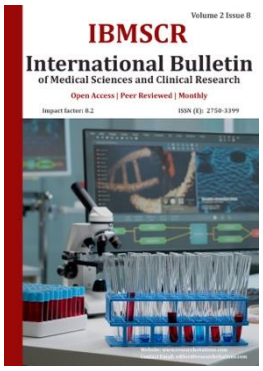

PURIFICATION OF PHASEOLUS VULGARIS LECTIN BY GEL FILTRATION CHROMATOGRAPHY

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Annotation. It is well established that gel filtration chromatography is an effective technique for the purification of proteins by removing low-molecular-weight impurities. In this study, gel filtration chromatography was employed for the purification of lectin isolated from Phaseolus vulgaris L. seeds, with the aim of eliminating foreign low-molecular-weight and iodinated substances present in the crude extract. Lectin extraction was initially performed using a conventional saline buffer method. The purification process was carried out using a Bio-Rad BioLogic LP chromatography system equipped with a column packed with Sephadex G-75 gel. Tris-HCl buffer was used as the mobile phase, and the flow rate was maintained at 1.5 mL/min. The collected fractions were analyzed for protein content and lectin activity to identify lectin-rich fractions. The purified lectin was subsequently freeze-dried to obtain a solid form suitable for physicochemical characterization. Characterization included determination of protein and carbohydrate content, hemagglutination activity, and gel electrophoresis for molecular weight estimation. The results indicated successful purification of Phaseolus vulgaris lectin using gel filtration chromatography, yielding a biologically active lectin preparation with high purity. These findings confirm that Sephadex G-75 gel filtration is an efficient and reliable method for the purification of lectin from Phaseolus vulgaris seeds.

Keywords: Phaseolus vulgaris; lectin; gel filtration chromatography; Sephadex G-75; purification; freeze-drying.

Introduction. Lectins are non-immunoglobulin proteins capable of binding specific carbohydrate structures with high selectivity and stereospecificity without altering the covalent structure of the recognized glycosyl ligands [1]. Lectins, also referred to as hemagglutinins or agglutinins, are widely distributed in nature and are particularly abundant in leguminous plants, where they play important biological and physiological roles [2]. These proteins differ considerably in their molecular structure, molecular weight, amino acid composition, quaternary organization, and number of carbohydrate-binding sites per molecule [3].

Distinct glycan patterns are expressed on the surface of different cell types, especially under pathological conditions such as inflammation, infection, and cancer [4]. The specific recognition of carbohydrate epitopes by lectins has stimulated growing interest in their application as targeting ligands in drug delivery systems and biomedical research [5]. Based on their carbohydrate-binding specificity, lectins are classified into several groups, including mannose-binding, glucose-binding, galactose-binding, *N*-acetylglucosamine-binding, fucose-binding, sialic acid-binding, and *N*-acetylgalactosamine-binding lectins [6].

Phaseolus vulgaris L. (common bean) is one of the most widely cultivated leguminous plants and represents a rich natural source of biologically active lectins [7]. Raw mature bean seeds contain various anti-nutritional factors, such as protease inhibitors, lectins, phytic acid, saponins, and other secondary metabolites [8]. Despite their anti-nutritional effects when consumed in large amounts, lectins have attracted significant attention due to their diverse

biological activities, including antitumor, antiproliferative, immunomodulatory, antibacterial, antifungal, antiviral, and insecticidal properties [9–11]. Certain sugar molecules, such as galactose and lactose, can specifically recognize lectins expressed on the surface of cancer cells, highlighting their potential role in cancer diagnosis and therapy [12,13]. Consequently, lectins have been investigated for applications in prodrug design, targeted drug delivery, and the development of lectin-mediated carrier systems [14–16].

However, the biological application of plant lectins largely depends on the development of efficient purification techniques that ensure high purity while preserving biological activity. Traditionally, lectins have been purified using affinity chromatography, ion-exchange chromatography, and size exclusion (gel filtration) chromatography [17]. Among these techniques, gel filtration chromatography is particularly advantageous for separating proteins based on molecular size and for removing low-molecular-weight impurities without the need for harsh chemical conditions [18].

Lectin was extracted from *Phaseolus vulgaris* L. seeds using a conventional saline buffer method and subsequently purified by gel filtration chromatography employing Sephadex G-75 as the stationary phase. The purification process was performed using a Bio-Rad BioLogic LP chromatography system to effectively eliminate low-molecular-weight and foreign substances from the crude extract. The purified lectin fractions were further analyzed for protein content and biological activity, followed by freeze-drying to obtain a stable solid form. This study aims to evaluate the suitability of Sephadex G-75 gel filtration as a reliable and efficient method for the purification of lectin from *Phaseolus vulgaris* seeds while maintaining its structural integrity and biological functionality.

Materials and Methods

Phaseolus vulgaris L. (common bean) is an important leguminous crop widely cultivated for its nutritional and bioactive components. Dried *Phaseolus vulgaris* seeds were procured from a local market and authenticated at the Department of Botany of a recognized academic institution. Sephadex G-75 gel was purchased from Pharmacia Ltd. Polyacrylamide gel and sodium dodecyl sulfate were obtained from Sisco Research Laboratories (SRL), India. Tris-HCl, phosphate buffers, ammonium sulfate, and other analytical-grade reagents were procured from standard commercial suppliers. Red blood cells (RBCs) from albino rats were obtained from a certified laboratory animal facility. Double-distilled water produced using a Millipore purification system (USA) was used throughout the study.

Extraction of Lectin. Lectin extraction was carried out using a conventional saline buffer method with slight modifications. Briefly, dried *Phaseolus vulgaris* seeds were finely powdered (<1680 µm) and defatted using ice-cold petroleum ether (70% v/v) at room temperature (28°C). The defatted meal was air-dried and extracted with cold phosphate buffer (pH 7.2) in a ratio of 1:4 (w/v) under continuous stirring for 4 h.

The extract was centrifuged at 15,000 rpm for 15 min, and the supernatant was collected and further centrifuged at 10,000 rpm for 10 min to remove residual debris. The resulting supernatant was subjected to ammonium sulfate precipitation by adding solid ammonium sulfate to achieve 80% saturation and stored at 4–8°C for 12 h. After centrifugation at 15,000 rpm for 15 min, the precipitate was dissolved in phosphate buffer (pH 7.2) and dialyzed sequentially against distilled water and phosphate buffer (pH 7.2).

The resulting crude lectin extract was obtained as a viscous aqueous solution and preliminarily evaluated for hemagglutination activity prior to purification. The extraction procedure was performed in duplicate using different batch sizes.

Purification of lectin was carried out using gel filtration chromatography to remove low-molecular-weight impurities and foreign substances. The purification process was performed on a Bio-Rad BioLogic LP chromatography system. A glass column was packed with Sephadex G-75 gel previously swollen and equilibrated with Tris-HCl buffer.

The crude lectin extract was carefully loaded onto the column, and elution was performed using Tris-HCl buffer as the mobile phase at a constant flow rate of 1.5 mL/min. Fractions were collected automatically and monitored for protein content by measuring absorbance at 280 nm. Fractions exhibiting lectin activity were pooled and subjected to dialysis against distilled water to remove buffer salts and residual impurities.

The purified lectin solution was freeze-dried to obtain a stable solid product. The solution was filled up to 30% of the tray capacity in a freeze dryer, and temperature probes were inserted into each tray. Initial freezing was carried out at −42°C for 12 h, followed by primary drying under vacuum (100×10^{-3} mbar) for 20 h. Secondary drying was performed at 25°C for 24 h.

The lyophilized lectin powder was collected, gently sieved through a 40-mesh (400 µm) stainless steel sieve to break any agglomerates, and stored in airtight containers at 2–8°C until further analysis.

Hemagglutination activity was assessed using a 96-well round-bottom microtiter plate. Standard lectin and RBC suspension (100 µL each) were used as positive controls, while RBCs mixed with saline served as negative controls. Freeze-dried lectin samples were serially diluted in saline, and an equal volume of RBC suspension was added to each well.

The plate was incubated at room temperature for 1 h and examined under a compound microscope (1000× magnification) for visible agglutination. One hemagglutination unit (HU) was defined as the reciprocal of the highest dilution showing visible agglutination.

Freeze-dried lectin equivalent to 30 HU was prepared in phosphate buffer (pH 6.8) and further diluted to 10 HU. The samples were incubated in buffers of varying pH values (3.0–9.0) for 48 h at room temperature (25°C). Hemagglutination activity was measured at 280 nm using an ELISA reader.

Lectin solutions equivalent to 10 HU were incubated at temperatures ranging from 27°C to 100°C. After incubation, hemagglutination activity was determined spectrophotometrically at 280 nm.

Sugar specificity of the purified lectin was evaluated using D-galactose as an inhibitor. Serial dilutions of D-galactose (100 mM) were prepared in phosphate-buffered saline (pH 7.2) and mixed with freeze-dried lectin (30 HU). The mixture was incubated with trypsinized New Zealand White rabbit erythrocytes at 37°C for 30 min. The minimum concentration of sugar required to inhibit hemagglutination was determined.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The molecular weight and homogeneity of lectin were determined by SDS-PAGE using a 13% resolving gel and a 4% stacking gel. Electrophoresis was performed under reducing conditions, and protein bands were visualized using standard staining procedures.

Sodium content was determined using flame photometry. Freeze-dried lectin samples were ashed in a silica crucible, dissolved in concentrated hydrochloric acid, diluted with distilled water, and analyzed after calibration using a standard lectin sample.

Protein content of the purified and freeze-dried lectin was determined using the Lowry method. Lectin standard solutions were prepared using bovine serum albumin (BSA) as a reference protein and serially diluted in phosphate buffer (pH 6.8) to obtain known concentrations.

Lowry reagent was freshly prepared and added to the standard and sample solutions, followed by incubation at room temperature for color development. Subsequently, Folin–Ciocalteu reagent was added, and the mixture was incubated for an appropriate time until the blue color developed. The absorbance was measured at 660 nm using a UV–visible spectrophotometer.

Protein concentration of the purified lectin samples was calculated from the standard calibration curve plotted between absorbance and protein concentration. All measurements were carried out in triplicate, and the results were expressed as mean ± standard deviation.

Results and Discussion

The results of this study demonstrate that *Phaseolus vulgaris* seeds are a valuable source of biologically active lectin. Optimization of extraction time and precipitation conditions significantly improved protein yield and activity. Gel filtration chromatography using Sephadex G-75 provided an efficient and gentle purification approach, ensuring both high purity and functional integrity of the lectin molecule.

The observed temperature and pH stability profiles suggest that the isolated lectin is suitable for further pharmaceutical and biotechnological investigations, particularly in applications requiring activity under physiological conditions. The molecular weight determined by SDS-PAGE supports the identity of the isolated protein as a typical legume lectin.

Overall, the applied methodology offers a reliable framework for the isolation and purification of plant lectins with potential applications in drug development, diagnostics, and biotechnology.

Purification of the Isolated Lectin Compound. It is well known that gel filtration chromatography can be used to remove unwanted low-molecular-weight impurities from protein preparations. In this study, gel filtration was employed to purify the isolated lectin by eliminating foreign iodinated substances. The purification process was carried out using the Bio-Rad BioLogic LP chromatography system.

The column was packed with Sephadex G-75 gel, and the flow rate of the Tris-HCl buffer was set at 1.5mL/min. The results of the chromatographic purification are shown in the figure below.

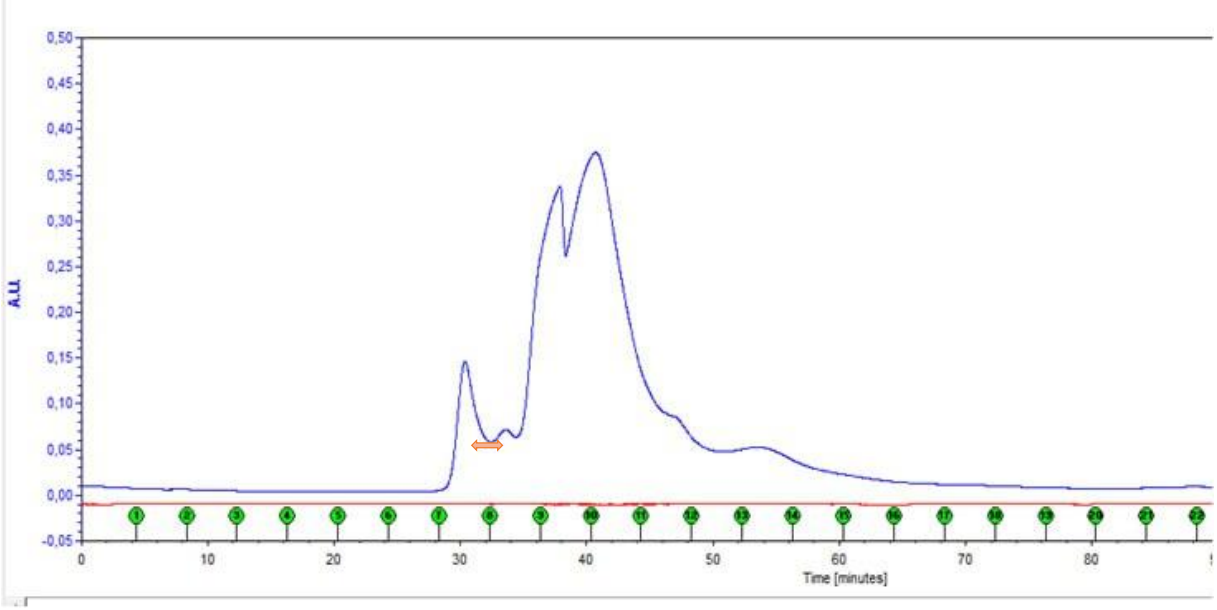

Figure 1. Chromatogram of lectin isolated from *Phaseolus vulgaris*, obtained using the Bio-Rad BioLogic LP chromatography system

The marked area in this figure represents the fraction that exhibited high hemagglutination activity.

SDS-PAGE Analysis of Lectin. The electrophoretic analysis of dry lectin samples on polyacrylamide gel was carried out using the Laemmli method [1]. The procedure was performed using the Mini-PROTEAN II Electrophoretic Cell (BIO-RAD, USA).

Two types of gels were prepared:

- Resolving gel: Tris-HCl buffer (pH 8.8) 244 mM; Bis-acrylamide 10%; TEMED 0.08%; APS 0.08%; SDS 0.01%
- Stacking gel: Tris-HCl buffer (pH 6.8) 125 mM; Bis-acrylamide 4%; TEMED 0.05%; APS 0.05%; SDS 0.01%

The electrode buffer consisted of Tris-OH 25 mM, glycine 192 mM, and 0.2% SDS.

Sample preparation included 60 µg of protein in 20 µL volume. Before loading onto the gel, samples were denatured at 90°C for 3 minutes in a solution containing 0.004% bromophenol blue (Sigma, USA), 10% mercaptoethanol, and 4% SDS.

After electrophoresis, the gels were stained with Coomassie Brilliant Blue G-250 aqueous solution. The destaining buffer consisted of acetic acid, ethanol, and water in a 1:1:8 ratio.

The electrophoresis was run under the following current conditions:

- 40 mA during stacking phase
- 80 mA during resolving phase

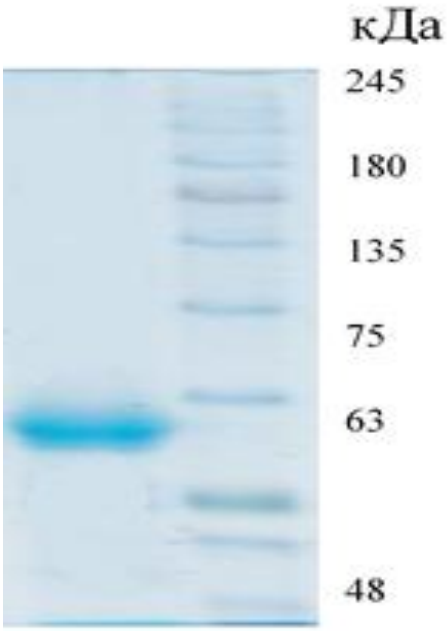

Figure 2. Molecular weight of lectin isolated from *Phaseolus vulgaris* determined by SDS-PAGE

As shown in Figure 3, the molecular weight of the isolated lectin was found to be approximately 62–63 kDa.

Conclusion. In this study, a series of experimental steps were conducted to extract, purify, and characterize the physicochemical properties of lectin derived from the seeds of *Phaseolus vulgaris* (common bean). Based on the results obtained, the following conclusions were drawn:

- The optimal incubation time for protein extraction was determined to be 10 hours, during which the maximum protein content in the extract reached 42.7 mg;
- The most effective ammonium sulfate concentration for protein precipitation was found to be 75–80%, corresponding to the highest hemagglutination activity;
- The biological activity of lectin was shown to be dependent on temperature and pH. Maximum activity was observed at 20–25°C and in the pH range of 5.0 to 8.0. Elevated temperatures and extreme acidic or alkaline conditions significantly reduced activity;
- The purified lectin was successfully separated using Sephadex G-75 gel filtration, and fractions with high hemagglutination activity were isolated;
- SDS-PAGE analysis revealed that the molecular weight of the lectin is approximately 62–63 kDa.

These findings suggest that the lectin extracted from *Phaseolus vulgaris* seeds possesses high biological activity and may serve as a promising candidate for further exploration in the fields of pharmaceuticals and biotechnology.

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