A novel phytolectin purified from Eucalyptus vicina: properties and immunomodulatory activity

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Research Article

Keywords: Eucalyptus vicina. Immunomodulatory. Purification of lectin. Phagocytic Activity. Carbon Clearance Test

Posted Date: April 24th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6311146/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at The Protein Journal on June 11th, 2025. See the published version at https://doi.org/10.1007/s10930-025-10277-6.

Abstract

This study characterizes a novel lectin isolated from *Eucalyptus vicina* (EVL) fruits, an area of limited prior research. The lectin (EVL) was purified from fruit extract via ammonium sulfate precipitation followed by single-step ion-exchange chromatography. EVL exhibits a strong affinity for fetuin and demonstrates potent hemagglutination activity against rabbit and all human blood types. Size exclusion chromatography revealed a single 57 kDa peak in solution. Hemagglutination activity was optimal at alkaline pH (8-10) and was not affected by the presence of metals. Notably, EVL retained significant agglutination activity after one hour at 70°C, indicating thermostability. Furthermore, *in vivo*immunomodulatory effects were investigated using a carbon clearance assay. EVL administration resulted in a statistically significant, dose-dependent increase in phagocytic activity compared to the control group, suggesting its potential immunomodulatory properties.

1. Introduction

Lectins are glycoproteins found in plants, animals, and microorganisms (Rüdiger and Gabius 2001). They are particularly abundant in plant fruits and storage organs (Lis and Sharon 1981; Rüdiger and Gabius 2001), although they are present across diverse families and classes, albeit not in all species (Lis and Sharon 1981). Historically, they were referred to as hemagglutinins due to their ability to agglutinate erythrocytes from humans (Procópio et al. 2017) and rabbits (Gardères et al. 2015). Lectins are characterized by their specific binding to carbohydrate residues, such as those found on cell membranes and cell walls (Sharon and Lis 1990; Cummings 1996). This interaction can influence cellular physiology and metabolism. It is important to distinguish lectins from other carbohydrate-binding proteins or enzymes as Sharon and Lis (1990) and Cummings (1996) emphasize that lectins do not possess enzymatic activity related to carbohydrates. The current interest in lectin research stems largely from their ability to "read" the information encoded in the three-dimensional structure of carbohydrates (Ambrosi et al. 2005). These protein-sugar interactions are crucial for a wide range of molecular recognition and cell signaling processes (Ambrosi et al. 2005), and a deeper understanding of these interactions is essential for advancing biological applications, especially given the challenges in developing effective carbohydrate-based therapies. Plant lectins exhibit a broad spectrum of biological activities, including cytotoxic, immunomodulatory, vasorelaxant, and anticancer effects (Procópio et al. 2017). Of particular interest is their role in immunomodulation (Souza et al. 2013). Phagocytosis, a vital host defense mechanism against both infectious and non-infectious agents (Platt and Fineran 2015), is often influenced by lectin interactions with carbohydrates on immune cell surfaces. These interactions can trigger the release of specific cytokines, initiate signal transduction pathways, and ultimately shape immune responses against pathogens or cancerous cells (Souza et al. 2013). While the importance of protein-carbohydrate interactions in diseases like cancer has been extensively investigated (Hakomori 1989; Reis et al. 2008), their role in infectious diseases remains less explored. The significant body of work by Hakomori (1989) demonstrating aberrant glycosylation patterns in cancer cells has contributed to the intense focus on protein-carbohydrate interactions in oncology. This research has, in turn, highlighted the broader biological significance of lectins (Souza et al. 2013). Some plant lectins, such as Concanavalin A (ConA) from jack bean (*Canavalia ensiformis*) (Hakomori 1989), are known to stimulate the immune system. In this study, we aim to purify and characterize a novel lectin from the fruits of *Eucalyptus vicina*, a medicinal plant used traditionally in the northeast of Algeria and possessing significant applications in disease biology. Furthermore, we investigate its *in vivo* immunomodulatory effects in rates using the carbon clearance assay and assessing phagocytic activity.

2. Experimental

2.1. Materials and methods

Ethics statement:

Swiss albino rats weighing 230 ± 2 g were used in the experiment. The standard laboratory conditions consisted of a temperature of 25 ± 2°C and a 12-hour light/12-hour dark cycle. The rats were provided with a regular mouse pellet meal and water ad libitum. The Institutional Project Committee (PRFU, D01N01UN250120200001) approved the *in vivo* experimental methodology. This study's experimental protocols necessitated compliance with the Guidelines for Reporting Animal Research.

2.2. Plant materials

Eucalyptus vicina. Fruits were collected at full maturity, as indicated by capsule browning and partial opening, during October 2022 from a privately owned grove near the village of El Khroub (Lemridj), Constantine Province, Algeria (36°20'20"N latitude and 6°36'40"E longitude) (Fig. 1).

2.3. Molecular identification

Due to the morphological similarities between *Eucalyptus vicina* and other *Eucalyptus* species in the Constantine region, particularly given the known intra-species variability within *Eucalyptus* species (Jacobs 1982), molecular identification was performed to confirm the species identity of the collected fruit samples. DNA extraction, amplification, and sequencing: Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54 ºC. Primers of ITS-p5-fwd and ITS-p4-rev (Cheng et al. 2016) were employed for plant samples. PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms. The obtained DNA sequence search was conducted against the GenBank nucleotide (nt) database (Zhang et al. 2000), using standard parameters, including a gap existence penalty of 5 and an extension penalty of 2. The top matches were recorded based on percent identity, query coverage, E-value, and total alignment score. Only sequences with an E-value of 0.0 and high sequence similarity (> 97%) were considered for species identification. The accession numbers of closely related sequences were retrieved from GenBank for further comparison. The phylogenetic tree was constructed using Clustal Omega (Madeira et al. 2024) and visualized with MEGA 11 software.

2.4. Extraction and protein purification

Eucalyptus vicina fruits were collected in October and thoroughly cleaned with distilled water before being dried at 40°C for 48 hours in a shaded area. The dried fruits were powdered and then suspended in 0.1M phosphate buffer solution (pH 7.4) at a concentration of 10% (w/v) with a volume of 100 mL. The suspension was homogenized and centrifuged at 10,000 xg for 20 minutes. The supernatant was collected, and ammonium sulfate was added to achieve 70% saturation by adding solid ammonium sulfate in accordance with approved processes. The resulting precipitate was redissolved in 16ml of phosphate buffer solution (7.4). The partially purified extract was dialyzed against 2 liters of 0.1M phosphate buffer solution (pH 7.4) with four buffer changes over 48 hours using a dialysis membrane with a molecular weight cutoff of 22 kDa. 1.5 mL of the dialyzed fraction was loaded onto a DEAE-Cellulose column (1 cm x 10 cm) pre-equilibrated with 0.1 M phosphate buffer solution (pH 8.5). The column was washed with ml of increasing gradient of NaCl (0.1M, 0.5M, 1M, 1.5M) at a flow rate of 12 mL/hour. Fractions of 4 mL were collected until the absorbance at 280 nm reached 0.05. Protein concentrations were determined using the Bradford assay with BSA as a standard (Bradford 1976).

2.5. Hemagglutination assays

Hemagglutination assays were performed using two-fold serial dilutions of the lectin in standard microliter plates (Preetham et al. 2020). Equal volumes (50 µL) of the serially diluted lectin and using phosphate buffer solution (pH 7.4) as a diluent, suspension of erythrocytes (3% v/v for rabbit and for human ABO blood types) were mixed and incubated at room temperature for 1 hour. Agglutination was assessed visually. Rabbit erythrocytes were used to determine the lectin's hemagglutinating activity. Hemagglutination assays were also conducted using human erythrocytes of blood types A, B, AB, and O to determine its ability to agglutinate different ABO blood groups.

2.6. Characterization of the purified lectin

2.6.1. Molecular weight determination by SDS-PAGE

The molecular weight of the purified lectin was estimated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli and Favre (Laemmli 1973). A 14% resolving gel and a 3.48% stacking gel were used. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. Destaining was performed to visualize the protein bands. The molecular weight of the lectin was determined by comparing its electrophoretic mobility to that of a protein marker with known molecular weights (Prestained Protein Ladder, Bio-Rad). A standard curve was generated by plotting the electrophoretic mobilities of the marker proteins against their respective molecular masses, and the molecular weight of the lectin was then estimated from this curve using linear regression analysis in GelAnalyzer 23.1.1.

2.6.2. Metal cation requirement assay

The effect of various metal ions on the hemagglutination activity of *Eucalyptus vicina* lectin (EVL) was investigated. The purified lectin was treated with EDTA at a 1:1 ratio (v/v). The lectin (EVL) at a concentration of 1 mg/mL was then incubated with 100 mM solutions of the following metal chlorides: $CaCl_2$, $MgCl_2$, $FeCl_2$, Back $^{2+}$, $SnCl^2$, $ZnCl^{2+}$, $CoCl^{2+}$and $MnCl^{2+}$. After 1 hour, 50 µL of the purified lectin was added to a well, followed by the addition of 50 µL of one of the metal solutions. Finally, 50 µL of a 3% (v/v) suspension of rabbit erythrocytes in phosphate-buffered saline (PBS), pH7.4 as diluent, was added to each well. Hemagglutination was assessed visually after a further 1-hour incubation at room temperature.

2.6.3. Heat stability assay

The thermal stability of the lectin was investigated by assessing its hemagglutination activity after exposure to different temperatures. 200 µL of the purified lectin at a concentration of 1 mg/mL was incubated for 1 hour over a temperature range of 30°C to 100°C in 10°C increments. After incubation, the lectin samples were immediately cooled on ice for 5 minutes to halt further changes. The remaining hemagglutination activity of each sample was then determined using the standard microtiter plate assay with rabbit erythrocytes, as described previously (Sampaio et al. 1998).

2.6.4. Effect of pH on lectin stability

The pH stability of *Eucalyptus vicina* lectin was evaluated across a pH range of 1 to 12. The lectin (1 mg/mL) was incubated at 4°C for 24 hours in PBS buffer spanning pH 1 to pH 12. After incubation, hemagglutination activity was assessed at each pH using the two-fold serial dilution method (Necib et al. 2014).

2.6.5. Sugar binding specificity assay

The sugar binding specificity of *Eucalyptus vicina* lectin was investigated using a hemagglutination inhibition assay. Serial two-fold dilutions of each sugar (starting at 400 mM) were prepared in PBS. 25 µL of each sugar dilution was mixed with 25 µL of the lectin solution. After a 30-minute incubation at room temperature, 50 µL of a 3% suspension of rabbit erythrocytes in PBS (pH 7.4) was added to each well. Hemagglutination was assessed visually by observing the presence or absence of red blood cell clumping. Hemagglutination titers were compared to a control without added sugar to determine the minimum inhibitory concentration (MIC). The MIC was defined as the lowest sugar concentration required to completely inhibit hemagglutination.

2.7. Immunomodulatory test

Male Albino Wistar rats were housed in conventional cages under a 12-hour light/dark cycle at a controlled temperature of 21 ± 1°C and provided with standard rodent chow and water ad libitum. This study was conducted in accordance with the guidelines for animal care and use established by the of the Algerian Association of Animal Experimentation Sciences. The method described by Cheng and Li (2005) was followed with the following modifications: Four groups of seven rates each were used. Group 1 (control) received intraperitoneal injections of 0.5 ml of 0.9% NaCl. Groups 2, 3, and 4 received intraperitoneal injections of EVL dissolved in 0.9% NaCl at doses of 30, 50, and 100 mg/kg body weight, respectively. After 48 hours, all groups were injected intravenously via the tail vein with 0.1 mL/100 g body weight of a carbon suspension, prepared by mixing 3 mL of black carbon ink, 4 mL of 0.9% saline, and 4 mL of 3%

gelatin solution. Blood samples (30 µL) were collected from the orbital plexus at 5 minutes (t1) and 10 minutes (t2) post-injection. Erythrocytes were lysed by the addition of 4 mL of 0.1% sodium carbonate solution. Absorbances were measured at 670 nm using a spectrophotometer. The liver and spleen were then excised and weighed separately. The 90% splenic macrophage and 10% liver Kupffer cell distribution was previously reported by (Vikasari et al. 2015). The rate of carbon clearance (κ), the half-time of carbon clearance (t1/2), and the phagocytic index (α) were calculated as follows:

$$\varvec{K} = \frac{(\varvec{l}\varvec{n}\varvec{O}\varvec{D}1 \; - \; \varvec{l}\varvec{n}\varvec{O}\varvec{D}2)}{(\mathbf{t}2 \; - \; \mathbf{t}1)}$$

where OD1 and OD2 represent the optical densities at times t1 and t2, respectively

$$\varvec{t}1/2 = 0.693/\varvec{K}$$

$$\propto = \sqrt[3]{\varvec{K}} \; \frac{\varvec{B}\varvec{o}\varvec{d}\varvec{y}\,\varvec{w}\varvec{e}\varvec{i}\varvec{g}\varvec{h}\varvec{t}\,\varvec{o}\varvec{f}\,\varvec{}}{\varvec{L}\varvec{i}\varvec{v}\varvec{e}\varvec{r} \; + \; \varvec{S}\varvec{p}\varvec{l}\varvec{e}\varvec{e}\varvec{n}\,\varvec{w}\varvec{}}$$

2.8. Statistical analysis

Data are presented as mean ± standard deviation. Statistical significance between groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's HSD test. All statistical analyses were performed using GraphPad Prism 8. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Molecular identification

The final DNA sequence obtained (see supplementary material) was submitted to the NCBI BLASTn database (Zhanget al. 2000), to determine its taxonomic identity by comparing it against the GenBank nucleotide (nt) database. The BLAST search revealed that the sequence had the highest similarity to species within the Eucalyptus genus. The top match was *Eucalyptus vicina* (GenBank accession HM116971.1) with a 99.29% identity and 87% query coverage, followed by *Eucalyptus camaldulensis* (99.28% identity, 85% query coverage) and *Eucalyptus tereticornis* (99.42% identity, 85% query coverage). All high-scoring alignments had an E-value of 0.0, confirming a strong and reliable match. These results indicate that the analyzed sequence is most likely derived from *Eucalyptus vicina*, though minor variations suggest potential genetic similarity with other *Eucalyptus* species. In addition, the maximum likelihood (ML) phylogenetic analysis (see supplementary material) revealed that the obtained sequence clusters closely with *Eucalyptus vicina*, supporting the BLASTn results. The short branch lengths indicate minimal genetic divergence, suggesting a high degree of similarity to known *Eucalyptus vicina* sequences. The strong bootstrap support further confirms the reliability of this relationship. These findings suggest that the sample belongs to *Eucalyptus vicina* or a closely related variant within the same clade.

3.2. Hemagglutination assays of purified lectin from *Eucalyptus vicina* fruits

The *Eucalyptus vicina* fruit extract exhibited strong hemagglutination activity with rabbit red blood cells. Following maceration in PBS (pH 7.4), 64 mL of extract was obtained, with a protein concentration of 0.0120 mg/mL and a specific hemagglutination activity of 512 U/mg protein. This corresponds to a total hemagglutination activity of the purification profile of the *Eucalyptus vicina* lectin (EVL) is summarized in Table 1.

Table 1
Purification profile and protein content of *Eucalyptus vicina* fruits

Samples	Volume (mL)	Protein concentration (mg/ml)	Total activity Titer	Specific activity (U/mg)	Total activity	Protein yield (%)	Purification fold
Crud extract (1/10)	64	12.10^{-3}	512	$42.6.10^{-3}$	393.21	100	1
Ammonium sulfate precipitate 70%	16	$36.39.10^{-1}$	1024	$28.23.10^{-3}$	5962.13	303	2
Ion chromatography (DEAE-Cellulose)	4	$12.33.10^{-1}$	4096	$332.19.10^{-3}$	1966.08	102	8

Ion-exchange chromatography of the 70% saturated ammonium sulfate precipitate of the *Eucalyptus vicina* lectin (EVL) yielded a single peak with hemagglutination activity (Fig. 2). This peak eluted with a linear gradient of 0.1M to 1.5M (0.9% NaCl). The *Eucalyptus vicina* lectin did not agglutinate erythrocytes from any of the ABO blood types (A, B, AB, and O). The importance of dimerization for lectin hemagglutinating activity has been previously noted (Loris et al. 1998; Srinivas et al. 2001).

3.3. Characterization of the purified lectin

The purification of the lectin from *Eucalyptus vicina* fruits is summarized in Table 1. SDS-PAGE of the purified lectin under reducing conditions (with β-mercaptoethanol) revealed a single protein band (Fig. 3), indicating the purity of the preparation. The molecular mass of the lectin was estimated to be 57 ± 2.0 kDa (mean ± SD, n = 4). SDS-PAGE analysis revealed a single protein band with a molecular mass of 57 kDa, indicating the high level of purity in the

sample. This molecular weight is similar to the dimeric lectin from *Phaseolus vulgaris* (64 kDa, composed of two 32 kDa subunits) This observed dimeric structure is common among lectins, which often exist as dimers or tetramers (Etzler 1985).

Table 2
Inhibition of *Eucalyptus vicina* lectin hemagglutinating activity by carbohydrates and glycoproteins. (+) Inhibition; (-) No inhibition. MIC: Minimum inhibitory concentration.

Carbohydrates	Hemagglutinating activity	MIC (mM)
Glucose		/
Saccharose		/
Maltose		/
Galactose		/
Mannose		/
Fucose		/
cellulose		/
Arabinose		/
Fructose		/
Sorbitol		/
Ribose		/
Xylose		/
Mannitol		/
Raffinose		/
Fetuin	+	25mM
Inulin		/
Mucin		/
BSA	+	100mM
Ovalbumine		/
Casein	+	400mM

3.3.1. Metal cation

Requirement assay

Neither treatment with 100 mM EDTA nor the addition of any of the tested metal cations affected the hemagglutination activity of the *Eucalyptus vicina* lectin (Table 3).

Table 3
Effect of metals on the hemagglutinating activity of the lectin. (+) Inhibition; (-) No inhibition.

Metals(100Mm)	Hemagglutinating activity
$CaCl_2$	-
$MgCl_2$	-
$SnCl_2$	-
$FeCl_2$	-
$BaCl_2$	-
$ZnCl_2$	-
$MnCl_2$	-
$CoCl_2$	-

3.3.2. Heat stability assay

The thermal stability of *Eucalyptus vicina* lectin (EVL) was assessed by measuring its hemagglutination activity after incubation at various temperatures. EVL retained approximately 100% of its initial hemagglutination activity after incubation at temperatures up to 50°C. Between 50°C and 70°C, an 80°C increase in hemagglutination activity was observed. Above 70°C, the hemagglutination activity of EVL progressively decreased. At 80°C, the lectin retained 50% of its initial activity, and at 100°C, the activity was reduced to less than 25%. The heat stability profile of EVL showed that it retained full hemagglutination activity up to 50°C. Above this temperature, activity progressively decreased, with 50% activity being retained at 80°C and near-complete loss of activity at 100°C. The thermal stability of lectins can be influenced by factors such as metal ions, sialic acid content, and the presence of stabilizing interactions within the protein structure (Schwarz et al. 1993; Nomura et al. 1998; Singh et al. 2010; Pompeu et al. 2015).

3.3.3. pH stability assay

The effect of pH on the hemagglutination activity of the *Eucalyptus vicina* lectin was investigated. The lectin exhibited no detectable hemagglutination activity at pH values below 6.0. Optimal hemagglutination activity was observed at pH 8.0, and activity was maintained up to pH 10. Above pH 10, the hemagglutination activity of the lectin decreased. The optimal pH for EVL activity was between 8.0 and 10.0. Activity was lost below pH 6.0, likely due to subunit dissociation and/or the leaching of divalent cations crucial for structural stability and function (Schwarz et al. 1993; Loris et al. 1998; Srinivas et al. 2001; Naseem and Khan 2005).

3.4. Immunomodulatory effect

The effect of *Eucalyptus vicina* lectin (EVL) on carbon particle clearance was investigated *in vivo*. rates treated with EVL at doses of 30, 50, and 100 mg/kg body weight showed significant differences in carbon clearance compared to the control group (ANOVA, n = 10 per group). The phagocytic index was significantly increased in the treated groups ($p < 0.005$), showing a dose-dependent increase (Fig. 6). *In vivo* administration of EVL to rates resulted in a dose-dependent increase in the phagocytic index ($p < 0.005$), suggesting an immunostimulatory effect. Glycosylation analysis revealed that EVL is an O-glycoprotein, as demonstrated by its inhibition by fetuin. This O-glycosylation and fetuin inhibition are shared characteristics with the lectin from the macro-fungus Pholiota aurivella (Singh et al. 2009) and Annona muricata seeds (Damico et al. 2003), suggesting similar carbohydrate-binding specificities. This study reports the isolation and characterization of a novel lectin (EVL) from the fruits of *Eucalyptus vicina*, a member of the Myrtaceae family. EVL exhibited hemagglutination activity against rabbit erythrocytes, with a titer of 1/4096. The 70% ammonium sulfate precipitation of EVL is consistent with the precipitation behavior of the lectin from Phaseolus vulgaris fruits (Basheer et al. 2013), suggesting similar surface properties. This increase in phagocytic activity may be due to the interaction of EVL with macrophages or other cells of the reticuloendothelial system (Goldman et al. 1976; Vargas-Albores et al. 1993; Maldonado et al. 1994; Necib et al. 2014). While the precise mechanisms by which lectins modulate immune responses are not fully understood, carbohydrate recognition plays a crucial role (Hakomori 1989; Souza et al. 2013). Further research is needed to elucidate the mechanisms by which EVL exerts its immunomodulatory effects and to explore its potential therapeutic applications.

4. Conclusions

A novel 57 kDa phytolectin was purified from *Eucalyptus vicina* fruit (EVL). This lectin exhibits fetuin-binding specificity, agglutinates human ABO and rabbit blood erythrocytes, and demonstrates thermostability and pH independence. Furthermore, it shows immunomodulatory activity, potentially through interactions with phagocytic cell surface glycans.

Abbreviations

EVL *Eucalyptus vicina lectin*

DNA Deoxyribonucleic acid desoxyribonucleic

PCR Polymerase chain reaction

ITS1F Internal Transcribed Spacer 1 Forward (primer)

ITS1 Internal Transcribed Spacer 1

rDNA Ribosomal DNA

DEAE-Cellulose: Diethylaminoethyl-Cellulose

NaCl Sodium chloride

BSA Bovine serum albumin

SDS-PAGE Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

EDTA Ethylenediaminetetraacetic acid

Declarations

Data availability

All data supporting the findings of this study are available within the paper, with the Excel sheet containing the Blastn database search results and the Word document containing DNA sequence of *Eucalyptus vicina* and the phylogenetic analysis provided as Supplementary Information.

Acknowledgments

The authors thank the Algerian Association of Animal Experimentation Sciences for their ethical guidance. We also acknowledge the laboratory and veterinary staff for their technical assistance and expertise, respectively. Finally, we thank the institutions that provided the necessary facilities and resources for this study.

CRediT authorship contribution statement

SB and AB conceptualization, SB methodology, AB software, SB, AB and IT validation, BY, ASI formal analysis, SB, IT, YN investigation, YB resources, SB, AB, and ASI data curation, SB writing-original draft preparation, SH, SB, AB, IT, and ASI writing-review and editing, MTB and YN visualization, YB supervision, SB project administration, AB funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: The author(s) received no specific funding for this work.

Declaration of competing interest

The authors declare there are no conflicts of interest related to this study.

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Figures

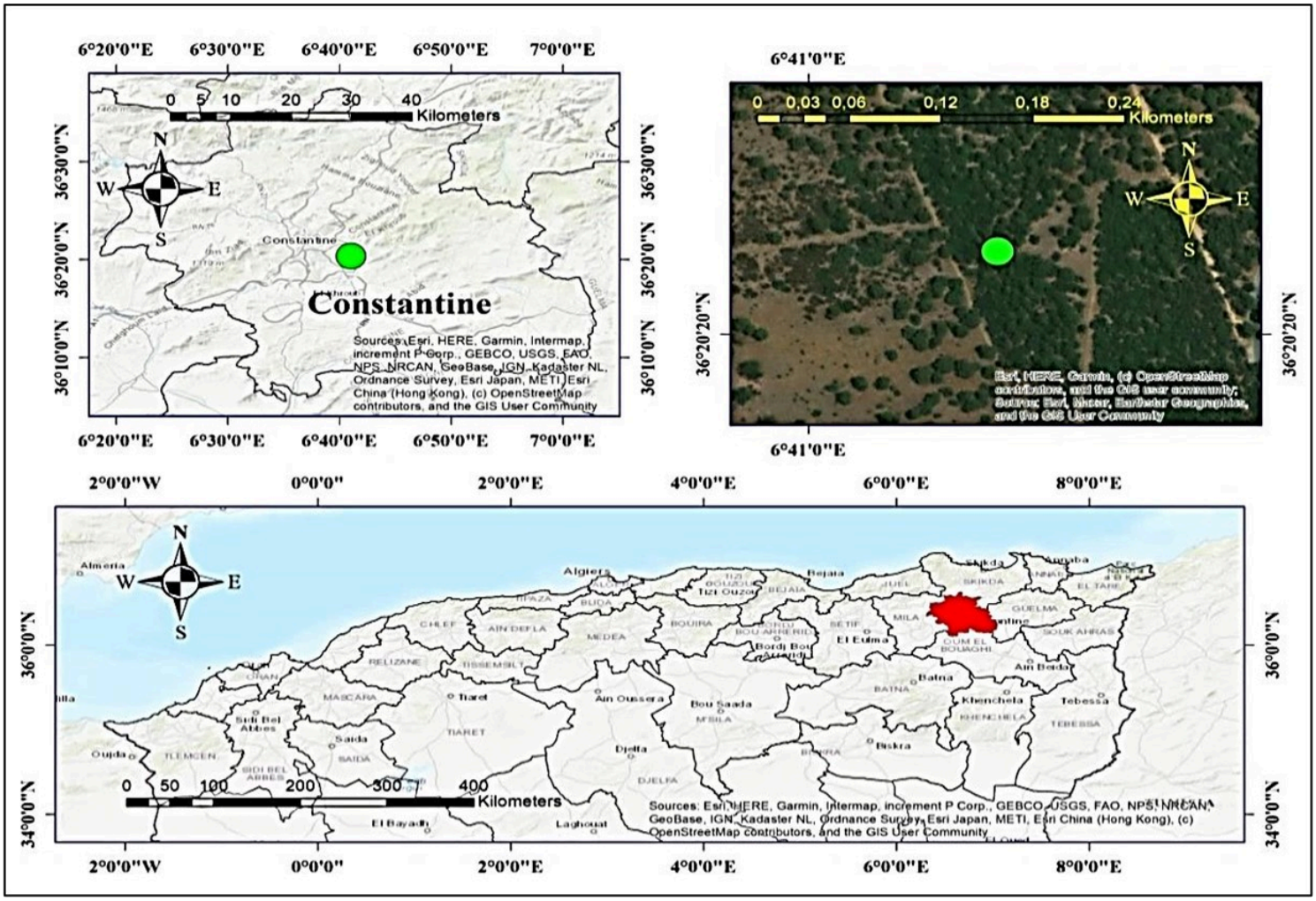

Figure 1

Location of *Eucalyptus vicina* fruits harvest, Constantine, El Khroub (Lemridj)

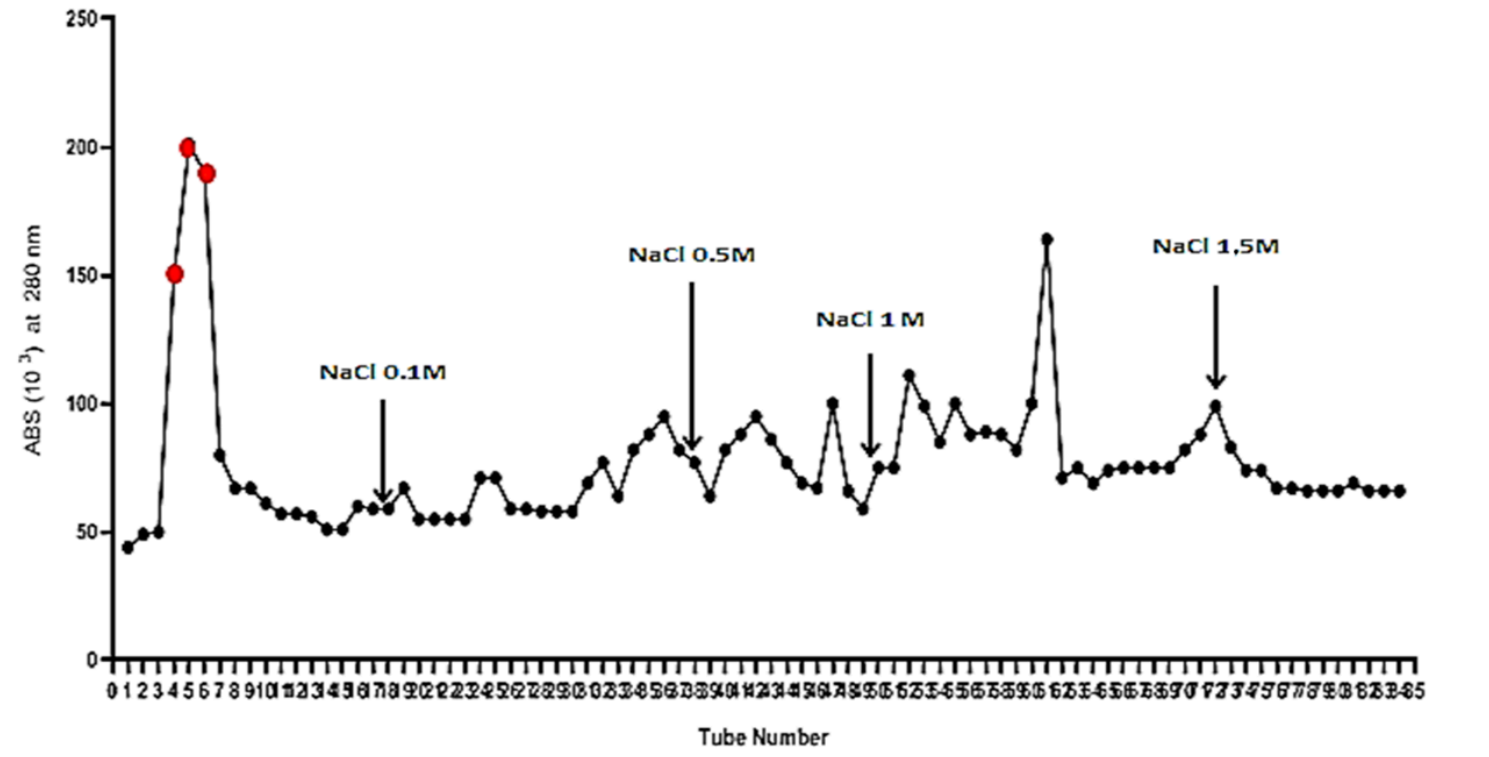

Figure 2

Chromatographic profile of the 70% ammonium sulfate precipitate of *Eucalyptus vicina* lectin (EVL) after ion-exchange chromatography on DEAE-cellulose, showing a single peak with hemagglutination activity.

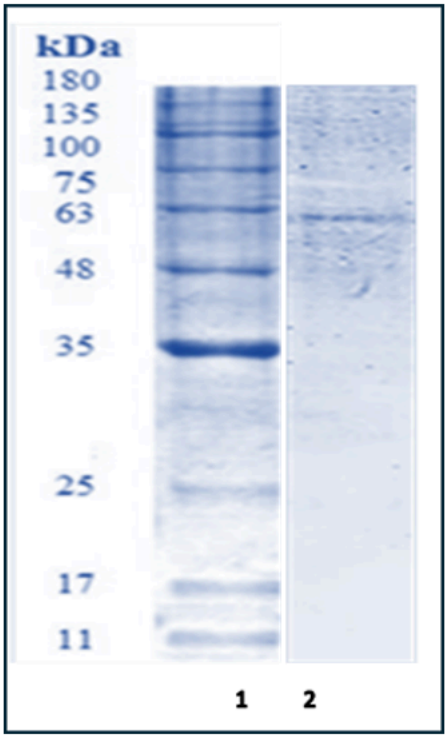

Figure 3

SDS-PAGE (14% polyacrylamide gel) of *Eucalyptus vicina*lectin after ion-exchange chromatography. Line 1: Prestained BlueRay PS-103 protein marker. Line 2: Purified *Eucalyptus vicina* lectin. The lectin's interaction with glycoproteins (BSA, Casein, Fetuin) suggests receptor differentiation, with minimum inhibitory concentrations of <0.0125 g/mL (BSA), <0.05 g/mL (Casein), and <0.00003 g/mL (Fetuin) using a starting concentration of 0.1 g/mL. This indicates a higher affinity for Fetuin compared to Casein or BSA (Table 2).

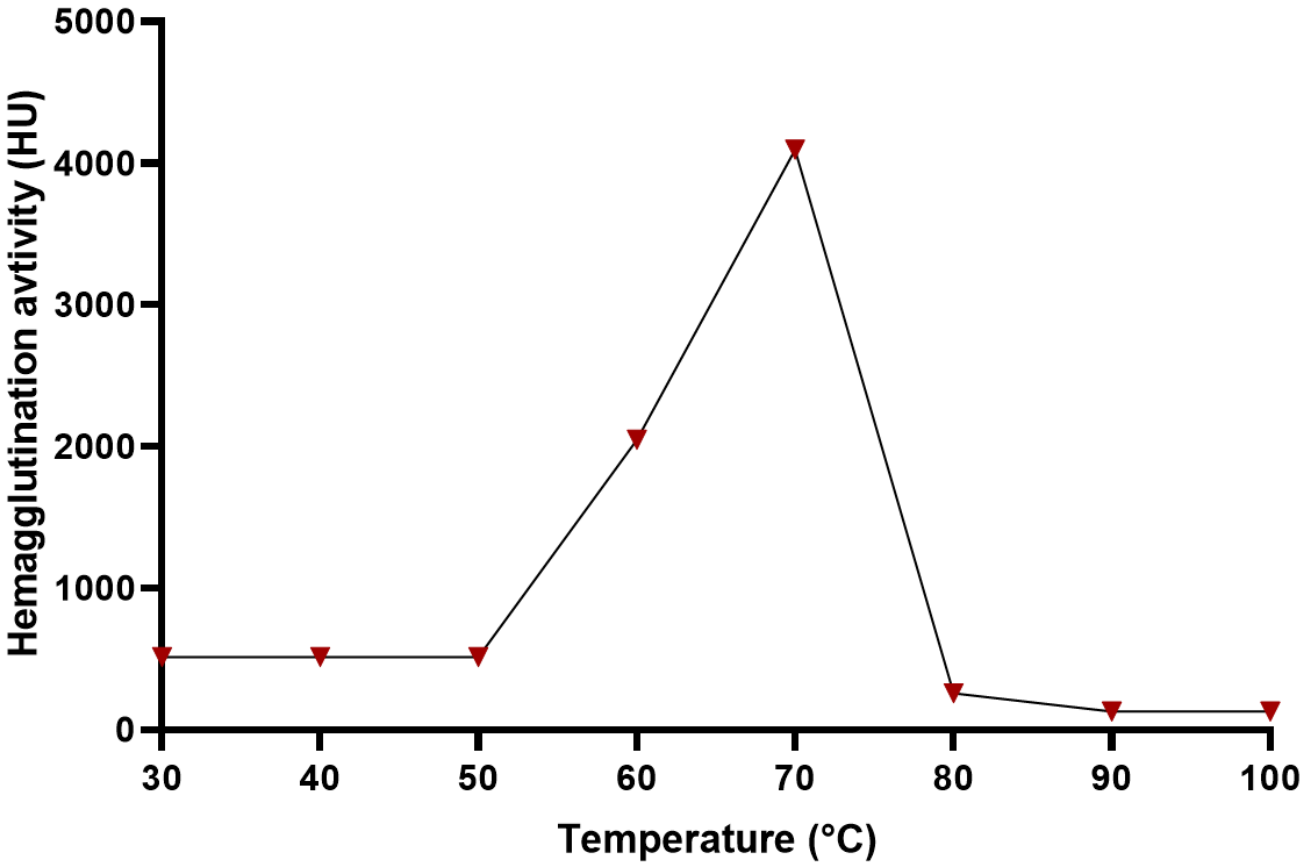

Figure 4

Effect of Temperature on agglutinating activity of the purified lectin

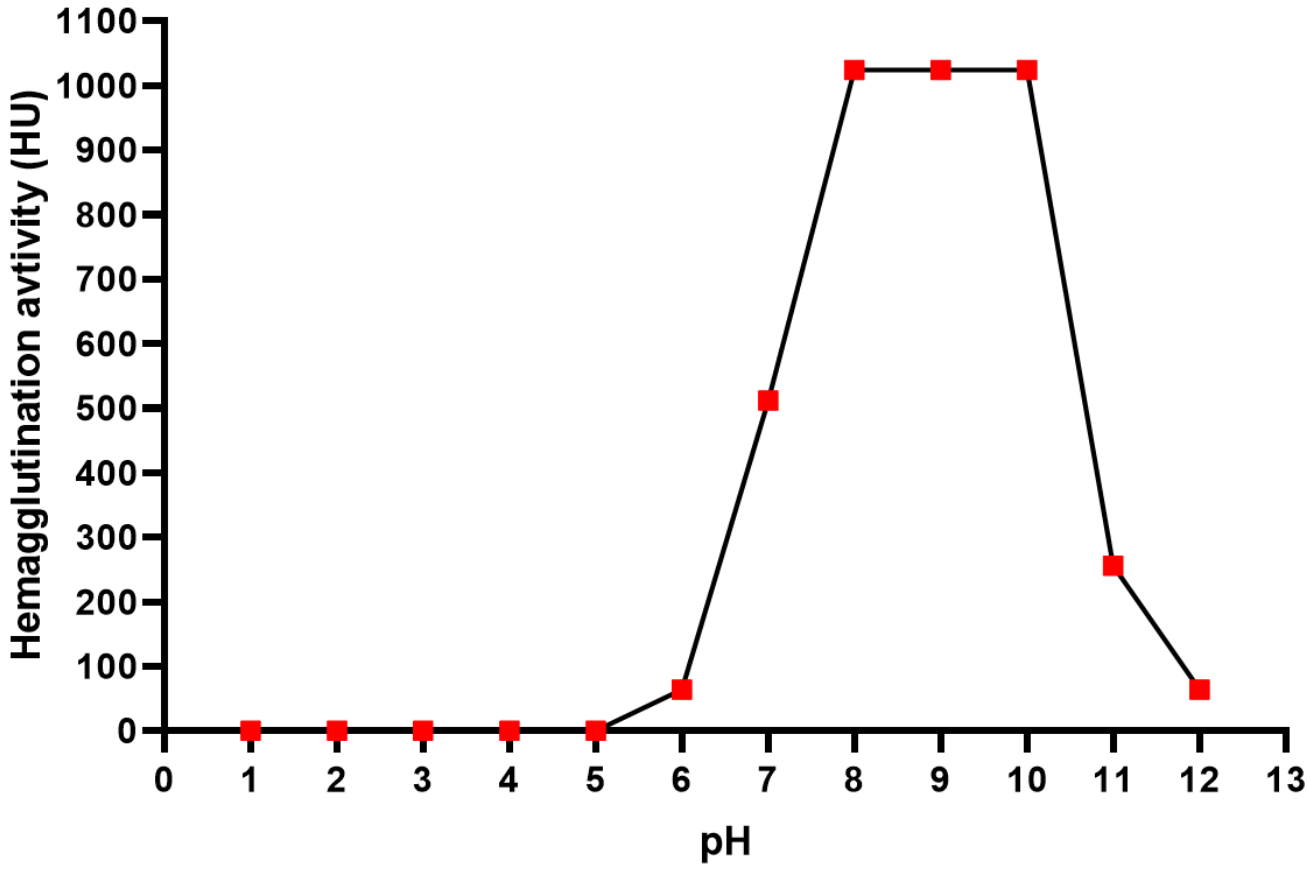

Figure 5

Impact of pH on the hemagglutinating activity of pure lectin.

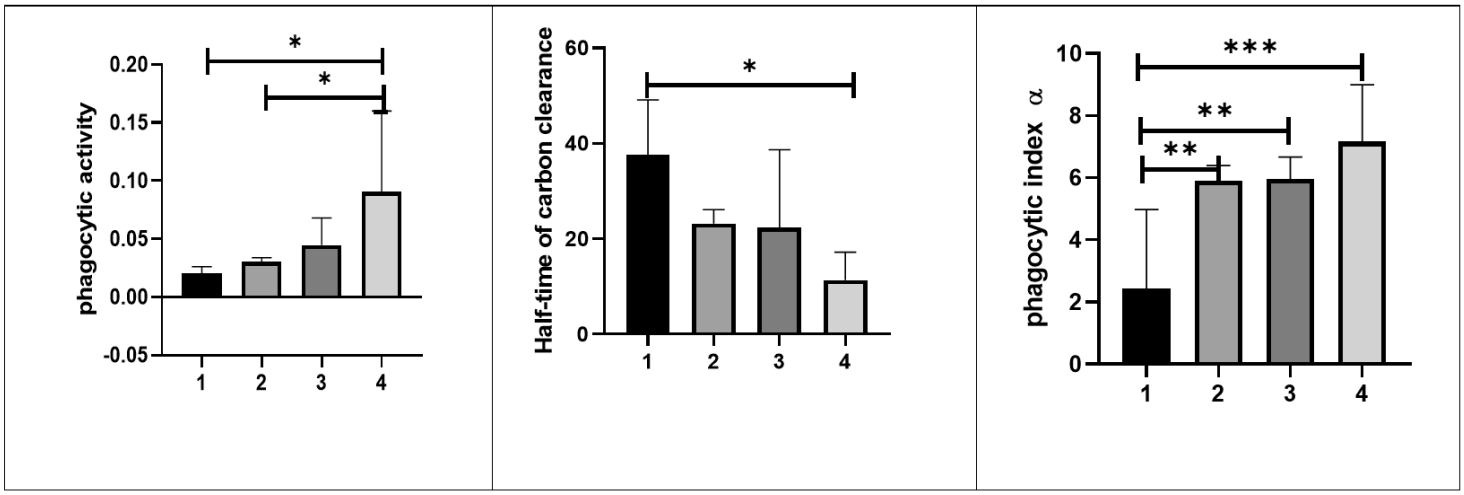

Figure 6

Impact of *Eucalyptus vicina* lectin on the half-life (t1/2) of carbon clearance, phagocytic activity, and phagocytic index. P < 0.05, with *: significant, **: very significant, and ***: extremely significant in comparison to the control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- V9Y04V52016AlignmentDescriptions.csv
- SupplementarymaterialTheproteinJ..docx