# ISOLATION AND CHARACTERIZATION OF PEANUT (*Arachis hypogaea*) LECTIN

## THESIS SUBMITTED TO
## National Institute Of Technology,Rourkela
## For The Partial Fulfillment Of
## The Master Degree In Life Science


**SUPERVISED BY:**

**DR. SUJIT K. BHUTIA**

**ASST. PROFESSOR**

**SUBMITTED BY:**

**CHANDAN KANTA DAS**

**ROLL NO: 410LS2050**

**DEPARTMENT OF LIFE SCIENCE**

**NATIONAL INSTITUTE OF TECHNOLOGY**

**ROURKELA-769008**

# DEPARTMENT OF LIFE SCIENCE
# NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA-769008

.....

Dr. Sujit Kumar Bhutia
Assistant Professor.

Ref. No.

Date: .....

## CERTIFICATE

This is to certify that the thesis entitled "**ISOLATION AND CHARACTERIZATION OF PEANUT (*Arachis hypogaea*) LECTIN**" which is being submitted by **Mr. Chandan Kanta Das**, Roll No. **410LS2050**, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by him under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. Sujit Kumar Bhutia
Assistant Professor,
Department of Life Science
National Institute of Technology
Rourkela – 769008, Odisha, India.

Phone no: 91-661-2462686 Email: sujitb@nitrkl.ac.in

DEDICATED TO

MY

BELOVED PARENTS

## DECLARATION

I do hereby declare that the Project report entitled **"ISOLATION AND CHARACTERIZATION OF PEANUT (*Arachis hypogaea*) LECTIN"** submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfillment of the Master Degree in Life Science is a faithful record of bona fide and original research work carried out by me under the guidance and supervision of **Dr. Sujit Kumar Bhutia**, Assistant Professor, Department of life Science, NIT, Rourkela.

**Date:** **CHANDAN KANTA DAS**

**Place:**

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At the end, I bow down my head to the almighty whose omnipresence has always guided me and made me energized to carry out such a project.

**Date:** **CHANDAN KANTA DAS**

**Place:**

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# 1. ABSTRACT

Plant lectins which belong to a class of glycoprotein have a wide spectrum of biological significance. In this thesis, the isolation and characterization of the lectins (AHL and PNA) from the peanut (*Arachis hypogaea*) have been reported. The lactose binding lectins were purified from the peanut seeds by performing affinity chromatography using a lactamyl column. Affinity chromatography products were dialyzed and SDS-PAGE was done to identify the fractions' molecular weight. Furthermore, Haemagglutination assay was performed for crude,20% cut,60%cut, affinity portion using AB+ve and O +ve blood group.

**Keywords**: peanut lectin, AHL, PNA, Affinity chromatography,SDS-PAGE, Haemagglutination assay

# INTRODUCTION

Lectins are sugar-binding proteins or glycoproteins which bind to specific mono or oligosaccharides without altering the bound ligand and having the ability to agglutinate cells, which are extensively found in plants, vertebrates, and invertebrates (Van Damme et al., 2004). They are most abundant in the plant kingdom, and are found in seeds, leaves, barks, tubers, rhizomes, roots, bulbs, depending on the plant species (Adenik et al., 2009; Wong et al., 2008; Audrey and Sharon., 2002; Thakur et al., 2007).The broad study on plant lectin is due to their highly specific interaction with carbohydrates and the biological effects based thereon. The high impacts of plant lectins on biological research is due to two main reasons. First plant lectins are a readily accessible carbohydrate binding proteins. Secondly, plant lectins are particularly suited for the analysis, isolation and purification of animal and human glycoconjuates because the latter compounds are the natural targets of most carbohydrate binding proteins present in plants. Lectins helps in differentiating between the malignant and normal cells based on their agglutination behavior. This property is due to variable glycosylation on the cell surface associated with malignancy, invasion and metastasis (Hakomori., 2002; Kim et al., 1997 ). Lectins can be used for the detection of changes of glycan in certain disease processes which involve enhanced sialylation, increased branching pattern of complex sugars and fucosylation. Identification of these altered structural profiles of glycans by lectins provides valuable disease biomarkers (Strauchen., 1984; Comunale., 2006). Lectins were first described in 1888 from castor bean extracts. Most of the lectins agglutinate (clump together) red blood cells. The father of immunology, Ehrlich has studied that feeding very little amounts of lectin bearing seeds to rabbits increased partial resistance to the toxicity, showing lectins are also immunogenic (able to induce antigen antibody reactions).High levels of lectins may be found in legumes, dairy, grains, and plants in the family of nightshade. Other foods contain lectins but are less well studied and the amounts of lectins present are not thought to be as high or as potentially toxic.

Lectins derived from leguminous plants are most extensively studied (Sharon et al., 1990).These glycoproteins may comprise up to 3% of the weight of a mature seed. Research highlighting protein and gene sequencing has revealed that most of the leguminous lectins are made up of either two or four protomers of about 30kDa. Undoubtedly all legume letins are related at the

molecular level but show a significant variation in carbohydrate binding specificity (Table-1) which certainly contributed to the success of legume lectins as therapeutic tools.

Table-1: Legume Lectin and their Sugar Specificity

| Lectin | Sugar specificity |
|---|---|
| 1. *Arachis hypogaea* (peanut) | Galactose |
| 2. *Bandeiraea simplicifolia* | Galactose |
| 3. *Bauhinia purpurea alba* | N-acetyl galactosamine |
| 4. *Dolichos biflorus* | N-acetyl galactosamine |
| 5. *Glycine max* (Soybean) | N-acetyl galactosamine |
| 6. *Lotus tetragonolobus* | Fucose |
| 7. *Phaseolus lunatus* (Lima bean) | N-acetyl galactosamine |
| 8. *Phaseolus vulgaris* (PHA-E; Kidney bean) | Galactose |
| 9. *Ricinus communis* (Castor bean) | Galactose |
| 10. *Sophora japonica* (Pagoda tree) | N-acetyl galactosamine |
| 11. *Ulex europeus* (Gorse) | Fucose |
| 12. *Vigna radiata* (Mung bean) | Galactose |
| 13. *Wisteria floribunda* | N-acetyl galactosamine |
| 14. *Concavalin ensiformis* (Jack bean) | Glucose/mannose |
| 15. *Lens culinaris* (Lentil bean) | Glucose/mannose |
| 16. *Pisum sativum* (Garden pea) | Glucose/mannose |

Priority to legume lectins are given because of their abundance in many crop plants and their involvement in the symbiosis between legumes and the nitrogen fixing bacterium *Rhizobium* (Diaz et al., 1989). Lectins are generally widely distributed among leguminous plants (Toms., 1971). Collectively, the legume lectins display many similar properties like metal ion-binding, tetrameric glycoproteins possessing identical or nearly identical subunits (Liener., 1976; Lis., 1972). Depending upon their common source and upon their possessing similar physical, chemical, and biological properties, many of the legume lectins are homolog (Foriers., 1977). The in vitro function of these proteins is well documented and the molecular basis of this interaction has been studied with a variety of biophysical techniques. The legume lectins form a large family of homologous proteins. From 50 legume lectin sequences, all show pair wise sequence identities not lower than 35%. Recently, it has been suggested that also in the animal kingdom legume lectin homologues may be present (Fiedler., 1994)

The peanut agglutinin (PNA) from peanut *(Arachis hypogaea* L.), which is the first lectin to be fully studied in this plant is highly specific for the tumor-associated T-antigenic disaccharide Gal (βl-3) GalNAc. PNA is a tetrameric protein with a molecular weight of 110 kDa. PNA especially binds malignant cells; as a result, this lectin has been extensively used as a probe for detecting malignant phenotypes in several tissues (Langkilde et al., 1992). The present investigation was focused to purify and characterize a lactose-binding lectin with similar properties from seeds of peanut. The structure, composition, biological activity of the lectin have been determined. There are various molecular heterogeneity within various purified PNA preparations (Dumont and Nardelli., 1979; Miller., 1983; Pueppke., 1981). Studies also have shown that the heterogeneity of the isolectin population in peanut varieties is basically due to the dissimilar composition of the subunits of isolectins, which exhibits changes in physicochemical characteristics and in biological properties, that is, mitogenic acitvity (Miller., 1983; Pueppke., 1981).

## 3. REVIEW OF LITERATURE

Lectins are sugar-binding proteins or glycoproteins which bind to the specific sugar moieties without altering their structure and having the capacity to agglutinate cells, which widely exist in invertebrates, vertebrates and plants (Van Damme et al., 2004). Legume lectins are most extensively studied. Concanavalin A (Con A), a tetrameric protein from the jack bean was the first lectin to be completely studied (Becker et al., 1975). The structure, function, and properties of this lectin were fully studied in 1970. Subsequently, lectins from animals, cereals and viral sources were analyzed (Marcia et al., 2002; Liu et al., 2007).The peanut agglutinin (PNA) which is the first lectin to be fully studied in this plant is specific for the tumor-associated T-antigenic disaccharide Gal(βl-3) GalNAc, . PNA is a tetrameric protein with a molecular weight of 110 kDa. PNA generally binds malignant cells of breasts; so, this lectin has been extensively used as a probe for detecting malignant phenotypes in several tissues (Langkilde et al., 1992). PNA generally agglutinates neuraminidase-treated human erythrocytes (Sun et al., 2008).But, *N*-acetyl Neuraminic acid (NeuAc) on the surface of the erythrocytes is removed after the treatment of neuraminidase, then only AHL could agglutinate these three types of human erythrocytes. In fact, agglutination property of AHL is similar to that of the lectin PNA previously found in peanut. These two lectins are different because AHL is a monomer protein with a molecular weight of 29 kDa, whereas PNA is a tetrameric protein with molecular weight 110 kDa. AHL could also recognize and agglutinate several kinds of tumor cells.

The agglutinin specificity of PNA for terminal *β*-D-galactosyl residues has enabled its purification by Sepharose 6B column chromatography, Sepharose-coupled on *α*-aminocaproyl-β-D-galactopyranosylamine by affinity chromatography, lactosaminylAE-P-150, agar polyacrylamide beads and lactosyl- Sepharose (Gray and Baues., 1977; Sutoh et al., 1977; Fish et al., 1978). Other studies have shown that the heterogeneity of the isolectin population in peanut varieties is generally due to the dissimilar composition of the isolectins subunits, which show differences in biological properties and physicochemical characteristics (Miller., 1983; Pueppke., 1981). Although each subunit has the same characteristic tertiary fold that is generally found in other legume lectins, the structure of PNA shows an unusual quaternary arrangement of subunits (Banerjee et al., 1994,1996). The isoforms of lectins shows differences in thermal

stability. At pH 7.4, the temperature of maximal stability of the peanut agglutinin tetramer has been calculated to be -33 °C (Reddy et al., 1999).

A large family of homologous proteins is formed by the legume lectins. From the 50 determined legume lectin sequences, all show sequence identities not lower than 35%. Animal kingdom legume lectin homologues are also studied recently (Fiedler., 1994, 1995). There are 11 members of the legume lectin family whose crystallographic co-ordinates have been submitted at the protein data bank. From these only crystallographic coordinates of *Arachis hypogaea* has shown below (Tables- 2 ).

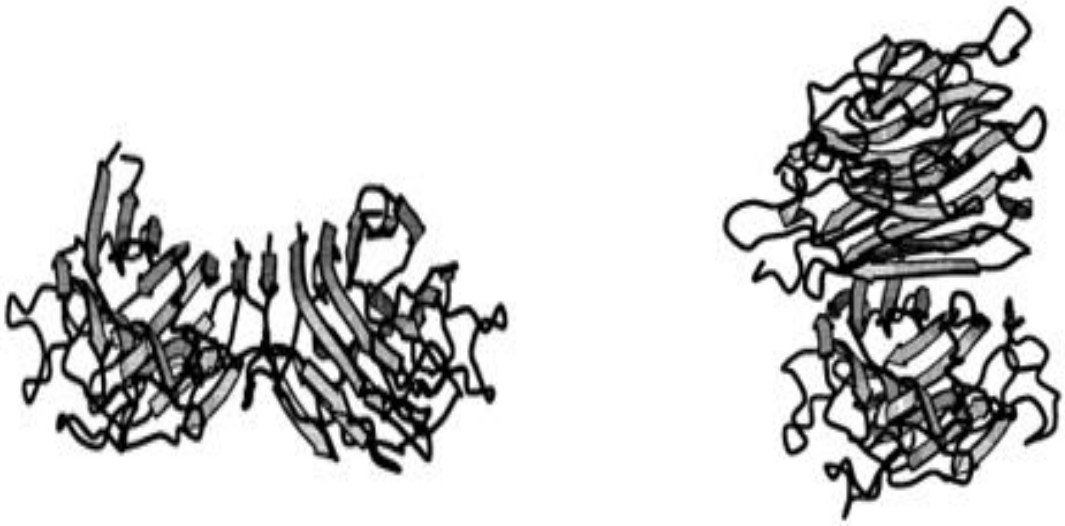
Table-2: Crystal structures of *Arachis hypogaea* lectin–carbohydrate complexes

| Species | Bound carbohydrate | Resolution | R-factor | Rfree-factor | PDB code |
|---|---|---|---|---|---|
| *Arachis hypogaea* | Galb1–4Glc _Lactose. | 2.2 | 0.164 | nr a | 2PEL |
| *Arachis hypogaea* | Galb1–3GalNAc _T-antigen disaccharide. | 2.4 | 0.17 | 0.251 | 1TEP |

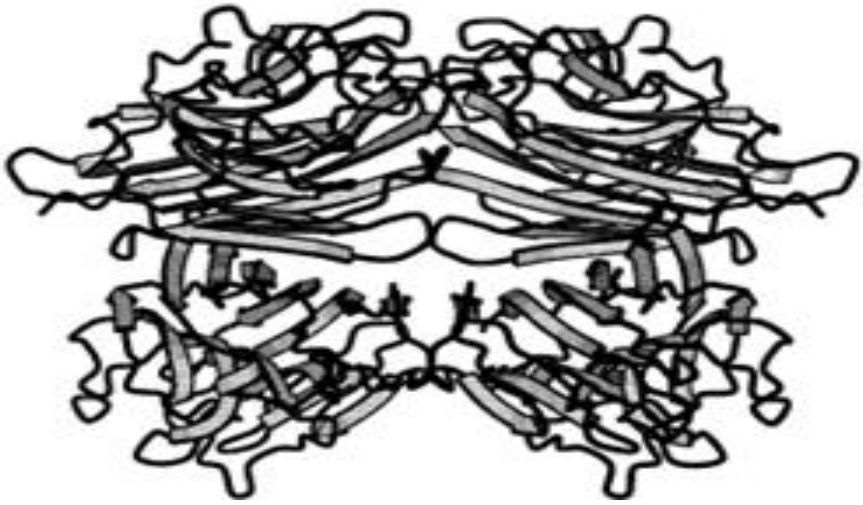
Not surprisingly, the monomer of legume lectins is structurally well conserved and it consists of two large β-pleated sheets that form a scaffold on which the carbohydrate binding region is grafted . The same topology and architecture is found in a wide variety of carbohydrate recognizing proteins such as the galactins (Lobsanov., 1993), serum amyloid protein (Emsley., 1994), Charcot–Leyden crystal protein (Leonidas., et al., 1995), the lectin-like domains Wing-1 and Wing-2 attached to the catalytic domain of *Vibrio cholera* neuraminidase (Crennell., 1994).The topology of the legume lectin fold, which is complex and is structurally related to the jelly-roll topology commonly found in viral coat proteins (Chelvanayagam. et al., 1992, Argos.et al., 1980). The structure of the legume lectin monomer is usually described as consisting of two

β-sheets. Peanut lectin or PNA was the second tetrameric lectin whose structure was determined. In 1982, its crystallization was reported but the refined structure at 2.2A° was only studied recently (Banerjee., 1994). Although it has some homology with other known legume lectins, but this structure could only be solved by replacing with isomorphs. The tetramer of peanut lectins generally consist of two GS-IV type dimmers depicted in (Fig- 1). GS-IV type dimmers containing two monomers each associate in a similar fashion but not identical to the canonical dimmer. However, no continuous 12-stranded β-sheet is formed along the dimmer interface. The two N-terminal strands of the two monomers are intercalated by a series of 6 water bridges. As the internal symmetry of PNA does not form a closed point group, its tetramer is thus different among all homo tetrameric proteins. PNA is not a glycoprotein. Therefore, its unique quaternary structure is due to the intrinsic properties of the protein itself. Modeling studies of the peanut agglutinin tetramers (Banerjee. et al., 1996) suggested that the important factor in the choice of the type of subunit association is the amount of non-polar surface area buried upon oligomerisation.

Peanut agglutinin plays an important role for the detection of mature and immature thymocytes prior to bone marrow transplantation (Reisner. et al., 1979). It also helps in the detection of malignant tumors (Zebda. et al., 1994).The carbohydrate specificity for PNA was determined at atomic resolution as a complex with lactose, which is generally involved in 9 hydrogen bonds with the residues stretching from the four loops. The galactose residue of lactose is attached to the protein through hydrogen bonds and water bridges. It has also stacking interaction with a tyrosyl residue from loop C. The PNA-T-antigen crystal structure (Ravishankar. et al., 1997) described the similarity in the mode of binding of T-antigen and lactose. Two additional water bridges present in the binding site of the PNA-lactose complex contribute the 20 fold higher affinity for T-antigen. PNA-lactose structure reveals these water molecules are not involved in any bonding and was thereafter proven to be critical in the binding of this sugar (Sharma., 1996). Complex of PNA with T-antigen confirmed the importance of this residue and showed the involvement of the same water molecule in linking both Leu-212 and Asn-41 to T-antigen (Ravishankar. et al., 1999).



a. Canonical Dimmer b. GS4 Dimmer



c. Peanut Agglutinin Tetramer

Fig–1: a. Canonical Dimmer, b. GS4 Dimmer, c. Tetramer of PNA

PNA shows an important principle of quaternary association in globular proteins. Generally, most of the legume lectins unfold and dissociate as single entities (Surolia. et al., 1996; Ahmad. et al., 1998). The common chaotrope of PNA, guanidine hydrochloride (GdnHCl) induces unfolding and forms a substantially unfolded intermediate monomer species, which contains its carbohydrate specificity without losing its tertiary structure. The existence of such a molten

globule like structure for PNA determines that the monomers of legume lectins are competent to bind sugars and oligomerization is responsible for the stability and necessary spatial disposition of sugar binding sites for manifestation of their respective biological activities. Peanut (*Arachis hypogaea*) agglutinin generally recognizes the Thomsen-Friedenreich antigen, a T-antigenic determinant (Galb1–3GalNAc) which is chemically well studied tumor-associated antigen having link to malignancy in man (Irimura. et al., 1975; Salunk. et al., 1985). These structures are generally found as *O*-linked glycans by poorly differentiated cells and tumor cells, but these cells are absent or modified in normal cells( Springer., 1984).The tumor-associated Thomsen-Friedenreich binding antigen from peanut does not associate with either GalNAc or other galactose derivatives with bulky C-2 substituent (Swamy et al., 1991; Lotan et al., 1975; Reisneret al.1979; Pereira et al., 1976; Younget al., 1984). It has been found that the interaction of dansylamido, acetamido group or other bulky hydrophobic C-2 substituents which are present on galactose, is controlled by a few residues which constitute a hydrophobic patch within GalNAc-specific lectins. The outer covering of the skin (epidermis), is generally made up of multiple layers of keratinocytes. Proliferation in the basal layer of cells undergo terminal differentiation as they move through the supra basal layers to the surface of the skin (Watt., 1989). A wide variety of markers of terminal differentiation have been described ( Watt., 1989). when keratinocytes are disaggregated with trypsin and EDTA, PNA binding sites are preserved (Morrison et al., 1988; Watt., 1983 ) and thus by flow cytometric method, PNA can be used to study keratinocyte sub populations (Jones and Watt., 1992, ). The difference between basal and supra basal keratinocytes due to PNA binding is observed early in the epidermal development with the onset of stratification (Watt et al., 1989). Immunoelectron microscopy shows that the glycoproteins are mostly found on the cell surface microvilli and absent in desmosomal junctions. PNA-binding glycoproteins plays a crucial role in cell-cell adhesion, but so far there has not been any data that directly support the idea (Morrison et al., 1988; Watt et al., 1989). CD44, a multifunctional polymorphic integral membrane glycoprotein share a number of features of the keratinocyte PNA-binding glycoprotein, that is a receptor for hyaluronan (Lesley et al., 1993b).

# OBJECTIVES OF THE STUDY

- **ISOLATION AND PURIFICATION OF LECTIN FROM PEANUT (*Arachis hypogaea*)**

- **MEASUREMENT OF CONCENTRATION OF PROTEIN (LECTIN)**

- **CHARACTERIZATION OF PROTEIN (LECTIN)**

  1. **HAEMAGGLUTINATION ASSAY**

  2. **SDS- PAGE**

# 4. MATERIALS AND METHODS:

## 4.1. MATERIALS :

Dried peanuts (*Arachis hypogaea*) were taken from sector-2 market of Rourkela, Odisha, India and were used for all the experiments in this study. Human blood type AB and O were obtained from healthy donors with the help of the pathologist of CWS hospital, Rourkela, Odisha, India.

## 4.2. CHEMICALS :

Sodium hydroxide (NaOH), Sodium carbonate ($Na_2CO_3$), glycine, Cupper sulphate ($CuSO_4$),Potassium sodium tartarate ($KNaC_4H_4O_6$) were purchased from SRL Pvt. Ltd., Mumbai. Acrylamide, bisacrylamide, Ammonium persulphate (APS), Sodium dodecyl sulphate (SDS), N,N,N',N'-tetramethylenediamine (TEMED), Bovine serum albumin (BSA), Tris were purchased from Sigma Aldrich, USA. Folin-Ciocalteau phenol reagent, Potassium Dihydrogen Phosphate ($KH_2PO_4$), Potassium hydrogen phosphate ($K_2HPO_4$) were purchased from S.D. fine Chem. Ltd., Mumbai. Acetic acid, Bromophenol blue, agarose were purchased from Himedia, Mumbai. Glycerol was purchased from RANKEM Pvt Ltd. Ethanol purchased from Trimurty Chemicals, India. Pre stained molecular weight marker was purchased from Bio-Rad, India. Methanol, Silver nitrate, Sodium thiosulphate were purchased from Nice chemicals Pvt. Ltd. India.

## 4.3. PREPARATION OF LACTAMYL SEPHAROSE AFFINITY MATRIX:

**METHODOLOGY:**

About 8g of sepharose 4B was washed by PBS in filtration unit and stored in 40ºC in alcohol.

**EPOXY ACTIVATION OF SEPHAROSE 4B:**

About 8g of sepharose 4B was suspended in 12 ml of distilled water and mixed well through pipette. Then 5.2ml of 2M NaOH was added to it. About 1.3ml of epichlorohydrin was added so that the final concentration of the various components were 30% (v/v) sepharose, 5% epichlorohydrin, 0.4M NaOH. Then the suspension was incubated at 40ºC for 2 hours with continuous shaking. It was then transferred to a glass filter funnel and the gel was washed with 500ml of distilled water.

**PREPARATION OF AMINO SEPHAROSE 4B:**

Epoxy activated sepharose 4B was suspended in 1.5 volume of concentrated ammonium solution (12ml).The suspension was then incubated at 40ºC for 1.30 min. It was then again transferred to glass filter funnel and the gel was washed with distilled water.

**COUPLING OF LACTOSE WITH AMINO SEPHAROSE 4B:**

8g of dried amino sepharose 4B was suspended in 6ml of 0.2M $K_2HPO_4$ buffer containing 208mg lactose and 102mg of $NaCNBH_3$.The suspension was then incubated at room temperature for 10 days with occasional shaking. Then the free amino groups which remained in the gel were acetylated by adding 2ml of acetic anhydride. The suspension was incubated at room temperature for 1 hour. The lactamyl sepharose 4B thus obtained was subsequently washed with distilled water, 0.1 M NaOH, distilled water and 10 mM PBS. Then it was stored in distilled water with traces of sodium azide at 4ºC.

**4.4. EXTRACTION AND PURIFICATION OF LECTIN:**

50g of peanut was taken and broken down into 1mm pieces by mortar pestle. Then the broken peanut was soaked overnight in 100ml of 10mM PBS at pH 7.2. The soaked peanut was grinded well by grinder with that of the PBS in which it was soaked. Then the grinded mixture

was centrifuged at 7500rpm at 4ºC for 20min. Supernatant obtained by centrifugation was then used for purification of lectin. Proteins in the supernatant were precipitated by salting out method using ammonium sulphate (20-60% saturation). After 60% cut off, the precipitate or the pellet was resuspended and dialyzed for 5days against 10mM PBS (pH 7.2) till all the salts to be released. Then it was applied to affinity chromatography on a galactoside agarose column which was pre equilibrated with the same buffer. Unbound materials were washed from the column by passing PBS (10mM at pH 7.2), until the O.D. 280 of eluents was below 0.01. Then 50ml of 0.4M lactose was passed through the column and the O.D. at 280nm of the lactose eluents were taken. Then these fraction which showed absorbance at 280nm, were collected and dialyzed extensively against 10mM PBS (pH-7.4) at 4ºC overnight to remove the lactose. Then protein estimation was done by Lawry method. Then total 13.5ml of effluent collected after adsorption, washing and elution was assayed for haemagglutination activity (HA).

**4.5. HEMAGGLUTINATION ASSAYS:**

About 1ml of human blood was collected in presence of anticoagulant from the CWS hospital. Then it was centrifuged at 1000rpm for 5min at room temperature. The pellet was taken and 10ml of PBS (10mM at pH 7.2) was added to it and again centrifuged at 1000rpm for 5min at room temperature. Then pellet was collected. 100µl of pellet was taken and added to the 10ml PBS (10mM at pH 7.2). Then these samples were taken in 96 well "U" bottom micro titer plate with serially dilution by taking the mixture of PBS(10mM at pH 7.2) and blood sample as negative control. The HA assay was repeated for two times by taking AB and O blood groups separately.

**4.6. ELECTROPHORESIS:**

The purity of the lectin was determined by SDS-PAGE, using 12% total concentration polyacrylamide as the resolving gel and 5% polyacrylamide as the stacking gel. The molecular weight of peanut lectin was determined by SDS–PAGE under reducing condition using beta mercaptoethanol and SDS. Then the protein was heated at 100ºCfor 5 min in a loading buffer containing 5% β mercaptoethanol and 2% SDS. The separated proteins were stained by silver staining method.

# 5. RESULTS

## 5.1. DETERMINATION OF PROTEIN CONCENTRATION:

Determination of the concentration of the proteins (crude, 20% cut, 60% cut, affinity) were done by Lowry method which is depicted in Table- 3.

Table-3: concentration of proteins:

| Sample | Volume (ml) | Concentration (mg/ml) | Total concentration (mg/ml) |
|---|---|---|---|
| Crude | 72 | 42.84 | 3048.48 |
| 20% cut | 67 | 33.44 | 2240.48 |
| 60% cut | 27 | 30.36 | 819.672 |
| Affinity | 13.5 | 0.3448 | 4.654 |

## 5.2. HAEMAGGLUTINATION ASSAY:

To characterize the protein, Haemagglutination Assay was performed by using human erythrocyte suspension (AB and O RBC). The assay was carried out in 96 well "U" bottom micro titer plate by serially diluting the lectin sample and allowing it to incubate for 2 hours. Then it was found that there was no agglutination reaction rather RBCs were settled at the bottom (Fig- 2, 3). This ensured that *Arachis hypogaea* lectin could not agglutinate the human RBCs.

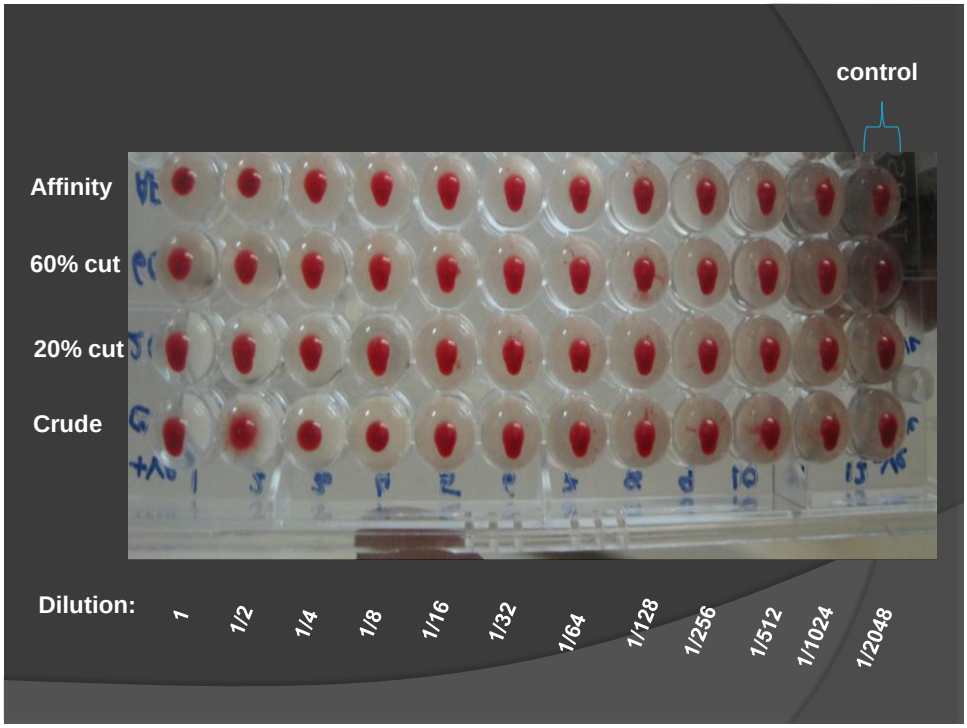



**Fig-2: HAEMAGGLUTINATION ASSAY FOR AB +VE BLOOD GROUP**

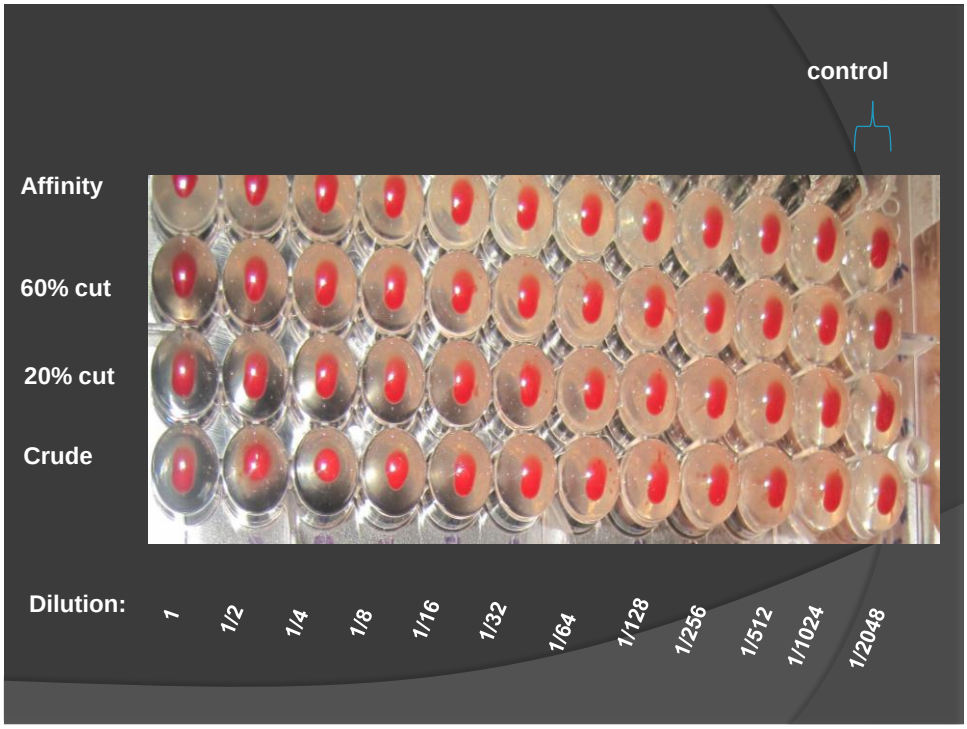



**Fig-3: HAEMAGGLUTINATION ASSAY FOR O+VE BLOOD GROUP**

### 5.3: SDS-PAGE:

To determine the molecular size of the protein, 12% SDS-PAGE gel was run following which silver staining was performed. Then the bands were visualized by gel documentation system (Bio-Rad) which showed that there were two intense bands in affinity protein, one was 29 kDa which represents the only subunit of AHL lectin while the other was 28kDa representing the monomeric subunit of homotetrameric PNA lectin (Fig-4).

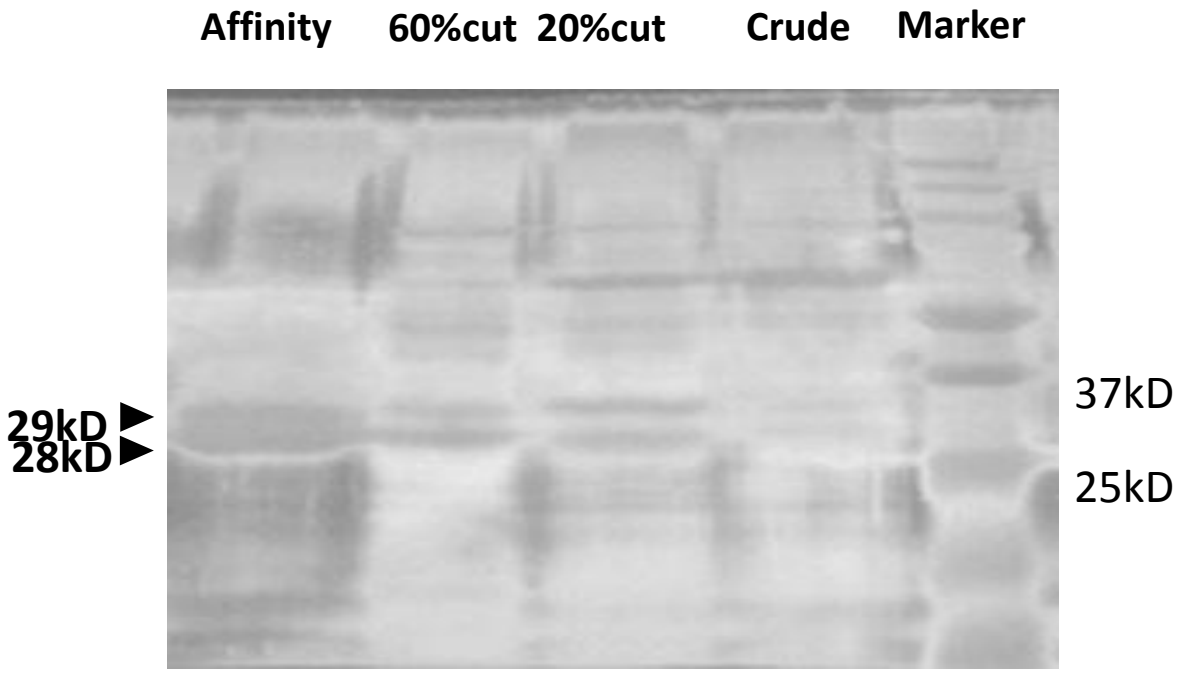



**Fig-4: SILVER STAINED SDS-PAGE (12%) ELECTROPHORETOGRAM**

## 6. DISCUSSION

Lectins are proteins or glycoprotein which bind to the sugar moieties without altering the sugar structure. Peanut lectins have agglutination activity which helps in differentiation of normal and malignant cells. It has also antitumor, antifungal, antibacterial and antiviral activity which enhance the importance of this lectin. In the present study two lectins from peanut (*Arachis hypogaea*) have been purified by affinity chromatography. SDS-PAGE analysis showed two bands with a molecular mass of 29 kDa and 28kDa. The 29 kDa band corresponds the AHL while the 28kDa band corresponds to the PNA because PNA is a homotetramer of molecular weight 110 kDa. Peanut lectins could not have the ability to agglutinate three types of RBC and they also could not distinguish human A,B and O RBC types because peanut lectin could not bind with NeuAc which is highly expressed on the surface of human erythrocytes. But some authors suggest that peanut lectin can only agglutinate human erythrocytes type if we treat neuraminidase to the RBC which removes the *N*-acetyl Neuraminic acid (NeuAc).

## 7. CONCLUSION

Two lactose binding lectins (AHL, PNA) were successfully isolated and purified from the seed of peanut (*Arachis hypogaea*). The purity of these proteins was confirmed by SDS-PAGE and their properties were studied by haemagglutination assay which shows that these proteins could not agglutinate the human erythrocytes until the erythrocytes were treated with neuraminidase. Further study needs to determine the function and biochemical characteristics of these lectins in more detail.

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