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Study of Lectin Like Protein from *Terminalia catappa* (TC) Seeds for Its Physicochemical and Antimicrobial Properties †

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Abstract: Lectins are a diverse group of proteins crucial in numerous biological activities. They exist in plants, animals, and microorganisms, each with unique structural and functional characteristics. Their ability to exhibit hemagglutination and specifically bind with carbohydrates allows lectins to participate in processes like cell adhesion, immune responses, and intracellular signaling pathways. Lectins are particularly noted for their roles in counteracting viral diseases, regulating blood sugar levels, and fending off pathogens, and preventing cancer progression. These natural compounds offer potential therapeutic benefits in various healthcare applications. *Terminalia catappa* (TC), known as Indian almond, is a large tropical tree containing flavonoids, tannins, saponins, and phytosterols with medicinal values. This research aimed to investigate the partial purification and characterization of lectins from TC seeds. The process involved extracting and partially purifying the lectin, testing it for hemagglutination assay, temperature and pH stability, EDTA dependence, effect of metal ions, specific sugar determination, and antibacterial activity. Hemagglutination activity was observed in human blood group B+. The findings suggest TC seed lectin is remarkably stable within a moderate temperature range and across a broad pH spectrum. The dependence on EDTA for hemagglutination activity indicates a potential metalloprotein nature, with notable interactions with various metal ions, except Hg^{2+}. While the initial antimicrobial assessment against common bacteria yielded limited results, further studies hold promise for uncovering the full potential of TC seed lectin in healthcare and therapeutic advancements.

Keywords: lectin; *Terminalia catappa*; hemagglutination; characterization

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1. Introduction

Terminalia catappa (TC) is a large tropical tree in the Combretaceae family, also known as the lead wood tree family. It is native to Asia, Australia, the Pacific, Madagascar, and Seychelles [1]. All parts of the *Terminalia catappa* plant have been utilized in traditional medicine due to their antimicrobial, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer properties [2]. Plants have a diverse group of lectins that vary in their molecular structures, biochemical properties, and carbohydrate-binding specificities [3]. Their affinity for carbohydrates enables them to bind to glycoconjugates on cell surfaces, facilitating cell agglutination, recognition, or the modulation of cellular signals [4]. Proteins with hemagglutinating activity, later identified as sugar-specific and named lectins, have been known to exist since the early 19th century [5]. Plant lectins exhibit broad activity against red blood cells (RBCs), irrespective of their origin. These molecules are widely used in studying metastatic conditions based on glycosylation patterns, as well as in the context of benign tumors. Additionally, some plant lectins have demonstrated antifungal properties [6].

The objective of this research was to study the partial purification and characterization of lectins extracted from TC seeds. The process involved isolating and purifying the lectin, followed by a comprehensive assessment. This included testing for hemagglutination activity, evaluating stability across different temperatures and pH levels, determining EDTA dependence, studying the impact of metal ions, identifying specific sugar interactions, and exploring potential antibacterial properties.

2. Methods

2.1. Extraction of Lectin-Like Protein from TC Seeds

Extraction of TC seeds lectin was carried out according to process by Dongre, P., et al., 2019 [6] with little modifications. TC seeds were collected from Mumbai, Maharashtra in India. Seeds were ground using coffee grinder. After that 50 mM phosphate buffer saline (pH 7.2) was added. Then, the homogenized powder in buffer was left 48 h for complete extraction at 4 °C. After filtration, the filtrate was centrifuged at 4000× *g* rpm for 20 mins. re-extraction was performed, and the combined supernatants underwent fractional precipitation at 30% to 90% saturation.

2.2. Partial Purification of Lectin-Like Protein from TC Seeds

1. **Ammonium Sulphate Precipitation** was carried out by the procedure outlined by Dongre, P., et al. in 2019 [6].
2. **Dialysis method** The precipitate from the ammonium sulfate precipitation was dissolved in extraction buffer, placed in a 12 kDa molecular weight cut-off dialyzing bag, and dialyzed for 12 h against distilled water in an ice bath with stirring. The distilled water was changed every 3 h [7].
3. **Determination of Protein content** the protein concentration of lectin was determined by using Lowry Method [6].

2.3. Hemagglutination Activity

Hemagglutination activity test was performed as per method of Bashir, H., *et al.* 2010 [8] with minor adjustment. 100 µL of lectin is mixed with 100 µL 5% erythrocytes suspension incubate at room temperature.

2.4. Temperature, pH, EDTA and Metal Ions Effect on TC seeds lectin

The temperature stability of the hemagglutinating activity of TC seeds lectin was determined by incubating lectin solution at different temperatures 20 to 100 °C [9]. pH stability of TC lectin was studied using hemagglutinating activity by incubating the lectin solutions for 1 h with buffers of different pH values ranging from pH 2 to pH 10 [9]. Lectin was incubated with EDTA and the hemagglutinating activity was assessed before and after addition of Ca^{+2}, Mg^{+2}, and Hg^{+2} ions [9].

2.5. Specific Sugar Determination

D-Glucose, D-Galactose, D-Dextrose, and D-Lactose sugar samples were prepared. These samples were mixed with serially diluted lectin solution and incubated at 37 °C for 30 min and then subsequently mixed with RBC suspension [10].

2.6. Antimicrobial Activity

The agar-well diffusion method was used to test the antimicrobial activity against *E.coli* and *S. aureus*. 100 µL of lectin solution was filled in 6 mm wells, and the plates were refrigerated overnight for diffusion and then incubated at 37 °C for 24 h [11].

3. Results and Discussion

The lectin from TC seeds was partially purified using ammonium sulfate precipitation and dialysis. It strongly agglutinated human erythrocytes, especially those of blood group B, as shown in Figure 1. The results of our study align with other studies on lectins from *Chenopodium quinoa* seeds studied by Pompeu, D. G et al. in 2015 [12] and seeds of *Jatropha curcas L* lectin studied by Phadke, R. M., & Pai, K. (2019) [18]. It was found that blood group B exhibits maximum hemagglutination activity, Lectins from *Phaseolus vulgaris* [6] and *Indigofera heterantha* [14] demonstrated activity towards Human Blood group A. Furthermore, lectins from Indian borage leaves (*Plectranthus amboinicus*) exhibit higher hemagglutination activity with 2% chicken native erythrocytes [13].

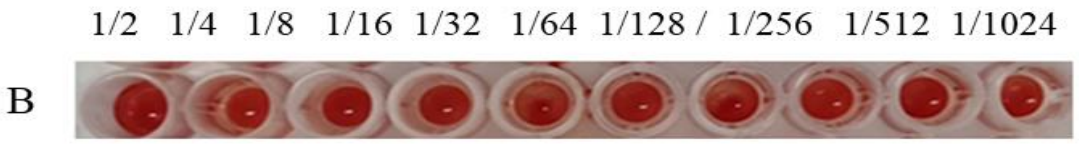
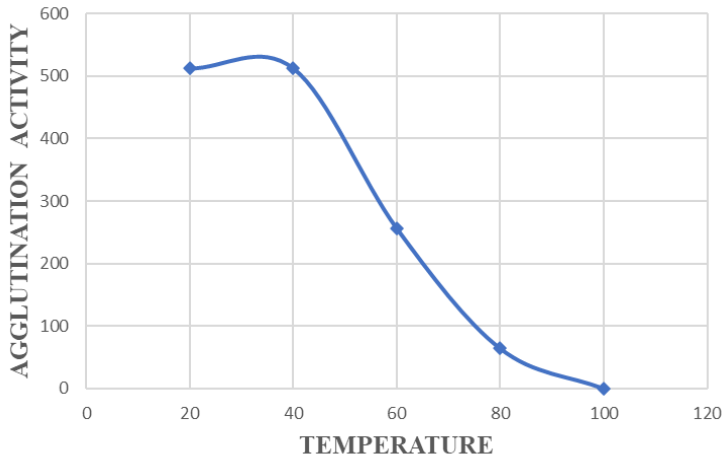


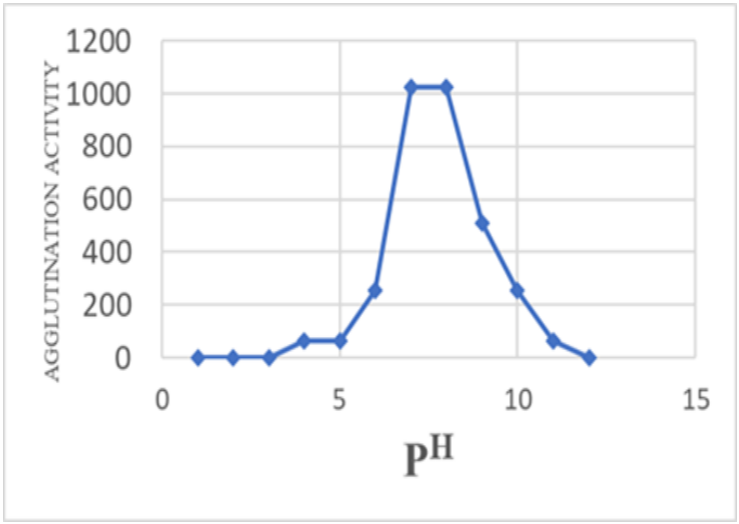
Figure 1. Hemagglutination activity of Blood group B.

TC seeds lectin peak activity occurs at pH 7–8 and temperatures of 20–40 °C, as indicated in Figure 2a,b. Based on literature surveys, similar pH stability results were found *for Indigofera heterantha* lectin (pH 2–9) [14], *Chenopodium quinoa* seeds lectin (pH 2–10) [12], *Jatropha curcas* L. *(*4–10) [17] and *Bryothamnion* triquetrum (pH 4–11) [15]. Similar temperature stability results were also obtained for lectin from the leaves *of Euphorbia tithymaloides* (L.) [10], *Indigofera heterantha* [14], *Jatropha curcas* L. [17] and *Phaseolus vulgaris* seeds [6].

TC seeds lectin binds specifically to galactose and lactose as shown in Table 1, and is responsive to Ca^{2+} and Mg^{2+}, but not Hg^{2+}. Similarly, Acorn Barnacle *Balanus rostratus* [16], *Erythrina speciosa* [9], *Jatropha curcas* L. [17], *Synadenium carinatum* [18] bind towards Galactose sugar and soyabean (*Glycine max*) lectin binds towards N-acetyl galactosamine and D-Galactose [8]. Bhattacharyya et al., 1985 [22] highlighted the metalloprotein nature of lentil lectin (LcH), which relies on metal ions like Ca^{2+} and Mn^{2+} for its saccharide-binding activity. Interestingly, D. biflorus lectin is unique in requiring only a single cation to maintain its binding activity, unlike most lectins that depend on a combination of Ca^{2+}, Mn^{2+}, Mg^{2+}, or Zn^{2+} [21].



(**a**)



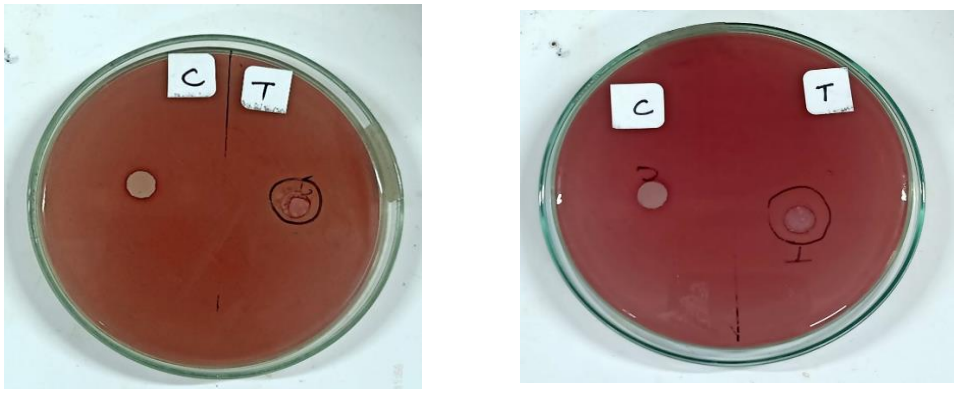
(**b**)

Figure 2. (**a**,**b**) Graph of Temperature and pH against agglutination activity.

Table 1. Hemagglutination inhibition assay on sugar.

Sugar	D-Glucose	D-Galactose	D-Dextrose	D-Lactose
Hemagglutination Activity	No Inhibition	Inhibition	No Inhibition	Inhibition

TC seeds lectin exhibits antimicrobial activity against *E. coli* and *S. aureus*, as illustrated in Figure 3a,b. Lectin from the tubers of *Xanthosoma violaceum* exhibits antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* but not *Pseudomonas aeruginosa* [9], a maize lectin-like protein displays antifungal activity against *Aspergillus flavus* [20], Notably, the *Indigofera heterantha* lectin exerts a significant antibacterial effect on four strains: *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* [14].



(**a**) (**b**)

Figure 3. (**a**,**b**) Antimicrobial activity of *E.coli* and *S. aureus*.

4. Conclusions

In this study, lectin isolated from the TC seeds was partially purified using the ammonium sulfate precipitation method at 30–90% saturation, followed by dialysis. The partially purified TC seed lectin showed strong agglutination with human erythrocytes, particularly with the Blood group B+ compared to other blood groups. Its maximum activity was observed at a pH range of 7–8 and a temperature range of 20–40 °C. The lectin's

activity was inhibited at highly acidic and basic pH levels, and its hemagglutination activity started to decrease at 60 °C, becoming inactive at 100 °C. It specifically binds to galactose and lactose and is responsive to Ca^{2+} and Mg^{2+} ions, but not to Hg^{2+} ions. A initial study indicated the presence of antimicrobial activity against *E. coli* and *S. aureus*, which requires further detailed investigation**.**

The literature and reviews clearly demonstrate the pivotal role of lectins in medicinal, molecular, and cellular biology. These versatile proteins have numerous applications, from treating a variety of illnesses and serving as potential therapeutic agents, to acting as crucial biomarkers for disease diagnosis. The increasing focus on lectins in recent research underscores their significant potential. To fully realize their benefits for future applications in food and medicine, it is essential to undertake comprehensive experimental research on the plants that produce these proteins. This research could lead to innovative therapeutic strategies, improved diagnostic tools, and enhanced food security. Understanding the diverse roles and mechanisms of lectins will be key to advancing their use in both medical and agricultural fields.

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