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GALOCTOSE SPECIFIC LECTIN EXTRACTION AND PURIFICATION FROM *ADATHODA VASICA*

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ABSTRACT

Lectin was isolated from *Adathoda vasica* leaves. The galactose specific lectin from *Adathoda vasica* leaves was purified by 80 % ammonium sulphate precipitation, followed by dialysis against 50 mM Tris HCl pH (7.6) and sephadex G-100 column (16 mm dia m x 22 cm) chromatography. The Flow rate of 1.5 mL 12min−1 fraction showed hemagglutinating activity against on rabbit 4% erythrocytes. The *A. vasica* lectin pH dependent assays showed best hemagglutinating activity at pH 5.0–7.0; being decreased at acidic/ alkaline conditions and by EDTA treatment. *A.vasica* lectin is a tetramer at pH 7.2 and a dimer at pH 4.0. Human erythrocytes from ABO system, cow, sheep, Goat, mice, rabbit, big were collected and titrated against *Adathoda vasica* lectin result confirmed that the rabbit erythrocyte specificity for AVL. The results for molecular mass determinations were about 20 kDa. This new methodology is useful and easy, with low costs, for lectin purification in large amounts.

Key Words:- Lectin isolation, Lectin purification, Protein purification.

INTRODUCTION

Lectins are a class with several structure-related proteins, which possess considerable specific binding capacity for carbohydrates molecules (Elgavish and Shaanan, 1997). They are binding proteins of non-immune origin, with highly ordered three-dimensional structures that can be associated as dimeric or tetrameric complexes (Sharon N, 1993). It is difficult to discuss carbohydrates without reference to lectins. Lectins are defined as proteins that preferentially recognize and bind carbohydrate complexes protruding from glycol lipids and glyco proteins (Mody, *et al*., 1995; Gorelik *et al*., 2001; Bies *et al*., 2004; Minko, 2004). The term lectin is derived from the Latin word legere meaning ‘‘to choose’’or‘‘select’’, and has been generalized to n compass all non-immune carbohydrate specific agglutinins regardless of blood-type specificity or source (Sharon and Lis, 2004). The interaction of lectins with particular carbohydrates can be as specific as the interaction between those of antigen and antibody or substrate and enzyme (Minko 2004). Lectins bind not only to oligosaccharides on cells but also to free-floating glycans including monosaccharides. Lectin– monosaccharide interactions, however, are relatively weak with dissociation constants often on the order of micro molar to milli molar range (Bouckaert *et al*., 2005; Rabinovich *et al*., 2007). Lectins were initially found and described in plants, but in subsequent years multiple lectins were isolated from microorganisms and also from animals (Bies *et al*., 2004; Sharon and Lis, 2004). The leguminous plants, particularly their seeds, are recognized lectins sources (Carlini CR, Grossi-de-Sa 2002). Generally, the lectin basic monomer is described as containing two main β -sheets and another one smaller (about to 20–30 kDa).

This folding and the features of the carbohydrate-binding site, which consists of a shallow groove on the

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superimposed β-sheets, are very similar in all leguminous lectins (Loris *et al*., 1998; Vijayan and Chandra 1999; Sharon and Lis 2002) Due to the specific binding properties, the lectins can be considered important biotechnological tools with wide applications, such as the ability of agglutinate complex carbohydrates, glycoproteins, erythrocytes, vegetative cells, lymphocytes, fibroblasts, spermatozoids, fungi and bacteria (Singh *et al*., 1999; Vijayan and Chandra 1999). Interestingly plant and animal lectins show no primary structural homology, yet they demonstrate similar preferential binding to carbohydrates. This suggests that animal and plant lectin genes may have co-evolved, thus highlighting the importance of lectin–carbohydrate interactions in living systems (Gorelik *et al*., 2001; Wormald and Sharon 2004). During the past several years, however, many primary and three-dimensional structures of lectins have been elucidated. It was observed that lectins from diverse sources lacked primary sequence similarity but shared similarities in their tertiary structures (Sharon and Lis, 2004).

Structural studies conducted on animal lectins suggested that the carbohydrate-binding activity of most lectins was generated by limited aminoacid residues designated as the carbohydrate recognition domain (CRD) (Sharon and Lis, 2004). The CRD typically recognizes the terminal non-reducing carbohydrate residues of cell membrane glycoproteins and glycolipids (Mody *et al*., 1995). Lectin CRDs also may discriminate between omeric isomers as a function of their specificities. For example, the lectin concanavalin A specifically binds the α-anomer of glucose and mannose, but not the β-anomer of either (Mody *et al*.,1995; Nimrichter *et al*., 2004; Kerrigan and Brown, 2009; Ruseva *et al*.,2009).

Within the animal lectins, several highly conserved CRD amino acid sequences have been identified, thus allowing investigators to categorize the majority of these lectins in to structurally related families and super families (Sharon and Lis, 2004). C-typelectins (CTLs) are the most abundant of all animal lectins, and the CTL super family is grouped in to three families: selectins, collectins and endocytic lectins (Stood 1989; Varki 1994; Tedder *et al*., 1995; Rabinovich *et al*., 2007; Kerrigan and Brown; 2009). A majority of CTLs are large, asymmetric, have one or more CRDs and existasCa2+-dependent proteins found in secrete dorbound forms (Drickamer and Taylor, 1993; Barondes *et al*., 1994; Drickamer, 1995; Cooper and Barondes, 1999; Minko, 2004; Sharon and Lis, 2004; Chou *et al*., 2009; Malik *et al*., inpress; Saravanan *et al*., inpress).

The lectin of *Macrotyloma axillare* was previously isolated by affinity chromatography using antigen A1 + substance H coupled to Sepharose 4BTM resin. It has *N*-acetyl galactosamine (GalNac) specificity, similar amino acid composition and the same N terminal sequence (Haylett and Swart 1982) as the lectin isolated from *Dolichos biflorus* (DBL) (Etzler and Kabat 1970; Etzler and Talbot 1977). Such as DBL is specific for A1 human erythrocytes, being therefore useful for routine blood group identification at blood banks (Sharon and Lis 2004; Henry 1996), in addition to other lectin biotechnology applications that include the GalNac recognition (Lis and Sharon 1981; Bies Lehr 2004). The affinity chromatography techniques generally are the first choice for lectin purification, in accordance to their binding features (Lis and Sharon 1981) but this method can be very expensive and, sometimes, the procedure does not supply enough amounts for industrial applications.

The present work proposes a new preparative methodology for the purification of *A.vasica* leaf lectin, based on extraction and ammonium sulphate precipitation followed by sel filtration chrom atography. Such simple and versatile methodology yields active lectin amounts applicable for preparative and industrial purposes. *A.vasica* Lectin properties were studied, such as thermal and pH-dependent stability; molecular mass determination by SDS-PAGE (denatured form) and by molecular exclusion (native form), since such studies and properties were not fully explored for *A.vasica* Lectin.

MATERIALS AND METHODS

Plant leaf materials *Adathoda vasica* was collected from Trichy. Various blood samples such as A^{+ve}, B^{+ve} and O^{+ve} were collected. Cow, goat, chicken, sheep, mice and rabbit bloods were collected from slaughter house. Microtitre V-plates, sephadex G- 100, glass column (16 mm dia m x 22 cm), Tris-HCl, EDTA, Acrylamide, Bis-Acrylamide, SDS, Posphate Buffer, Ammonium sulphate, Glucosamine hydrochloride, Galactosamine hydrochloride, N-Acetyl-d-galactosamine, N-Acetyl-d-glucosamine, Sialic acid, L(-) Fucose, Dextrose, Dulcitol, Mannitol, Salicin, Sorbitol, Trehalose, Melibiose, Fructose, (L)- Arabinose, Rhamnose, Xylose, Inositol, Lactose, Galactose, Adonitol, Cellobiose, Glucose, Mannose, Raffinose, Sucrose, Maltose. These above chemicals and all other analytical chemicals were purchased from Himedia chemicals, Bombay.

Collection and storage of blood samples

Various blood samples (O+ve, O-ve, A_1+ve, B+ve, Rabbit, Sheep, Cow and Chicken (RBC) were collected to check the blood group specificity of lectin. Clotting was prevented by adding 500µl EDTA to each 2ml blood sample and then centrifuged at 10.000rpm for

10 min for removal of plasma from blood. Equal volume of PBS (pH 7.4) was added and stored at 4˚C. 40µl of RBC was added to 960µl of PBS as a 4% RBC stock solution.

Lectin extraction from *A.vasica* leaves - crude extract

Leaf sample (100g) was homogenate with 125ml of 1x PBS using pestle and mortar. The crushing process was carried out in chilled conditions. The extract was centrifuged (10,000 rpm for 15 min) to remove debris for the removal of insoluble fibers and the crude extract was stored at 4˚C. The supernatant of extract was divided in to two aliquots: one for protein estimation and the other for haemagglutination assay.

Ammonium sulphate precipitation and dialysis

The extracted lectin was undergon to incubate various concentration (30, 40, 50, 60, 70, 80) of ammonium sulphate solution for overnight at 4ºC .The precipitate was collected by centrifuged at12, 000 rpm for 15 min to precipitated the proteins and discarded the supernatant. The precipitated proteins were exhaustively dialyzed against 50 mM Tris- HCl (pH 7.6) using with dialysis membrane and magnetic stirrer at 4ºC for 48 hrs with changing the buffer at once in 4 hrs interval. Re suspended partially purified proteins were applied for next step.

Column chromatography

The partially purified proteins were applied to a Sephadex G-100 column (16 mm diam x 22 cm).Pre washed by 50 mM Tris–HCl, (pH 7.6). Partially purified proteins were subjected in to Sephadex G-100 column. The column was eluted with 50 mM Tris–HCl, (pH 7.6) (the method was done at 18 °C (A/C) of under this condition).The every fractions were collected and monitored by UV assay (at 280 nm). Lectin active proteins were collected for protein estimation, Haemagglutination assay by titerplate method and stored at 4 °C.

Quantification of protein (Lowry *et al*., 1951)

The protein concentration of the fractions was measured using bovine serum albumin (BSA) as the standard. The absorbance was read at 660 nm.

Assay for lectin (hemagglutinating) activity

Agglutination of the red blood cells by the crude extract and the various fractions that were obtained during purification was estimated as described by Bing *et al*., (1967).

A serial two-fold dilution of the lectin solution in U-shaped microtitre plates (100 µl) was mixed with 50 µl of a 4% suspension of human erythrocytes in phosphate buffered saline, pH 7.2 at room temperature (the erythrocytes of human blood group A,B and O). The plate was left undisturbed for 1h at room temperature in order to allow for agglutination of the erythrocytes to take place. The hemagglutination titre of the lectin expressed as the reciprocal of the highest dilution exhibiting visible agglutination of erythrocytes was recorded as one hemagglutinating unit. Specific activity is the number of hemagglutination units per mg protein (Wang *et al*., 2000).

Blood group specificity (Screening) (Mirela *et al*., 2003)

Haemagglutination activity was assayed for partially purified lectin in a microtitre plate by serial dilution on 50µl of PBS (pH 7.4). A 50 µl of 4% human RBC (A, B and O) and animal (sheep, rabbit, cow, goat, chicken and rat) was added respectively and the specificity of lectin was observed.

Divalent cations requirement (Mirela *et al*., 2003)

The lectin sample was incubated in 0.005M EDTA. An aliquote of mixture was treated with equal volume of different divalent metal ions such as 0.01M $CaCl_2$, $Mncl_2$ and $Mgcl_2$. The mixture was incubated for 1 hr in room temperature. Followed by 4% of RBC was added and haemagglutination activity was observed after 1 hr.

Effect of temperature on hemagglutinating activity

The effect of temperature on the agglutinating activity of the lectin from *A.vasica* was determined by carrying out assay at different temperatures according to the method described by Patrick *et al*., (2007). The purified lectin was incubated in a water bath for 2hrs at various temperatutres: 10, 20, 30,40,50,60,70,80,90 and 100°C, and then cooled to 20°C. Hemagglutination assay was carried out as previously described.

Effect of pH on hemagglutinating activity (Mirela *et al*., 2003)

The effect of pH on the activity of the lectin from *A.vasica* was determined by incubating the lectin in the following buffers at different pH values: Glycine buffer (pH 2.0 to2.8), Citrate buffer, (pH 3.0 to 5.8), Phosphate buffer (pH 6.0 to 8.0), Tris - Hcl buffer (pH 8.2 to 9.0), Glycine-NaoH buffer (pH 9.2 to 10.4).After incubation for 2 hrs at room temperature. The samples were readjusted to pH 7.4 by dialysis and assayed for the haemagglutination activity. The control values were the agglutination titre of the lectin in PBS, pH 7.2.

Inhibition of lectin-induced hemagglutination by carbohydrates

The haemagglutination inhibition tests to investigate inhibition of lectin induced haemagglutination by various carbohydrates were performed in a manner analogous to the haemagglutination test. Serial twofold dilutions of sugar samples were prepared in phosphate-buffered saline. All of the dilutions were mixed with an equal volume (25 µl /25: l) of a solution of the lectin with hemagglutination units. The mixture was allowed to stand for 30 min at room temperature and then mixed with 50 µl (50: l) of a 4% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture, which completely inhibited hemagglutination units of the lectin preparation, was calculated (Wang *et al*., 2000).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed in 7.5% (w/v) polyacrylamide slab gel at pH 8.9 (Trudel and Asselin, 1990).PAGE was performed under denaturing conditions in 7.5% (w/v) polyacrylamide gels at pH 8.9. Samples were not heated prior to electrophoresis. Pre-stained molecular weight markers (Bio-Rad) were lysozyme (20,700), soybean trypsin inhibitor (29,100), carbonic anhydrase (34,000), ovalbumin (52,000), bovine serum albumin (70,000), b-galactosidase (121,000), and Myosin (205,000).

Protein staining after electrophoresis

Protein staining was done using aqueous silver nitrate (Blum *et al*., 1987 and modified Claimer *et al*., 2012).

RESULTS AND DISCUSSION

A complete and in-depth discussion of the biological significance of lectins is not within the scope of this paper, however, a discussion of a few mammalian system examples is warranted. Endogenous lectins mediate biological processes such as cell–cell self-recognition, cell–extracellular matrix (ECM) interactions, gamete fertilization, embryonic development, cell growth, cell differentiation, cell signaling, cell adhesion and migration, apoptosis, immune modulation and inflammation, host–pathogen interactions, glycoprotein folding and routing, mitogenic induction and homeostasis (Mody *et.al*., 1995; Gorelik *et.al*., 2001; Minko, 2004; Nimrichter *et.al*., 2004; Sharon and Lis, 2004; Wormald and Sharon, 2004; Rabinovich *et.al*., 2007; Chou *et.al*., 2009; Kerrigan and Brown, 2009; Malik *et.al*., in press; Ruseva *et.al*., 2009; Saravanan *et.al*., in press; Yamanaka *et.al*., 2009).

Adhatoda vasica lectin has a specific for the rabbit blood group and it has 4 and 1.8 mg protein. The purification folds at11.59% and 74.26% in the 80% of the ammonium sulphate saturation and column sephadex G – 100 column chromatography respectively. The specific activity was calculated by Haemagglutination assay, Its conform that maximum activity was observed at above mentioned saturation 1/16, 1/128 HU (Haemagglutination Unit). Lectins may present in leafs, seeds, fruits or different parts of the plant. It may be similar or different. Similarly in leaf crude extract agglutination activity was found which has ability to agglutinate native RBC. *Adathoda vasica* leaf crude extract had an ability to agglutinate only the rabbit blood so it's a blood group specific lectin and both leaf lectins are multivalent lectins because only the multivalent lectins can agglutinate blood groups tested for haemagglutination.

In the purification of lectin from *A. vasica* the column chromatography on Sephadex G-100 (Fig: 1) removed proteins with low molecular weight. Seven fractions were collected and identified high absorbance at 280 nm UV spectrometer (Shimadzu -1800). Based on the Haemagglutinating activity used as a main fraction of among the seven fractions, Subsequent purification resulted in 74.26 -fold purification and an overall yield of 155%. The effect of Haemagglutination also proved that the purified protein was lectin. Moreover, gel filtration chromatography on Sephadex G-100 confirmed the apparentmolecular weight of the chitin deacetylase as 20kDa, purification fold at 74.26 indicating that the purity of the product was high.

A positive pattern which indicated agglutination was a uniform coating of the bottom of the well by erythrocytes while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric clear zone of equal size to the blank. From above definition one can conclude that leaf lectin doest require trypsinized RBC for agglutination but in case of seed lectin RBC need to be trypsinized for agglutination. All the necessary reaction of leaf lectin was carried with native RBC only. Looking for blood group specificity of leaf lectin shows that it agglutinate all blood group from human to animals, leaf lectins doest require any metal ions for agglutination.

The effect of divalent metal and EDTA stability of *A. vasica* was metal independent lectins. It shows agglutination in presence of metal ions such as Mn^{2+} and Ca^{2+}. This quote was proved on this study results and shows that leaf lectin are metal ion independent lectins. The metals confer a high degree of structural stability, protecting the lectins against heat inactivation and hydrolysis by proteolytic enzymes, The *A.vasica*

hemagglutination activity on EDTA treatment and in the presence Ca (II) and Mn (II) metal ions activity is null before EDTA treatment and the hemaglutination activity recovery is uncompleted after addition of any divalent metal ions. Conformation studies using various physiochemical methods have led to the conclusion that binding of metal causes an alteration in the environment of the transition metal site which in turn is important for the creation or stabilization of the saccharine binding.

The effect of temperature stability leaf lectin were subjected to moist heat treatment for 2 hrs at 100˚C and found stable but the *Adhatoda vasica* and lectins are denatured at 100˚C in this result ambient temprature at 50˚C However, some lectins exhibit a remarkable degree of thermo resistance. Under these circumstances, substantial purification of a target lectin can be accomplished by heating a crude extract at a temperature where the target lectin is stable, but extraneous protein are denatured and precipitate from solution.

The effect of pH range *A. vasica* leaf lectin (Table.3) agglutinate native RBC using with following four different buffers namely, Glycine-HCl (pH 2 to 2.8), Citrate Buffer (pH 3 to 5.8), Phosphate Buffer (pH 6 to 8.0), Tris Buffer (pH 8.2 to 9.0), Glycine Buffer (pH 9.2 to 10.4) over this experiment observed the effect of pH on *A. vasica* leaf lectin in which complete agglutination was found at pH 5-7 and partial agglutination at pH 4. There was no agglutination above pH 8, and this experiment shows agglutination of trypsinized RBC took place over the range pH 4-10.

The hapten inhibition studies to define the sugar specificities of the crude extract (the phosphate buffered saline extract) of *A. vasica* showed that Glucosamine-HCl, N- Acetyl D Glucosamine, N- Acetyl Galactosamine, Galactosamine- HCl, maltose, fructose, sucrose, dextrose, L fucose, rhamnose, arabinose, trehalose, salicin, sialic acid and adonitol had no effect on the hemagglutinating activity. Dulcitol and sorbitol enhanced the activity of the lectin while mannose, lactose, glucose, rhamnose, raffinose, xylose, melibiose, sorbitol and cellobiose slightly inhibited the activity of the lectin. The activity of the lectin was completely inhibited by galactose with minimum inhibitory concentration of 50mM and 25mM respectively.

Purified Lectin proteins from *A.vasica* were electrophoresis on SDS Polyacrylamide gel and it's stained by aqueous Silver stain. Molecular weight determination for the haemagglutinin of leaf lectin was performed. The most highly expressed protein come in the range of 20 KDa protein can be confirmed as lectin of purification step.

Table1. Purification status of *Adhatoda vasica* leaf lectin

Fractions	Volume (mL)	Protein ($mg.mL^{-1}$)	Total protein (mg)	Total activity[a] (H.U.) mL^{-1}	Specific activity[b] HAU	Purification Fold
Crude extract	50	12.3	615	1287	2.092	100
$(NH_4)2SO_4$ fraction. (60%)	12	4	48	1164	24.25	11.59
Sephdox G-100	3	1.8	5.4	839	155.37	74.26

[a] - The total activity was obtained by multiplication between the HAU total mass for each step.
[b]- The hemagglutinating activity unit (HAU) was calculated using the reciprocal of the highest title with visible hemagglutination on Rabbit erythrocytes.

Table 2. The effect of divalent metal ion and EDTA on *A. vasica* Lectin

Treatment	HAU
PBS	156 ± 12.5
0.005M EDTA	0
0.01M $MnCl_2$	49 ± 7
0.01M $MgCl_2$	0
0.01M $CaCl_2$	26 ± 5

Table 3. Effect of temperature on hemagglutinating activity of *A. vasica* Lectin

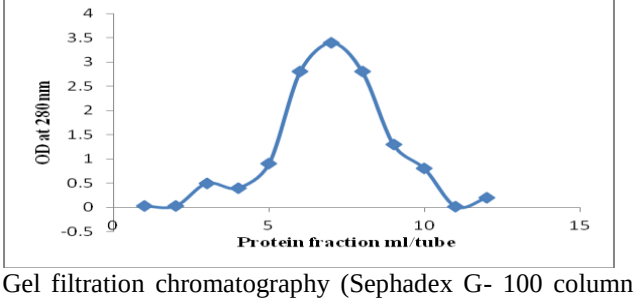
Temprature **(°C)**	30	40	50	60	70	80	90	100
Hemagglutinating activity (number of units)	12	12	12	4	2	0	0	0

Table 4. Effect of various carbohydrates on hemagglutination induced by A. vasica lectin (27 hemagglutinating units)

Sugar/Conc. In mM	100mM	50mM	25mM	12.5mM	PBS
Glucosamine hydrochloride	+	+	+	+	+
Galactosamine hydrochloride	+	+	+	+	+
N-Acetyl-d-galactosamine	+	+	+	+	+
N-Acetyl-d-glucosamine	+	+	+	+	+
Maltose	+	+	+	+	+
Sialic acid	+	+	+	+	+
L(-) Fucose	+	+	+	+	+
Dextrose	+	+	+	+	+
Dulcitol	+	+	+	+	+
Mannitol	+	+	+	+	+
Salicin	+	+	+	+	+
Sorbitol	+	+	+	+	+
Trehalose	+	+	+	+	+
Melibiose	+	+	+	+	+
Fructose	+	+	+	+	+
(L)- Arabinose	+	+	+	+	+
Rhamnose	+	+	+	+	+
Xylose	+	+	+	+	+
Inositol	+	+	+	+	+
Lactose	+	+	+	+	+
Galactose	-	-	-	+	+
Adonitol	+	+	+	+	+
Cellobiose	+	+	+	+	+
Glucose	+	+	+	+	+
Mannose	+	+	+	+	+
Raffinose	+	+	+	+	+
Sucrose	+	+	+	+	+

Note. +, hemagglutination activity; _, no hemagglutination activity; PBS, phosphate-buffered saline

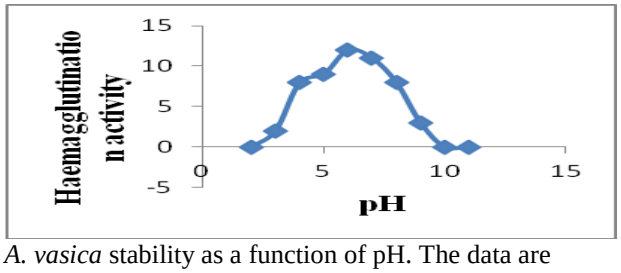
Fig 1. Column chromatography of *A. vasica* extract on Sephadex G-100.



Gel filtration chromatography (Sephadex G- 100 column chromatography in system)

Flow rate of 1.5 mL 12min−1 and detection at 280nm U.V Spectrophotometer reciprocal of Haemagglutination unit.

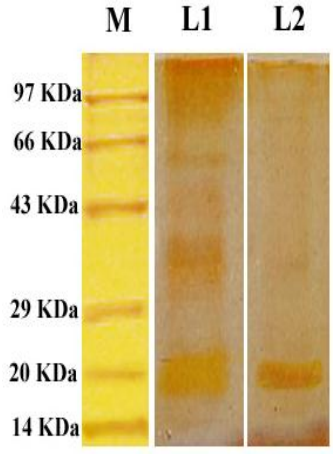
Fig 2. Effect of pH on hemagglutinating activity of *A. vasica* Lectin.



A. vasica stability as a function of pH. The data are represented by average standard deviation (*n = 3). The hemaglutination assay was conducted at pH 7.4.*

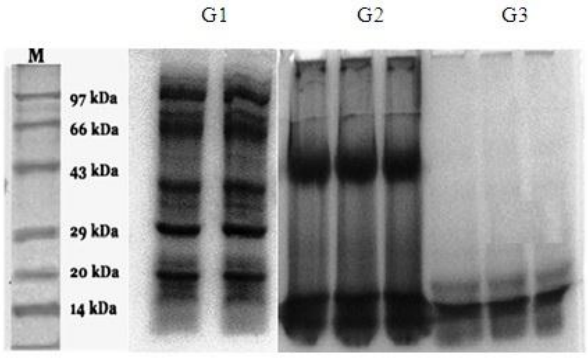
1.GLYCINE-HCL	(pH 2 to 2.8)
2.CITRATE BUFFER	(pH 3 to 5.8)
3. PHOSPHATE BUFFER	(pH 6 to 8.0)
4. TRIS BUFFER	(pH 8.2 to 9.0)
5. GLYCINE BUFFER	(pH 9.2 to 10.4)

Fig 3. Sodium Dodecyl sulphate Polyacrylamide gel electrophoresis of *A. Vasica* Lectin



Silver stained M- Marker protein, Lane 1 – Partial purified *A. vasica* Lectin, Lane 2- purified *A. vasica* lectin

Fig 4. Sodium Dodecyl sulphate Polyacrylamide gel electrophoresis of *A. Vasica* Lectin
Different purification stages



M- Marker protein, G1 – *A. vasica* Lectin Crude extract separated gel, G 2- *A. vasica* Lectin Partial purifiedseparated gel,G3- purified *A. vasica* lectin separated gel

CONCLUSION

In this study, a new extracellular lectin has been extracted from *Adathoda vasica*, purified lectin 11.59%, 74.26% fold at 24.5 and 155 yield through 80% Saturation of ammonium sulfate precipitation and Sephadex G-100 column chromatography respectively. This glyco protein exhibits different properties, not as thermostability only 50°C is a maximum temperature and stable of haemagglutinin activity and above 80°C inhibited in this activity, the lectin activity was enhanced at very high acidic pH, inhibited at high basic pH but stable at physiological pH range of 5 - 7 against on rabbit erythrocyte. Thus, it has potential application in the effective haemagglutination activity characterized by various aspects like blood group specificity, divalent metal ion and EDTA, sugar specificity against on rabbit erythrocytes. Its result that rabbit blood group specificity and galoctose sugar binding specificity in 50mM and 25mM. Molecular weight of lectin was done by the method of SDS PAGE and staining was done by silver stain method. The structure and the active center of need to be studied further.

The proposed purification methodology is easy to perform, avoiding the use of the expensive column gel filtration chromatography. Large amounts of *A.vasica* lectin can be purified by this new methodology and the yields are comparable with earlier methodology values reported for other lectin isolation. Thus, the industrial process for *A. vasica* lectin purification can be adopted using the methodology described herein, with economic advantages, because the equipments and reagents used are widespread at biotechnology factories. Due to the direct industrial applicability of the *A. vasica* lectin purification, provided that the *A. vasica* lectin has the same Galactose specificity as does rabbit blood type specificity. *A. vasica* lectin has a significant thermal stability (up to 50-60 ◦C for 30min), what could enable the thermal treatment as the first step of purification, since most of the total activity was recovered with a substantial increment of the specific activity. As verified for other legume lectins, the*A. vasica* lectin is Ca(II), Mn(II) and pH dependent for hemagglutinating activity, with full activity in the pH range from 6.0 to 8.0. In this pH range, the *A. vasica* lectin tetramer prevails at neutral to slightly alkaline range (>6.0), while the dimeric form prevails in the acid pH range (<4.0).

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