strobili. The micro-climate of bagged strobili is likely to be more humid, hence they showed a longer receptive time. When receptive, the cone scales open until they are at nearly right angles to the cone axis, exposing the ovules (Figure 1). The nucellus secretes a pollination droplet between the arms of the micropyle, and pollen lodges on the sticky surface of the droplet which is fairly high in glucose, fructose and sucrose content[3]. The droplet is withdrawn, apparently reabsorbed, drawing any trapped pollen into a depression on the surface of the nucellus called the nucellar or pollen chamber. The middle cell layer in the micropylar neck enlarges, closing the neck. If pollen is plentiful, four to ten pollen grains are lodged in each pollen chamber.

Controlled pollination did not reveal any incompatibility. There was successful cone setting (72.86 to 80.0%, 80.0 to 82.35% and 81.54 to 84.71% at lower altitude (900 m a msl) and 76.92 to 83.33%, 73.08 to 76.36% and 75.93 to 78.18% at higher altitude (1900 m a msl) was recorded after six months of pollination. However, after 18 months, cone setting was 41.43 to 53.75%, 48.57 to 57.65 and 49.23 to 61.18 at lower altitude and 51.85 to 58.33%, 40.74 to 54.55% and 40.00 to 43.64% at higher altitude; and after 26 months it still got reduced to 38.57 to 51.25%, 47.14 to 52.94% and 49.23 to 60.00% (at lower altitude) and 51.85 to 58.33%, 38.89 to 50.91% and 36.00 to 42.59% (at higher altitude). Seed set varied from 78.00 to 83.33%, 76.00 to 80.67% and 86.00 to 88.00% (at lower altitude) and 76.67 to 84.67%, 68.66 to 76.00% and 68.00 to 78.66% (at higher altitude), as recorded in the Pauri, Badiyargarh and Srinagar provenances, respectively with respect to open pollinated seed setting (Table 1).

Various provenances of *P. roxburghii* and their cross-ability patterns were reviewed by Kedarnath[4]. Dogra[5] recognized seven altitudinal provenance types in blue pine (*Pinus wallichiana*), and observed a weak reproductive barrier between the populations growing at lower and higher altitudes of both moist and dry zones, but a strong reproductive barrier between the moist and dry arid zones of blue pine of Himachal Pradesh[5]. Yet *P. wallichiana* has been earmarked potentially the most valuable pine for interspecific breeding[2,6–10]. Blada[11] made many crosses between Swiss stone pine *P. cembra* and other white pines, but succeeded only in the *P. cembra* × *P. wallichiana* cross after a series of tests. However, work on improvement on chir pine through crossing of even desired individuals was not done prior to this study. Successful intraspecific hybridization by pollinating with pollen grains of desired superior provenances led us to believe that by applying this method one can get the superior quality cones and seeds on easily approachable selected trees. This can be undertaken on a mass scale in the seed orchards of chir pine, as the reproductive system of chir pine is exclusively sexual.

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Purification and characterization of a galactose-specific lectin from the stems and leaves of *Dolichos lablab* (Indian lablab beans)

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Seeds of the Indian lablab beans (*Dolichos lablab*) contain two distinct lectins which differ in their sugar specificities, viz. the glucose/mannose-specific lectin and the galactose-specific lectin. Stems and leaves of the three-week-old plants contain proteins that strongly agglutinate rabbit erythrocytes but not human erythrocytes, and the agglutinating activity is inhibited by galactose, its derivatives, lactose and N-acetylgalactosamine. However, the lectin does not bind to the conventional Sepharose-derivatized galactose or lactose gel and can be purified to homogeneity by ion exchange chromatography and gel filtration. The lectin eluted as a single symmetrical peak from a Biogel P-200 column with a molecular mass of 66 kDa. Purified stem and leaf lectin is a glycoprotein with 3% reducing sugar and is dissociated into two subunits in SDS–PAGE with molecular masses of 48 kDa and 20 kDa, respectively. The lectin cross-reacts with an antibody to the glucose/mannose-specific seed lectin, suggesting similar antigenic sites in these two lectins. Antibodies raised against the purified galactose-specific seed lectin show specific reactivity with the seed lectin as well as with the stem and leaf lectin, suggesting that they are related proteins.

PLANT lectins are a diverse group of proteins/glycoproteins that have the ability to agglutinate eryth-

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rocytes. By virtue of their abundance in the legumes these have been largely purified from the legume seeds and are well characterized[1]. Pioneering work on several legume lectins was done by Surolia and his coworkers in India[2]. Seeds of the *Dolichos lablab* (Indian lablab beans) contain two distinct sugar-specific lectins that have been purified and studied in our laboratory[3,4]. *D. lablab* seeds have also been found to contain a tetrameric lectin that agglutinates human O^+ erythrocytes[5]. Another lectin called FRIL has also been reported recently from *D. lablab* that preserves haematopoietic progenitors in suspension culture[6]. Lectins with distinct sugar specificities have also been purified from other parts of some legume plants such as the stems, leaves and roots[7–9]. The galactose-specific lectin from the seeds of lablab beans could not be purified by affinity chromatography on conventional Sepharose-derivatized galactose or lactose gels and has been purified by gel filtration. The galactose lectin was shown to cross-react with the antibody to the mannose-specific seed lectin[4]. When the seeds of the lablab beans were grown to plants, protein extracts from the stems and leaves of three-week-old plants showed strong haemagglutinating activity with rabbit erythrocytes that was inhibited by galactose, its derivatives and lactose. The objective of the present study was to isolate this lectin in pure form from the stems and leaves and to compare its properties with those of the galactose-specific and mannose-specific seed lectins. This is a report on the purification, physico-chemical properties, carbohydrate-binding specificity and immunological properties of this lectin.

Seeds of *D. lablab* were procured locally. They were grown into plants under natural conditions in a well-protected area in the University of Hyderabad for 3–4 weeks. Stems and leaves were separated and frozen in liquid nitrogen and stored at – 80°C. Sepharose 6B, divinyl sulphone and all sugars used in the study were purchased from Sigma Chemical Co (St. Louis, USA). All other reagents used in the study were of high purity and were procured from local firms.

All operations were carried out at 4°C. The protocol published by Talbot and Etzler[10] was followed for extraction of the proteins. Typically 200 g of the frozen material was homogenized in a blender with five-fold excess (w/v) of 0.1 M potassium phosphate buffer, pH 7.2 containing 150 mM isoascorbic acid and 2 mM thioglycollic acid. After stirring for two hours, the particulate matter was removed by centrifugation at 7227 *g* for 20 min and the clear supernatant was subjected to 0–40% ammonium sulphate fractionation. The supernatant obtained from this step was subjected to 40–80% ammonium sulphate fractionation. Both ammonium sulphate-precipitated pellets were dissolved in 40 mM Tris-HCl buffer, pH 7.4, and dialysed extensively against the same buffer.

Haemagglutinating acitivity at all steps of purification was monitored by using trypsin-treated rabbit erythrocytes as described earlier[11]. Agglutination was also tested using human A, B, O and AB erythrocytes. Sugar-inhibition studies were carried out for the 40–80% ammonium sulphate-precipitated fraction and the purified lectin employing a wide variety of sugars as described earlier[11] and the extent of inhibition is expressed as mM concentration of the sugar required to cause inhibition.

From the seeds of the lablab beans, the galactose-specific lectin was purified by gel filtration[4] and mannose-specific lectin was purified by affinity chromatography on Sepharose-mannose gel[11]. The homogeneity of the purified lectins was tested by native polyacrylamide gel electrophoresis at pH 8.3 and antibodies were raised for the purified lectins.

Affinity chromatography on Sepharose-divinyl sulphone-galactose and Sepharose-divinyl sulphone lactose gels: Sepharose affinity adsorbents (5 ml each) containing galactose and lactose sugars linked via divinyl sulphone were prepared as described in ref. 11, except that instead of mannose, galactose and lactose were used. The 40–80% ammonium sulphate-precipitated protein obtained above was passed through these gels equilibrated with 40 mM Tris-HCl buffer, pH 7.4 (column buffer). The gels were washed extensively with the same buffer and sequentially eluted with 0.2 M galactose, 0.2 M lactose in column buffer and 0.1 M acetic acid, respectively.

DE-52 cellulose chromatography: Most of the lectin activity was detected in the 40–80% ammonium sulphate fraction. The dialysed fraction was applied to a DE-52 cellulose gel (35 ml) equilibrated with column buffer at a flow rate of 30 ml/h and after washing the gel with the column buffer, bound protein was eluted with one column volume of 0.1 M, 0.2 M, 0.3 M and 0.4 M NaCl each in column buffer. Most of the protein was eluted in the 0.4 M NaCl fraction.

Chromatography on Biogel P-200: Protein recovered in the 0.4 M NaCl eluate was concentrated and dialysed against 25 mM Tris-HCl buffer, pH 7.4 and applied to a Biogel P-200 column (90 ml) equilibrated with column buffer. Fractions (2.0 ml) were collected and the absorbance monitored at 280 nm. The gel was calibrated with standard proteins and the elution volumes of each were determined. The native molecular mass of the stem and leaf lectin was calculated from the calibrated curve prepared.

Protein in the column fractions was monitored by measuring the absorbance at 280 nm. Protein was also estimated using Folin's reagent[12]. Carbohydrate in the purified protein was analysed by phenol-sulphuric acid method[13].

Biogel P-200 eluted fractions that contained the purified lectin were subjected to SDS-PAGE under reducing conditions according to Weber and Osborn[14].

Antiserum to the purified glucose/mannose-specific lectin was obtained as described earlier[3]. For immunodiffusion experiments, 1% agar plates were prepared in PBS, and holes were punched. Antiserum to the seed lectin was placed in the central well in an agar plate. Affinity-purified mannose-specific lectin and purified galactose-specific stem and leaf lectin were placed in neighbouring wells. Antibodies were raised for the purified galactose-specific seed lectin in a rabbit according to standard methods. For immunodiffusion experiments, antiserum to the galactose-specific seed lectin was placed in the central well and the purified galactose-specific seed lectin and the purified stem and leaf lectin were placed in separate wells. Agar plates were placed at 4°C and the precipitin line formation was visualized.

When the stem and leaf protein extracts were subjected to ammonium sulphate precipitation, and the dialysed fractions analysed for their agglutinating activity with untreated and trypsin-treated rabbit erythrocytes, the 40–80% fraction showed strong agglutinating activity with trypsin-treated erythrocytes and contained most of the lectin activity. The biological activity of this fraction was found to be inhibited by galactose, and lactose; hence an attempt was made to purify the lectin by passing the 40–80% fraction through sepharose-divinyl sulphone galactose gel and sepharose-divinyl sulphone lactose gel. Both columns were extensively washed with column buffer and all the protein and activity applied was recovered from both columns in the unbound fraction itself and no protein could be eluted with the sugars (0.2 M galactose and 0.2 M lactose) or by lowering the pH (elution with 0.1 M acetic acid) (data not shown). This suggests that the protein fails to bind to these two affinity adsorbents. Similar results were obtained when we tried to purify the galactose-specific seed lectin[4].

These gels, however, bound the galactose/lactose-specific seed lectin from the *Momordica charantia* effectively (data not shown), suggesting that the gels do have the derivatized galactose and lactose sugars. As the lectin agglutinates rabbit erythrocytes, erythrocyte membrane proteins were prepared and coupled to Affigel-10 as described earlier[15]. The stem and leaf lectin like the galactose seed lectin also failed to bind on this gel (data not shown). As none of the classical affinity adsorbents proved effective to purify the stem and leaf lectin, we resorted to purify the lectin by conventional chromatography.

When the 40–80% ammonium sulphate fraction was passed through DE-52 cellulose gel, the lectin activity could be retained on the gel and could be eluted only when 0.4 M NaCl was applied to the gel (Figure 1). The eluates were concentrated, dialysed against 25 mM Tris-HCl buffer (pH 7.4) and subjected to gel filtration on Biogel P-200. From Figure 2 *a* it is apparent that the lectin eluted as a single peak from the gel and was found to exhibit a native molecular mass of 66,000 as determined from the calibrated curve obtained (Figure 2, inset). Table 1 shows the purification of the stem and leaf lectin. From 200 *g* of the fresh stem and leaves, about 3 mg of purified lectin could be obtained.

Purified stem and leaf lectin did not agglutinate untreated or trypsin-treated human A, B, O and AB erythrocytes. It was found to be a glycoprotein with 3% carbohydrate and dissociates into two subunits of molecular mass 48 kDa and 20 kDa, respectively under reducing conditions in SDS-PAGE (Figure 2 *b*).

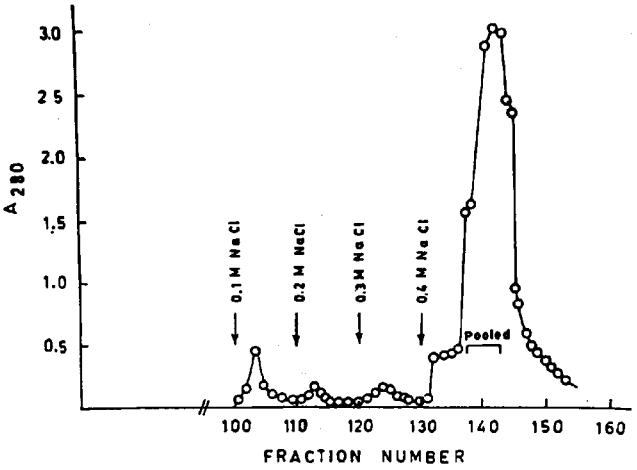

Figure 1. Elution profile of the stem and leaf lectin from DE-52 cellulose gel. 40–80% ammonium sulphate fraction (47 mg protein) was loaded on DE-52 gel (2.1 cm × 10 cm) equilibrated with 40 mM Tris-HCl buffer, pH 7.4. 4 ml fractions were collected and absorbance monitored at 280 nm. Stepwise elution was performed using 0.1 M, 0.2 M, 0.3 M and 0.4 M NaCl in column buffer.

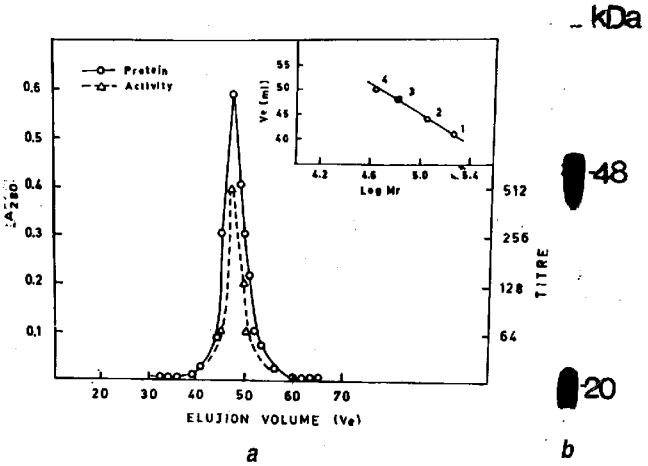

Figure 2. *a*, Gel filtration of the lablab bean stem and leaf lectin. 0.4 M NaCl eluate from DE-52 gel (9 mg/ml) in 25 mM Tris-HCl buffer, pH 7.4 containing 0.15 M NaCl was loaded onto a column (1.4 cm × 86 cm) of Biogel P-200 and eluted with the same buffer. 2 ml fractions were collected and elution was followed by monitoring the absorbance at 280 nm. (Inset) Plot of elution volume (*Ve*) vs log *Mr*. (o), Standards: 1, myosin (2,05,000); 2, β-galactosidase (1,16,000); 3, Bovine serum albumin (66,000); and 4, ovalbumin (45,000). • Stem and leaf lectin. *b*, SDS–PAGE pattern of the purified stem and leaf lectin.

Table 1. Purification of lablab bean stem and leaf lectin from 200 g of freshly frozen material

Step	Volume (ml)	Protein (mg)	Activity (U)	Specific activity (U/mg)	Recovery (%)	Fold purification
Crude extract	901	317	30604	114	100	1
Ammonium sulphate (40–80%)	20	47	25600	545	71	4.8
DE-52 eluate (0.4 M NaCl)	35	18	22400	1244	62	11
Biogel P-200	12	3	21000	7000	58	61

Table 2. Inhibition of haemagglutinating activity of stem and leaf lectin with various sugars. Four haemagglutinating units of the P-200 eluate of purified lectin, were used in the assay. 0.01 to 0.1 ml of 0.1 M stock solutions of various sugars were also used

Sugar	Inhibitory concentration (mM) Biogel P-200 eluate
Galactose	2.5
Lactose	2.5
Methyl α-D-galactopyranoside	2.5
6-*O*-methyl D-galactopyranoside	2.5
N-Acetylgalactosamine	2.5

4-*O*-D-galactopyranosyl, D-galactopyranose, D-mannose, D-glucose, L-fucose, cellobiose and *N*-acetylglucosamine were found to be non-inhibitory at 0.1 M concentration.

The galactose-specific lectin from lablab beans exhibited a native molecular mass of 1,20,000 and dissociates into two subunits with molecular mass of 48 kDa and 20 kDa, respectively and is possibly tetrameric in nature[4]. The mannose-specific seed lectin is also a tetramer. However its subunits are considerably smaller, with molecular mass of 15 kDa and 12 kDa, respectively[11]. The native molecular mass of the stem and leaf galactose-specific lectin seems to be half that of the seed lectin and appears to be made of two chains only. On the other hand, the mannose-specific seed lectin is a tetramer and exhibits a subunit molecular mass of 15 kDa and 12 kDa, respectively[11].

Among a number of animal and human A, B, O, erythrocytes tested for haemagglutinating activity of the stem and leaf lectin, only trypsin-treated rabbit erythrocytes were agglutinated by the lectin. The inhibitory effects of various sugars on the agglutinating activity of the stem and leaf lectin purified on Biogel P-200 are shown in Table 2. Among the various sugars tested, lactose, galactose and derivatives of galactose and *N*-acetylgalactosamine are inhibitory to the lectin acitivity. While galactose and lactose are comparable in their potency, *N*-acetylgalactosamine is more inhibitory than these two sugars. (However, due to the non-availability of the *N*-acetylgalactosamine-derivatized gel, this was not tested to purify the galactose-specific lectins.) Additionally, galactose substituted with methyl groups at either position 1 or 6 was found to be more inhibitory compared to free galactose, which might suggest that the lectin might have a hydrophobic region in the sugar binding site.

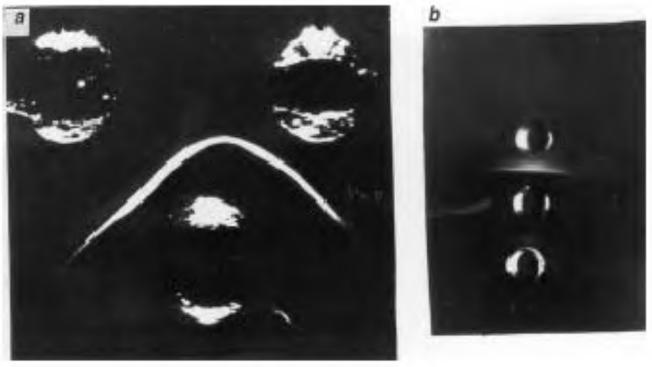

Figure 3. Immunodiffusion of the stem and leaf lectin. ***a***, Central well contained 20 µl of antiserum to the purified glucose/mannose-specific seed lectin. Wells contained 20 µg each of purified mannose-specific seed lectin and purified stem and leaf lectin; ***b***, Central well contained 20 µl of antiserum to galactose-specific seed lectin. Wells contained 20 µg each of the purified mannose-specific seed lectin and stem and leaf lectin.

The immunoreactivity of the stem and leaf lectin was tested against an antiserum to the glucose/mannose-specific seed lectin. From Figure 3*a* it is apparent that the antiserum against the glucose/mannose-specific seed lectin reacts with the purified glucose/mannose-specific seed lectin as well as with the isolated galactose-specific lectin from stems and leaves, suggesting that both lectins exhibit antigenic similarities. In order to check if an antiserum for the galactose-specific seed lectin cross-reacts with the mannose-specific lectin as well as with the galactose-specific lectin from stems and leaves, antiserum to purified galactose-specific seed lectin was raised in a rabbit. From Figure 3*b* it is clear that the antiserum cross-reacts with the mannose-specific lectin as well as with the galactose-specific lectin from stems and leaves. Additional precipitin line seen in Figure 3*b* may possibly be due to the interaction of the antibody with serum glycoproteins. Inclusion of inhibitory sugars up to 0.1 M concentration in the agar did not alter the precipitin line formations (data not shown).

Vegetative lectins have been characterized only from a few legume plants. It has been reported that the stem and leaf lectin from *Dolichos biflorus* cross-reacts with the seed lectin antibody[7]. The seed lectin is a tetramer composed of apparently equal amounts of two closely related subunits I and II. Extensive biochemical studies carried out on the cross-reactive material suggest that

the cross-reactive material is a dimer containing a subunit identical in electrophoretic mobility to subunit I of the seed lectin and another subunit of higher molecular mass than the seed lectin subunit. The subunits may represent different degrees of completion or modification of a common polypeptide chain[16]. Leaf and seed lectins that exhibited similar properties have also been identified from *Griffonia simplicifolia* and *Phaseolous vulgaris*[17,18]. Leaves and flowers of *Sophora japonica* also contain galactose lectins[19].

Seeds of only a few legumes have been known to contain two distinct sugar-specific lectins[20]. *D. lablab* seeds contain two lectins with distinct sugar specificities[3,4]. The results obtained in this study clearly demonstrate that the stems and leaves of the lablab bean seeds contain strong agglutinating activities that can be inhibited by galactose and lactose. The general properties of the galactose-specific seed lectin and the stem lectin are similar and possibly the stem and leaf lectin represents the dimeric form of the tetrameric galactose specific-seed lectin, as the native molecular mass of the stem and leaf lectin is about half that of the native molecular mass of the galactose-specific seed lectin. The fact that the antibody to the mannose-specific seed lectin cross-reacts with the galactose-specific seed lectin as well as with the stem and leaf lectin suggests that the lectins have related antigenic sites. Additional evidence for this comes from the fact that the galactose-specific seed lectin antibody also cross-reacts with the mannose-specific seed lectin (data not shown) and with the galactose-specific lectin from stems and leaves. Further, while the seeds express both the mannose and galactose-specific lectins, the stems and leaves seem to contain predominantly galactose-specific lectin only. Considering the experimental data on the binding abilities as well as the physico-chemical and biological properties of the galactose-specific seed lectin and the stem and leaf lectin, it is evident that both lectins are unusual and related. Our future work aims at understanding the structural similarities of the two galactose-specific lectins as well as the mannose-specific lectin.

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Colony morphology mutants of chemolithotrophic *Acidithiobacillus ferrooxidans* are associated with altered genomic distribution of family 1 repetitive DNA sequence

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Two classes of colony morphology mutants, large spreading and non-spreading small colonies, were isolated from *Acidithiobacillus ferrooxidans* in thiosulphate–agarose and tetrathionate-sulphide–agarose medium, respectively. The spreading colony morphology mutants exhibited an increased chemotaxis toward thiosulphate compared to the wild type or the non-spreading mutants. Inward primers designed from the ends of two distinct families of repetitive DNA elements of *A. ferrooxidans* produced PCR amplicons of 1.3 and 1.4 kb, respectively, from both the wild type as well as mutant strains. Restriction fragment length polymorphism compared

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