

Isolation And Characterization Of A Sugar Specific Lectin From *Tamarindus Indica* Exhibiting Strong Hemagglutination Activity

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Abstract

Lectins are carbohydrate-recognizing proteins known for their capacity to agglutinate erythrocytes through specific glycan interactions. Screening of native plant species is an effective approach for identifying novel lectins with potential biomedical relevance. The present investigation aimed to evaluate hemagglutination activity from *Tamarindus indica* seeds collected from the Karnataka University Dharwad region and to characterize the most potent lectin source based on carbohydrate specificity, thermal and pH stability, and molecular mass determination. Fresh *Tamarindus indica* seeds were prepared and assessed for their ability to agglutinate human erythrocytes. The extract from *Tamarindus indica* seeds, which exhibited the reactivity, was selected for further biochemical analyses. Total protein content was quantified, sugar inhibition assays were performed, and the influence of pH and temperature on lectin activity was evaluated. SDS-PAGE was used to determine the approximate molecular weight of the lectin in crude and partially purified fractions. The screened extracts demonstrated hemagglutinating ability, showing the strongest agglutination, particularly against A erythrocytes (titer value: 48). The crude extract contained 10 mg/mL of protein. Glucose inhibited hemagglutination at 500 mM, confirming binding specificity toward monosaccharides. The lectin exhibited maximum activity at neutral pH and remained functionally stable across pH 4–10. Temperature stability assays showed that activity was retained between 40–70 °C and at room temperature but was completely lost at 90°C. SDS-PAGE revealed distinct protein bands between 35–45 kDa, indicating the molecular mass of the lectin. The lectin derived from *Tamarindus indica* seeds is pH and heat-tolerant, preferentially agglutinates A group erythrocytes, and possesses characteristic monosaccharide specificity. These features suggest strong potential for application in diagnostic and suggesting potential biochemical and biomedical relevance.

Keywords: *Tamarindus indica* seeds, Hemagglutination, Sugar specificity, Ph, Temperature

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I. Introduction

Lectins are a diverse group of carbohydrate-binding proteins ubiquitously expressed and isolated from viruses, bacteria, fungi, animals, and plants. Among these sources, plants represent one of the richest reservoirs of lectins, particularly in their seeds, where lectin accumulation occurs during seed maturation and typically declines upon germination (Naithani et al., 2021). Although seeds remain the primary source, lectins have also been reported in nearly all plant tissues, including seeds, bark, roots, and tubers. Plant lectins are distinguished by their ability to bind specific carbohydrate structures and glycoconjugates with high affinity and selectivity, without catalyzing chemical modifications (Peumans & Damme, 1998). Based on conserved sequence features and structural motifs, plant lectins are currently grouped into seven major families, including the well-studied legume lectins, having -domain lectins, monocot mannose-binding lectins, and type II ribosome-inactivating proteins, which collectively represent the largest and most diverse lectin groups (Bojar et al., 2022). Legume lectins in particular exhibit high sequence homology across species yet display remarkable variability in carbohydrate specificity. Typically, lectins exist as homo- or hetero-oligomers consisting of two or four subunits, each containing a single carbohydrate-binding site with defined specificity (Lagarda-Diaz et al., 2017a).

Functionally, plant lectins are integral to defense mechanisms against fungal pathogens, bacteria, phytophagous insects, and other environmental threats. Nearly all lectins possess hemagglutination activity and many display potent antitumor, antiviral (including anti-HIV), immunomodulatory, and mitogenic effects on human lymphocytes (Lannoo & Van Damme, 2014). Their ability to recognize subtle glycan alterations on cell surfaces has also enabled lectins to become valuable tools in cancer research. In photodynamic therapy (PDT), porphyrin-based photosensitizers are effective anticancer agents; however, their tumor selectivity is often

insufficient (Xu et al., 2025). Since certain lectins exhibit preferential binding to tumor-associated glycans, they have been explored as potential targeting moieties to enhance porphyrin accumulation in cancer cells, thereby improving PDT specificity. These expanding biomedical applications have intensified research efforts focused on the isolation, purification, and characterization of lectins from diverse biological sources (Glowacka-Sobotta et al., 2024).

Within the legume family (Fabaceae), which includes species such as *Trigonella foenum-graecum* known for its medicinally active seeds, *Tamarindus indica* has emerged as a plant of significant biochemical interest. *T. indica*, commonly known as tamarind, is a long-lived tropical tree traditionally believed to be native to India but botanically traced to sub-Saharan Africa (Kumar et al., 2024). It is now widely cultivated across South Asia, Southeast Asia, Africa, and Central America. The tree grows up to 20–24 meters, producing acidic pulp-rich pods that enclose several hard, protein-rich seeds. Tamarind holds considerable importance in food, traditional medicine, and industry. Historically, various plant part including seeds, bark, pulp, and seeds have been used for treating digestive disorders, inflammation, diabetes, skin infections, and febrile illnesses. Seeds specifically exhibit antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, anticancer, and anti-venom activities, and are used in pharmaceutical formulations, cosmetics, ophthalmic gels, and sustained-release drug delivery systems (Paun et al., 2020).

From a molecular perspective, tamarind seeds constitute a promising source of lectins and other bioactive proteins. The N-acetylglucosamine-specific lectin, structurally related to chitinase class III, has previously been isolated and biochemically characterized from *T. indica* seeds (Cavada et al., 2020). This lectin is implicated in defense signaling and glycan recognition and has been structurally studied through crystallographic analyses. Notably, preliminary investigations including those from our laboratory have indicated the presence of additional lectin isoforms in tamarind seeds, including a novel mannose/maltose-binding lectin exhibiting strong hemagglutinating activity, suggesting a more diverse lectin repertoire than currently documented (Katoch & Tripathi, 2021).

Tamarindus indica seeds were selected for the present study due to their abundance, underutilization as a biological resource, and previous reports indicating the presence of lectins in leguminous seeds. Despite the extensive use of *T. indica* in traditional medicine and food systems, limited information is available on the biochemical characterization of seed lectins from this plant. Therefore, the present investigation was undertaken to isolate and characterize lectins from *T. indica* seeds,

II. Materials And Methods

Collection of Plant Material and Chemicals

Fresh seeds of *Tamarindus indica* were collected during March 2024 from the Botanical Garden, Karnataka University, Dharwad, India (15°26'28.5"N, 74°59'2.1"E). The seeds were washed thoroughly, air-dried, and immediately processed for lectin extraction. For hemagglutination studies, Human blood samples were collected through the civil Hospital, Dharwad. All chemicals used were of analytical grade. Ammonium sulfate, sodium chloride, dialysis membrane (MWCO 50 kDa), pH buffers (2–12), acrylamide, bisacrylamide, β -mercaptoethanol, Coomassie Brilliant Blue R-250, urea, thiourea, DTT, CHAPS, IPG strips, glycerol, SDS, and TEMED were procured from Himedia (India) and Sigma-Aldrich (USA). Additional reagents such as EDTA, trypsin, bovine serum albumin (BSA), folin ciocalteu reagent (FCR), and other electrophoresis-grade chemicals were purchased from Sisco Research Laboratories (SRL) and Himedia. Sugars used for inhibition assays including D-glucose, D-mannose, D-ribose, D-fructose, L-arabinose, lactose, and maltose were obtained from Sigma-Aldrich. All glassware and plasticware used were of sterilizable laboratory quality.

Extraction of Lectin from *Tamarindus indica* Seeds

30 g of *Tamarindus indica* seeds free of seed coat were ground and removed fat by washing with hexane for 1 h, followed by four parts (w/v) of 1:2 ratio EtOH + CHCl₃ for 30 min. Lectin extraction was carried out following a modified protocol described by (Katre et al., 2005). Approximately 20 g of Dried *Tamarindus indica* seeds were fine powdered and separated through 50 mm sieve. Then, powder was mixed with 20 mL of phosphate-buffered saline (PBS; 100 mM, pH 7.2) supplemented with 200 mM EDTA and 200 mM PMSF to inhibit proteolysis. The homogenate was kept under gentle stirring at 4 °C overnight. The mixture was filtered through muslin cloth and centrifuged at 5000 rpm for 20 min at 4 °C to remove insoluble debris. The clear supernatant was collected and stored at 4 °C for subsequent purification.

Purification of Crude Extract & Estimation Protein concentration

Dialysis was performed to eliminate low-molecular-weight impurities. The crude extract was transferred into dialysis tubing (MWCO 50 kDa) and dialyzed against normal saline for 24 h at 4 °C with intermittent buffer changes. Ammonium sulfate precipitation was carried out by gradually saturating the dialyzed extract to 0–100% saturation at 4 °C. The precipitated protein fraction was collected by centrifugation at 6000 rpm for 30 min. The

resulting pellet was resuspended in normal saline and dialyzed thoroughly to remove residual ammonium sulfate. This ammonium sulfate fraction (ASF) served as the partially purified lectin sample for further characterization (M. Osman et al., 2016). The protein content of the samples obtained during the purification process was determined by the method of (Lowry et al., 1951) using bovine serum albumin as the standard. Readings at 280 nm were also used to determine the protein content of the column eluates.

Preparation of Trypsinized Erythrocytes

Blood samples of Human (A, B, O) were collected into tubes containing 4% sodium citrate. The erythrocytes were pelleted at 1500 rpm for 5 min, washed thrice with isotonic saline, followed by washing with PBS. The packed erythrocytes were adjusted to OD 2.5 at 660 nm. Trypsinization was performed by incubating erythrocytes with 0.025% trypsin at 37 °C for 1 h. Excess trypsin was removed by repeated saline washing, and the final erythrocyte suspension was adjusted to OD 3.5 at 660 nm. This suspension was used for hemagglutination and inhibition assays (Zhang et al., 2000).

Haemagglutination test

Hemagglutination activity of *Tamarindus indica* lectin was assessed in a 96-well U-bottom microplate using a two-fold serial dilution assay with trypsinized human erythrocytes. For each row, 100 µL of standard lectin and 100 µL of RBC suspension were added to the first well as the positive control. The final well of each row received 100 µL of RBC suspension and 100 µL of saline, serving as the negative control. All intermediate wells were filled with 100 µL each of RBCs, saline, and lectin solution. To initiate the assay, 10 µL of the normalized crude freeze-dried lectin was dispensed into the first well and serially diluted across subsequent wells. The plate was incubated at room temperature for 1 hour and then visually inspected for agglutination. Both the lowest and highest dilutions showing agglutination were recorded. The highest dilution that still produced visible hemagglutination was defined as the hemagglutination titer, and the minimum lectin concentration capable of causing agglutination was designated as one hemagglutinating unit (1 HAU).

Sugar Specificity Assay

Sugar inhibition assays were performed as described earlier (Kurokawa et al., 1976). Two-fold serial dilutions of each selected sugar were prepared in normal saline. A fixed amount of lectin corresponding to 4 HAU was added to each sugar dilution and incubated at 37 °C for 1 h. After incubation, 50 µL of 2% trypsin-treated erythrocytes was added, and the mixtures were kept at room temperature for 30 min. The minimum sugar concentration that completely inhibited hemagglutination was recorded as the inhibitory concentration.

Effect of pH & Temperature Stability of Lectin

The pH stability was done to observe the optimum pH for maximum activity of lectins present in the seeds of the partially purified plant lectin. The pH dependence of seeds lectin was determined by using buffers ranging from pH 1–10. For pH 1: 0.1N HCl, for pH 2 & 3: 0.2M glycine - HCl buffer, for pH 4 & 5: 0.2M sodium acetate buffer, for pH 6 & 7: 0.2M sodium phosphate buffer, for pH 8: 0.2M Tris HCl buffer, for pH 9: 0.2M glycine-NaOH buffer and for pH 10: 0.2M carbonate-bicarbonate buffers were used. 100 µL of lectin was incubated with 100 µL of different buffer solutions for 30 minutes at room temperature and then assayed for agglutination with 2% erythrocytes (Suseelan et al., 1997a). Thermal stability was tested by incubating 100 µL aliquots of lectin at temperatures ranging from 30–90 °C for 30 min. Following heat treatment, samples were cooled to room temperature, and hemagglutination activity was evaluated using the standard assay (Wong & Ng, 2006a).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Lectin purity and molecular weight were analyzed by SDS-PAGE following Laemmli's discontinuous buffer system (Laemmli, 1970a). A 10% resolving gel and 4% stacking gel were prepared. Approximately 25 µg of protein was loaded per well. Electrophoresis was performed at 70 V for stacking and 100 V for resolving. Gels were stained using 0.1% Coomassie Brilliant Blue R-250 (50% methanol, 10% acetic acid) and destained until clear band resolution was obtained. Pre-stained molecular weight markers (20–140 kDa) were used for calibration. Proximate Molecular weights were determined using the Bio-Rad Protocol Bulletin 6210.

Statistical analysis

The statistical analyses were conducted using Excel Office-2019. A p-value of ≤ 0.05 was considered statistically significant. All experiments were performed in triplicate, and results are expressed as mean \pm standard deviation (SD), with error bars in the graphs representing the SD.

III. Results

Selection of sample

The seeds sample of the medicinal plant *Tamarindus indica*, from Karnatak University Dharwad was selected for screening due to its traditional medicinal relevance and limited prior research on its seeds proteins. Preliminary assays showed strong hemagglutination activity against all blood groups, making the seed extract a suitable candidate for further lectin characterization.



Fig. 1 Collection of seed samples and initial steps involved in lectin extraction from *Tamarindus indica*. (a) Collect of fresh dried seed sample of *Tamarindus indica* (b) weighing of seeds sample (c) extraction of lectin from seed sample

Agglutination Assay

Tamarindus indica exhibited agglutination with all tested erythrocytes and showed no strict discrimination among blood groups. As shown in Table 1, the strongest hemagglutination was observed with A erythrocytes, whereas B and O groups displayed comparatively weaker reactions. In Fig. 2, A, B, O group erythrocytes formed characteristic carpet patterns up to dilutions of 6, 5 and 5 respectively beyond these points, button formations appeared, indicating the loss of agglutination. Although *Tamarindus indica* did not exhibit absolute blood group specificity, it demonstrated varying degrees of affinity, with the highest titre observed against Blood group A erythrocytes (1:48) and the lowest at 1:24. Based on these findings, A group erythrocytes were used for all subsequent analyses, as they provided maximum hemagglutination activity. Protein estimation of the dialyzed extract using the Biuret method revealed a total protein concentration of 10 mg/mL Table 2.

Table 1 Agglutination study of lectins of *Tamarindus indica* seeds with human erythrocytes.

Sl. No	Erythrocytes								Agglutination
1		A							+++
2		B							++
3		O							++

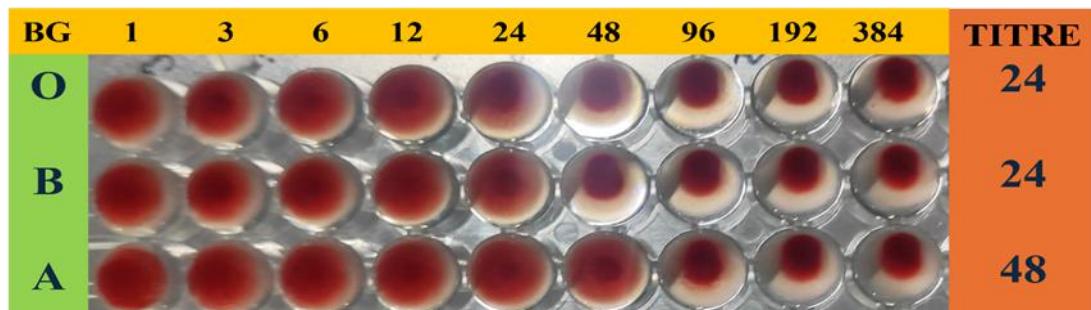


Fig. 2. Hemagglutination activity of *Tamarindus indica* lectin with different human blood group erythrocytes.

Table 2 Protein concentration in seeds of *Tamarindus indica*

	Protein (mg/ml ⁻¹)	HAU/ml A	HAU/ml B	HAU/ml O	SA A	SA B	SA O
Dialysed extract	10	48	24	24	4.8	2.4	2.4

Note: HAU-Hemagglutination Unit, SA-Specific activity.

Sugar specificity assay

To evaluate the carbohydrate-binding preference of the lectin, a sugar inhibition assay was performed using a panel of carbohydrates (Table 3). Among all the tested carbohydrates, only glucose was able to inhibit hemagglutination, with a minimum inhibitory concentration (MIC) of 500 mM. None of the other monosaccharides or disaccharides produced any detectable inhibition. These findings indicate that the lectin exhibits a strong affinity for simple sugars, particularly glucose, while showing no recognition of more complex carbohydrates. This monosaccharide specificity may also contribute to its selective interaction with certain human blood groups.

Table 3 Inhibition of agglutination with different sugars by lectins of *Tamarindus indica* seeds

Sl. No	Sugar	Minimum concentration required to inhibit the hemagglutination (mM)
1	Fructose	No Inhibition
2	Sucrose	No Inhibition
3	Maltose	No Inhibition
4	Arabinose	No Inhibition
5	Xylose	No Inhibition
6	Lactose	No Inhibition
7	Glucose	Inhibition (500mM)

Effect of pH & Temperature

To know the optimum pH for lectin activity, lectin was extracted in different buffers with varied pH ranging from 1 to 14. The hemagglutination activity was retained and showed stability up to pH ranging from 4 to 10. However, there was a gradual decrease in activity when the pH was below 4. The lectin retained activity up to 75% at pH 3 and up to 60% at pH 2. Agglutination activity was lost below pH 4 and above pH 10 as mentioned in Fig. 3 (a). To determine the stability of lectin activity over different temperatures, lectin was extracted and incubated at different temperatures from 30 to 90 °C for one hour, and then, the hemagglutination activity was determined. As depicted in Fig.3 (b), Lectin from seeds of *Tamarindus indica* shows 100% agglutination between temperatures ranging from 40- 70°C when tested after being heated for 1 h at temperatures above 70°C. Below 40°C was stable as the temperature increases, the activity decreases and is finally lost at 90°C. According to the observed result, lectin was stable for a long period, but it was thermally inactive after heating at 90 °C, but the same activity remained for several days. This may be attributed to the inactivation of proteases that are present in the extract. Furthermore, lectin activity was also stable for at least seven days when it was kept at room temperature.

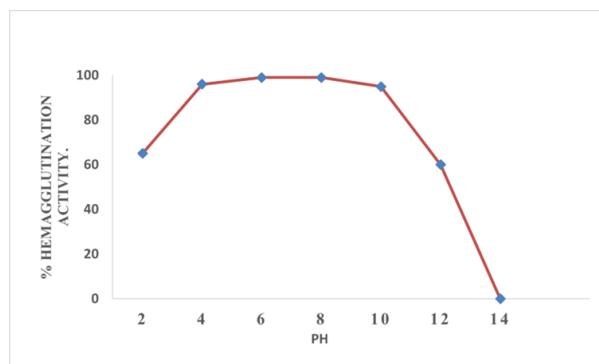


Fig.3 (a). Effect of pH on Agglutination Activity of the Lectin of *Tamarindus indica* seeds

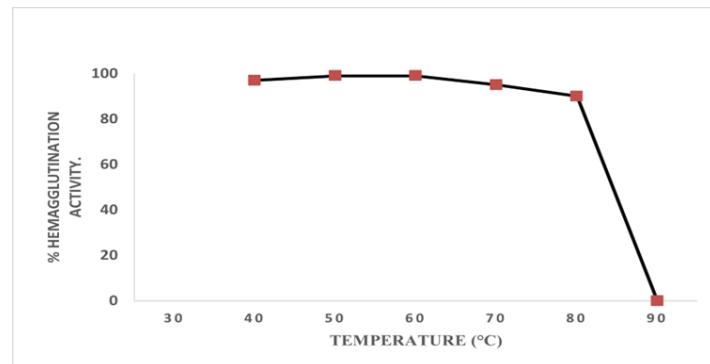


Fig. 3 (b). Effect of temperature on Agglutination activity of lectin of *Tamarindus indica* seeds

SDS-PAGE

The analysis of partially purified lectin. SDS-Polyacrylamide gel electrophoresis of crude and ammonium sulphate precipitated fractions was performed to analyze the number of proteins present in the samples. As shown in Fig. 4, after 30-60 % of ammonium sulphate precipitation, the number of protein bands was reduced significantly (Lane-3) compared to the crude sample (Lane-1). The common protein bands that are present in all the fractions are near the molecular weight, ranging from 35 to 45kDa. These could be the protein bands which may be associated with lectin activity.

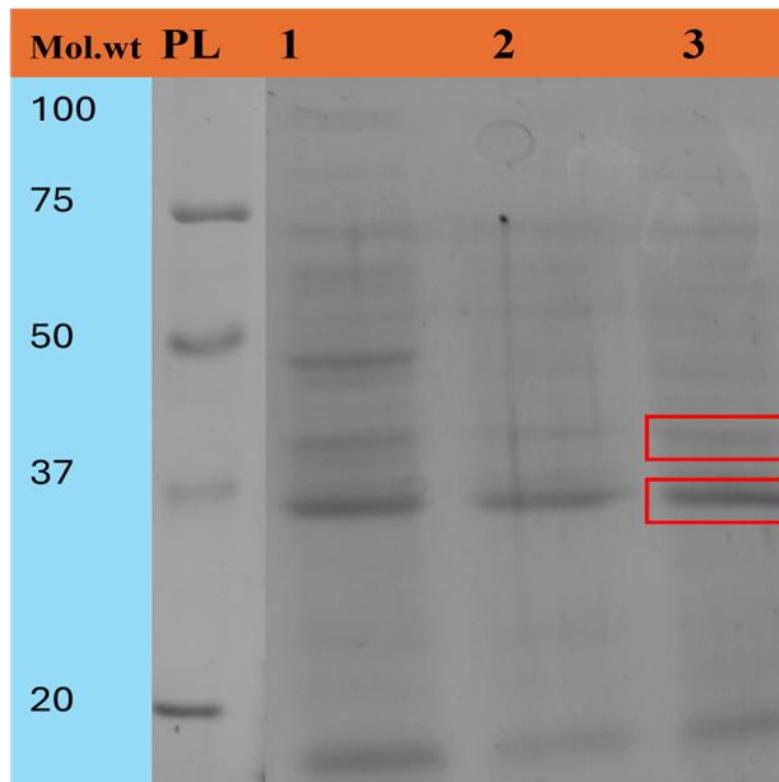


Fig. 4 SDS-PAGE analysis of proteins precipitated from the *Tamarindus indica* seed crude extract. Common bands that are present in crude extract (lane-1), 0-30% (lane-2), and 30-60% (lane-3) ammonium sulphate precipitated proteins are closely associated with molecular weight from 35 to 45kDa.

IV. Discussion

Lectins are widely distributed carbohydrate-binding proteins, particularly abundant in leguminous seeds, where they are believed to play important roles in storage and defense. In the present study, a sugar-specific lectin was isolated and partially characterized from *Tamarindus indica* seeds, and its hemagglutination activity, carbohydrate specificity, physicochemical stability, and molecular weight were evaluated and compared with previously reported plant lectins. The seed extract exhibited strong hemagglutination activity against all tested human erythrocytes (A, B, and O), with the highest titer observed for blood group A. Similar patterns of differential but non-blood-group-specific agglutination have been reported for several legume lectins, including those from *Trigonella foenum-graecum*, *Vigna mungo*, and *Canavalia* species, where variations in agglutination strength were attributed to differences in erythrocyte surface glycan composition rather than strict antigen specificity (Lagarda-Diaz et al., 2017). Previous studies on *T. indica* seeds have also demonstrated hemagglutinating activity, although differences in erythrocyte preference and activity levels suggest the presence of multiple lectin isoforms or variations due to extraction conditions (M. E. M. Osman et al., 2016).

Carbohydrate inhibition assays revealed that hemagglutination activity was specifically inhibited by glucose at a concentration of 500 mM, whereas other tested mono- and disaccharides failed to inhibit agglutination. This finding indicates that the lectin characterized in this study exhibits glucose specificity. Earlier reports on *T. indica* seed lectins have described lectins with N-acetylglucosamine or mannose/maltose specificity, highlighting the diversity of lectin types within the same plant species (Cavada et al., 2020b). The requirement of a relatively high glucose concentration for inhibition is consistent with observations for several plant lectins, where multivalent interactions between lectins and cell-surface glycoconjugates compensate for low monosaccharide affinity (Wong & Ng, 2006). The present results therefore support the existence of a distinct glucose-specific lectin in *T. indica* seeds.

The lectin demonstrated considerable stability across a broad pH range (4–10), with maximum hemagglutination activity near neutral pH, and retained activity under moderately acidic conditions. Such pH tolerance is a characteristic feature of many seed lectins and has been reported for lectins isolated from *Dolichos biflorus*, *Vigna unguiculata* and *Phaseolus* species (Suseelan et al., 1997). Thermal stability studies showed that the lectin retained activity between 40 and 70 °C but lost activity at 90 °C, indicating moderate heat tolerance. Similar thermal behavior has been reported for legume lectins, which are generally stable at elevated temperatures but undergo irreversible denaturation at higher temperatures due to disruption of their tertiary structure (Laemmli, 1970). The observed stability at room temperature for several days further suggests that the lectin is structurally robust and resistant to degradation.

SDS-PAGE analysis of the crude and partially purified fractions revealed protein bands in the molecular weight range of 35–45 kDa, which corresponds well with the reported molecular weights of many legume lectins. Lectins from *T. indica* and related Fabaceae members typically exhibit monomeric subunits within this range, although minor variations have been attributed to glycosylation or isoform heterogeneity (Naithani et al., 2021). The reduction in the number of protein bands following ammonium sulfate precipitation indicates partial enrichment of the lectin fraction and supports the association of these bands with hemagglutination activity.

Overall, the findings of this study are consistent with previous reports on legume seed lectins while also demonstrating distinct features, particularly glucose specificity and stability characteristics. These results contribute to the growing understanding of lectin diversity in *Tamarindus indica* seeds and provide a biochemical basis for future detailed purification and functional studies.

V. Conclusion

The present study demonstrates that *Tamarindus indica* seeds are a valuable source of a biologically active, sugar-specific lectin with strong hemagglutination activity. The lectin exhibited differential agglutination of human erythrocytes, with the highest activity toward blood group A, and showed specific inhibition by glucose, indicating a distinct carbohydrate-binding preference. The lectin displayed considerable stability across a wide pH range and moderate thermal conditions, and SDS-PAGE analysis revealed protein bands in the 35–45 kDa range, consistent with typical legume lectins. Comparison with earlier studies suggests that *T. indica* seeds contain diverse lectin isoforms with varying sugar specificities and biochemical properties. These findings enhance the current understanding of *T. indica* seed lectins and provide a foundation for further purification, structural characterization, and exploration of potential biochemical and biomedical applications.

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Conflict of Interest Authors have declared that no conflicts of interest exist.

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