml)	1.15	16	13.91	100
Ammo-nium sulphate fraction	0.545	32	58.72	47.39
Affinity chroma-tograph y frac-tion	0.65	64	98.46	56.52

Table- 2. Blood gp specificity of LPL

Blood group	**Titre value**
A	64
B	64
AB	64
O	64
Rat	32
Chicken	64

Table-3: Inhibition of the hemagglutinating activity of LPL by common sugars.

Sugar (200mM)	Haemagglutination (+/-)
Glucose	+
Fructose	-
Lactose	-
Xylose	-
Arabinose	-
Maltose	-
Dextrose	-
Mannose	+
Galactose	-
N-acetyl-galactosamine	-
Mellibiose	-

References

Ahmed, M, Trisha, UK, Shaha, SR, Dey, AK, Rahmatullah, M. An initial report on the antihyperglycemic and antinociceptive potential of Lablab purpureus beans. World Journal of Pharmacy and Pharmaceutical Sciences 2015; 4(10): 95-105.

Alwana A,Deignana T,O ́Sullivane M,Kellyb J,O ́ Farrellya C (1998) Quantitative assay of Salmonella adherence to intestinal epithelial cells, A new method for assessing novel intervention products. Journal of microbiological methods.33:163-170.

Blum, H.; Beier, H.; Gross, H.J. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis, 1987, 8, 93-99.

Carvalho AS, Silva MV, Gomes FS, Paiva PMG, Malafaia CB, Silva TD, Vaz AFM, Silva AG,. Arruda IRS, Napoleão TH, Carneiro-da-Cunha MG, Correia MTS, Purification: characterization and antibacterial potential of a lectin isolated from Apuleia leiocarpa seeds. *Int. J. Biol. Macromol*. 75 (2015)402–408.

Charungchitrak, Sarinya & Petsom, Amorn & Sangvanich, Polkit & Karnchanatat, Aphichart. (2013). Antifungal and antibacterial activities of lectin from the seeds of Archidendron jiringa Nielsen. Food Chemistry. 126. 1025-1032. 10.1016/j.foodchem.2010.11.114.

Chrispeels, M.J.; Raikhel, N.V. Lectins, lectin genes, and their role in plant defense. Plant Cell 1991, 3, 1–9.

Cummings, R.D.; Kornfeld, S. Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized Phaseolus vulgaris leukoagglutinating and erythroagglutinating lectins. J. Biol. Chem. 1982, 257, 11230–11234.

Devi R.P,Sudhakar L.G.R,Vasudhevan,I and M.Vijayakumar (2011).Biological properties of hemolytic lectin from *Acacia melanoxylon*.International journal of biological .technology.2(2):64-68.

Dhuna, V., Bains, J. S., Kamboj, S. S., Singh, J., Kamboj, S., & Saxena, A. K. (2005). Purification and characterization of a lectin from Arisaema tortuosum Schott having in-vitro anticancer activity against human cancer cell lines. Journal of Biochemistry and Molecular Biology, 38, 526–532.

Fakhoury, AM, Woloshuk, CP. Inhibition of growth of Aspergillus flavus and fungal alpha-amylases by a lectin -like protein from Lablab purpureus. Mol Plant Microbe Interact 2001; 14(8):955-961.

Gautam, A. K., Sharma, D., Sharma, J. and Saini, K.C., Legume lectins: Potential use as a diagnostics and therapeutics against the Cancer, *International Journal of Biological Macromolecules*, 10.1016/ j.ijbiomac.2019.09.119, (2019).

Guran,A., Ticha,M., Filka,K. and Kocourek,J. (1983) Isolation and properties of a lectin from the seeds of the Indian bean or lablab (Dolichos lablab L.). Biochem. J., 209, 653–657.

Habib, MAM, Hasan, R, Nayeem, J, Uddin, N, Rana, S. Anti-inflammatory, antioxidant and cytotoxic potential of methanolic extract of two Bangladeshi bean Lablab purpureus L. sweet white and purple. International journal of pharmaceutical sciences and research 2012; 3(3): 776-781.

Imberty, A.; Gautier, C.; Lescar, J.; Perez, S.; Wyns, L. An unusual carbohydrate binding site revealed by the structures of two Maackia amurensis lectins complexed with sialic acid-containing oligosaccharides. J. Biol. Chem. 2000, 275, 17541–17548.

Islam B and Khan A U, Lectins: To Combat Infections, Protein Purification, Dr. Rizwan Ahmad (Ed.), 2012; ISBN: 978-953-307-831-1.

Kabir S R, Islam M F, Alom M J, Zubair M A and Absar N, Protein Peptide Lett., 2012, 19(3), 360-368.

Kaneda, Y.; Whittier, R.F.; Yamanaka, H.; Carredano, E.; Gotoh, M.; Sota, H.; Hasegawa, Y.; Shinohara, Y. The high specificities of Phaseolus vulgaris erythro- and leukoagglutinating lectins for bisecting GlcNac or beta 1 –6-linked branch structures, respectively, are attributable to loop B. J. Biol. Chem. 2002, 277, 16928–16935.

Kaur M, Singh K, Rup P.J, Kamboj S.S, Saxena A.K, Sharma M, et al., A tuber lectin from Arisaema jacquemontii Blume with anti-insect and anti-proliferative properties, J. Biochem. Mol. Biol. 39 (4) (2006) 432–440.

Kaur, M.J.Singh,S.S. Kamboj,J. Singh,A. Kaur, Sood S.K. and Saxena, A.K., (2005).Isolation and characterization of two N-acetyl-D-lactosamine specific lectins from tubers of *Arisaema intermedium* Blume and *A.wallichianum* Hook f.Indian Journal of Biochemistry & Biophysics.42.34-40.

Liener IE. Nutritional significance of lectins in the diet. In the Lectins, I.E. LIener, N. Sharon, and I.J. Goldstein, eds (San Diego: Academic Press, Inc.) 1986, 527-552.

Leopoldo, Paulo & Xavier-Filho, J & Lima, Maria. (1994). Lectins of Lablab purpureus seeds. Journal of the Science of Food and Agriculture. 65. 179 - 183. 10.1002/jsfa.2740650209.

Maass BL, Knox MR, Venkatesha SC, Angessa TT, Ramme S and Pengelly BC. Lablab purpureus-a crop lost for Africa? Trop Plant Biol 2010; 3(3):123–135.

Mo, H., Lin, Z. and Sun,C. (1990) A new lectin from Dolichos lablab beans. In Kocourek,J. (ed.), Lectins: Biology, Biochemistry, Clinical Biochem. 7. Sigma Chemical Co., St. Louis, pp. 35–40.

Moreira,R.A.,Monteiro,A.C.O.,Horta, A.C.G.,Oliveira,J.T.A., and Cavada,B.S., (1996).Isolation and characterization of *Dioclea altissima* var.megacarpa seed lectin.Phytochemistry,46,139-144.

Öberg F, Sjöhamn J, Fischer G, Moberg A, Pedersen A, Neutze R, Hedfalk K. Glycosylation increases the thermostability of human aquaporin 10 protein. *J.Biol. Chem*. 286 (2011) 31915–31923.

Peumans, W.J.; Van Damme, E. Lectins as plant defense proteins. Plant Physiol. 1995, 109, 347.

Puppeke SG. Purification and characterization of a lectin from seeds of the winged bean, Psophocarpus tetragonolobus (L) DC. Biochem. Biophys. Acta., 1979; 581:63-70.

Quinn J, Etzler M, Marilynn E. Isolation and characterization of a lectin from the roots of Dolichos biflorus. Arch. Biochem. Biophys. 1987; 258:535-544.

Rahman, S. A., Akhter, M.S. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (Lablab purpureus L. sweet 'white'). J Adv Biotechnol Exp Ther. 2018; 1(2): 49-54.

Roh, K. S. and D. J. Lee (2002) Purification and some properties of lectin from *Canavalia ensiformis* L.*Kor. J. Biotechnol. Bioeng.* 17: 484–489.

Rudiger, H. (1993) Isolation of plant lectins. In H. J. Gabius and S. Gabius (Eds.), Lectins and glycobiology (pp. 32–46). Berlin: Springer Verlag.

Saha R.K., Tuhin S. H. M., Jahan, N., Roy, A. and Roy P. Antibacterial and Antioxidant Activities of a Food Lectin Isolated from the Seeds of Lablab purpureus American Journal of Ethno medicine, 2014, Vol. 1, No. 1, 008-017.

Shet, MS and Madaiah, M, J.Sci Food Agric., (1987),41,287.

Sheahan CM. Plant guide for lablab (Lablab purpureus). USDA-Natural Resources Conservation Service, Cape May Plant Materials Center 2012.

Vázquez L, Maldonado G, Agundis C, Pérez A, Cooper EL, Zenteno E. 1997. Participation of a sialic acid-specific lectin from freshwater prawn Macrobrachium rosenbergii hemocytes in the recognition of non-self cells. Journal of Experimental Zoology 279:265–272.

Vogt G, Woell S, Argos P. Protein thermal stability hydrogen bonds, and ion pairs. *J. Mol. Biol*. 269 (1997) 631–643.

Ynalvez R A, Fuentes L M and Sanchez C V, J Plant Sci., 2011, 6(3), 124-136.