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Isolation and Purification of Lectin from Soybean (Glycine Max) Mahabeej Js-335

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Abstract
Plant lectins have a wide spectrum of biological significance. In this project work the isolation and purification of the lectins (SBL) from the Soybean (Glycine max) Mahabeej JS-335 have been reported. The lactose binding lectins were purified from the Soybean seeds by performing affinity chromatography using a lactamil sephadex 100G. Affinity products were dialysed against PBS and SDS-PAGE was done to identify the fractions 'molecular weight. This work explored different techniques of protein (lectin) purification and describing their characteristics.

Keywords: Soybean lectin, SBL, Affinity chromatography, SDS-PAGE

Introduction
The word "Lectin" has been derived from the Latin word which means "I choose". Because lectins are very specific to the site to which it binds. Lectins are carbohydrate-binding proteins which bind to glycoproteins, glycolipids, and also polysaccharides (Goldstein and Hayes, 1978) [3] which mediates various kind of biological processes by binding to different sugar moiety (Lis & Sharon, 1998; Vijayan & Chandra, 1999) [10]. Lectins are highly diverse in structure, molecular weight, composition, and number of sugar binding sites present per molecule (Laija, *et al*. 2010) [7]. Lectins are a well-known proteins which has highly specific carbohydrate-binding. Lectins have non-immune origin and can binds to oligosaccharides, glycoproteins, glycolipids and different polysaccharides without changing their covalent structure (Kocourek & Horejsi, 1981; Barondes, 1988) [6]. The lectins were first described by Stillmark in 1888 when he was working with castor bean extracts and showed agglutination reaction. On the basis of their carbohydrate binding specificity, lectins can be classified into many groups such as mannose-binding, glucose-binding, galactose-binding, N-acetylglucosamine- binding, N-acetylgalactosamine- binding, fucose-binding, and sialicacid-binding.

Lectins are widely distributed in nature and found in all forms of life including plant products such as fruits, vegetables but nuts, grains, beans and seeds contains high lectin amount (Lis and Sharon, 1986) [9]. Researchers has great interest and lectins has been studied and isolated from various sources including plants, animals, fungi, lichens and bacteria (Liener 1976; Hapner and Robins 1979; Damjanov 1987; Sharon and Lis 1989) [4, 2]. It has been reported that lectins have been found mostly in seeds of Legumes and other plant species (Liener 1976; Pueppke 1979; Quinn *et al*.1987) [12, 15]. The interest on plant lectin study is due to their high specificity on carbohydrates (Willy J. Peumans *et al*., 2010). Lectins have also been isolated from vegetative tissues of plants like leaves, stems, barks, and roots (Callow 1975; Goldstein and Haye 1978; Quinn and Etzler 1987) [3, 15]. Lectins are also suited for analysis and isolation of animals and human glycoconjugates. The mature seed contains about 3% of the weight of it (Laija *et al*., 2010) [7]. The plant lectins are stable proteins which can be characterized without affecting their sugar binding properties.

The biological activities like anti-tumor, anti-proliferative, immune potentiating, antibacterial, antifungal, anti-insect, and antiviral activities have been found in lectin Microbes carry lectins, which help them for attachment to the host cells. The lectins consumption disturbs normal growth in humans as well as in other animals. It has been reported that the lectins influences the nutrient intake (Liener I.E., 1986) [8]. Lectins causes morphological injury in the small intestinal mucosa due to its adhesion to the mucosal surface. But the injury can be prevented by the simultaneous administration of saccharides having specific affinity for the lectins or by treatment of the foods containing lectins with

Heat. Lectins are found commonly in most legumes and their toxic effects have been seen. (Liener, I. E. 1986) [8]. Pusztai *et al*. reported that beans having higher content of lectins causes most serious damages to the luminal surface of intestine in rats compared to those having lesser lectin contents, Lectins isolated from bean species has influenced the intestinal structure and function negatively (Liener,I.E.,1986) [8] leading to diseased situations.

Some lectins show anticancer property *in vivo* as well as *in vitro*, as a result they can be used as therapeutic agents for tumor inhibition as it causes apoptosis. Lectins has affinity to bind with ribosomes as a result it inhibits protein synthesis. Lectins can decreases the telomerase activity and suppresses angiogenesis.

Lectin can be used as probes for the characterization and isolation of simple and complex sugars (Rudiger and Gabius, 2001) [16]. Lectins can be used in immunological studies as a tool (Moreira *et al*., 1991) [11]. Dietary lectins can cross the gastrointestinal barrier and enters the circulation intact (Pusztai *et al*.1989) [13], and also be able to interact with synovial tissues directly. Lectins has the tendency of alternating interleukin production which affects the body immune system. Various plant lectins has the tendency to bind with the intestinal mucosa which disturbs the functions of intestine and may causes enlargement of pancreas (Pusztai and Bardocz, 1996) [14]. Plant lectins plays a vital role to defense itself from the predators is not new.

Lectins are used in the activation of lymphocytes and for induction of proteins like enzymes, interleukins or cytokines (Kilpatrick, 1991) [5]. To determine the 3-dimensional structure of carbohydrate binding site of lectins NMR and XRD are used. Lectins have many applications starting from identification of microorganisms to cancer research. Mitosis (cell division) can be enhanced with pokeweed lectin (PWA). Lectins has been used as carriers in drug delivery such as delivery of chemotherapeutic agents. The lectins isolated from different plant species often varies greatly in their molecular structure and specificity. Many legume species contain proteins which are clearly related to the lectins lacking carbohydrate binding activity.

3. Objectives

- Isolation of Lectin from Soybean (Glycine max) Mahabee J JS-335
- Measurement of concentration of proteins
- SDS-PAGE for Purification of Lectin

4. Biology of the Species

4.1 Classification

Kingdom: -	Plantae
Subkingdom: -	Tracheobionta
Superdivision: -	Spermatophyta
Division: -	Magnoliophyta
Class: -	Magnoliopsida
Subclass: -	Rosidae.
Order: -	Fabales.
Family: -	Fabaceae.
Genus: -	Glycine.
Species: -	max.
Brand: -	Mahabee J JS – 335.

5. Materials and Methods

5.1 Chemicals

Sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3), glycine, Copper sulphate ($CuSO_4$), Potassium sodium tartrate ($KNaC_4H_4O_6$). Acrylamide, bisacrylamide, Ammonium persulphate (APS), Sodium dodecyl sulphate (SDS), N,N,N‘,N‘-tetramethyl enediamine (TEMED), Bovine serum albumin(BSA), Tris. Folin-Ciocalteau phenol reagent, Potassium Dihydrogen Phosphate (KH_2PO_4), Potassium hydrogen phosphate (K_2HPO_4). Acetic acid, Bromophenol blue, agarose Glycerol, Ethanol, Pre stained molecular weight marker, Methanol, Silver nitrate, Sodium thiosulphate.

5.2 Sample Collection

The Soybean (Glycine max) MahabeeJ JS-335 seeds were collected for isolation and purification of Lectins from a local food store situated At. Krushi Seva Kendra, Osmanabad (MS). The chemical used in the study are supplied by Bio-Era and Emcure Pharmaceuticals.

5.3 Seed Coat Removal

Soybean seeds were taken and grinded in a mixer for removal of seed coats and 50gms of uncoated seed were taken for the study. The uncoated seeds were soaked in PBS for overnight. Then the seeds are grinded with minimum volume of PBS and the pastes were collected in 50 ml centrifuge tubes and were centrifuged by the Remi centrifuge R-4C with 10,000rpm, at 4 ˚C for 40 mins. The supernatant were taken after centrifuge and measured by a measuring cylinder. Some supernatant were stored in an Centrifuge tube as crude at 4 ˚C and the remaining were taken as salting out Process.

5.4 Dialysis

When the ionic strength of a protein solution is increased by adding salt, the solubility decreases, and protein precipitates. The salt molecules compete with the protein molecules in binding with water. The concentration of salt requires for precipitation of the protein out of the solution is varies greatly in different proteins. It is also used to concentrate dilute solutions of proteins. Ammonium sulphate salt was taken in the salting out process. According to the salt chart ammonium sulphate were added to the crude by pinch wise and continues stirining was done by magnetic stirrer.

Then the sample was stored for overnight at 4 °C and in the next day the sample was taken for centrifuge, then supernatant and pellet was collected. The supernatant was taken and ammonium sulphate salt was added in pinch wise and continues stirining was done by magnetic strirer.

Similarly supernatant was collected and measured ammonium salt was added for dialysis and stored at 4 ˚C overnight. The pellet was collected after centrifuge and undergo dialysis in PBS for 3-4 days.

5.5 Preparation of Lactamil sephadex 100 G

5.5.1 Coupling Of Lactose With Amino Sephadex 100g

4gms of Suction dried amino sephadex 100 G was suspended in 3ml of 0.2M K_2HPO_4 buffer, which was contain 51mg $NaCNBH_3$ and 104 mg of Lactose. The Suspension was incubated at room temperature for 10 days with occasionally shaking. The free amino groups which remained in the gel were acetyled by adding 2 ml of acetic anhydride. The suspension was incubated in the room temperature for 1 hour. The lactamil sephadex 100G thus obtained was subsequently washed with distilled water, 0.1 M NaOH, distilled water and 10 mM PBS subsequently. It was stored in distilled water with traces of sodium azide at 4 ˚C.

5.6 Affinity Chromatography
The lactamil sephadex column washed by PBS solution (pH7.2) and O.D of the washed PBS was measured at 280nm. When the OD value decreases and tend to zero then the Protein sample was passed through lactamil sephadex beads and the elute sample was collected and its O.D was determined at 280nm.
Lactamil sephadex beads were again washed with PBS solution (pH7.2) and the O.D of the washed PBS was measured at 280nm. When the OD value decreases and tends to zero the 20ml 4M lactose solution was loading on lactose sephadex beads and O.D of the eluent was measured at 280nm. The eluent was collected for dialysis in PBS (pH 7.2) and stored at 4 ˚C for 1day.

5.7 Determination of Concentration of Protein
The concentration of crude, dialysed, and purified sample mesure concentration of protein affinity were measured by Lowry method using bovine serum albumin as the standard protein (Lowry *et al*., 1951).

5.8 Lowry's Method
REAGENT A = Sodium hydroxide (0.5%)
Sodium carbonate (2%) make it upto 1 litre
REAGENT B1 = 1% Copper sulphate
REAGENT B2 = 2% Sodium potassium tartrate
REAGENT C = A: B1: B2=100:1:1
BSA STANDARD = 1mg/ml
Folin ciocalteu's reagent = 1N (5 ml solution +5 ml distill water)
Take different concentration of BSA solution from stock solution and add distill water to it and made up to 2ml.Abrus protein taken unknown quantity dissolved in 1ml distill water. And add reagent C of 5 ml and protein of 0.5ml. Mixed properly and incubate for 10 mins. Then 0.5 ml of Folin reagent was added and incubate for 30min. Take OD at 750nm.

5.9 SDS-Page
The molecular mass of the subunits of the lectins was estimated by SDS-PAGE (12%). The poly acrylamide gel electrophoresis was done according to the protocol given in the Book "Molecularcloning" by Sambrook & Russell. The mixture of 10µl of sample, 10µl of sample loading buffer were added to the well. Crude, dialysed, and purified affinity sample was allowed for SDS PAGE isolation. The gel was silver stained to make the bands visible.

6. Result
6.1 Determination of Protein Concentration
Determination of the concentration of the proteins (crude dialysed sample, purified sample) were done by Lowry method which is depicted in Table- 1.

Table 1: concentration of proteins

Sample	Volume (ml)	Concentration (mg/ml)	Total Concentration (mg/ml)
Crude	85	18.46	1569.1
Dialysed sample	78	15.77	1230.06
Purified sample	61	6.60	402.6

7. Discussion
Many reports have been suggested about many structural and biochemical uses of plant lectins and their increasing use as biotechnological tools. According to early investigators the role of lectins in plant is defense against seed predators and during seedling development (Quinn *et al*., 1987; Naeem *et al*., 2001) [15]. Lectins are abundantly present in Legumes. In seeds, lectins accumulate in the protein bodies to form well-characterized storage proteins.
Lectins can be readily separated by size exclusion chromatography or by affinity chromatography (Sharon and Lis 1972). Spilatro and Anderson (1989) reported regarding isolation and purification of soybean lectin several studies. In 1952 Soybean lectin (SBL) was originally purified by Leiner and Pallansch and the same lectin was again purified on affinity column with immobilized amino caproyl-β-D-galactosylamine coupled to sepharose by Sharon and Lis (2004). In the present study the affinity elusion profile showed a single peak.
The purified SBL obtained when treated with denaturing and reducing conditions in the presence of sodium dodecyl sulphate (SDS) and 2-β, mercaptoethanol according to Laemmli (1970), the lectin moved and a single band was found.

8. Summary and Conclusion
In summary, SBL were successfully isolated and purified from the seed of Soybean (Glycine max) by affinity chromatography. The SDS-PAGE of SBL showed the single band.

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