Isolation and characterization of lectins from red seeds of "*Abrus precatorius*"

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CERTIFICATE

This is to certify that the thesis entitled "ISOLATION AND CHARECTERIZATION OF AGGLUTININ FROM RED SEEDS OF *Abrus precatorius*" which is being submitted by Miss SNEHA PRASAD, Roll No. 410ls2068, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafied research work, carried out by her 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.

Sujit K Bhutia

Date: 03/05/12

DECLARATION

Myself Sneha Prasad hereby declare that this project entitled-Isolation and characterization of lectins from red seeds of "*Abrus precatorius"* has been carried out by me at National Institute of technology, Rourkela under the guidance of Dr. Sujit Kumar Bhutia, Assistant Professor,NIT Rourkela. This thesis has not formed the basis for the award of any Degree /Diploma/ Associateship/ fellowship or other similar title to any candidate in any university.

Date:03/05/12
Place:Rourkela

SNEHA PRASAD

CONTENTS

SUBJECT	Page No.
ACKNOWLEDGEMENT	i
LIST OF FIGURES	ii
LIST OF TABLES	iii
ABBREVIATION	iv
ABSTRACT	1
INTRODUCTION	2-6
REVIEW OF LITERATURE	7-10
OBJECTIVES	11
METHODS AND MATERIALS	12-16
RESULT	17-20
DISCUSSION	21
CONCLUSION	22
REFERENCE	23-25

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NIT Rourkela SNEHA PRASAD

LIST OF FIGURE

SL. NO.	PARTICULARS	PAGE NO.
1.	Protein data bank structure of agglutinin.	8
2.	Red Abrus seed	15
3.	After ammonium sulphate fractionation	15
4.	Dialysis	15
5.	Lactamyl Sepharose affinity column	15
6.	Doing haemagglutinin assay	16
7.	Loading the sample ,PBS and RB	16
8.	After silver staining	16
9.	Lectins are desorbed with 0.4 M lactose in PBS	17
10.	Haemagglutinin assay	19
11.	SDS PAGE	20

LIST OF TABLES

SL NO.	PARTICULARS	PAGE NO
1.	Protein estimation by taking OD at 280 nm	18

ABBREVIATION

PBS: Phosphate Buffer Saline

et al: And others

Rpm: Rotation Per minute.

Conc: Concentration

Hrs: Hours

L: litre

Mg: Milli gram

pH: Hydrogen concentration

NaOH: Sodium hydroxide

Na_2CO_3 : Sodium carbonate

APS: Ammonium per sulphate

TEMED: N,N,N',N'-tetramethylenediamine

$KNaC_4H_4O_6$: Potassium sodium tartarate

SDS-PAGE: Sodium Dodecyl sulphate Polyacrylamide Gel Electrophoresis.

BSA: Bovine serum albumin

KH_2PO_4: Potassium Dihydrogen Phosphate

K_2HPO_4: Potassium hydrogen phosphate

$(NH_4)_2SO_4$: Ammonium Sulphate

Pvt .Ltd: Private limited

kDa: kilo dalto

ABSTRACT

The study was done to isolate lectin from red seeds of *Abrus precatorius.* The protein was purified by using affinity chromatography. The activity of the lectin was determined by haemagglutinin assay and the purity of the protein was tested by Sodium dodecyl sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The activity of lectins is quantified by their ability to agglutinate erythrocyte. The eluted protein exhibited agglutinating activity when reacted against different types of fresh erythrocytes. In SDS-PAGE in affinity 2 bands were observed of 34kD and 31kD of heterotetrameric agglutinin.

Key words: lectin,affinity chromatography, SDS-PAGE, heterotetrameric

1.INTRODUCTION

1.1 Lectin Overview:

Lectins can be defined as carbohydrate binding protein which binds reversibly to specific mono- di- or oligosaccharides without altering the structure of bound ligand. Most convenient sources of lectins are the plant. Plant lectin has been attracting much attention because of their ease of isolation and their usefulness as reagents for glycoconjugation in solution and on the cell.

These plant lectins are characterized as secretory proteins i.e. they can enter the secretory system and accumulate in vacuoles (Windholz et al, 1983). In the cell wall and intercellular spaces for example the well-known lectins phytoheamogglutinin, concavalin A, soybeans, agglutinin, pea, and flavin are all present relatively high levels and accumulate in vacuole in the cotyledons (1-8% of the total protein) and lower levels in embryonic axes of the seeds. During seed development together with more abundant seed storage proteins these lectins are synthesized during germination and seedling growth this lectins and storage protein broken down to provide amino acids for the growing seedlings and also found on vegetative part of plants.

The impact of the plant lectins on biological research the legume lectin monomer is structurally well conserved. It consists of two β pleated sheets which form a scaffold on which the carbohydrate binding region is grafted. The same architecture and topology is found in a wide variety of carbohydrate recognizing proteins such as the galactins. Serum amyloid protein, the lectin-like domains Wing-1 and Wing-2 attached to the catalytic domain of *Vibrio cholera* neuraminidase w25x and Charcot–Leyden crystal protein w26x (Sharon et al, 1990). The topology of the legume lectin fold, is complex and is structurally related to the jelly-roll topology commonly found in viral coat proteins w27, 28x. The architecture of the legume lectin monomer is usually described as consisting of two β-sheets. The vast majority of all currently known plant lectins can be classified into a relatively small number of families of evolutionary and structurally related proteins.

7 **lectin families are distinguish namely**:

i) Legume lectins.

ii) Monocot mannose binding lectins.

iii) Chitin binding proteins which possess hevein domain.

iv) Type II Ribosome Inactivating proteins.

v) Cucurbitaceae phloem lectins.

vi) Jacalin family.

vii) Amaranthaceae lectins.

i) **Legume lectins**:It is the best known lectin family. Classical legume lectins have been found exclusively in members of the leguminosae. Protein and gene sequencing has demonstrate that all legume are built up of either two or four protomers of about 30kDa in the so called two chain legume lectins the protomers are further cleaved into two smaller polypeptide. All legume lectins show a remarkable variation in carbohydrate binding specificity (Stillmark et al, 1888).

ii) **Monocot mannose binding lectins:**The monocot mannose binding proteins are a relatively new group of lectins. Since the initial discovery of a lectin with an exclusive specificity towards mannose in snowdrops bulbs similar proteins have been found in a species of the monocot families alliaceae, araceae, ochidaceae, iliaceae and bromeliaceae due to their exclusive specificity towards mannose several monocot mannose binding proteins have become important tools (Budavari et al, 1989).

iii) **Chitin binding proteins containing Hevein domain:**The term 'hevein domain' refers to hevein a small 43 amino acids reside protein found in the latex of the rubber tree. Hevein is a merolectin composed of a single chitin binding domain, which is derived from a larger precursor molecule (Fiedler et al, 1994).

iv) **Type II Ribosome Inactivating Proteins**.The type II ribosome inactivating proteins are chimerolectins composed of a polynucleotide adenosine glycosidase domain (A chain), carbohydrate binding domain (B chain) both the chains are synthesize on a single precursor molecule (Davis jh, 1978). Since the A and B chain remain linked through an interaction of disulphide bridge, the building blocks of type 2 RIP corresponds to an [A-S-SB] pair.

v) **Cucurbitaceae phloem lectins:**Cucurbitaceae contains high concentration of lectin that binds oligomers of GlcNAc. Cucurbitaceae phloem lectins are dimeric proteins composed of 2 identical subunits of about 24 kD (Dreisbach et al, 1987).

vi) **Jacalin family**:The galactose specific lectin from seeds of jackfruit is one of the classical non-legume plant lectins. All moraceae lectins are built up of two identical protomers each protomer contains a small and a large subunit both derived from a single precursor through a complex post transcriptional modification of the primary translation product (Gosselin et al, 1984).

vii) **Amaranthaceae lectins**:Molecular cloning and x-ray diffraction angles revealed that amaranthin the seed lectin from *Amaranthus caudarus* does not resemble any other plant lectin with respect to its amino acid sequence and 3 dimensional structures. Based on these data amaranthin is now considered as the prototype of the amaranthin lectin family (Fiedler et al, 1995).

1.2 PROPERTIES OF LECTIN:

Lectins have precise carbohydrate specificity and can be blocked by simple sugars and oligosaccharides. Because of the specificity each lectin has affinity towards a specific carbohydrate structure; 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, have a similar specificity towards monosaccharide, may differ in their affinity for a particular disaccharide, oligosaccharide or glycopeptide (Reynolds et al, 1982).

Lectin vary in composition, molecular weight subunit structure and number of sugar sites binding site 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.

Although lectins are found ubiquitously in plant species, they have various structures and particular activities. Thus purification and characterization of lectins from a variety of plant species interests researches in the field of glycobiology.

1.3 PHYSIOLOGICAL ROLE OF PLANT LECTIN:

Plant lectin receptors are glycoconjugates possessing a carbohydrate moiety with a structure of complementary to that of binding site of the lectin. Different types of glycoconjugates with identical carbohydrates can act as receptor for the same lectin. To understand the role of plant lectin it is important to identify their receptor. The broad spectrum of carbohydrate binding specificities can be regarded as evolutionary adaptation of the plant to cope with the problems inherent to the recognition of a wide diversity of microbial and animal glycans.

Several lectins affect the growth and development of insect upon oral uptake. Both the specificity of plant lectins and their deleterious effect on insects or animals indicates that they play a very protective role against predators. Several example of lectin mediated resistance are found in nature for lectin mediated resistance are found in nature for instance, black locust and elderberry accumulate large quantities of black lectins cause the severe toxicity. When the plant lectin is attacked by insects and higher animals the lectins act as specific defense proteins.

1.4 IN BIOTECHNOLOGY:

Specific proteins like enzyme, cytokines or interleukins (Bermer et al Used in basic and applied biotechnology and biomedical research. Lectin is used as tools in biotechnology process, recombinant DNA technology and improves the transgenic or quality of transgenic plants. As bioactive compound in biotechnology for bioactive proteins in which tools is based on specific carbohydrate binding activity of lectin. Biological effects of lectins on cells, tissues and organs, used as inducers of specific process in animal or human cells (Kilpatrick et al, 1991). Activation of lymphocytes with mitogenic lectins.

As tools it is used to activate any glycoconjugate containing and carbohydrate with structure that is complementary to its binding site of lectins (Oswa and Tsuji, 1987). Carbohydrate detection technology to check specificity of lectins used in histopathology also plant lectin is a powerful tools for the isolation and fractionalization of glycoconjugates and for the study of oligosaccharides and glycopeptides in lectin affinity chromatography it is used as for the purification of glycoconjugates and offers many advantage (Schumacher et al,1991; Gabius and Gabius,1991). Improve the availability and properties of plant lectins through

biotechnology by lectin engineering, in production of unglycosylated lectin and in production of truncated lectin.

1.5 AS PLANT DEFENSE PROTEINS:

Type 2 RIPs are known to be potent cytotoxic agents. The sugar-binding B chain binds to a (glycoconjugate) receptor on the cell surface, thereby promoting the uptake of the **A** chain. After its entry into the cell the A chain catalytically inactivates eukaryotic ribosomes by cleaving the N-glycosidic bond of a single adenosine residue of the large rRNA. In principle, type 2 RIPs is extremely toxic to all eukaryotes if they reach the cytoplasm.

1.6 ANTIVIRAL ACTIVITY:

Plant lectins have been reported to prevent viral infection replication or symmetric spread.

1.7 AS ANTIBACTERIAL ACTIVITY:

The cell wall of bacteria not only precludes any interaction between the glycoconjugates on their membrane and carbohydrate binding proteins but also prevents these proteins from penetrating the cytoplasm. Plant lectin cannot alter the structure/ permeability of the membrane or disturb the normal intracellular process of invading microbes. Lectin play a role in the plant defense against bacteria through an indirect mechanism that is based on interactions with cell wall carbohydrate or extracellular glycans.

1.8 INSCET ACTIVITY OF PLANT LECTIN:

The epithelial cells along the digestive tract of phytophagous insects are directly exposed to the contents of the diet which are specific target sites for plant defense proteins. Since glycoproteins are major constituents of membranes, gut luminal side is literally covered with potential binding sites for dietary lectins. One can easily imagine that when the binding of a lectin to a glycoprotein receptor provokes a local or systemic deleterious effect the insect may be repelled, retarded its growth, or even killed.

2. REVIEW OF LITERATURE

2.1 LECTIN:

The word lectin is derived from the Latin word *legere*, which means, "to select". Stillmark first described lectin in 1888 while working with Castor bean extracts (Stillmark et al, 1988). Lectins are the ubiquitous protein or glycoprotein substances usually of plant origin and of non-immunoglobin in nature which specifically bind to the sugar moieties of glycoconjugates without altering any of the recognized glycosyl ligands (Goldstein et al, 1980). Presence of at least one catalytic domain that binds reversibly to a specific carbohydrate is only prerequisite for a protein to be named as lectin (Damme EJM et al, 1997). Lectins bind with the carbohydrate by the principle of luck and key mechanism. Therefore availability, stability and suitability for chemical modifications made plant lectins important tools of cell biology and glycosciences.

2.2 TYPES OF PLANT LECTIN:

Divided into four parts according to structure (Goldstein et al, 1980).

(i) **Merolectin**: Single carbohydrate binding domain, do not show agglutination properties, e.g.; mannose binding protein from orchids.

(ii) **Hololectin:** Two or more carbohydrate binding domain, e.g. majority of plant group lectins.

(iii) **Chimerolectin:** composed of carbohydrate binding domain tandemly arrayed with an unrelated domain, later domain acts independly with carbohydrate binding domain, e.g. Ribosomal inhibitory protein (RIP).

(iv) **Super lectin:** Special type of chimero lectin, e.g.: lectin from tulip bulbs (TxLC-1).

Ribosome Inhibitory Proteins (RIPs): Lectins which is capable of inactivating ribosome, and accordingly are grouped as ribosome-inactivating proteins (RIP). Subdivided into two groups: RIP I and II. RIP I consists of 25-30 kD single peptide (e.g. tricosanthin, momorcharin) while RIP II consists of two chains, enzyme A chain and lectin B chain. These two chains are linked by disulfide bonds (Goldstein et al, 1980). Type-I RIPs exhibit low

toxicity to a whole cell system as comparison to Type-II RIP due to lack of internalization facility.

2.3 Abrus precatorius: Plant of the leguminosae family with characteristics red and black seeds. It is most common in dry areas at low elevation throughout the tropical and subtropical climates. *Abrus precatorius* is known as "Wild Liquorice" in India. The plant is known for its varied spread uses in traditional medicine (Ndamba J et al, 1994, and Ghosal S, 1971). Owing to their spectacular appearance, the seeds are used for jewelry, beadwork, and also the various ornaments (Chopra et al, 1956). *Abrus* seeds contain the toxic lectins, namely abrin (ABR) and the relatively less toxic agglutinin known as *Abrus* agglutinin (AGG). Abrin is a 63 kD heterodimeric glycoprotein, but agglutinin is a heterotetrameric glycoprotein having molecular weight of 134 kD. Both of these lectins belong to the ribosome inactivating proteins-II (RIP-II) family, and consist of a toxic subunit A chain (molecular weight 30 kD (Lin EY et al, 2004) a galactose-binding B subunit (molecular weight 31 kD, (Lin EY et al, 2004) connected by a single disulfide bond (Olsnes et al, 1974). The protein synthesis inhibitory concentration for *Abrus* agglutinin is (IC50=3.5 nM) is weaker than Abrin (IC50=0 .05 NM). Lethal dose to mice is very less (LD50=5 mg/body weight compared to 20 µg/kg body weight for Abrin (Hegde et al, 1991). A full length cDNA clone of *Abrus* agglutinin contains an open reading frame of 1641 base pairs which corresponds to 547 amino acid residue protein of a single peptide and a linker region of two amino acids. In this protein there are 13 amino acid conserved residues, which are involved in catalysis (Liu CL et al, 2000).

2.4 Structure of *Abrus* agglutinin with domains:

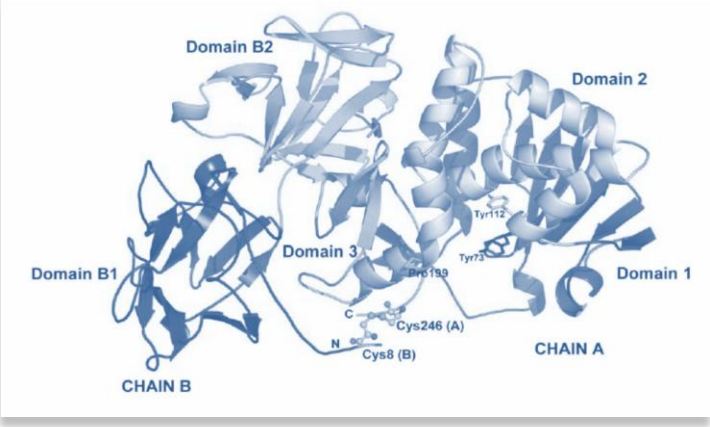

FIG 1: Protein data bank structure of agglutinin.

A cartoon representation, illustrating the *Abrus* agglutinin molecule with domains. The

disulfide bond between the toxin and the lectin chain at their respective C and N terminal is Shown in ball and stick model (FIG: 1).

Decreased toxicity of *Abrus* agglutinin than abrin is due to substitution of Asn200 in abrin-A with Pro199 in agglutinin, though the overall protein folding of agglutinin-I is similar to that of abrin

2.5 Prevention of disease:

Both the lectins trigger apoptosis by caspase-3 activation, involves potential damage to the mitochondrial membrane, and reactive oxygen species production (Narayanan et al, 2004). Moreover, in vitro growth inhibitory properties of Abrus lectins have been evaluated in various tumor cells. For instance, ABR causes cell death of Dalton's lymphoma (DL), as is evident from typical morphological changes connected with apoptosis. Necrotic cell death is dominant when a higher dose of ABR is used. ABR stimulates apoptosis by stimulating the expression of caspase-3, at the equal time blocking the expression of Bcl-2 (Ramnath et al, 2007). Interestingly, AGG in a heat-denatured condition (prepared by maintaining it at 50°C for 30 minutes and then immediately at 100^0 C for 2 minutes in a water bath) exerts similar types of cellular growth inhibition potential as demonstrated in DL cells (Ghosh et al, 2007).

2.6 Properties:

ABR showed greater cytoagglutination against human cultured cell lines derived from acute lymphoblastic leukemia and adult T cell leukemia, and less agglutination against normal lymphocytes (Kaufman et al, 1975). In the late 1960s and at first part of 1980s, the effect of Abrus protein extract on growth inhibition of Yoshida sarcoma and Ehrlich ascites tumors in mice were investigated (Reddy et al, 1969 and Lin et al, 1982). Denatured AGG, like native AGG, was able to drop the DL tumor cell number in vivo, and also significantly increased the median survival time of DL bearing mice (Ghosh et al, 2007). The in vivo tumoricidal property of lectins against DL and EAC induced tumors was thought to be mediated through apoptosis (Ghosh et al, 2007 and Ramnath et al, 2007). A tryptic digested Abrus agglutinin peptide fractions obtained from 10 kD molecular weight cut off membrane permeate(10 kMPP), was found to have anti proliferative activity (1–10 µg /ml) on several tumor cell lines in vitro without having any cytotoxic effect on normal cell lines with dose of 100 µg/ml (Bhutia et al, 2008).

2.7 Lectins and immunomodulation:

Lectins are one of the potent candidates for immunomodulators. Lectins are known to be Polyclonal activators of lymphocytes and work through the induction of a variety of cytokines and chemokine which vary from cell to cell. Treatment of AGG induces the expression of activation markers (CD71, CD25) in T and B cells implying that mitogenic stimuli of agglutinin influences not only proliferation but also the activation status of splenocytes (Ghosh et al, 2009). In vivo administration of native and heat-denatured AGG showed macrophage and NK cell activation (Ghosh et al, 2007). Similarly ABR also augments the humoral and cell-mediated immune response of the host.

3.OBJECTIVE

- Isolation and purification of lectin from red seed of *Abrus precatorius.*
- Measurement of concentration of protein
- Characterization of protein:

    a. Haemagglutinin assay.

    b. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

4. MATERIALS AND METHODS

4.1 Sample collection:

Dry Red *Abrus* seeds were collected from deep jingle of Angul,Odisha,India.

4.2 Chemical:

Acrylamide, bisacrylamide, Sodium dodecyl sulphate (SDS), Ammonium persulphate (APS),N,N,N',N'-tetramethylene diamine (TEMED), Bovine serum albumin (BSA), Tris were purchased from Sigma Aldrich, USA. Sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3), Potassium sodium tartarate ($KN_aC_4H_4O_6$), glycine, Copper sulphate ($CuSO_{4)}$ were bought from SRL, Sysco Research laboratories Pvt. Ltd., Mumbai. Folin-Ciocalteau phenol reagent, Potassium hydrogen phosphate (K_2HPO_4) and Potassium Dihydrogen Phosphate (KH_2PO_4) were purchased from S.D. fine chem. Ltd., Mumbai. Bromo phenol blue, acetic acid, agarose were purchased from Himedia, Mumbai. Glycerol was purchased from RANKEM Pvt Ltd. Pre stained molecular weight marker was purchased from Bio-Rad, India. Methanol, Silver nitrate, Sodium thiosulphate were purchased from Nice chemicals Pvt. Ltd. India. Ethanol purchased from Trimurty Chemicals of India.

4.3 Purification of Abrus agglutinin:

Abrus agglutinin was purified from the seeds of red *Abrus precatorius* following the methods described by (Hegde *et al*, 1991). In brief, 100 gms of red *Abrus* seeds were taken and decorticated. The decorticated seeds (76 Gms) were soaked in PBS overnight and crushed in minimum volume of PBS in a blender for 5 minutes followed by centrifugation at 7000 rpm for 15 minutes. The supernatant was collected and exposed to ammonium sulfate fractionation (first 0-30% and then 30-90%). The precipitate formed by 90% ammonium sulfate cut was dissolved in minimum volume of water and dialyzed against PBS. The dialyzed sample was loaded onto Lactamyl Sepharose affinity column and eluted with 0.4 M lactose solution. The affinity elusion profile showed a single peak. The activity of the lectin was determined by haemagglutinin assay and the purity of the protein was tested by Sodium dodecyl sulphate gel electrophoresis.

4.4 **Protein estimation by Lowry et al, (1951):**

Procedure: Aliquots from suitably diluted samples were made up to 0.5 ml with distilled water. To this 5 ml of Biuret reagent was added, vertexed and incubation was done at room temperature for 10 minutes. A volume of 0.5ml of Folin-Ciocalteau phenol reagent was added, vertexed and incubation was done for 30 minutes. The absorbance (A) of the blue colour thus developed was read against a suitable blank at 700 nm in a Systronic spectrophotometer. Bovine serum albumin was used as a standard.

4.5 Haemagglutination assay*:*

Human erythrocyte suspension (10^6 cells/ml) of blood was used for the haemagglutination assay. The assay was carried out in a 96 well round bottom microtitre plate. Agglutinin solutions were serially diluted (double dilution) and 100µl volume of agglutinin solutions at different concentration were added to 100µl of cell suspension.

4.6 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (Laemmli, 1970): (SDS-PAGE)

Polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). Stacking gel (5%) and resolving Gels (12%) were prepared. The electrode buffer (pH 8.3) contained 0.025 M Tris, 0.192 M glycine, and 0.1% SDS. Gel loading buffer (5x) contained 50 Mm Tris-Cl (p^H 6.8), 100 mM β-mercapto ethanol, 2% (w/v) SDS. 0.1% bromophenol blue, 10% (v/v) glycerol. Proteins in the sample buffer were boiled for 3 min before electrophoresis. Electrophoresis was carried out until the bromphenol blue marker reached the bottom of the gel. The stacking gel was run at 90v and resolving gel was at 140v.

4.7 Silver staining

The gel was fixed in the solution for 1 hour containing methanol (50%), acetic acid (12%), and formaldehyde (37%). After that washing were done in 50% ethanol for about 10 minutes and continued for 3 times. After that pretreatment was done in Sodium thiosulphate (0.02%) for 1 minute. It was rinsed with distilled water 20seconds and continued 2 times. Impregnation was done solution containing silver nitrate (0.2%).37% formaldehyde (0.075%) for 20 minute. Distilled water rinsing was done for 20 seconds .Now the gel was transferred to developing solution containing Sodium carbonate (6%), Sodium thiosulphate (mg) and 37% formaldehyde (0.05%). After that rinsing was done in distilled water, and the solution

was kept in stopping solution for about 10 minutes. Stopping solution contained Methanol (50%), acetic acid (12%) and the rest volume was made by distilled water.

After that the photo graph was taken in Bio-Rad Gel Documentatin system. The molecular weight was determined by applying the quantity one software. Also the photograph was taken in Nikon handi cam.

FIGURES RELATED TO EXPERIMENT

FIG 2: **Red *Abrus* seed**

FIG 3: **After ammonium sulphate fractionation**

FIG 4: **Sample in dialysis tube Dialysis**

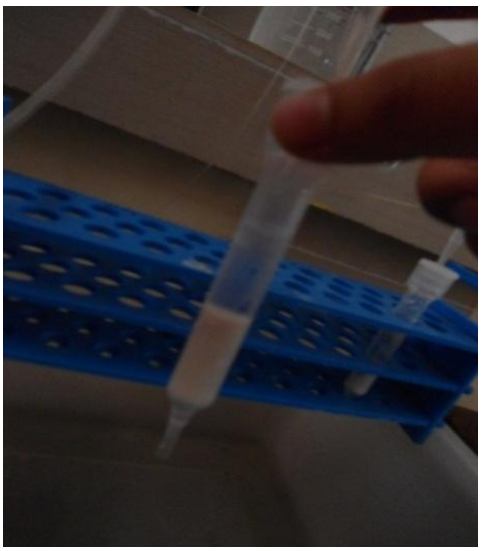

FIG 5: **Lactamyl Sepharose affinity column**

FIG 6**: Haemagglutinin assay**

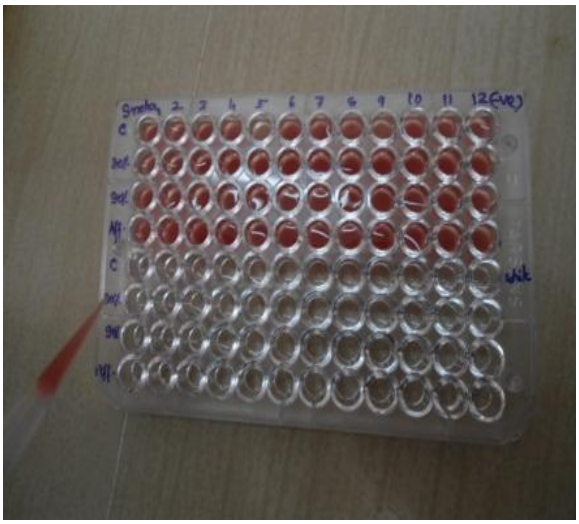

FIG 7: **Loading the sample,PBS and RBC**

FIG 8: **After silver staining**

5. RESULTS

5.1 Purification of Agglutinin:

Red *Abrus* seed extract was precipitated 30% to 90% by ammonium sulphate fraction method. It was dialyzed for 3 days till the $BaCl_2$ gives no precipitate with ammonium sulphate. Dialyzed supernatant was loaded on lactamly sepharose 4B column in the method of affinity chromatography. The unbound proteins were drained out during the washing of lactamyl beads with PBS solution at each 10mins interval. Those proteins were binds with lactamyl Sepharose beads, containing metal ions (Ca^{2+} or Mg^{2+}ions). The solutions of eluted protein had bound on lactose sugar than lactamly beads. Lactose was removed from the protein by the method of dialysis in PBS solution (pH-7.2) for 1day. Hence purified protein was obtained.

a) ELUSION PROFILE OF ABRUS LECTIN FROM LACTAMYL SEPHAROSE AFFINITY MATRIX

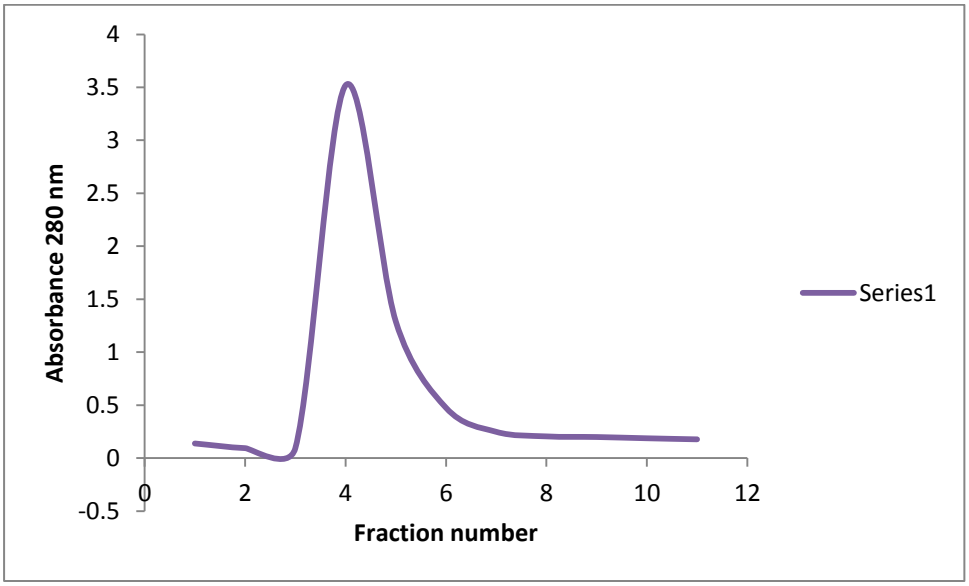

Fig 9 : Lectins are desorbed with 0.4 M lactose in PBS

5.2 Protein estimation by taking od at 280nm.

TABLE NO 1:

SAMPLE	VOLUME	OD AT 280 nm	CONCENTRATION (mg/ml)	TOTAL CONCENTRATION (mg)
CRUDE	33ml	126.6	101.28	3342.33
30%	32ml	101.7	81.38	2603.45
90%	30ml	3.19	2.55	76.5
AFFINITY	15ml	1.096	0.876	13.14

Sample contains total protein content of 3342.33mg. In affinity protein content is 13.14mg which is our required protein lectin.

5.3 Haemagglutination Assay:

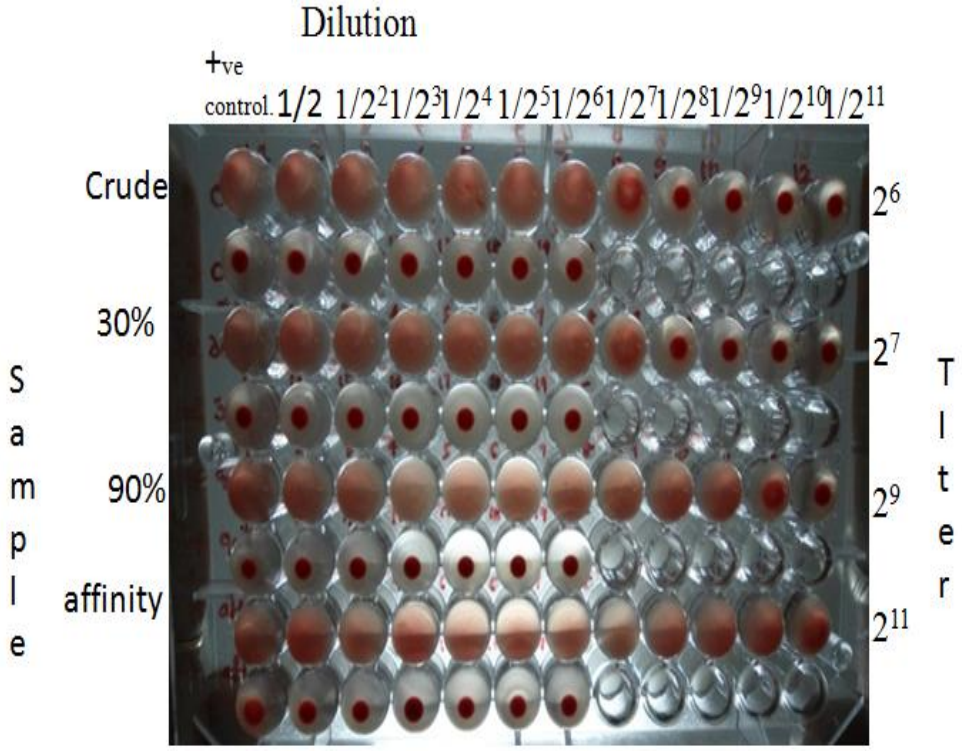

FIG: 10 **Haemagglutinin assay**

Haemagglutination activity of affinity sample. Shows the higher titer value in comparison to crude, 30%, 90%.The titer value of affinity sample is 2^{11}.Shows the highest agglutination activity.

5.4. SDS-PAGE ANALYSIS OF AGGLUTININ

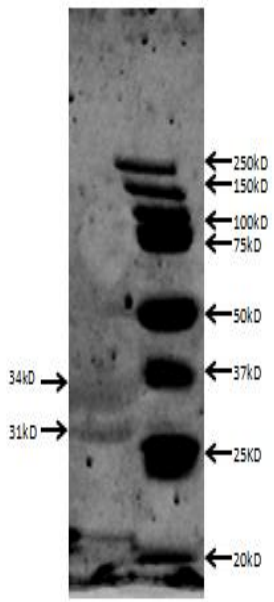

FIG 11: **SDS PAGE**

Lane 1 represents the heterotetrameric agglutinin protein isolated from affinity sample. Having molecular weight of 34kD and 31kD. Lane 2 represent the Bio Rad prestained protein molecular weight markers (FIG 11).

6. DISCUSSION

Affinity chromatography eluted samples were successfully used to purify two lectins from *Abrus precatorius*. Polyacrylamide gel has been used to purify lectins from ***Abrus precatorius*** **(**Tomita et al, 1972), so its usefulness for D-galactose binding lectin is well established. There are several related reports indicating that lectins which bind D-glucose can be purified by affinity techniques on Sephadex (Agrawal et al, 1967).

The lectin agglutinin showed high agglutinating activity in human erythrocytes, giving the idea that it has a binding activity in cancer cell lines and at last leads to cytotoxicity. When we compare the four sample crude, 30% fractionation, 90% fractionation and at last affinity sample, interestingly the agglutinating activity of affinity sample showed the titre value 2^{11} which is highest among the four samples. It gives the idea our affinity sample may contain desired protein agglutinin. *Abrus* seeds contain the toxic lectins, namely abrin (ABR) and the relatively less toxic agglutinin known as *Abrus* agglutinin (AGG). Abrin is a 63-kDa heterodimeric glycoprotein, although agglutinin is a heterotetrameric glycoprotein with a molecular weight of 134 kD. Both of these lectins belong to the ribosome inactivating proteins-II (RIP-II) family, and consist of a toxic subunit A chain (molecular weight 30 kD (Lin et al) a galactose-binding B subunit molecular weight 31 kD (Lin et al, 2004) a connected by a single disulfide bond (Olsnes et al, 1974).

The lectin agglutinin mol.wt 134 kD yielded peptides of approximately molwt.34 kD and 31 kD respectively from β-mercaptoethanol-sodium dodecyl sulfate-polyacrylamide gels. From the molecular weights of the subunits obtained after reduction and electrophoresis on sodium dodecyl sulphate polyacrylamide gels, we assumed that Agglutinin is probably a hetero tetramer heterotetrameric glycoprotein. ABR exhibits antitumor activity in mice at a sub-lethal dose and able is to decrease solid tumor mass improvement, as is evident from the study related to murine DL and the Ehrlich's ascites carcinoma (EAC) model (Ramnath et al,2007). Besides efforts to render lectin application amenable to clinically beneficial immunomodulation, we also need to turn our attention to the idea that the plant lectin may mimic endogenous lectins in their inherent role to participate in immune modulation by protein-carbohydrate interactions.

7. CONCLUSION

Applying the step wise fractionation methods to isolate agglutinin from *Abrus precatorius* we reached in the result of SDS-PAGE which gave our searching protein agglutinin (heterotetramer) having the molecular weight 34kD and 31kD respectively. This tumor targeting peptide from *Abrus precatorius* belong to ribosome inactivating proteins-II (RIP-II), needs further application to confirm antiproliferative activity of Abrus agglutinin in different cancer cell lines if it success there will be change in the concepts of phytochemicals in the world of cancer biology.

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