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Purification of a Seed-Derived Lectin from Medicinally Important Plant *Terminalia Catappa* (TC)

Fakeha Mohammed Rehan Shaikh¹ & Ashish Sambhaji Uzgare^{1*}

¹Department of Chemistry, Wilson College (Autonomous), Chowpatty Seaface Road, Mumbai 400007, Maharashtra, India

*Corresponding Author: ashish.uzgare@wilsoncollege.edu

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Abstract: Lectins represent a diverse class of carbohydrate-binding proteins distributed across plants, animals, fungi, and marine organisms. Their strong affinity for specific sugar residues enables them to participate in essential biological events, including cell signaling, immune modulation, and defense against pathogens. Among them, plant lectins have received considerable attention due to their medicinal attributes, such as antifungal, antiviral, anti-inflammatory, and antitumor activities. Harnessing these therapeutic potentials, however, requires reliable and efficient methods for their extraction and purification. In the present study, a lectin-like protein was extracted from *Terminalia catappa* seeds using phosphate-buffered saline (PBS) and purified through sequential ammonium sulphate precipitation, dialysis, gel filtration, and affinity chromatography. The crude extract had a protein concentration of 1.5 mg/mL, which was reduced to 0.9 mg/mL following gel filtration and 0.7 mg/mL after affinity chromatography. The appearance of a single, well-defined peak confirmed the homogeneity of the purified lectin and corresponded with an increase in its specific activity.

Keywords: Chromatographic Purification, Lectin, *Terminalia catappa*

I. INTRODUCTION

Plants contain a wide variety of lectins, which differ in their molecular structures, biochemical characteristics, and specificities for carbohydrate binding (Shaikh and Uzgare, 2024). The concentration of plant lectins varies depending on the plant part. Lectins extracted from the seeds of specific plants induce the highest levels of erythrocyte agglutination, while the lowest levels of agglutination are observed with lectins from plant bulbs and leaves (Zubcevic *et al.*, 2016). Plant lectins are recognized for their therapeutic potential, which includes antiviral, anticancer, and immunomodulatory activities (Sharon and Lis, 2004). Owing to their distinctive properties, lectins are utilized as tools in biochemistry, cellular biology, immunology, and related fields for both analytical and preparative applications (Shaikh and Uzgare, 2025). *Terminalia catappa* L., also known as Indian Almond, is a member of the Combretaceae family (Shaikh and Uzgare, 2025). The *Terminalia catappa* Linn. plant has been the subject of numerous pharmaceutical studies due to its diverse range of chemical constituents (Terças *et al.*, 2017). The method of lectin purification includes dialysis, gel filtration, electrophoresis, and affinity chromatography (Shaikh and Uzgare, 2025; Roopashri *et al.*, 2023). Traditional purification

methods, particularly affinity column chromatography, have played a key role in isolating a wide variety of lectins and glycoproteins (Welch *et al.*, 2019). Due to lectins ability to bind to carbohydrates and other glycoproteins, affinity chromatography is the primary method used for purifying lectins. This binding property is being investigated for interactions with substances such as N-acetylglucosamine, derived from galactose, mannose, and proteins among others (da Silva *et al.*, 2023). The origins and applications of lectins are examined, highlighting their unique carbohydrate-binding domains and the role these play in advancing affinity-based methods for more efficient and effective lectin purification. The purity of lectins is evaluated based on overall yield and purification factors for various purification strategies, along with the required steps for their isolation. New approaches are described for improving lectin separation and purification (Nascimento *et al.*, 2012).

The aim of this study is to purify the lectin like protein from *Terminalia catappa* seeds by using various chromatographic techniques.

II. MATERIALS AND METHODS

Chemical and Reagents: The seeds of *Terminalia catappa* were collected from Mumbai, Maharashtra, India. Send it for identification. Analytical-grade reagents and solvents were procured from Himedia, SRL, Loba Chemie, PCL, and Chemsworth (India).

Extraction of lectin: Extraction of lectin was performed as per the method of Shaikh and Uzgare, (2024).

Purification steps

Precipitation and dialysis were performed according to the procedure of Arekal, *et al.*, (2023) with minor adjustments. Proteins from the extract were fractionated using ammonium sulphate precipitation at varying salt concentrations (20-80 %). The precipitates were dissolved in phosphate buffer saline (PBS) and dialyzed at 4°C for overnight.

Gel filtration After dialysis, the lectin was loaded onto a Sephadex G75 column for gel filtration, following minor adjustments to the procedure of Hong *et al.*, (2015). The active fraction was concentrated, loaded onto the Sephadex G75 column, and eluted with PBS. Absorbance was monitored at 280 nm, with an elution rate of 2 mL/min. lectin hemagglutination activity was checked.

Affinity chromatography Lactamyl- Seralose CL-4B column was prepared for affinity chromatography according to the protocol of Konozy *et al.*, (2002). A 15 mL lectin-rich fraction was loaded to the Lactamyl Seralose CL-4B column and eluted with 0.2 M lactose in saline. The resulting 3 mL fractions were analyzed for protein concentration using a Systonic UV spectrophotometer at 280 nm. After that lectin hemagglutination activity was checked.

Lectin Hemagglutination activity

A hemagglutination activity test was performed. A total of 100 µL of lectin is mixed with a 100 µL 5% erythrocytes suspension incubated at room temperature (Shaikh and Uzgare, 2024).

Paper Chromatography

Circular paper chromatography was performed according to procedure of Giri *et al.*, (1954). For the preliminary detection of amino acids present in *Terminalia catappa* seeds lectin.

III. RESULTS AND DISCUSSION

Lectin was extracted from TC seeds using PBS and purified through ammonium sulphate precipitation, dialysis, and gel filtration with Sephadex G75. Figure 3.1 demonstrates the peak, indicating successful purification of the lectin.

Terminalia catappa seed lectin maximum Hemagglutination activity is visible in Human Erythrocyte B. It confirmed that

this lectin is specific to Blood Group B. After gel filtration lectin was loaded in Lactamyl-Seralose CL-4B for Affinity chromatography this step confirms lectin is highly purified because impurity and another protein doesn't bind to this column and wash away Figure 3.2 shows the successful purification of lectin by using affinity chromatography and Table 1 Show the Hemagglutination activity of lectin. Purity of protein was confirmed by PAGE electrophoresis.

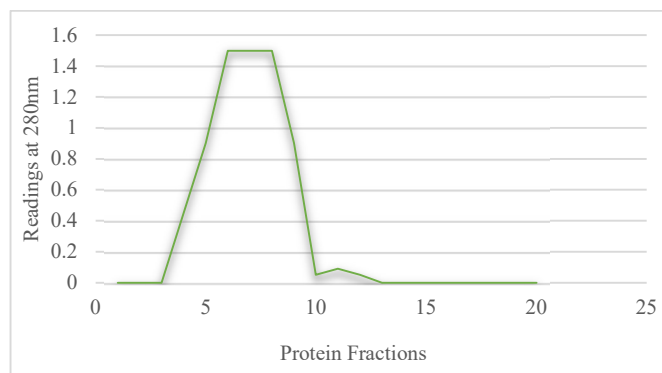


Figure 3.1. Elution profile on Gel Filtration

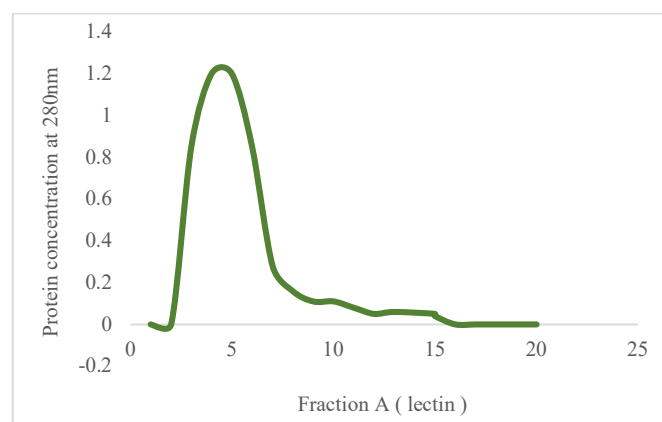


Figure 3.2. Elution profile on Affinity Chromatography

TABLE 1
Hemagglutination Activity of Lectin

Blood Group	A	B	O
Hemagglutination Activity	-	++	+

Strong Activity ++, Mild Activity +, No Activity -

Our findings align with previous studies on lectins, such as the one from *Erythrina indica* leaves, which was isolated using affinity chromatography on Lactamyl-Seralose CL-4B and gel filtration with Sephadex G-100. This lectin agglutinated all human RBC types, with a slight preference for blood group O (Konozy *et al.*, 2013). A novel Entadin lectin was isolated and purified from *Entada rheedii* seeds via ammonium sulfate precipitation and lactose affinity chromatography. It showed the highest agglutination titer against human blood group B red blood cells, and its hemagglutination activity was inhibited by lactose, cellobiose, and galactose (Naik *et al.*, 2020). Lectin from *Artocarpus* species was purified using ammonium sulfate and gel filtration with Sephadex G-200, demonstrating

hemagglutination with human blood group O (Krupa *et al.*, 2021). A lectin from *Erythrina costaricensis* seeds was isolated using O-alpha-D galactosyl polyacrylamide, galactose-derivatized Sepharose, and ConA-Sepharose affinity chromatography, which agglutinated human and rabbit erythrocytes but not others (Perez *et al.*, 1995). A lectin from *Psophocarpus palustris* seeds was purified using gel filtration on a Sephadex G-150 column and specifically agglutinated red blood cells of human type A, without affecting type B or O (Kuku *et al.*, 2005). Lectin from *Lepechinia bullata* seeds was purified by anion exchange chromatography on diethyl aminoethyl (DEAE)-Sephadex, followed by affinity chromatography (Wilches *et al.*, 2017). A lectin from *Phaseolus vulgaris* cv. "Anasazi beans" were isolated through a combination of affinity chromatography on Affi-gel blue gel, FPLC-ion exchange chromatography on Mono S, and FPLC gel filtration on Superdex 200 (Sharma *et al.*, 2019). While, *Parkia biglobosa* seeds were purified by ammonium sulfate precipitation and affinity chromatography on a Sephadex G-100 column, with the *P. biglobosa* lectin (PBL) strongly agglutinating rabbit erythrocytes (Silva *et al.*, 2013).

Circular paper chromatography was performed to the preliminary detection of amino acid present in *Terminalia catappa* seeds lectin. Rf Value were calculated by using formula:

$Rf = \frac{\text{Distance traveled by the solvent front}}{\text{Distance traveled by the compound}}$

$Rf_1 = 3/4 = 0.75$ (Leucine)

$Rf_2 = 2.5/4 = 0.62$ (Phenylalanine)

$Rf_3 = 2/4 = 0.50$ (Tryptophan or Tyrosine)

Leucine typically shows higher Rf values due to its non-polar nature, while aromatic amino acids like Phenylalanine and Tryptophan exhibit moderately lower Rf values due to their interaction with the stationary phase (Harborne, 1998; Sadasivam and Manickam, 1996). The overlap between Tryptophan and Tyrosine in some systems is acknowledged, but further confirmation through UV absorbance or ninhydrin reaction intensity can aid in distinguishing them.

These values were compared with standard Rf ranges reported in literature, confirming the preliminary identification of the amino acids present in lectin isolated from *Terminalia catappa*.

IV. CONCLUSION

This study successfully showed purified lectin-like protein from the seeds of *Terminalia catappa*, a plant known for its therapeutic properties. The lectin was extracted using phosphate-buffered saline (PBS) and purified through a multi-step process, including ammonium sulfate precipitation, dialysis, gel filtration with Sephadex G-75, and affinity chromatography using Seralose CL-4B. The purified lectin demonstrated hemagglutination activity with human blood group B, confirming its biological activity. The findings highlight the potential of *Terminalia catappa* seeds as a source

of bioactive lectins, which could have significant applications in medicine, particularly in immune modulation and pathogen defense. Circular paper chromatography of *Terminalia catappa* seed lectin showed three Rf values: 0.75, 0.62, and 0.50, likely corresponding to Leucine, Phenylalanine, and either Tryptophan or Tyrosine. These identifications match standard amino acid Rf values reported in similar solvent systems. Leucine, being non-polar, traveled furthest, while aromatic amino acids moved less. The results suggest the presence of hydrophobic and aromatic residues in the lectin protein. Future studies could focus on further characterization of the lectin's structure and its specific interactions with carbohydrates to explore its full therapeutic potential.

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