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Isolation & Purification of Lectin from *CICER ARIETINUM & ARACHIS HYPOGAEA*

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ABSTRACT: Lectins are carbohydrate-binding proteins abundantly present in legume seeds and are known for their significant biological activities, particularly hemagglutination. The present study aimed to isolate and partially purify lectins from the seeds of *Cicer arietinum* (chickpeas) and *Arachis hypogaea* (peanuts). Extraction was carried out at 4°C, followed by partial purification using ammonium sulfate precipitation at 30%, 60%, 70%, and 90% saturation levels, and subsequent dialysis. Protein concentration was estimated using the Folin–Lowry method, which revealed that the partially purified lectin fractions exhibited higher protein content in *Cicer arietinum* (chickpeas) lectin at 98 µg/mL and at 79 µg/mL in *Arachis hypogaea* (peanuts) lectin than the crude extracts 0.3 ug/ml of *Cicer arietinum* (chickpeas) and *Arachis hypogaea* (peanuts). Hemagglutination assays demonstrated significant activity, with maximum activity observed in the 90% saturation fraction of *Cicer arietinum* (chickpeas) lectin and in the 60% saturation fraction of *Arachis hypogaea* (peanuts) lectin. The results confirm that *Cicer arietinum* and *Arachis hypogaea* are rich sources of biologically active lectins and highlight their potential for further biochemical and biomedical applications.

KEY WORDS: *Cicer arietinum* (chickpeas), *Arachis hypogaea* (peanuts), lectin, hemagglutination assay, ammonium sulfate precipitation.

I.INTRODUCTION

Lectins are a diverse group of carbohydrate-binding proteins that play a fundamental role in biological recognition processes. Unlike enzymes, lectins do not catalyze modifications of the carbohydrates to which they bind; rather, they specifically recognize and reversibly bind to distinct sugar moieties, thereby mediating cellular interactions and molecular signaling events. Through this selective carbohydrate recognition, lectins participate in various biological processes including cell–cell communication, immune regulation, host–pathogen interactions, and glycoprotein trafficking [1]. Lectins are widely distributed in nature and have been identified in plants, animals, fungi, bacteria, and viruses. Plant lectins, in particular, have been extensively investigated due to their abundance and structural diversity. They are commonly found in edible plant sources such as beans, chickpeas, soybeans, peanuts, lentils, wheat, and other legumes. For instance, red kidney beans are known to contain significant levels of lectins, while *Arachis hypogaea* (peanut) and *Cicer arietinum* (chickpea) are important dietary sources of lectin-containing proteins [2]. These legumes are not only rich in protein, dietary fiber, vitamins, and essential minerals, but also contain bioactive compounds that influence metabolic processes and gut health. Despite their nutritional value, certain lectins and related antinutritional factors may exert adverse physiological effects when consumed in raw or improperly processed forms. For example, raw soybeans contain high levels of trypsin inhibitors and lectins, which can impair protein digestion and reduce growth performance in experimental animals [1]. Similarly, the presence of biologically active lectins in red kidney beans necessitates adequate cooking to inactivate potentially toxic components. Groundnuts, commonly referred to as peanuts, belong to the genus *Arachis* within the family Fabaceae and represent a major legume crop cultivated for human consumption and livestock feed. They are valued for their high content of mono- and polyunsaturated fatty acids, protein, antioxidants, and micronutrients, which contribute to their recognized health benefits [3]. Lectins have also been isolated and characterized from various plant species using biochemical techniques such as affinity chromatography. For example, a mitogenic lectin was successfully isolated from garden pea (*Pisum Sativum*) using Sephadex-based affinity purification methods [4]. In general, lectins are glycoprotein or proteins capable of specifically binding saccharine and can be extracted from seeds of numerous plant species [2].Due to their specificity and biological activity; lectins have attracted considerable scientific interest. They have been widely

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studied for their involvement in cellular communication, immune modulation, inflammation, and disease mechanisms. Furthermore, lectins have demonstrated promising applications in biotechnology and medicine, including targeted drug delivery, cancer diagnostics and therapeutics, and antimicrobial strategies [5].
This study provides a comprehensive review of the occurrence, classification, biological functions, and biotechnological applications of lectins. By synthesizing findings from previous research, it aims to highlight the significance of lectins in nutrition, health, and disease, while identifying areas for further scientific investigation.

II.RELATED WORK

Researcher worldwide studied that Lectins are carbohydrate-binding proteins found in plants, animals, fungi, and microorganisms that specifically and reversibly bind sugars and play important roles in **cell recognition, immune response, and biological interactions.** Studies have shown that plant and fungal lectins, particularly from ***Phaseolus vulgaris***, possess defensive, antimicrobial, antifungal, and potential anticancer properties, highlighting their significance in biological and biomedical research. These articles present works on highlight the significance of lectins in nutrition, health, and disease, while identifying areas for further scientific investigation [15-29].
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III. SIGNIFICANCE OF THE SYSTEM

The study of lectins is significant because these carbohydrate-binding proteins play important roles in **cell recognition, immune regulation, and biological interactions**. Understanding their occurrence and properties in plant sources can help improve **nutritional quality, reduce antinutritional effects, and explore their potential applications in biotechnology and medicine**.

IV. MATERIALS & METHODOLOGY

Collection and Preparation of Seed Samples
Seeds of *Cicer arietinum* (chickpea) and *Arachis hypogaea* (peanut) were collected (Fig 1) from a local grocery store situated at Aurangabad and cleaned to remove dust and impurities. The seeds were air-dried and ground into a fine powder using a laboratory grinder (Ken Star SCCA/BT/2013-14). The powdered samples were stored in airtight containers at 4°C until further use [1].

Fig 1: The *Cicer arietinum* (chickpeas) and *Arachis hypogaea* (peanuts) seeds

Lectin isolation from collected seeds:
Approximately 25 g of seed powder was suspended in 250 mL of 50 mM phosphate buffer (pH 7.2). The homogenate was maintained at 4°C overnight to ensure complete extraction of soluble proteins, including lectins. Cold extraction conditions were used to preserve protein stability and biological activity, as recommended in standard protein purification procedures [6]. Following extraction, the mixture was filtered through muslin cloth to remove insoluble materials. The filtrate was then centrifuged at 8000 rpm for 20 minutes at 4°C to separate the soluble protein fraction from particulate matter. The resulting supernatant served as the crude protein extract. Partial purification of lectins was achieved by fractional precipitation using ammonium sulfate at increasing saturation levels of 30%, 60%, 70%, and 90%. Ammonium sulfate precipitation is widely employed in protein purification due to its ability to selectively precipitate proteins based on differences in solubility [7]. Each saturation level was allowed to equilibrate, and the precipitated proteins were collected by centrifugation. The

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pellets obtained at each fraction were resuspended separately in 15 mL of phosphate buffer (pH 7.0). The resuspended fractions were subsequently subjected to further purification and characterization procedures, including dialysis and hemagglutination assays, to evaluate lectin activity and determine carbohydrate-binding specificity. Affinity chromatography techniques, particularly those utilizing carbohydrate-based matrices such as Sephadex, are commonly employed for lectin purification due to their high specificity for glycan-binding proteins [8].

Partial purification of Lectin:

Partial purification of lectin was performed using ammonium sulfate precipitation based on the principle of "salting out." When the ionic strength of a protein solution is increased by the addition of neutral salts, protein solubility decreases, resulting in precipitation. This occurs because salt ions compete with protein molecules for water molecules, thereby reducing protein hydration and promoting aggregation [6]. Solid ammonium sulfate was gradually added to the crude extract in a pinch-wise manner with continuous stirring using a magnetic stirrer to achieve the desired saturation level according to standard ammonium sulfate precipitation charts. The solution was maintained at 4°C to preserve protein stability and biological activity. After complete dissolution of the salt, the mixture was stored overnight at 4°C to allow full precipitation of lectin proteins [6]. The following day, the sample was centrifuged to separate the precipitated proteins (pellet) from the supernatant. The supernatant was subjected to further ammonium sulfate fractionation by increasing salt saturation with continuous stirring. Each fraction was allowed to equilibrate overnight at 4°C before centrifugation. The pellets obtained at different saturation levels were collected and dissolved in phosphate-buffered saline (PBS, pH 7.2). To remove excess ammonium sulfate and other small molecules, the protein fractions were subjected to dialysis against PBS for 3–4 days at 4°C with periodic buffer changes, following the method described by [1]. Dialysis facilitates purification by allowing small ions to diffuse through a semi-permeable membrane while retaining high-molecular-weight proteins [9]. This partially purified lectin preparation was subsequently used for protein estimation and biological activity assays

Determination of concentration of protein:

The protein concentration of the partially purified lectin fractions was determined using the Lowry method, which is based on the reaction of peptide bonds with copper ions under alkaline conditions, followed by reduction of the Folin–Ciocalteu reagent [9]. Bovine serum albumin (BSA) was used as the standard protein for calibration. Absorbance was measured spectrophotometrically at 750 nm, and protein concentration was calculated using a standard calibration curve.

Assay of hemagglutination activity:

Hemagglutination activity, a characteristic property of lectins, was evaluated using normal human erythrocytes following standard protocols [10]. Fresh human blood samples were centrifuged at 3000 rpm for 4 minutes at 5–10°C to separate erythrocytes from plasma. The erythrocytes were washed several times with phosphate-buffered saline (PBS, pH 7.2) to remove plasma proteins and other interfering substances. A 3% (v/v) erythrocyte suspension was prepared in PBS. Hemagglutination assays were carried out in microtiter plates by mixing 50 µL of erythrocyte suspension with 50 µL of serially diluted lectin samples. The plates were incubated at room temperature for 1 hour, and agglutination was assessed visually. The hemagglutination titer was defined as the highest dilution of lectin that produced visible agglutination. This assay is widely used to determine lectin activity because lectins specifically bind to carbohydrate moieties present on erythrocyte membranes, leading to cross-linking and cell aggregation [1].

V. EXPERIMENTAL RESULTS AND DISCUSSION

Isolation and Partial purification of Lectin

Lectins were successfully isolated from the collected seeds of *Arachis hypogaea* (peanuts) and *Cicer arietinum* (chickpeas) (Fig. 2). Partial purification of the lectins was carried out using the ammonium sulfate precipitation method, which exploits the principle of "salting out" to selectively precipitate proteins based on their solubility at different salt concentrations. Ammonium sulfate was added stepwise to the crude protein extract to achieve 30%, 60%, 70%, and 90% saturation, and each fraction was collected separately after centrifugation The pellets obtained at each saturation level were dissolved in phosphate-buffered saline (PBS, pH 7.2) for further characterization and analysis. The partial purification process is illustrated in Fig.3. This fractionation approach

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allowed enrichment of lectin proteins while removing other soluble proteins and impurities, providing a foundation for subsequent protein quantification and functional assays such as hemagglutination.

Fig2: - Isolation of Lectin protein from seed sample

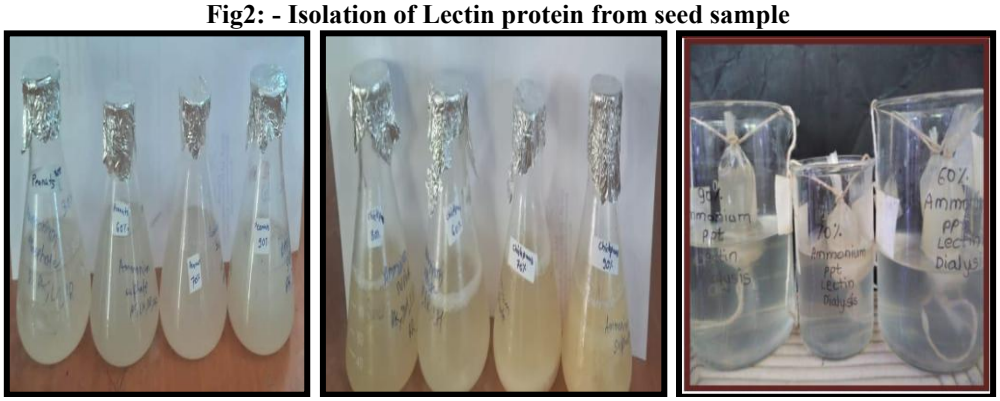

Fig3: - Partial purification of lectin protein

Determination of concentration of Lectin protein:

The protein concentration of the partially purified lectin fractions from *Cicer arietinum* (chickpeas) and *Arachis hypogaea* (peanuts) was determined using the Lowry method, with bovine serum albumin (BSA) as the standard [10]. For each ammonium sulfate saturation fraction (30%, 60%, 70%, and 90%), absorbance readings were measured, and protein concentration was calculated using the formula:

Protein concentration (µg/mL)= Slope O.D. – Intercept

The results are summarized in Table 1 and illustrated graphically in Fig. 6.

Table 1: - Protein Concentration in Chickpeas & Peanuts

Saturation %	Chickpeas	Chickpeas Protein concentration (ug/ml)	Peanuts	Peanuts Protein concentration (ug/ml)
30%	0.39	3 (ug/ml)	1.54	76 (ug/ml)
60%	0.72	22 (ug/ml)	1.49	**79 (ug/ml)**
70%	0.79	92 (ug/ml)	1.33	66 (ug/ml)
90%	0.97	**98 (ug/ml)**	1.22	52 (ug/ml)

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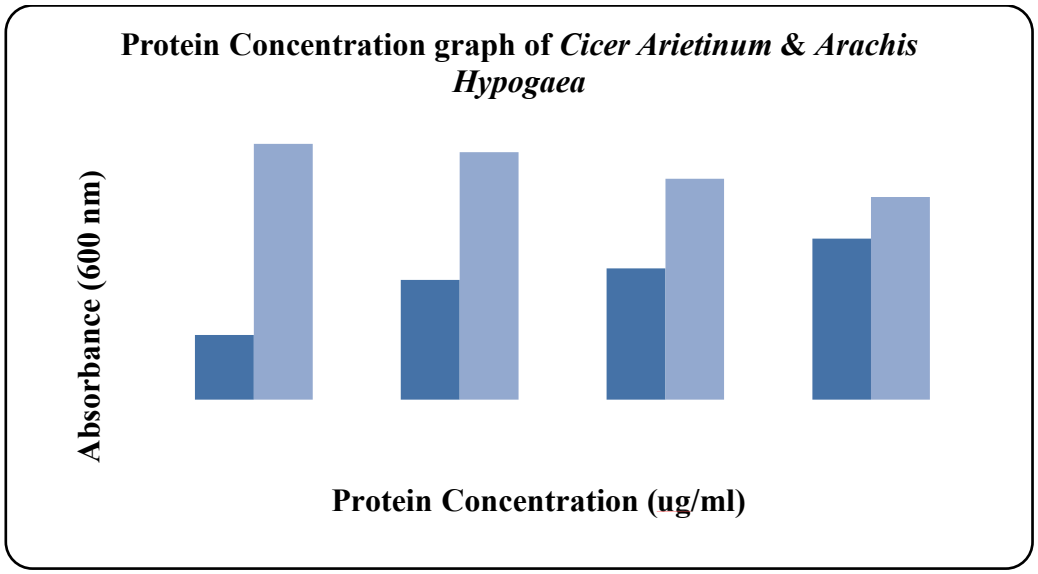

Fig 6: -Protein Concentration graph of *Cicer Arietinum* &*Arachis Hypogaea*

As observed, the protein concentration in chickpeas increased progressively with higher ammonium sulfate saturation, reaching a maximum of 98 µg/ml at 90% saturation. In contrast, the protein concentration in peanuts was highest at lower saturation (30–60%) and decreased at higher saturation levels. This difference may reflect variations in lectin solubility and binding affinity in the two species. **Fig. 6** shows the protein concentration graph of the partially purified lectins from *Cicer arietinum* and *Arachis hypogaea*, illustrating the differential precipitation profile across the ammonium sulfate fractions.

Assay of Lectin Protein Using Hemagglutination Activity

The hemagglutination activity of partially purified lectins from Cicer arietinum (chickpeas) and Arachis hypogaea (peanuts) was evaluated using human erythrocytes. This assay is based on the ability of lectins to bind specifically to carbohydrate moieties on the surface of red blood cells, resulting in visible agglutination. The hemagglutination assay results were consistent with the protein concentration measurements obtained by the Lowry method. The highest lectin activity corresponded to the fractions with the highest protein concentration: 98 µg/ml for the 90% ammonium sulfate fraction of chickpea lectin and 79 µg/mL for the 60% ammonium sulfate fraction of peanut lectin. This indicates a positive correlation between protein concentration and hemagglutination activity, demonstrating that the partially purified lectins retained their carbohydrate-binding functionality. Fig. 7 shows the protein concentration determination by the Folin–Lowry method, while Fig. 8 illustrates the hemagglutination activity of the lectin fractions.

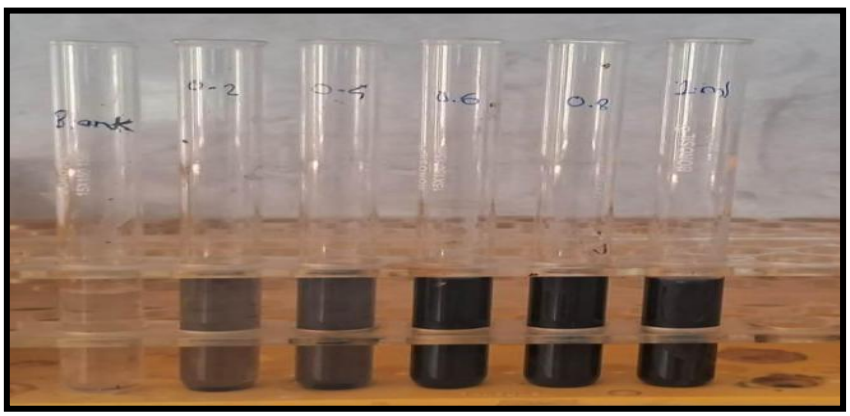

Fig 7: - Determination of protein by Folin Lowry method

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Fig 8: - Hemagglutination activity

V. CONCLUSION

Lectins are a diverse and biologically significant group of carbohydrate-binding proteins that play crucial roles in cellular recognition, immune modulation, and host–pathogen interactions. In this study, lectins were successfully isolated and partially purified from the seeds of *Cicer arietinum* (chickpeas) and *Arachis hypogaea* (peanuts) using ammonium sulfate fractionation followed by dialysis. The stepwise precipitation at 30%, 60%, 70%, and 90% ammonium sulfate saturation effectively enriched the lectin proteins while reducing the presence of other soluble proteins. Protein quantification using the Lowry method demonstrated that lectin concentration varied with saturation level, with chickpeas exhibiting a progressive increase in protein content up to 98 µg/mL at 90% saturation, while peanuts showed the highest protein content at lower saturation levels (30–60%), reflecting differences in solubility and lectin properties between the two species. Hemagglutination assays confirmed the biological activity of the partially purified lectins, with the highest agglutination observed in fractions corresponding to peak protein concentration. Overall, this study highlights that *Cicer arietinum* and *Arachis hypogaea* are rich dietary sources of bioactive lectins and that ammonium sulfate precipitation is a reliable method for their partial purification.

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