



**Research Paper**

**ISOLATION AND CHARACTERIZATION OF LECTIN FROM LEAVES OF  
*DREGEA VOLUBILIS***

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**Abstract**

An enzymic lectin with hemagglutination activity towards erythrocytes of human blood groups was isolated from leaves of wild plant *Dregea volubilis*. The lectin was isolated by following the conventional purification procedures using ammonium sulphate precipitation, DEAE cellulose chromatography followed by affinity chromatography on cross linked starch entrapped in agarose beads. The homogeneous protein on SDS-PAGE was designated as DVLL. The purified lectin expressed optimum activity at pH 5.0 at 45°C. Treatment of lectin with EDTA lost hemagglutinating activity and  $Mg^{++}$  were found to be necessary for the activity of purified lectin. Molecular weight of purified lectin was found to be 32.810 kD by SDS-PAGE and 35.481 kD by Sephadex G-75 gel filtration chromatography. Purified lectin (DVLL) was inhibited by  $\alpha$ -D-glucose and  $\alpha$ -D-mannose indicating the lectin to be glucose/mannose specific and was found to be glycoprotein in nature. DVLL expressed  $\alpha$  and  $\beta$  glucosidase activity with  $\alpha$ -pNPG and  $\beta$  pNPG substrates.

Key words: Lectins, Agglutinins, Hemagglutinins, Phytoproteins, *Dregea*.

**INTRODUCTION**

Plant lectins are a unique heterogeneous group of glycoproteins classified on the basis of their ability to recognise and specially bind the carbohydrate ligands. This most significant property of lectin, to bind with the specific carbohydrate residues, now a day is being utilized in lectin mediated drug delivery system (Michael, 1998) [1]. Property of lectins, to agglutinate lymphocytes, spermatozoa, and other cells has attributed great importance to the lectins (2). Sperm agglutination has been tested in the preparation of contraception devices (Oldham and Rosebruce, 1995) [3]. The present paper reports purification and characterization of lectin isolated from leaves of *D. volubilis* by following different procedures. Efficiency of each procedure was monitored by lectin activity and the recovery at every step. Homogeneity of each sample was tested by native PAGE and SDS-PAGE. Molecular weight of the purified fraction was determined by SDS PAGE and gel filtration chromatography on sephadex G-75 column.

**MATERIALS AND METHODS**

**Source of the lectin:** *Dregea volubilis* was identified and authenticated by the taxonomists of University Department of Botany, R.T.M. Nagpur University, Nagpur (India). Fresh active and fully mature green leaves of *Dregea volubilis* of uniform size were selected and used for preparation of extract.

**Chemicals:** BSA, M.W. markers, papain were obtained from Sigma Chem. Co. USA. Folin Ciocalteu reagent was purchased from Qualigens, Mumbai. Defatted starch, epichlorohydrin, ammonium sulphate, were obtained from E. Merck, Germany. DEAE Cellulose was purchased from Hi Media, Mumbai. Other chemicals were from Analytical reagent grade.

#### EXTRACTION AND PURIFICATION OF LECTIN

- 1. Preparation of extract and RBCs:** Preparation of extract and papain treated RBC was essentially done as described by Deshpande and Patil (2002) [4]. Fresh *Dregea volubilis* leaves were washed twice with distilled water. The adhered water was soaked by pressing the leaves between the folds of filter paper. Thirty g finely cut leave were crushed in 100 mL 20 mM sodium phosphate buffer, pH 7.0 containing 100 mM sodium chloride (PBS1). The slurry was kept on shaker at 100 RPM for 1h for complete extraction of proteins. The slurry was then passed through muslin cloth to remove the debris. The filtrate was centrifuged at 5000 RPM at cold condition for 20 min (Remi C24) to remove remaining debris before the supernatant was subjected to further purification procedure.  
Fresh blood of all the blood groups was collected from healthy donors in heparinised tubes from the out patients visiting the clinical biochemistry laboratory of University Department of Biochemistry, R.T.M. Nagpur University, Nagpur, and used for preparation of papain treatment as suggested earlier. Papain treated erythrocytes were used fresh for accurate results.
- 2. Proteins estimation:** Protein estimation was performed by the method of Lowry *et al.*, (1951) [5] using fat free BSA as standard protein using Gilford spectrophotometer (Germany).
- 3. Carbohydrate estimation:** This was performed by the method of Dubois *et al.* (1956) [6] using D- glucose as standard.

#### PURIFICATION:

**Precipitation of proteins:** Suitable aliquots of crude extract were subjected to ammonium sulphate precipitation by the method of Patil and Shastri (1982) [7]. The 0-90% precipitate after dialysis was were loaded on to DEAE cellulose column by the method described by Deshpande and Patil (2002) [4]. Further purification of the lectin was performed by affinity chromatography on cross linked starch entrapped in agarose beads as per the method of Sawhney *et al.*, (1988) [8]. All purification procedures were carried out at 4°C unless otherwise mentioned.

**Homogeneity of the sample:** This was tested by the SDS PAGE as described by Weber & Osborne (1969) [9], using Tris-glycine buffer, pH 8.3 with a constant current of 15 mA for 90 min. The gels were stained by coomassie brilliant blue for 45 min and destained in 7.5 % acetic acid at 37°C. Destaining was repeated at every 30 min interval till the colour was removed from the gels (Jana, 1998) [10]. The homogeneous purified *Dregea volubilis* leaf lectins were designated as DVLL.

**Molecular weight determination:** Molecular weight of the DVLL was determined on SDS PAGE and by gel filtration chromatography on sephadex G-75 using BSA- 66kD, pepsin- 34.7 kD, trypsinogen- 24 KD and lysozyme – 14.3 [9].

#### CHARACTERIZATION

**Agglutination inhibition assay:** This was performed as suggested by Kurokawa *et al.* (1976) [11]. Various pentoses, hexoses, di and tri saccharides along with glucose derivatives were used for agglutination inhibition assay. Inhibitory concentration of carbohydrate was determined as the concentration that prevents the RBC to agglutinate by DVLL.

**pH stability:** pH dependence of DVLL was determined by incubating DVLL with buffers ranging from pH 1.0 to 10 as per the method of Suseelan *et al.* (1997) with suitable controls [12].

**Temperature stability:** DVLL was mixed in PBS2 (20 mM sodium phosphate buffer pH 7.0 containing 0.9% sodium chloride) for determination of effect of temperature on

hemagglutination activity. Tubes containing equal quantity of PBS2 and DVLL containing 4.5 µg protein were exposed to the temperature ranging from 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95° C. Suitable controls were run simultaneously and the activity was compared with the expression of HAU by DVLL at 37°C. (Suseelan *et al.* 1997) [12].

**Metal ion dependency of DVLL:** This was determined by the method of Kawagishi *et al.* (1990) by chelating the metal ions initially with EDTA and then incubating the demetalized DVLL with various metal ions as described elsewhere [13].

**Blood group specificity of DVLL:** This was detected as described by Deshpande and Patil (2002), using 2 % suspension of papain treated RBC of all the blood group [4].

**Agglutination of erythrocytes of different animals:** DVLL was checked for the agglutination with erythrocytes of buffalo, bullock, chick, dog, goat, guinea pig, mice, rabbit, rat and owl (Deshpande and Patil (2002) [4].

**Determination of  $\alpha$  and  $\beta$  glucosidase activity:**  $\alpha$  and  $\beta$  glucosidase activity of DVLL was determined by method described by Herr (1979) using p-nitrophenyl- $\alpha$ -D-glucopyranoside and p- nitrophenyl-  $\beta$ -D-glucopyranoside as substrates. P- nitrophenol was used as standard. One unit of enzyme activity is defined as the amount that liberates one micromole of p- nitrophenol per min under experimental conditions [14].

**Thin layer chromatography of DVLL:** Major carbohydrate content of DVLL was determined by TLC (Upadhyay *et al.* 1997) using various carbohydrate as standards.  $R_F$  values were determined by the formula:  $R_F = \frac{\text{the distance travelled by the solute}}{\text{the distance travelled the solvent}}$  [15].

**Estimation of proteins in terms of amino acids:** Proteins in terms of amino acids were measured by the method described by Spice (1959) using purified tyrosine as standard [16].

**Estimation of tryptophan in DVLL:** Tryptophan content in DVLL was measured by the method described by Spande and Witkop (1967) using pure tryptophan as standard amino acid [17].

**Statistical analysis:** All results were statistically analysed by the method described by Walpole (1982). P value was set at < 0.05 [18].

## RESULTS

Results presented in Table 1 indicate that the proteins in crude extract of *D. volubilis* were precipitated between the concentration 0-90% ammonium sulphate saturation showing maximum HAU and considerable purification. Ammonium sulphate precipitated DVLL was loaded on to the DEAE cellulose column (length 20 cm, width 1 inch). DEAE cellulose chromatography elutes the DVLL with a gradient of sodium chloride of 100 – 500mM in the fraction from 16 to 32 showing the broad peak with a 3 ml fraction each (LKB Pharma, Amarsam). The peak fraction showed further purification with a commendable recovery of HAU. Further affinity chromatography of DVLL on cross linked starch agarose beads gave 94 fold purification with 31% recovery. Molecular weight of DVLL by SDS-PAGE was found to be 32.810 and by sephadex G-75 gel filtration it was 35.481 kD. The agglutination inhibition assay with various carbohydrates indicated that DVLL was inhibited by  $\alpha$ -D-glucose, and  $\alpha$ -D-mannose. Inhibitory concentrations of  $\alpha$ -D-glucose and  $\alpha$ -D-mannose were found to be 200 mM.  $\alpha$ -D- ribose,  $\alpha$ -D-arabinose,  $\alpha$ -D- xylose, D-ribulose,  $\alpha$ -D-galactose, D-fructose, disaccharides such as maltose, lactose, sucrose, trisaccharides as trehalose, raffinose, D-rhamnose, and D-fucose, did not inhibit the agglutination upto 400 mM concentration. Other derivatives of  $\alpha$ -D-glucose such as GluNAc (N-acetyl-D-glucosamine), Glucosamine hydrochloride,  $\alpha$  methyl-D-glucoside and 3-O- methyl glucose were found to inhibit agglutination. Inhibitory concentration of GluNAc was found to be 225 mM where as that of Glucosamine hydrochloride and  $\alpha$ -methyl-D-glucoside and 3-O-methyl glucose was observed to be 250 mm as shown in Table 2. Carbohydrate content of DVLL when estimated by the method of Dubois *et al.* (1956) has shown that DVLL contains quite high amount of hexose type carbohydrate indicating DVLL to be a glycoprotein in nature [6].

Table 3 shows the some physicochemical properties of DVLL. The pH dependency of DVLL appears to be between pH 4.0 to 7.0 only. At pH below 4.0 and above 7.0 DVLL appear to lose activity; the optimum activity was expressed at pH 5.0. Exposure of DVLL to various

temperature shows that DVLL could show maximum activity up to 45°C only. Purified DVLL was maximally active from temp 15 to 45°C only, where as 50 % activity was lost at 55°C. Effect of metal ions on DVLL activity indicated that Mg<sup>++</sup> ions are essential for agglutination by purified DVLL. When treated with EDTA DVLL lost activity completely. Among the chloride salts of metal ion tested Mg<sup>++</sup> ions were found to restore the agglutination activity as shown in table 4. The DVLL expressed α glucosidase activity 20 U/mL and β glucosidase activity of 35 U/mL. DVLL agglutinated papain treated red blood cells of all blood groups with similar efficiency and except bullock RBC of all the animals agglutinated by DVLL. Major carbohydrate content when detected by TLC the R<sub>F</sub> values of GLUNAc and DVLL were found to be similar indicating probably the carbohydrate moiety in DVLL to be GluNAc. Estimation of total amino acids in 100 µg DVLL was found to be 92 µg amino acids. The rest of the 8 µg would be occupied by carbohydrate residues in DVLL as it being glycoprotein in nature. The tryptophan content of DVLL was found to be 11 µg in 100 µg protein of DVLL.

Table1. Purification of lectin from leaves of *D. volubilis*

Fraction	HAU/g of fresh weight of leaf	Protein (mg) /g leaves	Specific activity	% yield	Fold purification
Crude fraction	868850	121.3	716	100	1
0-90% ASP	43425	5.2	8351	50	11.4
DEAE Cellulose fraction	39477	3.7	10669	45.4	14.8
Affinity Chromatography fraction	26979	0.4	67447	31	94

ASP= ammonium sulphate precipitated fraction

Table2: Physicochemical properties of lectin from leaves of *D. volubilis*

Property	Optimum parameter
pH dependence	4.0 to 7.0
Temp. dependence	45°C
SDS PAGE	Homogeneous
Molecular weight	32.810 kD by SDSPAGE and 33.481 kD gel filtration on sephadex G-75
Carbohydrate moiety	GluNAc
Blood group specificity	No
α -glucosidase activity	20 U/mL
β- glucosidase activity	35 U/mL
Metal ion dependence	Mg <sup>++</sup>
100µg lectin	11µg tryptophan and 92µg amino acids

Table 3. Inhibition of agglutination by lectin from leaves of *D. volubilis* by carbohydrates

Carbohydrate	Inhibitory Concentration (mM)
α-D-glucose	200
α-D-mannose	200
N-acetyl-D-glucosamine	225
Glucosamine hydrochloride	250
α-methyl-D-glucoside	250
3-O-methyl-D- glucose	250

Table 4: Effect of metal ions on agglutination by lectin from leaves of *D. volubilis*

Metal ion (1mM concentration: in terms of chloride salts)	Agglutination
Mg <sup>++</sup>	+
Ag <sup>++</sup>	--
Ba <sup>++</sup>	--
Ca <sup>++</sup>	--
Fe <sup>++</sup>	--
Hg <sup>++</sup>	--
Mn <sup>++</sup>	--
Sb <sup>++</sup>	--

+: Agglutination; -- : No agglutination

Table: 5 Agglutination of erythrocytes of various animals by lectin from leaves of *D. volubilis*

Animal species	Untreated erythrocytes	Papain treated erythrocytes
Buffalo	+	+
Bullock	-	-
Chick	+	+
Dog	+	+
Goat	+	+
Guinea pig	+	+
Mice	+	+
Owl	+	+
Rabbit	+	+
Rat	+	+

+ : Agglutination; - : No agglutination

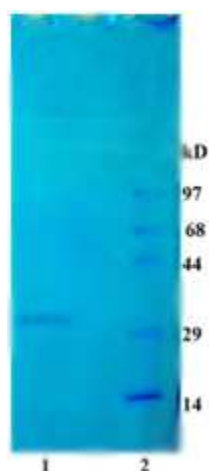


Fig.1. SDS PAGE of *D. volubilis* leaf lectin

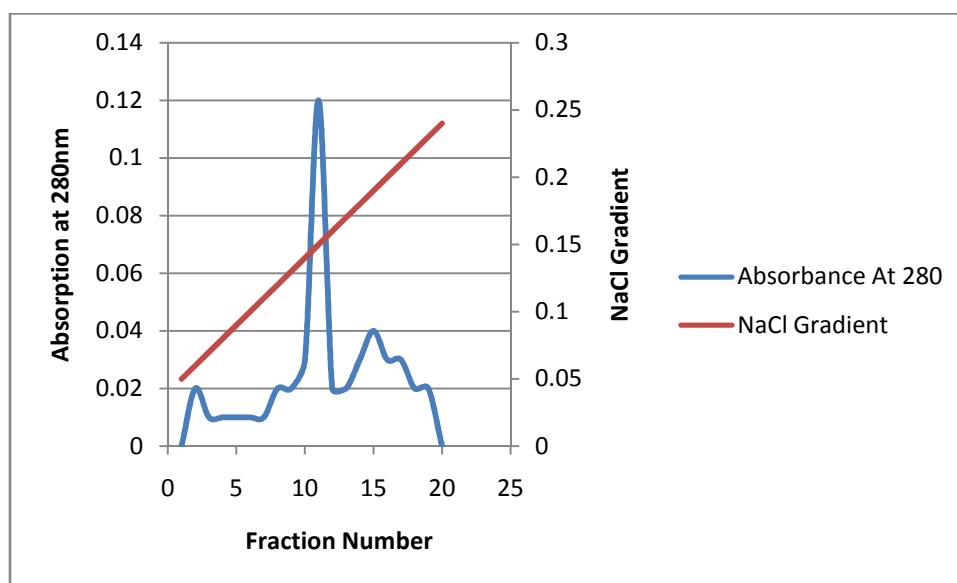


Fig. 2. DEAE cellulose chromatography of *D. volubilis* leaf lectin

## DISCUSSION

DVLL was purified to homogeneity by conventional purification methods followed by affinity chromatography on cross linked starch in agarose beads. Other herbal lectins purified to homogeneity by affinity chromatography are *D. lablab* lectins (Tulsi and Nadimpalli, 2002) [19] and *T. procumbans* lectins (Ramteke and Patil (2005) [20]. Molecular weight of DVLL by SDS PAGE was found to be 32.8 kD where as by sephadex G 75 gel filtration it was found to be 33.48 kD. Similar results for other lectins were observed with *Chenopodium amaranticolor* having Mr. 33 kD. (Suseelan and Mitra, 2001) [21]. The glycoprotein nature of DVLL was confirmed. Similarly many lectins have been tested positive for glycoprotein to be in nature. e.g. Rice lectin (Sureshkumar *et al.*, 1982 [22]; Indravathamma and Sheshadri, 1980 [23]).  $\alpha$ -D-glucose and  $\alpha$ -D-mannose were found to inhibit agglutination by DVLL indicating that DVLL is D-glucose and  $\alpha$ -D-mannose specific lectin. DVLL is active between pH 4.0 to 7.0 only for its activity; similar results are manifested by *V. mungo* lectin for its pH dependence (Suseelan *et al.*, 1997) [12]. DVLL was maximally active at temp 45°C. Similar temp stability was also shown by *Phaseolus mungo* lectin which was active till 40°C only (Sharma and Salahuddin, 1993) [24]. On treatment with EDTA DVLL lost activity where as  $Mg^{++}$  ions were able to restore activity. Similar type of results for  $Mg^{++}$  ions dependence were observed with Con A (Gold and Balding, 1975) [2] and *D. lablab* (Tulsi and Nadimpalli, 2002) [19]. DVLL agglutinated RBC of all the blood groups with similar efficiency indicating it to be non blood group specific. Similar blood group nonspecific lectins were WGA (Nagata and Burger, 1982) [25] and Soybean lectin (Nathan, 1977) [26]. DVLL agglutinated RBC of all the animals tested except bullock. This may be due to the absence of specific receptors on bullock RBC. Similar results were manifested by ricin, abrin, croton lectins (Tulsi and Nadimpalli. 2002) [19]. DVLL expressed  $\alpha$  and  $\beta$  glucosidase activities. Other lectins such as ricin, abrin and mistletoe lectin are also found to possess enzymic activity (Peumans *et al.*, 2000) [27]. Major carbohydrate content of DVLL was found to be GluNAc. Similar carbohydrate content was found in rice lectin (Indravathamma and Sheshadri, 1980) [23] and *Bauhinia purpuria* (Tulsi and Nadimpalli, 2002) [19]. Total amino acid content in 100  $\mu$ g DVLL was measured to be 92  $\mu$ g. The rest would probably constitute carbohydrate moiety in DVLL. Tryptophan content of 11  $\mu$ g per 100  $\mu$ g DVLL was measured indicating that DVLL has good aromatic amino acid content.

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