

PARTIAL PURIFICATION AND CHARACTERIZATION OF A NEWLY IDENTIFIED LECTIN-LIKE PROTEIN FROM *ELEUSINE CORACANA* (FINGER MILLET) SEEDS

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Abstract

Lectins are carbohydrate-binding proteins with significant biological activities, including antimicrobial, anticancer, and antioxidant properties. *Eleusine coracana* (finger millet), a nutritionally rich cereal traditionally valued in India, has not been extensively explored for lectin properties. This study aimed to partially purify and characterize a lectin-like protein from *Eleusine coracana* seeds, with a focus on its hemagglutination activity, pH - temperature stability, sugar specificity, effects of metal ions and EDTA. Lectin partial purification was performed using ammonium sulphate precipitation followed by dialysis, yielding a protein concentration of 1 mg/mL. Hemagglutination assays revealed highest activity toward human blood group A+. The lectin was active within a pH range of 5-9, with maximum stability at pH 6-7, and retained high activity between 20-50 °C, with thermal stability up to 70°C. Sugar specificity assays showed mild hemagglutination inhibition by galactose. EDTA completely abolished hemagglutination activity, while Ca²⁺, Zn²⁺, and Mg²⁺ enhanced its activity, suggesting lectin's metal dependence. These results demonstrate presence of lectin with distinct stability and characteristics in *Eleusine coracana* seeds, providing a foundation for further purification, characterization and exploration of its potential biomedical and nutraceutical applications.

Keywords: *Eleusine coracana* seed; Lectin; Hemagglutination activity; Partial purification; Characterization

Introduction

Eleusine coracana (finger millet), also known as ragi, is a nutrient-rich cereal valued for its broad range of health benefits (Malleshi *et al.*, 2025). Finger millet, or ragi, has been cultivated in India since ancient times (Shobana, *et al.*, 2013). Finger

millet's health benefits are largely linked to its polyphenols, dietary fiber, and high calcium content. It exhibits various bioactive effects, such as anti-diabetic, anti-tumor, anti-atherogenic, antioxidant, and antimicrobial

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activities (Bruntha Devi *et al.*, 2014). Finger millet is traditionally malted, yielding a malt that is a good source of α - and β -amylases, proteases, and phytase, and that offers readily digestible carbohydrates and proteins with enhanced mineral bio-accessibility (Malleshi *et al.*, 2025). Proteins exhibiting hemagglutinating activity, later recognized as sugar-specific and termed lectins, have been known since the early 19th century (Sharon, 2007). Lectins are a distinct class of proteins widely distributed in nature, characterized by their ability to selectively recognize and bind carbohydrates and glycoconjugates via specific binding sites. They can be detected using hemagglutination assays and interact with a variety of carbohydrates present on cell surfaces (Shaikh & Uzgare, 2025b). Lectins occur in diverse sources such as plants, animals, viruses, and bacteria, but are particularly abundant in plants (Shaikh & Uzgare, 2025a). These plant lectins are noted for their bioactive properties, including antiviral, anticancer, and immunomodulatory effects (Sharon & Lis, 2004). Exhibiting broad activity toward red blood cells, plant lectins are widely applied in studies of metastasis and benign tumors via glycosylation analysis, and several have demonstrated antifungal effects (Shaikh & Uzgare, 2024a).

The aim of this study is to partially purify and characterize a lectin-like protein from *Eleusine coracana* (finger millet) seeds. Focusing on its temperature stability, pH stability, sugar specificity, and its responses to effect of temperature, pH, metal ions, and EDTA, assessed via hemagglutination assays.

Materials and Methods

Chemicals and reagents:

All reagents and solvents used were of analytical grade, sourced from Himedia, SRL, Loba Chemie, PCL, and Chemsouth (India). Human red blood cell samples were collected from the Blood Diagnostic Centre, Mumbai, Maharashtra, India.

Sample Collection:

Good-quality finger millet (*Eleusine coracana*) seeds were obtained from an agricultural store and identified by the Botany Department of Wilson College (Autonomous), Mumbai, India.

Lectin from *Eleusine coracana* (finger millet) seeds: Isolation

Lectin was extracted from *Eleusine coracana* seeds following the method of (Hiremath *et al.*, 2019) with slight modifications. High-quality seeds were soaked in distilled water at room

temperature for 3 days. 40 grams of the soaked seeds were ground into a fine powder using a mortar and pestle. The powder was defatted by mixing with petroleum ether at a 1:10 ratio and stirring for 4 hours. The defatted powder was then extracted in physiological saline at 4°C for 15 hours. The homogenate was centrifuged at 5,000 rpm for 10-15 minutes, and the extraction was repeated. The combined supernatants were subjected to fractional ammonium sulphate precipitation ranging from 20% to 80% saturation.

Partial Purification of Crude Extract:

The crude extract of *Eleusine coracana* seeds was partially purified using the procedure outlined below:

Ammonium Sulphate Precipitation:

Ammonium Sulphate Precipitation was carried using the method of (Dongre *et al.*, 2019). The crude extract was subjected to fractional ammonium sulphate precipitation between 20% and 80% saturation by slow addition of solid ammonium sulphate with continuous stirring at 4°C. Each fraction was centrifuged at 4,000rpm for 20 minutes, and the resulting pellets were resuspended in physiological saline and dialyzed extensively.

Dialysis:

The ammonium sulphate precipitate was dissolved in physiological saline, placed in a 12 kDa cut-off dialysis bag, and dialyzed against distilled water in an ice bath for 12 hours, with water changes every 3 hours. After dialysis, the lectin-containing fractions were centrifuged at 4000rpm for 15 minutes to remove any insoluble material. The clear supernatant was collected and used for further biochemical characterization. Protein concentration was determined by the Lowry method, and the partially purified lectin was stored at -4°C until further use (Shaikh & Uzgare, 2024a).

Protein Determination:

The protein content of the extract was measured using the Lowry method. Following reaction with Folin-Ciocalteu reagent, absorbance at 660 nm was recorded, and protein concentrations were calculated from a BSA standard curve (Lowry, 1951).

Hemagglutination Activity Assay:

A 2% human erythrocyte suspension in physiological saline was used for the hemagglutination assay. In a microtiter plate, 100 μ L of erythrocytes was combined with 100 μ L

of serially diluted lectin, and agglutination was assessed visually after 30 minutes of incubation at 37 °C (Shaikh, & Uzgare, 2025b).

Effect of pH:

To study the effect of pH on lectin activity, buffers ranging from pH 1 to 12 were prepared as described by (Shaikh, & Uzgare, 2025b). Buffers included 0.2 M sodium acetate-acetic acid (pH 1 to 6), 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate (pH 7 to 12). Serially diluted lectin solutions were mixed with the buffers, incubated at 37 °C for 30 minutes, and hemagglutination was evaluated using a 2% erythrocyte suspension.

Effect of Temperature:

Lectin activity was tested at temperatures between 20°C to 100°C according to (Jawade *et al.*, 2016). Samples were incubated for 30 minutes at different temperatures and subjected to hemagglutination assay with 2% erythrocytes.

Temperature Stability:

The thermal stability of the lectin was assessed following the method of (Qadir *et al.*, 2013). 100 µL of lectin extracted from *Eleusine coracana* seeds were incubated at temperatures ranging from 20°C to 100°C for 30 minutes. After incubation, a 2% red blood cell suspension, PBS pH 7 was added, and the mixture was allowed to stand at room temperature for 30 minutes to evaluate hemagglutination.

pH Stability:

The pH stability of the lectin was assessed following (Qadir *et al.*, 2013). 100 µL of *Eleusine coracana* lectin was incubated in buffers ranging from pH 1 to 12 (0.2 M sodium acetate-acetic acid for pH 1 to 6; 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate for pH 7 to 12) for 30 minutes. A 2% RBC suspension was then added, and hemagglutination was observed after 30 minutes at room temperature.

Sugar Specificity:

The lectin's sugar specificity was evaluated by inhibiting hemagglutination. Solutions of 10mM of different sugars - fructose, dextrose, galactose, lactose, mannose, and sucrose were mixed with equal volumes of serially diluted lectin and incubated at 37°C for 30 minutes. A 2% human erythrocyte suspension PBS pH 7 was then added. Sugars that prevent agglutination indicate the lectin's specific binding preference (Shaikh, & Uzgare, 2025b).

Effect of EDTA on Hemagglutination:

The effect of EDTA on lectin-induced hemagglutination was evaluated according to (Benseghir & Namulodi, 2017) Serially diluted lectin solutions (100 µL) were mixed with 100 µL of EDTA at concentrations of 10, 20, 40, 60, 80 and 100mM and incubated at 37°C for 30 minutes. A 2% erythrocyte suspension was then added. Positive controls (without EDTA) and negative controls (without lectin) were included to validate the assay.

Dependence of Hemagglutination activity on Metal Ions:

The influence of metal ions on lectin-induced hemagglutination was evaluated following standard procedures. Serially diluted lectin solutions (100 µL) were mixed with 100 µL of metal ion solutions (e.g., Ca^{2+} , Mg^{2+} , Ni^{2+} , Hg^{+2} Fe^{+2} and Zn^{2+}) at varying concentrations and incubated at 37°C for 30 minutes. A 2% erythrocyte suspension was then added, and hemagglutination was assessed (Sun *et al.*, 2019).

Results and Discussion

Isolation and Partial Purification:

Eleusine coracana seeds lectin was partially purified through ammonium sulphate precipitation (20-80%), followed by centrifugation and dialysis. Mentioned in Figure 1.

The protein concentration of the partially purified lectin extract was estimated to 1 mg/mL using the Lowry method. Concentration of serially diluted lectin of *Eleusine coracana* (Finger Millet) Seeds use in all hemagglutination assay were mentioned in Table 1. Hemagglutination assays were conducted on Human Rh blood groups A, B, and O. The lectin showed positive agglutination across the blood group system, as summarized in Table 2. All the experiments were done in triplicate.

Negative hemagglutination was indicated by button formation, while positive hemagglutination appeared as mat formation at the bottom of the wells. Among the blood groups, A+ exhibited the highest hemagglutination activity up to a 1/512 dilution, O+ showed activity up to 1/64, and B+ displayed very less hemagglutination activity till 1/8, as shown in Figure 2. In Figure 2 and Figure 5, the images show cropped regions of the original titre plates for clarity.

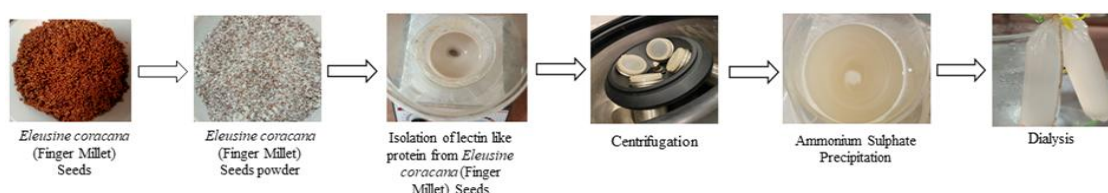
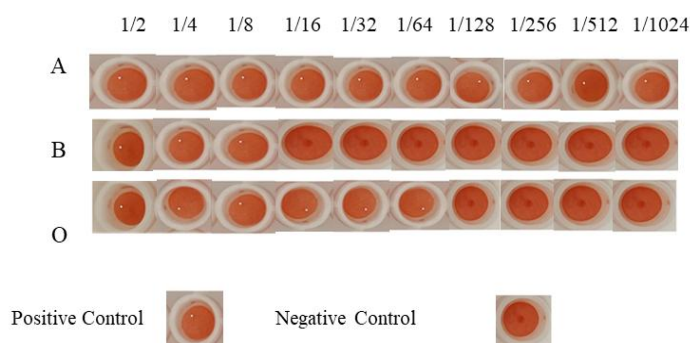
Table 1. Concentration of serially diluted lectin of *Eleusine coracana* (Finger Millet) Seeds in all Hemagglutination Assay

Dilution (Titer Value)	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Concentration of lectin in mg/ml	0.5	0.25	0.125	0.0625	0.03125	0.01562	0.00781	0.00390	0.00195	0.0009765
Concentration of lectin in µg/ml	500	250	125	62.5	31.25	15.62	7.81	3.90	1.95	0.97

Table 2. Hemagglutination assay of lectin of *Eleusine coracana* (Finger Millet) Seeds with Human RBCs

Human Blood Group	A	B	O
Hemagglutination Activity	+++	+	++

+++ is Strong Hemagglutination activity, ++ Mild Hemagglutination activity, + is Minor Hemagglutination activity

**Figure 1. Isolation of lectin like protein from *Eleusine coracana* (Finger Millet) Seeds****Figure 2. Hemagglutination activity of *Eleusine coracana* (Finger Millet) Seeds lectin with 2% RBCs**

Our findings are consistent with previous studies. Lectin from the seeds of *Crotalaria striata* exhibited hemagglutination activity towards blood group A⁺ (Khang *et al.*, 1990), and lectins from *Phaseolus vulgaris* (Red Kidney Bean) also demonstrated hemagglutination activity in human blood group A⁺ (Dongre *et al.*, 2019). (Zubcevic *et al.*, 2016) reported that plant lectins exhibit activity in blood groups A, B, and AB, with 40% of lectins showing activity in blood group A and 50% in other blood groups. Similarly, (Shaikh & Uzgar 2024a) showed that partially purified lectins exhibited activity towards blood group B⁺.

No hemagglutination activity was detected in the pH range of 1 to 4 during the pH effect test. Activity was evident between pH 5 to 10, with the highest hemagglutination observed at pH 6 and 7, as summarized in Table 3. This suggests that the lectin from *Eleusine coracana* seeds is active within the pH range of 5 to 10. Only minimal activity was noted at pH 10, while no activity was observed at pH 11 and 12, consistent with the titer values shown in Figure 3. The lectin was found to be stable between pH range 4 to 9, maximum stable at pH 6 and 7 and unstable at 12. The result is summarized in Table 3 and Figure 3. The titer value shown in

the figure 3 represents the hemagglutination activity of the lectin, determined using the twofold serial dilution method. These values were obtained from both the pH stability and pH effect experiments, indicating the highest dilution of the lectin sample that was still capable of causing visible agglutination of erythrocytes under the respective temperature conditions.

According to literature reports, lectins from various sources exhibit broad pH stability. For example, the lectin from black turtle bean (*Phaseolus vulgaris*) remained stable across a pH range of 2 to 10 (He *et al.*, 2015). An extracellular lectin obtained from the culture liquid of *Thermodesulfobacterium mobile* ECM-1128 was stable between pH 4-8 (Kobelev, & Bagaeva, 2015). Similarly, the lectin from *Phaseolus vulgaris* L. cv. white seeds (white kidney bean) showed stability across pH 4 to 11 (Al-Alwani *et al.*, 2013). Overall, lectins demonstrate remarkably high pH stability, often within the range of 4 to 10 (Konozy & Osman, 2024) and our results are consistent with the reported findings.

Effect of Temperature and Temperature Stability:

Temperature studies on Hemagglutination activity was carried out between the temperature range 20°C to 50°C. The Figure 4. value indicates that as the temperature changes above and below this range, lectin activity gradually decreases and is totally absent at 90°C. From the Table 4, we can deduce that *Eleusine coracana* (Finger Millet) seeds contain lectin which is active between temperatures 20°C to 50°C and as shown by the peaks in the curve as shown in figure 4. As the temperature exceeds 80°C, activity and stability gradually decrease and cease at 90°C. The lectin is stable between temperature range 20°C to 70°C with maximum stability between temperature range 20°C to 50°C and unstable at 80°C. Figure 4 and

Table 4. The titer value shown in the figure 4 represents the hemagglutination activity of the lectin, determined using the twofold serial dilution method. These values were obtained from both the temperature stability and temperature effect experiments, indicating the highest dilution of the lectin sample that was still capable of causing visible agglutination of erythrocytes under the respective temperature conditions.

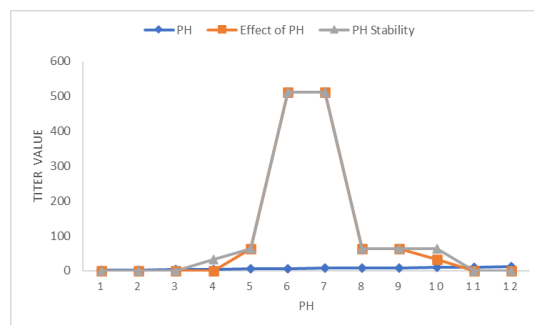


Figure 3. Graph of Effect of pH and pH Stability

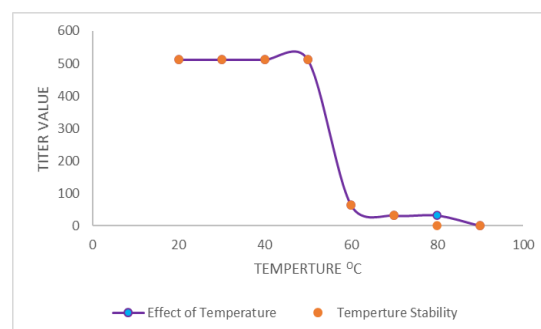


Figure 4. Graph of Effect of Temperature and Temperature Stability

Table 3. Effect of pH and pH stability of lectins from *Eleusine coracana* (Finger Millet) Seeds

pH	1	2	3	4	5	6	7	8	9	10	11	12
Effect of pH	-	-	-	-	+	++	++	+	+	+	-	-
pH Stability	-	-	-	+	+	++	++	+	+	-	-	-

++ is Strong Hemagglutination activity, + Mild Hemagglutination activity, - is No Hemagglutination activity

Table 4. Effect of Temperature and Temperature stability of lectins from *Eleusine coracana* (Finger Millet) Seeds

Temperature in °C	20°C	30°C	40°C	50°C	60°C	70°C	80°C	90°C
Effect of Temperature	++	++	++	++	+	-	-	-
Temperature Stability	++	++	++	++	+	+	-	-

++ is Strong Hemagglutination activity, + Mild Hemagglutination activity, - is No Hemagglutination activity

Our study aligns with previous lectin studies. Lectin from *Terminalia catappa* seeds shows temperature dependence between 20 to 60°C (Shaikh & Uzgaré, 2025b), lectin activities in Texas (*Quercus fusiformis*) crude extracts show temperature stability between 0 to 50°C (Ynalvez *et al.*, 2011), *Erythrina* (*Erythrina speciosa*) seed lectin (Konozy *et al.*, 2002) and leaf lectin of *Euphorbia tithymaloides* (L.) show activity up to 70°C (Jawade *et al.*, 2016).

Sugar Specificity:

Lectin concentration used was 1 mg/mL for all the experiment and 10mM sugar solution were prepared.

Assessing sugar specificity showed that galactose elicited mild hemagglutination inhibition. Further investigation using N-acetylgalactosamine is required, as the lectin exhibited hemagglutination specifically with blood group A, whose characteristic sugar is N-acetylgalactosamine a derivative of galactose. Testing with this sugar will help confirm the lectin's binding affinity. Sugars fructose, dextrose, mannose, and sucrose did not exhibit any inhibitory effect, as presented in Table 5, thereby excluding them as potential sugar moieties for lectin recognition.

As reported lectins from various plant sources exhibit distinct sugar specificities. For example, lectin from *Terminalia catappa* seeds displays specificity toward lactose and galactose, similar to that observed in *Eleusine coracana* (finger millet) seed lectin (Shaikh & Uzgaré, 2025b). Lectin from the Indian borage plant (*Plectranthus amboinicus*)

shows specificity toward dextrose (Arekal *et al.*, 2023), whereas a galactose-specific lectin has been isolated from *Chrysophyllum cainito* (Madayi *et al.*, 2016). Peanut lectin is also characterized by its affinity for galactose (Ravishankar *et al.*, 1999), and galactose-specific lectin activity has been reported from *Bosqueia angolensis* pulp (Adedugbe *et al.*, 2022).

Effect of EDTA on Hemagglutination:

No hemagglutination activity was observed at any of the tested EDTA concentrations (10, 20, 40, 60, 80, and 100 mM). the absence of hemagglutination activity in the presence of EDTA suggests that the *Eleusine coracana* (Finger Millet) seed lectin may be a metalloprotein, with its activity dependent on metal ions.

Effect of Metal Ions on Hemagglutination:

In the present study the hemagglutination activity of *Eleusine coracana* (Finger Millet) seed lectin is enhanced in the presence of metal ions such as Ca^{2+} , Zn^{2+} , and Mg^{2+} , but is inhibited by ions like Hg^{2+} , Fe^{2+} , and Ni^{2+} , as shown in Figure 5. In Figure 5, the positive control demonstrates lectin hemagglutination activity and is observed as a uniform mat formation for all metal ions tested. In contrast, the negative control shows button formation, indicating the absence of hemagglutination activity, which is also consistent and identical across all metal ions tested individually with RBC's alone. These observations suggest that the lectin may function as a metalloprotein.

Table 5. Sugar specificity test of *Eleusine coracana* (Finger Millet) seeds lectin

Name of the Sugars	Fructose	Dextrose	Galactose	Lactose	Mannose	Sucrose
Hemagglutination activity	++	++	-	+	++	++

++ is Strong Hemagglutination activity, + Mild Hemagglutination activity, - is No Hemagglutination activity

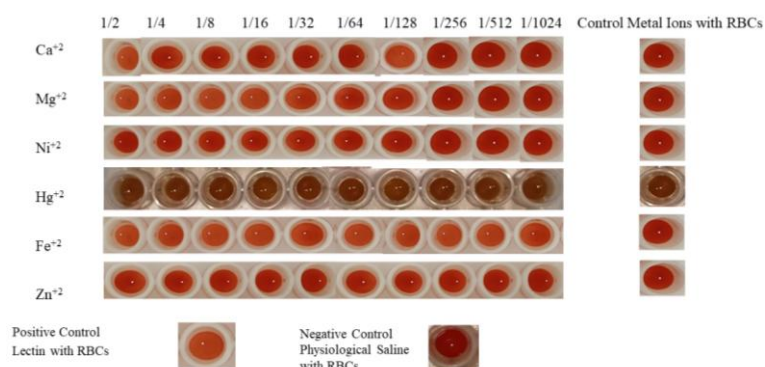


Figure 5. Hemagglutination activity on Metal ion

Our findings augment the reports on other lectins that displayed metal ion dependence. For instance, the lectin from *Zizyphus oenoplia* is a metalloprotein whose hemagglutinating activity is completely restored by 1 mM Mg^{2+} or Mn^{2+} (Butle & Patil, 2015). The lectin from *Chlorella sorokiniana* depends on Mg^{2+} and Ca^{2+} (Savitha *et al.*, 2023), while leaf and seed lectins from *Terminalia brownii* are active in the presence of Zn^{2+} and Mn^{2+} (Idries *et al.*, 2024). Similarly, the lectin from the edible wild mushroom *Hygrophorus russula* (Fr.) Quél. exhibits hemagglutinating activity stimulated by Cu^{2+} , Al^{3+} , Fe^{3+} , and Pb^{2+} , but inhibited by Hg^{2+} (Liu *et al.*, 2014). Likewise, lectins purified from the Surco and Vara (Furrow and Rod) varieties of *black Phaseolus vulgaris* are glycoproteins that require Ca^{2+} , Mn^{2+} , and Mg^{2+} ions for activity (Valadez-Vega *et al.*, 2022).

Conclusions

This study reports and confirms the presence of a biologically active lectin like protein in *Eleusine coracana* (finger millet) seeds. The partial purification and subsequent characterization revealed key functional properties of this protein. The lectin exhibited a distinct hemagglutination assay, showing specificity for human blood groups A+, while being less reactive towards group B+, which points to its selective recognition of surface glycans. The biochemical characterization identified the finger millet lectin as a robust protein, stable under physiological conditions of temperature between (20-50°C) and pH (5-9). Crucially, the hemagglutination activity was mildly inhibited by galactose. Additional studies using N-acetylgalactosamine are required, as the lectin showed hemagglutination toward blood group A erythrocytes. Since N-acetylgalactosamine is the defining terminal sugar of Human blood group A and structurally related to galactose, testing with it will clarify the lectin's binding affinity. Furthermore, the complete inhibition of activity by EDTA and its enhancement by metal ions like Ca^{2+} , Zn^{2+} , and Mg^{2+} provide compelling evidence that the *Eleusine coracana* lectin is a metalloprotein, dependent on divalent cations for its structural integrity and carbohydrate-binding function.

In summary, the lectin like protein from *Eleusine coracana* (finger millet) seeds characterization supports and adds a new dimension to the understanding of its nutritional and functional components, which are already renowned for their health benefits. These properties of this newly reported lectin make it a promising candidate for further exploration in biomedical research,

particularly in areas such as glycobiology, cancer research (for its specific binding properties), and as a tool for probing cell surface carbohydrates. Future work would aim at achieving full purification and sequencing to fully understand its structure-function relationship and pharmaceutical applications.

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Author Contributions

Conceptualization: F.M.R.S., A.S.U.; Methodology: F.M.R.S., A.S.U.; Investigation: F.M.R.S.; Data Curation: F.M.R.S.; Writing - Original Draft: F.M.R.S.; Writing - Review & Editing: A.S.U.; Supervision: A.S.U. All authors have read and agreed to the published version of the manuscript.

Ethics Statement

Ethical approval was not required for this study because it did not involve human participants, animals, identifiable personal data, or materials requiring institutional ethical review.

Declaration of Generative AI Use

The authors declare that no Generative AI tools were used in the preparation of this manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this work.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgement

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