

Isolation and Partial Characterization of Two Plant Lectins

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Abstract: Plant lectins with different sugar specificities were isolated from the seeds of *Artocarpus integrifolia* and *Canavalia gladiata* using a procedure that involved affinity chromatography. Of the two lectins isolated, protein content was found to be more in *Artocarpus integrifolia* than in *Canavalia gladiata* whereas carbohydrate content was more in *Canavalia gladiata*. Both the lectins were found to be stable up to 100°C. Lectin from *Artocarpus integrifolia* showed two bands with molecular weight 11 and 17 kDa. *Canavalia gladiata* also gave two dissimilar bands of molecular weight 26 and 17 kDa both in the presence and absence of β -mercaptoethanol. Carbohydrate staining of SDS gel indicated that both the bands are glycosylated *Canavalia gladiata* whereas only one band was glycosylated in *Artocarpus integrifolia*.

Key words: *Artocarpus integrifolia*, *Canavalia gladiata*, lectin, mannose, N-acetyl galactosamine

INTRODUCTION

Plants are the richest and most convenient source of lectins. Plant lectin has been attracting much attention because of their ease of isolation and their usefulness as reagents for glycoconjugates in solution and on cell surface. Lectins were discovered by their ability to agglutinate erythrocytes and probably the easiest and most convenient method of detecting lectin activity remains agglutination of a panel of human and/or animal red blood cells or other cells. Lectins are particularly prevalent in leguminous plants, where they are localized in the cotyledons of the seeds and roots. Lectins may comprise up to 3% of the weight of a mature seed.

Lectins have precise carbohydrate specificity and can be blocked by simple sugars and oligosaccharides. Because of the specificity each lectin has towards a particular carbohydrate structure, even oligosaccharides with identical sugar composition can be distinguished or separated. The actual structure recognized by the binding site of the lectin when it combines with its natural ligand is generally large and more complex than a single monosaccharide. Lectins, which have a similar specificity towards monosaccharides, may differ in their affinity for a particular disaccharide, oligosaccharide or glycopeptide.

Lectins vary in composition, molecular weight, subunit structure and number of sugar binding sites per molecule. By virtue of the abundance of lectins in legumes, most of the reports are confined to the biochemical analysis of lectins in the different members of the legume family. Only a few have been studied for their biological application.

In recent years, lectins have become very attractive due to their extensive use as probes for both the characterization and isolation of simple and complex sugars (Rudiger and Gabius, 2001; Sharon and Lis, 2002) and as useful tools in immunological studies (Moreira *et al.*, 1991). Considering the innumerable number of lectins available in the nature, the ease with which they could be prepared in the purified form, their amenability to chemical manipulation and the fact that they can be inhibited by simple sugars makes them attractive as an important tool in biological research. Although lectins are found ubiquitously in plant species, they have variable structures and specific activities according to the plants they originate from. Thus purification and characterization of lectins from a variety of plant species interests researchers in the field of glycobiology. This study reports the purification and characterization of lectin from *Canavalia gladiata* and *Artocarpus integrifolia*.

MATERIALS AND METHODS

Seeds of *Canavalia gladiata* and *Artocarpus integrifolia* were collected locally and used for the isolation of lectin. The present study was conducted at Regional Cancer Centre, Kerala.

Haemagglutination assay: The haemagglutination assay was carried out using the method described by Pueppke (1979). Human erythrocytes of A, B and O phenotypes were used. The assays were carried out in WHO plates with a final volume of 400 μ L in each well.

Decreasing concentration of the seed extracts was taken in the wells by two fold serial dilution with PBS to determine the agglutination titre. PBS was added in the control wells instead of plant extract. Washed erythrocytes were added at a concentration of 2% in PBS and incubated at room temperature for 2 h. The minimum concentration of the extract that gave haemagglutination was recorded as the haemagglutination titre.

Neuraminidase treatment of erythrocytes:

Neuraminidase treated erythrocytes were used for seed extracts which were found negative in direct haemagglutination assay. Packed human erythrocytes (0.5 mL) were washed in PBS (pH 7.4) three times and suspended in 2 mL of acetate buffered saline (pH 5.6). It was incubated for 30 min at 30°C with 20 µL of *Vibrio cholerae* neuraminidase. The cells were then washed two times in PBS and were then resuspended in PBS to make 2% w/v solution.

Isolation and purification of *Artocarpus integrifolia* lectin:

Ripened seeds from *Artocarpus integrifolia* were collected. Twenty five gram of the seeds were deskinning and ground to flour. The flour was defatted by soaking in petroleum ether for 24 h at room temperature. The defatted flour was stirred well on a magnetic stirrer with 250 mL of phosphate buffered saline overnight at 4°C. The extract was filtered and spun for 30 min at 10,000 g. The supernatant was collected and ammonium sulphate was added to the sample with constant stirring to a concentration of 40% saturation and kept overnight. The precipitates were removed by centrifugation at 20,000 g for 30 min. The clear supernatant was then adjusted to 60% saturation by further addition of ammonium sulphate with constant stirring and kept overnight. The precipitate thus formed was collected by centrifugation as above, dissolved in 20ml of PBS and then dialyzed extensively against three changes of PBS (pH 7.2). Any precipitate formed was removed by centrifugation and the clear supernatant was collected. The isolated lectin was then purified by affinity chromatography on cross-linked guar gum columns.

Soluble guar gum was purchased from Sigma Chemical Company. Insoluble guar gum was prepared by cross-linking the soluble polysaccharide using epichlorohydrin. A fine emulsion of 1.5 mL of epichlorohydrin in 25 mL of 3N Sodium hydroxide was prepared in a 100 mL-stoppered bottle by thorough mixing. The emulsion was then poured into 250 mL beaker followed by 10 g of guar gum powder, stirring the mixture vigorously until it solidified and kept at 40°C in water bath for 24 h with occasional stirring. Then it was kept at 70°C in a hot air oven for 8 h. The solidified material was then soaked in thrice the volume of water. It was then washed in distilled water several times until the

pH became neutral. It was transferred to 20 mL of PBS (pH 7.4) and was then homogenized in a blender to the correct particle size (about 300 µm). It was again washed in PBS (pH 7.4) several times. The homogenized guar gum was then packed to a height of 30 cm in a 2.5 cm diameter glass column equipped with flow adapter and equilibrated with PBS (pH 7.4).

The column kept at 4°C was run at a rate of 100 mL/h, first with PBS until the absorbency of the effluent at 280nm became equal to that of PBS. The isolated lectin solution was then applied to the top of the column. The column was then washed extensively with PBS until the absorbance of the effluent at 280 nm became equal to or less than 0.05. The absorbed lectin was then eluted using a solution of N-acetyl D-galactosamine (20 mM in PBS). The effluents were collected in 2 mL aliquots and the absorbance of each fraction was read at 280nm. The fractions with maximum absorbance were pooled and dialyzed against several changes of distilled water. The dialyzed jackfruit lectin was then lyophilized and kept in the dessicator.

Isolation and purification of *Canavalia gladiata* lectin:

Defatted, finely ground seeds of *Canavalia gladiata*, 50 g, were suspended in 0.15M NaCl and stirred overnight in the cold. The resulting suspension was and the residue discarded. The filtrate was then centrifuged for one hour at 10000 rpm and the residue discarded. The supernatant solution was made 30% saturated with ammonium sulphate by gradual addition of the solid salt. The mixture was stirred at room temperature for four hours and the precipitated proteins were removed by centrifugation at 10000 rpm for one hour and discarded. The protein was then precipitated by adding ammonium sulphate to 80% saturation. The precipitate was collected by centrifugation as above, dissolved in distilled water and dialyzed in cold against several changes of distilled water, and finally against 1.0M NaCl.

The isolated *Canavalia gladiata* lectin was then purified by the adsorption of lectin to sephadex G-75. A column of sephadex G-75 was prepared and equilibrated with 1.0 M NaCl and the flow rate was adjusted to 30-40 mL/h. The protein solution from above, in 1M NaCl, was applied to the column, which was connected to a reservoir of 1M NaCl. Fractions were collected every 15 min and monitored for protein by absorbance at 280nm. After the absorbance at 280 nm_≤0, 1 M solution of glucose in 1 M NaCl was added to elute the lectin from the dextran gel. The effluents were collected in 2ml aliquots and the absorbance of each fraction was read at 280 nm. The fractions with maximum absorbance were pooled and dialyzed to remove glucose and concentrated.

Sugar inhibition assay: The sugar inhibition assay was performed by setting up a series of wells containing twice

the minimum haemagglutination dose of lectin together with serial dilutions of the sugar under test. The test was carried out using different sugars such as fructose, glucose, galactose, mannose, fucose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, lactose, melibiose, raffinose, methyl- α -D-glucopyranoside and arabinose. The lowest sugar concentration in which total inhibition of haemagglutination occurs was recorded after 3 to 4 h incubation at room temperature.

Protein estimation: Protein concentration of the lectin was done by Lowry's *et al.*, (1951) method.

Estimation of carbohydrate content: The total carbohydrate content of the purified lectin was estimated by phenol sulphuric acid method (Dubois *et al.*, 1956).

Temperature and pH stability of the lectins: For the determination of heat stability, a solution of lectin (1 mg/mL) was prepared in PBS. Aliquots of the solution were transferred to tubes kept in waterbaths maintained at various temperatures from 30 to 100°C for a period of 15 min, cooled to room temperature and assayed for haemagglutinating activity. pH stability of the lectin was determined by treating the lectin (1 mg/mL) for a period of 24 h at 25°C using different buffers in pH range (2 to 12).

Molecular weight determination by Gel filtration and Sodium dodecyl sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE): Gel filtration was carried out using the method of Whittaker (1963) and Andrews (1964). The chemicals and standard markers were purchased from Sigma chemical company, USA.

Sephadex G-100 column was used for Jack fruit lectin and Sephadex G-200 for *Canavalia gladiata* lectin. All the procedures were carried out at 4°C. The presoaked Sephadex was carefully packed in a column of 90x1.5 cm leaving a small area for sample loading. The column was washed well with the buffer (PBS for Sephadex G-100, and 0.1 M solution of glucose in 1 M NaCl for Sephadex G-200) several times.

Electrophoretic analysis: SDS PAGE was done using the discontinuous buffer system in the slab gel. PAGE was carried out in accordance with the procedure of Laemmli and Favre (1973) using a 12.5% resolving gel and 1.5% stacking gel. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue. After destaining, the electrophoretic mobilities of the marker proteins and the purified lectin were determined. The molecular mass of the lectin was estimated from the standard curve plotting electrophoretic mobility against molecular mass.

Demonstration of glycoprotein: Glycosylation of the lectin was demonstrated in the gel by the method

Table 1: Inhibition capacity of common sugars on agglutination of erythrocytes

Sugar	Inhibitory concentration (mM)	
	JFL	CGL
Galactose	64	>200
Glucose	>200	64
Fructose	>200	125
Mannose	>200	4
Arabinose	>200	>200
Lactose	>200	>200
Melibiose	>200	>200
Raffinose	>200	>200
Methyl D-pyranoside	>200	>200
N-acetyl glucosamine	>200	125
N-acetyl galactosamine	16	>200

described by Zacharius *et al.* (1969) in SDS PAGE carried out in 12.5% gel. The gel after electrophoretic run was fixed in 12.5% Trichloro acetic acid for 30 min followed by distilled water rinses for 5 min. The gel was then immersed in 1% periodic acid solution prepared in 3% acetic acid for 1 h. Extensive washing of the gel with 10 changes of distilled water was done in the dark for 100 min. The gel was then kept in Fuchsin sulphate reagent (2% rosaniline in distilled water is decolourised with SO₂, treated with charcoal, and diluted 20 times with distilled water) in dark for 50 min. The gel was then washed with freshly prepared 0.5% Sodium metabisulphate for 30 min and washed extensively with distilled water. The gel was stored in 7% acetic acid and dried.

RESULTS

Crude extracts from the seeds of *Canavalia gladiata* and *Artocarpus integrifolia* were found to exhibit a reasonable agglutination activity. The carbohydrate specificity of the agglutinin in crude extracts was determined by hapten inhibition tests with a series of simple sugars. As shown in Table 1, N-acetyl galactosamine for *Artocarpus integrifolia* and mannose for *Canavalia gladiata* were found to be the best inhibitors.

When fractions precipitated with 60% Ammonium sulphate from N-acetyl galactosamine specific lectin was applied to the guar gum column, no agglutinability was found in the unbound peak, while activity was found in the N-acetyl galactosamine eluted peak. Maximum lectin activity was found in the mannose eluted peak of *Canavalia gladiata* when 80% Ammonium sulphate precipitated fraction was applied to Sephadex G-75 column. The lectin purified from *Artocarpus integrifolia* was named after the common name of the source as Jack fruit lectin and abbreviated as JFL. The lectin purified from the seeds of *Canavalia gladiata* was named after its botanical name as CGL (*Canavalia gladiata* lectin).

Of the two lectins isolated, protein content was found to be more in *Artocarpus integrifolia* than *Canavalia gladiata*. The carbohydrate estimation revealed maximum

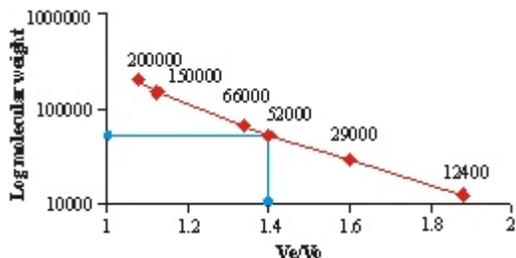


Fig.1: Molecular weight determination of JFL by gel filtration on Sephadex G-100

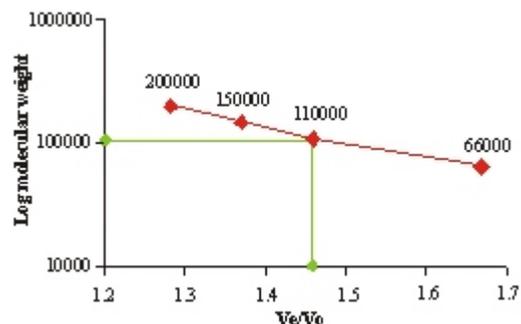


Fig.2: Molecular weight determination of CGL by gel filtration on Sephadex G-200

sugar content in *Canavalia gladiata* (16.3%). The carbohydrate content was less in *Artocarpus integrifolia* (5.5%). JFL was found to be stable in a broad range of pH (2-9). Purified CGL is active at a pH ranging from 6-8.2. The two lectins were found to be stable up to 100°C.

Both JFL and CGL eluted from guar gum column as well as Sephadex G-75 column were found to be homogenous by native gel electrophoresis where it gave a single sharp band (Plate 1a). JFL eluted as a single peak from Sephadex G-100 and was found to exhibit a native molecular mass of 52000 as determined from the calibrated curve obtained (Fig. 1).

On SDS PAGE both in the presence and absence of β -mercaptoethanol JFL showed two bands with molecular weight 11 and 17kDa (Plate 1a). CGL by gel filtration on Sephadex G-200 eluted as a single peak and the molecular weight was estimated as 1,10000 (Fig. 2).

CGL also gave two dissimilar bands of molecular weight 26 and 17 kDa both in the presence and absence of β -mercaptoethanol. Carbohydrate staining of SDS gel indicated that both the bands are glycosylated in CGL whereas only one band was glycosylated in JFL (Plate 1b).

DISCUSSION

The haemagglutinating activity of *Artocarpus integrifolia* was specifically and most effectively inhibited

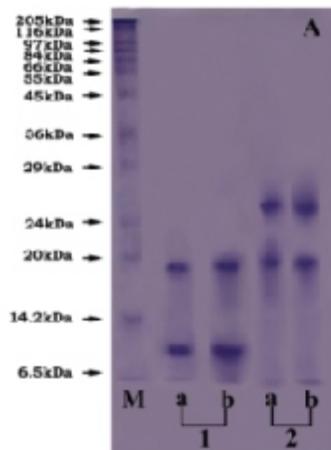


Plate 1a: A - SDS PAGE of purified lectins stained with Coomassie brilliant blue. - Molecular weight standards. Lane 1 a - Jackfruit Lectin with mercaptoethanol and Lane 1 b- Jack fruit Lectin without mercaptoethanol. Lane 2a - *Canavalia gladiata* lectin with mercaptoethanol and Lane 2 b- *Canavalia gladiata* lectin without mercaptoethanol



Plate 1b: B-Periodic acid Schiff's staining of SDS PAGE : Lane 1- Jack fruit Lectin and Lane 2- *Canavalia gladiata* lectin

by N-acetyl galactosamine and to a lesser degree by galactose, suggesting that the agglutinating factor was probably a lectin specific for N-acetyl galactosamine. Affinity chromatography is widely used for the isolation and large-scale production of lectins. For this reason, affinity chromatography on cross-linked guar gum was the method of choice for JFL isolation. Aberman *et al* (1991) isolated lectin from jackfruit seeds by affinity chromatography on a sorbent prepared from the egg white. Soluble guar gum contains galactomannan molecules with average molecular weight of 220kDa arranged as linear β (1-4) linked mannan chains which are substituted at every other mannose unit, on the average, with single galactose units in α (1-6) linkage

(Baker *et al.*, 1976). This dense distribution of terminal α -linked galactose units facilitated the single step affinity purification of galactose specific lectins on guar gum. The lectin jacalin from *Artocarpus integrifolia* was purified to homogeneity in a single step by preparative anion exchange high-performance liquid chromatography by De Simone *et al.* (1994). The present study found maximum lectin activity in the N-acetyl galactosamine eluted peak and no haemagglutinability was observed in any of the fractions, suggesting that the single step affinity purification of JFL in cross-linked guar gum is the better method. JFL was found to be thermally stable like many other plant lectins namely *Artocarpus lakoocha* and *Erythrina indica*. The protein content was found to be more in *Artocarpus integrifolia*. Jackfruit seeds are rich in protein and starch; one can reasonably assume that the cellular and subcellular localization of the storage parenchyma cells of jackfruit seeds is comparable to that of, for example, pea and jack bean seeds. The localization of the jacalin related lectins in the cytoplasm and vacuole contributes to the understanding of the molecular evolution of the family of jacalin related lectins (Chatterjee and Uhlenbruck, 1982; Majumder and Chatterjee, 1996; Peumans, 2000). Unusual carbohydrate binding specificity towards galactose and mannose was exhibited by jacalin, the seed lectin from *Artocarpus integrifolia* (Bourne *et al.*, 2002). Structural analysis has demonstrated that this may be due to the large size of the carbohydrate-binding site of jacalin that enables it to accommodate monosaccharides with different hydroxyl conformations.

The molecular weight of JFL has been reported as 39,500 Da with identical subunits of 10 kDa (Appukuttan and Basu, 1985). A molecular weight of 62, 65 and 56 kDa has been reported for JFL by different authors (Moreira and Ainouz, 1981; Namjuntra *et al.*, 1985; Misquith *et al.*, 1994). The gel filtration results of JFL used in the present study show that it contains 52 kDa protein, which yielded a single band in native gel electrophoresis. The two dissimilar bands of molecular weight 17 and 11 kDa both in the presence and absence of mercaptoethanol thus indicates that the protein should be a tetramer with no intersubunit disulphide bond. Two tetrameric lectins, jacalin and artocarpin for *Artocarpus integrifolia* have been reported (Pratap *et al.*, 2002). A molecular weight of 46,000 with two subunits 12,000 and 15,000 has been reported for jacalin by Young *et al.* (1989). Two subunits of molecular weight 11500 and 15000 daltons were reported for *Artocarpus integrifolia* by Sureshkumar *et al.* (1982). Moreira and Oliveira (1983) and Vijayakumar and Forrester (1986) reported a molecular weight of 39,500 for *Artocarpus integrifolia* lectin with a single polypeptide of 10,500 dalton.

Canavalia gladiata lectin agglutinated human type A, B and O erythrocytes almost equally. Specific

monosaccharides such as D-mannose have been shown to be potent inhibitors of the biological activities of *Canavalia gladiata* lectin, which is a member of the family Leguminosae. A glucose/mannose-binding lectin was isolated from the seeds of *Parkia discolor* (Mimosoideae) using affinity chromatography on Sephadex G-100 gel (Cavada *et al.*, 2000). Lectin from Japanese jack bean (*Canavalia gladiata* agglutinin, CGA) was purified by affinity chromatography on a maltamyl-sepharose column (Kojima *et al.*, 1991) and Sephadex G-50 by Yamauchi and Minamikawa (1990). In the present study Sephadex G-75 column was used for the isolation of *Canavalia gladiata*. Upon SDS PAGE, under both reducing and nonreducing conditions, CGL yielded two polypeptide bands of molecular weight 26 and 17kDa indicating that they are not linked by disulphide bonds. A lectin from Japanese jack bean (*Canavalia gladiata* agglutinin) was shown to have a protein subunit with a molecular weight of 30,000 (Kojima *et al.*, 1991), whereas CGL isolated in the present study exhibited a higher molecular weight of 1,10,000. Concanavalin isolated from *Canavalia ensiformis* by Sheldon *et al.* (1998) yielded five bands of molecular weights 78, 74, 54, 32 and 30 kDa on SDS PAGE.

Carbohydrate binding, particularly mono and disaccharides, is the defining feature of the lectins and is the basis of many methods of classification of plant lectins. The saccharide binding specificities of lectins can be utilised in the purification, characterization and sequencing of polysaccharides, polypeptides and glycoproteins.

The major advantage of the lectins is their ready availability and monospecificity. The parallel development of monoclonal antibodies will be expensive and time consuming for detecting the biological differences in cell population. The ready availability, ease of preparation in purified form and their unique properties to specifically attach with carbohydrate moieties opens the potential use of these lectins as diagnostic markers.

ABBREVIATIONS

CGL - *Canavalia gladiata* Lectin, JFL - Jack Fruit Lectin, SDS - Sodium dodecyl sulphate, PAGE - Polyacrylamide Gel Electrophoresis

REFERENCES

- Aberman, L.E., L.I. Petychna and M.D. Lutsyk, 1991. Production of lectin from jack fruit (*Artocarpus integrifolia*) seeds and its interaction with carbohydrates and glycoproteins. Ukr Biokhim Zh, 63: 70-76.
- Andrews, P., 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J., 91: 222.

- Appukuttan, P.S. and D. Basu, 1985. Four identical subunits in jack fruit seed agglutinin offers only two saccharide binding sites. FEBS Lett., 180: 331-334.
- Baker, C.W. and R.L. Whistler, 1976. Biochemical method of estimation of carbohydrates. Methods Carbohydr. Chem., 7: 152-156.
- Bourne, Y., C.H. Astoul, V. Zamboni, W.J. Peumans, L. Menu-Bouaouiche, E.J. Van Damme, A. Barre and P. Rougé, 2002. Structural basis for the unusual carbohydrate binding specificity of jacalin towards galactose and mannose. Biochem. J., 364: 173-180.
- Cavada, B.S., S.V.F. Madeira, J.J. Calvete, L.A. Souza, L.R. Bomfim, A.R. Dantas, M.C. Lopes, T.B. Grangeiro, B.T. Freitas, V.P. Pinto, K.B. Leite and M.V. Ramos, 2000. Purification, chemical and immunochemical properties of a new lectin from Mimosoideae (*Parkia discolor*). Prep. Biochem. Biotechnol., 30: 271-280.
- Chatterjee, B.P. and G. Uhlenbruck, 1982. Occurrence of an antihuman friedenreich like lectin in jack fruit seeds reacting with a receptor in anti EGG glycoprotein. Experientia, 38: 1225-1226.
- De Simone, S.G., R. Santos, M.F. Araujo and R.T. Pinho, 1994. Preparative isolation of the lectin jacalin by anion exchange high-performance liquid chromatography. J. Chromatogr. A, 688: 357-362.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.
- Kojima, K., H. Ogawa, N. Seno and I.L. Matsumoto, 1991. Purification and characterization of *Canavalia gladiata* agglutinin. Carbohydr. Res., 213: 275-282.
- Laemmli, U.K. and M. Favre, 1973. Maturation of the head of bacteriophage T4. I, 1973. DNA packaging events. J. Mol. Biol., 80(4): 575-599.
- Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 265: 1951.
- Majumder, M. and B.P. Chatterjee, 1996. Subcellular distribution of jacalin in *Artocarpus integrifolia* seed. Cytobios, 88: 201-208.
- Misquith, S., P.G. Rani and A. Surolia, 1994. Carbohydrate binding specificity of the B-cell maturation mitogen from *Artocarpus integrifolia* seeds. J. Biol. Chem., 269: 30393-30401.
- Moreira, R.A., I.L. Ainouz, J.T. De Oliveira and B.S. Cavada, 1991. Plant lectins, chemical and biological aspects. Mem. Inst. Oswaldo. Cruz., 2: 211-218.
- Moreira, R.A. and I.L. Ainouz, 1981. Lectins from seeds of jackfruit (*Artocarpus integrifolia* L.): Isolation and purification of two isolectins from the albumin fraction. Biol. Plantarum, 23: 186-192.
- Moreira, R.A. and J.T.A. Oliveira, 1983. Lectins from the genus *Artocarpus*. Biol. Plant., 25: 343-348.
- Namjuntra, P., P. Muanwongyathi and M.A. Chulavatnatol, 1985. Sperm-agglutinating lectin from seeds of jackfruit (*Artocarpus heterophyllus*). Biochem. Biophys. Res. Comm., 128: 833-839.
- Peumans, W.J., W. Zhang, A.C. Barre Houlès, P.J. Balint-Kurti, P. Rovira, P. Rougé, G.D. May, F. Van Leuven, P. Truffa-Bachi and E.J. Van Damme, 2000. Fruit specific lectins from banana and plantain. Planta, 211: 546-554.
- Pratap, J.V., A.A. Jeyaprakash, P.G. Rani, K. Sekar, A. Surolia and M. Vijayan, 2002. Crystal structures of artocarpin, a Moraceae lectin with mannose specificity, and its complex with methyl- α -D-mannose: Implications to the generation of carbohydrate specificity. J. Mol. Biol., 317(2): 237-247.
- Pueppke, S.G., 1979. Purification and characterization of a lectin from seeds of the winged bean, *Psophocarpus tetragonolobus* (L) DC. Biochem. Biophys. Acta., 581: 63-70.
- Rudiger, H. and H.J. Gabius, 2001. Plant lectins: Occurrence, biochemistry, functions and applications. Glycoconj. J., 18(8): 589-613.
- Sharon, N. and H. Lis, 2002. How proteins bind carbohydrates: Lessons from legume lectins. J. Agr. Food Chem., 50: 6586-6591.
- Sheldon, P.S., J.N. Keen and D.J. Bowles, 1998. Purification and characterization of N-glycanase, a concanavalin A binding protein from jack bean (*Canavalia ensiformis*). Biochem. J., 330: 13-20.
- Sureshkumar, G., P.S. Appukuttan and B. Debkumar, 1982. α -D-galactose specific lectin from jack fruit (*Artocarpus integrifolia*) seed. J. Biosci., 4(3): 257-261.
- Vijayakumar, T. and J.A. Forrester, 1986. Purification and physico chemical properties of lectins from the jack fruit (*Artocarpus integrifolia*). Biol. Plantarum, 28: 370-374.
- Whittaker, J.R., 1963. Phytohemagglutinins for carbohydrate purification. Anal. Chem., 35: 1950.
- Yamauchi, D. and T. Minamikawa, 1990. Structure of the gene encoding concanavalin a from *Canavalia gladiata* and its expression in *Escherichia coli* cells. FEBS Lett., 260: 127-130.
- Young, N.M., R.A.Z. Jhonston, A.G. Szabo and D.C. Watson, 1989. Homology of the D-galactose specific lectins from *Artocarpus integrifolia* and *Maclura pomifera* and the role of an unusual small polypeptide subunit. Arch. Biochem. Biophys., 270: 596-603.
- Zacharius, R.M., T.E. Zell, J.H. Morrison and J.J. Woodlock, 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem., 30: 148-152.