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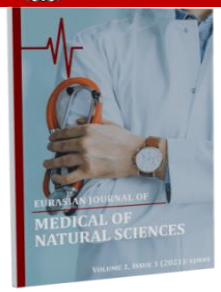

ISOLATION AND STUDY OF PHYSICOCHEMICAL PROPERTIES OF LECTIN EXTRACTED FROM *PHASEOLUS VULGARIS* SEEDS

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ABSTRACT

This study investigates the extraction and characterization of lectin from Phaseolus vulgaris seeds. Lectins are carbohydrate-binding glycoproteins known for their wide range of biological activities, including antimicrobial and anticancer effects. The seeds were extracted in phosphate-buffered saline, and incubation time was optimized. Maximum protein yield (42.7 mg) was obtained after 10 hours. Ammonium sulfate at 75–80% concentration was most effective for protein precipitation, coinciding with peak hemagglutination activity. Purification was performed using gel filtration on a Sephadex G-75 column. The biological activity of lectin was tested at different temperatures and pH levels. High activity was observed at 20–25°C and pH 5.0–8.0. SDS-PAGE analysis revealed the molecular weight of lectin to be around 62–63 kDa. The findings confirm that Phaseolus vulgaris lectin is a stable and active protein with promising applications in biotechnology and pharmaceutical research..

Relevance of the Topic. Lectin is a glycoprotein that binds specifically to carbohydrates such as mannose, galactose, fucose, and rhamnose. Depending on the type of carbohydrate base, lectins can exhibit a wide range of biological activities. To date, lectins have been widely used in medicine, diagnostics, pharmaceuticals, and agriculture.

Plant-derived lectins have shown antibacterial, antifungal, antiviral (including against the human immunodeficiency virus), anticancer, anti-inflammatory, and analgesic properties. These lectins can be extracted from various parts of plants, including leaves, seeds, stems, roots, and fruits. Among plants, legumes are considered one of the main sources for lectin extraction.

However, it has been found that many lectins derived from legumes can irritate the intestinal villi in humans, making them unsuitable for direct consumption in food. Therefore, thorough investigation is required before they can be used safely in medicine. [1-4]

Materials and Methods. Dried *Phaseolus vulgaris* seeds were ground into powder and passed through a 280-micron sieve. The resulting powder was mixed with phosphate-buffered saline (PBS, 10 mM, pH 7.0) and placed on a magnetic stirrer overnight at 4°C. The extract was then filtered and centrifuged. Ammonium sulfate was gradually added to the supernatant until saturation, and the solution was dialyzed in a dialysis bag for 24 hours to achieve desalting.

The obtained lectin was further purified using gel filtration. A 10 mg/mL solution of deionized lectin was filtered through a 0.45 μm membrane and subjected to gel filtration on a Sephadex G-75 column using Tris-HCl buffer at a flow rate of 1.5 mL/min. The lectin-containing fractions were lyophilized to obtain a dry powder. [5-6]

Determination of Hemagglutination Activity. Hemagglutination activity was tested using specialized Szyan titration microplates. A 0.5 mg/mL solution of lyophilized lectin was prepared. 50 μL of this solution was serially diluted with physiological saline, and 5 μL of a 2% rabbit erythrocyte suspension was added. The reaction was incubated for 45 minutes at room temperature.[3]

Results and Discussion. The optimal conditions for lectin extraction were identified. The table below summarizes the impact of incubation time on protein yield:

1-table

The effect of incubation time on the lectin extraction process

№	Volume of extract (ml)	Incubation Time (hours)	Volume of protein (mg)
1	750	2.0	29.1
2	750	4.0	36.1
3	750	6.0	38.6
4	750	8.0	41.4
5	750	10	42.7
6	750	12	42.7

These results indicate that increasing the incubation time has a positive effect on protein extraction, with the maximum protein concentration (42.7 mg) observed after 10 hours, which remained stable afterward.

In the next stage, vacuum filtration (Nutch method) was used to process the extract. After centrifugation (7000 rpm, 20 minutes), the supernatant was subjected to ammonium sulfate precipitation. The hemagglutination activity was highest when using 75–80% ammonium sulfate, which was identified as the optimal concentration for protein precipitation.

Additional Biological Activity Tests. Agglutination tests with trypsin-treated human erythrocytes confirmed strong lectin activity. Microscopy images showed erythrocyte clumping after 15 minutes, indicating strong hemagglutination. Thermal stability tests revealed high activity between 10°C–25°C; activity decreased at 50–60°C. pH stability tests showed maximum activity at pH 5.0–8.0.

The effect of incubation time on the extraction process was studied. As shown in the table, the extraction process is dependent on time. An increase in incubation time positively influenced the efficiency of lectin extraction. The data demonstrate that after 10 hours of incubation, the protein content reached **42.7 mg**, and this amount remained unchanged with further incubation.

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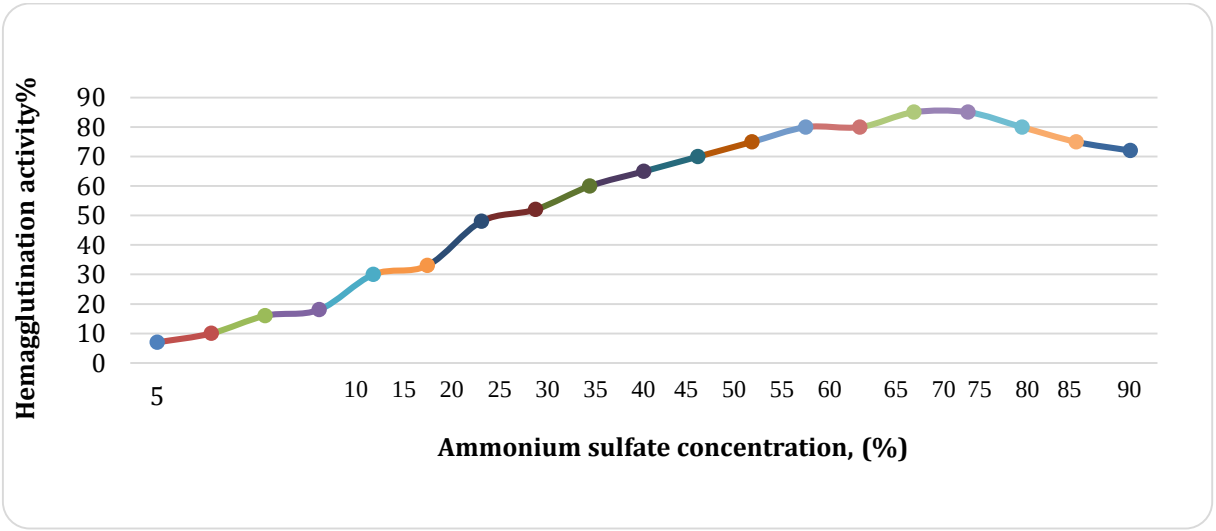
In the next stage of the study, the extract was filtered using the **Nutsch vacuum filtration method.** The extract was poured gradually and filtered in 50 mL portions, with the filter paper replaced after each portion. The process continued until all extract was processed.

The resulting filtrate was centrifuged at **7000 rpm for 20 minutes**, and the supernatant was separated. Proteins from the supernatant were then precipitated using **ammonium sulfate.**

The **optimal conditions for precipitation** were also determined in the course of this process.

1- graph

Effect of ammonium sulfate concentration on the precipitation of lectin from the extract



As shown in the linear graph above, the hemagglutination activity of lectin was used to determine the optimal concentration of ammonium sulfate for protein precipitation from the extract. The highest protein precipitation and a simultaneous increase in hemagglutination activity were observed at ammonium sulfate concentrations between 75% and 80%. Based on these results, this concentration range was identified as optimal for the precipitation of lectin from the *Phaseolus vulgaris* extract.

Determination of Lectin Activity via Hemagglutination. The biological activity of the lectin was assessed through agglutination testing. Trypsin-treated human erythrocytes were used in specialized microplates for the hemagglutination assay.

To perform the test, 50 µL of phosphate-buffered saline (PBS) was added to each well, followed by serial two-fold dilutions of the lectin extract (10 dilution steps). Next, 50 µL of a 2% erythrocyte suspension was added to each well. The mixtures were incubated at room temperature for 1 minute to 20–30 minutes and observed under a microscope.

The extent of agglutination was determined by visually assessing the clustering of red blood cells. If no agglutination occurred, the erythrocytes remained dispersed individually.

If agglutination was present, the red blood cells appeared clumped together, indicating lectin binding activity.

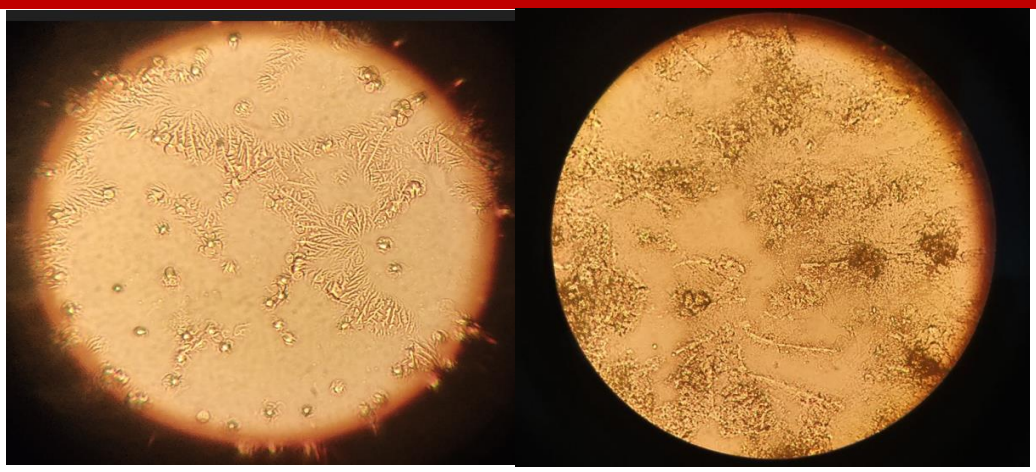


Figure 1. Microscopic appearance of the hemagglutination process

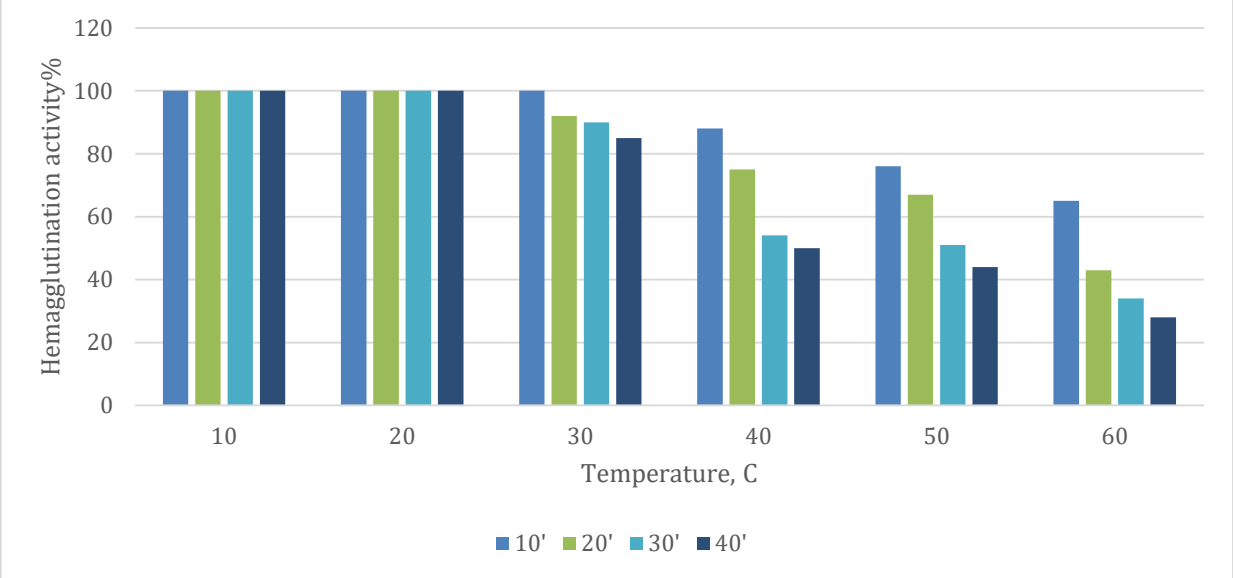
A – Initial state of erythrocytes before agglutination.

B – State after 15 minutes of incubation showing visible cell agglutination.

Study of the Effect of Temperature on Lectin Activity. The graph below illustrates the dynamics of changes in the hemagglutination activity of lectin at different temperatures over various time intervals. This allows for the assessment of how temperature influences the biological activity of lectin.

2-graph

Effect of temperature on lectin activity



This graph demonstrates that lectin activity is temperature-dependent. Hemagglutination activity was measured at various temperatures — 10°C, 20°C, 30°C, 40°C, 50°C, and 60°C — and across different time intervals (10, 20, 30, and 40 minutes) for each sample.

The results indicate that lectin retains high biological activity between 10°C and 20°C throughout the 10–40minute incubation period. However, as the temperature increases, a negative correlation is observed between temperature and lectin activity. Both prolonged incubation time and higher temperatures lead to a gradual decrease in hemagglutination activity.

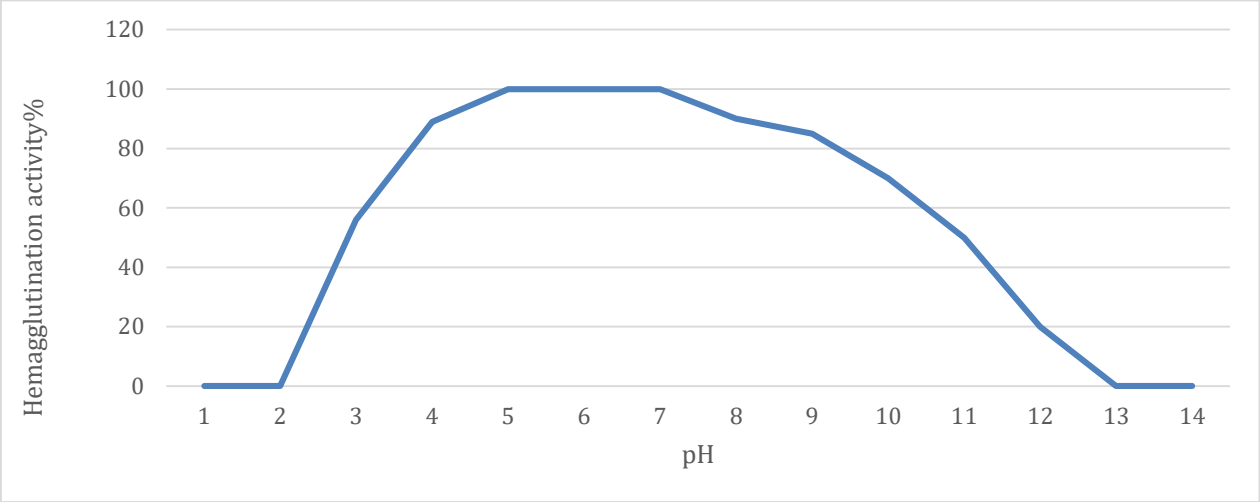
A sharp decline in activity is particularly noticeable at 50°C and 60°C, even within short exposure times. These findings confirm that lectin maintains its stability and biological activity up to approximately 25°C.

Study of the Effect of pH on Lectin Activity

The hemagglutination assay of lectin under various pH conditions demonstrates that the lectin remains active within a pH range of 3.0 to 11.0, as shown in graph 3.

3-graph

Study of the Effect of pH on Lectin Activity



Lectin hemagglutination activity increased with rising pH levels and reached its maximum between pH 5.0 and 8.0. However, in alkaline conditions ranging from pH 10 to 11, the activity dropped significantly, maintaining less than 50% of its initial agglutination capacity. These results indicate that both extremely acidic and highly alkaline environments adversely affect the hemagglutination activity of lectin.

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.

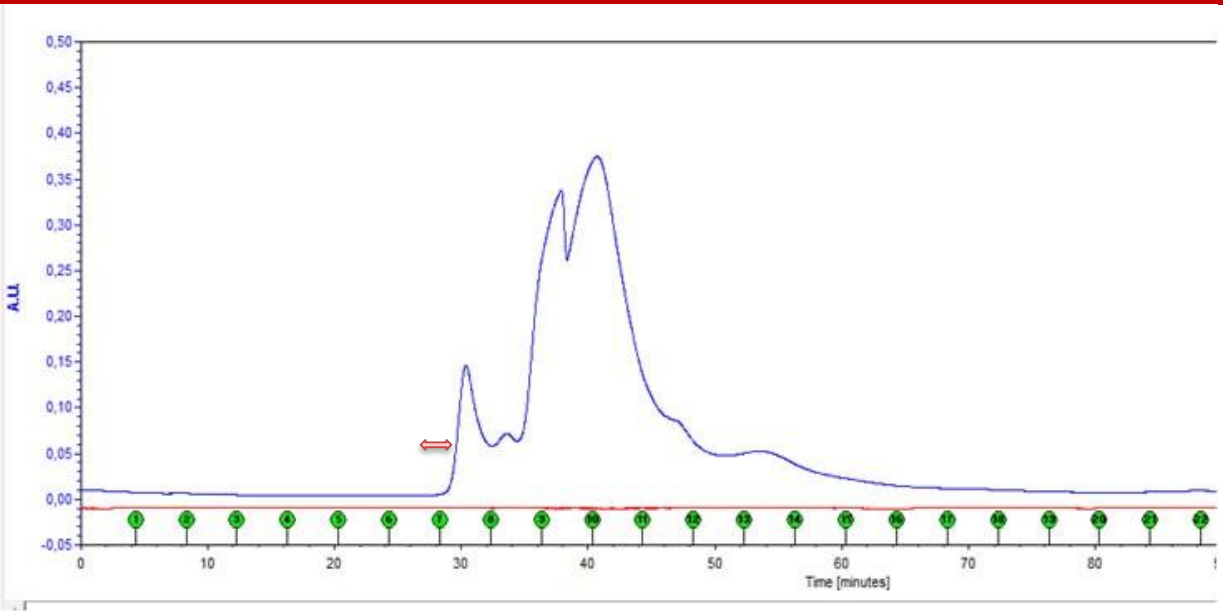


Figure 2. Chromatogram of lectin isolated from *Phaseolus vulgaris*, obtained using the Bio-Rad BioLogic LP chromatography system

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

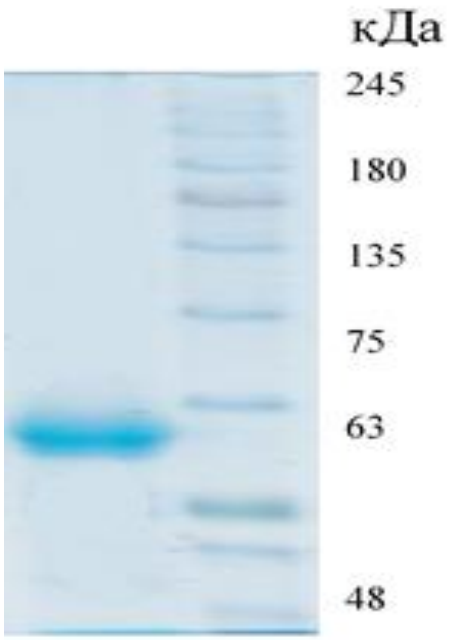


Figure 3. Molecular weight of lectin isolated from *Phaseolus vulgaris* determined by SDS-PAGE

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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