A galactoside-specific *Dalbergieae* legume lectin from seeds of *Vataireopsis araroba* (Aguiar) Ducke

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ABSTRACT

The *Dalbergieae* lectin group encompasses several lectins with significant differences in their carbohydrate specificities and biological properties. The current work reports on the purification and characterization of a GalNAc/Gal-specific lectin from *Vataireopsis araroba* (Aguiar) Ducke, designated as VaL. The lectin was purified from the seeds in a single step using guar gum affinity chromatography. The lectin migrated as a single band of about 35 kDa on SDS-PAGE and, in native conditions, occurs as a homodimer. The purified lectin is stable at temperatures up to 60 °C and in a pH range from 7 to 8 and requires divalent cations for its activity. Sugar-inhibition assays demonstrate the lectin specificity towards *N*-acetyl-D-galactosamine, D-galactose and related sugars. Furthermore, glycan array analyses show that VaL interacts preferentially with glycans containing terminal GalNAc/Galβ1-4GlcNAc. Biological activity assays were performed using three insect cell lines: CF1 midgut cells from the spruce budworm *Choristoneura fumiferana*, S2 embryo cells from the fruit fly *Drosophila melanogaster*, and GutAW midgut cells from the corn earworm *Helicoverpa zea*. *In vitro* assays indicated a biostatic effect for VaL on CF1 cells, but not on S2 and GutAW cells. The lectin presented a biostatic effect by reducing the cell growth and inducing cell agglutination, suggesting an interaction with glycans on the cell surface. VaL has been characterized as a galactoside-specific lectin of the *Dalbergieae* tribe, with sequence similarity to lectins from *Vatairea* and *Arachis*.

Keywords: Lectin; *Vataireopsis araroba*; *Dalbergieae*; Insect cells.

1. INTRODUCTION

Lectins are unique proteins in their capacity to read the glycocode, the code contained in the three-dimensional structure of glycans [1]. This property is related to their specific and reversible binding to carbohydrates [2] which allows them to elicit several biological activities [3]. In addition, lectins interact with hydrophobic molecules such as secondary metabolites, plant hormones, and antibiotics independently from their carbohydrate-binding properties [4, 5]. Plant lectins, in particular legume lectins, are the most studied group, and inside this group, *Dalbergieae* lectins (*Papilionoideae* subfamily) are being explored due to their variability in specificity, structural properties, and biological activities. To date, a total of 26 plant lectins have been purified from this tribe encompassing the following genera: *Andira, Arachis, Centrolobium, Lonchocarpus, Machaerium, Platypodium, Platymiscium, Pterocarpus,* and *Vatairea.* The characterization of these lectins allowed the division into three groups based on specificity, in particular the mannose/glucose-specific, the *N*-acetylgalactosamine/galactose-specific, and the *N*-acetylglucosamine-specific *Dalbergieae* lectins. Some of these proteins elicit interesting biological effects such as pro-and anti-inflammatory [6–8], nociceptive [9], anticancer properties [10, 11] and others [12]. Besides the above cited biological activities, some legume lectins are known to elicit an interesting insecticidal effect, most likely due to lectin interactions with glycoconjugates in the insects cells, thus affecting essential biological processes of the organism [13, 14].

Endemic from the Brazilian tropical forest of Southern Bahia and North of Espírito Santo *Vataireopsis araroba* (Aguiar) Ducke belongs to the *Dalbergieae* tribe of the *Papilionoideae* subfamily (*Leguminosae* family) and is a deciduous tree that can reach 20-35 meters in height. Its wood is yellow or dark brown with a density of 0.60 g/cm^3 and is considered fetid and with a bitter taste. Feathered leaves (up to 50 leaflets) and flowers in erect panicles are grouped at the end of the branches. Its flowering period from May to July begins with the fall of the leaves. Subsequent fruiting extends throughout the year [15]. Two applications of *V. araroba* are reported in literature. The first is its use in civil construction and wood industry, mainly due to its high resistance to shearing and cracking [16, 17]. The second application is aimed at the pharmacological industry, where studies carried out with compounds present in the bark of *V. araroba* show that it is effective against psoriasis [18, 19]. To date, there are no reports of lectin studies for this genus/species.

In this study, we present the purification, physicochemical and biological characterization, and a preliminary evaluation of the insecticidal potential of a GalNAc/Gal-specific lectin present in seeds of *Vataireopsis araroba*.

2. MATERIALS AND METHODS

2.1 Plant material and reagents

Mature seeds of *V. araroba* were collected at the Federal University of Ceara, Pici Campus. The reagents used in the experiments are of high purity, purchased from Merck (St. Louis, MO) and GE Healthcare (Little Chalfont, United Kingdom).

2.2 Protein extraction, hemagglutination, and sugar-inhibition assays

For the crude extract preparation, the seed coats of *V. araroba* seeds were removed, and the endosperm was ground in an electric grinder until a fine flour was obtained. Protein extraction was carried out using 150 mM NaCl (1:10 w/v) under constant stirring for 4 h at room temperature. Afterwards, the crude extract was clarified by centrifugation at 10,414 x *g* for 30 min at 4 ºC, followed by filtration through filter paper [20].

Hemagglutination assays were performed in 96-well plates, following the protocol of Cavada and Silva (with adaptations) [21, 22], these assays were performed to detect the lectin activity during the purification steps. Rabbit and human erythrocytes of types A, B, and O (both native and treated with papain and trypsin) were applied. The hemagglutination titer was calculated as the reciprocal of the highest dilution, with positive agglutination per mL (hemagglutination units/mL, H.U./mL) and the specific activity as the hemagglutination units (H.U.) per milligram of protein (H.U./mg P).

To determine the binding specificity of the lectin, the following sugars/glycoproteins were tested: D-glucose, D-mannose, α-methyl-D-glucopyranoside, α-methyl-D-mannoside (α-mm), D-galactose, *N*-acetylgalactosamine (GalNAc), α-lactose, *N*-acetylglucosamine (GlcNAc), L-rhamnose, L-fucose, ovalbumin, and porcine mucin. Briefly, in 96-well plates, sugars and glycoproteins at initial concentrations of 100 mM for sugars and 1 mg/mL for glycoproteins were serially diluted in 150 mM NaCl and the diluted protein extract (at 4 H.U./mL) was added to each well. The minimal inhibitory concentration (MIC) for each sugar/glycoprotein was defined as the lowest concentration able to completely inhibit lectin agglutination. Wells containing only the saline solution, sugars, and erythrocytes were used as controls [20]. This experiment was repeated with the purified lectin.

2.3 VaL purification

VaL was purified by affinity chromatography on a cross-linked guar gum polysaccharide matrix as previously reported [23,24,25]. The matrix was pre-equilibrated with 150 mM NaCl, 5 mM $CaCl_2$, and 5 mM $MnCl_2$ and the extract was applied and kept undisturbed for 4 hours. Subsequently, all non-retained materials were washed with the equilibration solution. Then, the retained lectin was eluted with the second eluent (100 mM glycine-HCl pH 2.6 + 150 mM NaCl). A constant flow of 1 mL per minute was set during the entire chromatography using a peristaltic pump. Immediately after elution, the bound material was dialyzed against distilled water and freeze-dried. All fractions were monitored by spectrophotometry at 280 nm [22]. The purification data were compiled in Table 1.

2.4 SDS-PAGE and molecular mass determination

SDS-PAGE was performed according to the method described by Laemmli (1970) [26] with modifications. The lyophilized protein fractions were solubilized at a concentration of 4 mg/ml in sample buffer, followed by heating at 100 °C for 10 min after addition of 2% 2-mercaptoethanol. The electrophoretic run was performed in a Mini-Protean II mini-gel system (Bio-Rad; Milan, Italy) with an electric current of 30 mA, 120 V, and 10 W per gel. Proteins in the gel were stained using Coomassie Brilliant Blue G-250. The Prestained Protein Molecular Weight Marker (Thermo Scientific) with 6 protein bands ranging from 20 to 120 kDa was used.

The apparent molecular mass of VaL was estimated by size exclusion chromatography coupled to an ultra-performance liquid chromatography system (UPLC). VaL was solubilized in 50 mM Tris-HCl pH 7.6 containing 150 mM NaCl at a final concentration of 4 mg/mL. and the same buffer was employed throughout the size exclusion chromatography under a constant flow of 0.6 mL/min. A BioSuite™ 250 silica SEC matrix, 5 µm HR 7.8 x 300 mm was applied. Proteins with different masses (GE Healthcare) were used to build a calibration curve: ribonuclease A (13,700 Da), carbonic anhydrase (29,000 Da), ovalbumin (44,000 Da), and conalbumin (75,000 Da). VaL molecular mass was calculated as explained in the online resource 1.

2.5 Protein and carbohydrate quantification

The Bradford assay was applied to quantify soluble proteins. Calculations were made using bovine serum albumin as standard protein [27]. The presence of glycans in VaL structure was checked by the Periodic acid-Schiff method [28]. The percentage of neutral sugars in VaL was estimated by Dubois method using glucose as a standard [29].

2.6 Thermal stability, pH stability, and divalent cations dependence

To determine the lectin thermal stability, a 1 mg/mL VaL solution in 150 mM NaCl was prepared and subjected to varying temperatures, ranging from 30 to 100 °C, for one hour with 10 °C intervals. At each point, an aliquot was applied in hemagglutination assays.

The pH stability of VaL was obtained by submitting a 1 mg/mL solution of VaL (in 150 mM NaCl) to hemagglutination assays after dialysis against the following buffers: 100 mM glycine pH 3.0; 100 mM sodium acetate pH 4,0; 100 mM sodium acetate pH 5.0; 100 mM sodium citrate pH 6.0; 20 mM sodium phosphate pH 7.0; 100 mM Tris-HCl pH 8.0; 100 mM glycine pH 9.0 and 100 mM glycine pH 10.0, all containing 150 mM NaCl.

The dependence on divalent cations was analyzed by dialysis of the lectin solution against 100 mM EDTA + 150 mM NaCl for 24 h. Subsequently, additional dialysis was performed against 150 mM NaCl to remove excess EDTA. The activity of VaL before and after the addition of $CaCl_2$ and $MnCl_2$ (both at 10 mM) was determined [20].

2.7 Amino acid sequencing

The partial protein sequence of VaL was obtained following the procedures described by Shevchenko and collaborators (2006) [30]. From the SDS-PAGE, bands around 35 kDa were excised, treated with ammonium bicarbonate and acetonitrile. Endoproteinase Asp-N, chymotrypsin, trypsin, and Glu-C solutions were used separately for digestion, and the obtained peptides were extracted by formic acid. After drying in SpeedVac, the peptides were solubilized in trifluoroacetic acid and separated in a BEH300 C18 column coupled to the nanoAcquity™ system (Waters), also connected to a nanoelectrospray mass spectrometer (SYNAPT HDMS System). The double or triple-charged precursor ions, which were fragmented by collision-induced dissociation (CID) using argon as the collision gas, were selected, processed, and analyzed by Proteinlynx (Waters). The CID spectra were interpreted manually using the Peptide Sequencing tool of the MassLynx 4.1 (Waters) software. The peptide sequences were submitted to BLASTp in the database of non-redundant proteins [31]. Then, multiple sequence alignments were performed using the ClustalOmega and ESPript 3.0 tool with the sequences of proteins that had the highest percentage of identity in BLASTp [32, 33].

2.8 Glycan array

The carbohydrate specificity of VaL was tested by glycan array screening. Synthetic glycan microarrays were prepared as previously described [34]. Tested carbohydrates/oligosaccharides are depicted at online resource 2. For VaL detection, the lectin was labeled with Alexa Fluor TM 555 succinimidyl ester (NHS) (Fisher Scientific) following the manufacturer's instructions. Excess of labeling reagent was removed employing Amicon 10 kDa devices and buffer exchanged to TSM (Tris 25 mM, 150 mM NaCl, pH 7.5) containing 4 mM $CaCl_2$. Lectin solution (1.5 µM) was incubated on the glycan microarray in the dark at room temperature for one hour. The glycan array was washed with TSM containing 0.01% Tween 20 and water. The slide was dried in a slide spinner and fluorescence was analyzed in an Agilent G265BA microarray scanner (PerkinElmer). Relative Fluorescence Units (RFU) with local background subtraction were averaged from four replicate spots and reported as histograms with the standard deviation (SD) of the mean.

2.9 Insect cell assays

CF1 (midgut, *Choristoneura fumiferana, Lepidoptera*), S2 (embryo, *Drosophila melanogaster, Diptera*) and GutAW (midgut, *Helicoverpa zea, Lepidoptera*) cells were maintained under standard growing conditions at 27 ºC in serum-free Sf-900 (Gibco®) for S2, IPL-41 supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) for CF1 and serum-free EX-CELL® 420 (Sigma Aldrich) for GutAW [35, 36].

Cell viability assays were performed in 96-well plates. Briefly, 90 µl of each specific medium was added to each well followed by a sterile solution of VaL (in phosphate-buffer saline pH 7.4) to a final concentration ranging from 0.65 µM to 2.6 µM or medium (control). Next, cells were added to a final number of 20,000 cells per well and the plates were incubated for 24h, 48h, and 72h. At the set time points, the plates were removed from the incubator and the cell number was estimated with the PrestoBlue® dye (Thermo Fisher Scientific, United States) according to the manufacturer protocol. The fluorescence was measured in an

Infinite M200 PRO (Tecan, Switzerland) with excitation wavelength set at 560 nm and emission wavelength at 590 nm. The cell number in each well was determined using a previously prepared standard curve. A total of 3 biological replicates were employed each with 3 technical repetitions. Pictures were taken in a Nikon Ti wide-field microscope (Nikon) using a 40x objective lens.

3. RESULTS

3.1 Hemagglutination activity in *V. araroba* seed extract

The protein extract from *V. araroba* seeds agglutinated rabbit and human erythrocytes, both native cells and cells treated with papain and trypsin. Sugar-inhibition assays using the protein extract suggested a strong specificity for *N*-acetylgalactosamine (GalNAc), with MIC of 0.4 mM D-galactose (Gal) and α-lactose (Lac) also inhibited the extract activity, indicating specificity for galactosides. In addition, L-rhamnose and porcine mucin also inhibited the hemagglutination (Table 2). The purified lectin presented the same sugar-inhibition results as the crude protein extract.

3.2 Isolation and purification of VaL

It was possible to isolate a lectin from seeds of *Vataireopsis araroba* (named VaL) to a high degree of purity in a single chromatographic step. A guar gum gel affinity chromatography was used, the lectin was eluted with 100 mM glycine pH 2.6 + 150 mM NaCl (Fig 1). The extract was clear, without pigments, and rich in proteins (Online resource 3). Table 1 summarizes the purification progress.

3.3 Physicochemical characterization

SDS-PAGE demonstrated that VaL has an apparent molecular mass of 36 kDa (Fig 1) while the size-exclusion chromatography revealed a molecular mass of 79 kDa (Online resource 4), indicating that the lectin is a dimer under the tested conditions. VaL strongly reacted with the Periodic acid-Schiff reagent (Fig 1) and further analysis indicated a neutral sugar content of 0.6% per milligram of protein. VaL presented full hemagglutination activity at 30-60 ºC, with a 50% reduction at 70 ºC and a total loss at ≥ 80 ºC or more (Online resource 5a). For pH stability, VaL showed 100% HA at pH between 7 and 8. Below or above these values, lectin activity is absent or significantly reduced (Online resource 5b). When treated with EDTA, the lectin activity was reduced, indicating that VaL is a metalloprotein. The addition of Ca^{2+} and Mn^{2+} to demetalized VaL reestablished 50% of the initial activity with the addition of ions separately and 100% with the addition of both divalent cations (Online resource 5c).

3.4 Glycan array

The fine specificity of the studied lectin was evaluated by employing a glycan array including 144 synthetic glycans from different origins including mammalian, plant and invertebrate glycans [34]. The glycan structures were robotically arrayed on N-hydroxysuccinimide (NHS) activated glass slides forming 7 identical subarrays per slide. Fluorescently labeled VaL was incubated at room temperature in the dark and

the slide was washed with binding buffer and water. The bound fluorescence on the array surface was analyzed by a microarray scanner and the positive RFU values of replicate spots were represented as histograms. The binding profile for VaL is depicted in Fig 2 and indicates that VaL interacts preferentially with glycans containing terminal GalNAc/Galβ1-4GlcNAc with binding also being verified, albeit at lower RFU levels, towards glycans that contain fucosylated LacdiNAc (LDNF) epitopes. However, glycans with LewisX epitopes showed no interaction with the lectin.

3.5 Sequence analysis and alignment

MS/MS analysis of seven peptides obtained by digestion of VaL with different proteases yielded a total of 109 amino acid residues (Online resource 6). Judging from sequence alignments with other lectins, this partial sequence of VaL corresponds to 45.41% of the predicted total sequence. The alignment in the non-redundant database (BLASPp) revealed a degree of similarity above 70% with the sequences of *Vatairea macrocarpa* lectin (VML) (ID: 4XTM_A), *Vatairea guianensis* lectin (VGL) (ID: P86893.1), and *Robinia pseudoacacia* bark agglutinin I (RPbAI) (ID: BAA36414.1), all of them are galactose-specific *Dalbergieae* lectins (Fig 3).

3.6 Insect cells assays

The effect of VaL on 3 insect cell lines, CF1 (midgut, *Choristoneura fumiferana*), S2 (embryo, *Drosophila melanogaster*) and GutAW (midgut, *Helicoverpa zea*) was evaluated by checking the viability and morphology of the cells. From the 3 tested cell lines, VaL only demonstrated noticeable effects to CF1 cells by presenting a biostatic effect and inducing cell aggregation. VaL did not affect or induce agglutination of S2 and GutAW cells. In neither of the tested cell lines, debris was observed.

Incubation of CF1 cells with different concentrations of VaL (0.65 μM to 2.6 μM) reduced the cell numbers compared to the control (Fig 4a). This reduction in cell number was observed for all time points and is probably related to the aggregation effect that has been induced (Fig 4b-c). When VaL was assayed at 1.3 and 2.6 μM, the cell number remained close to the initial 20,000 cells, indicating a biostatic effect, while at the lowest dose of 0.065 μM, the cell number was statistically different from the control after 48 and 72h incubation with about 26,000 viable cells per well, a smaller number in comparison to the control cells. Microscopy visualization did not indicate any morphological changes in the cells.

4. DISCUSSION

The lectins from *Dalbergieae* plants purified until now allowed a classification into three groups according to their specificity, namely the mannose/glucose-specific, the *N* acetylgalactosamine/galactose-specific, and the N-acetylglucosamine-specific lectins. Sugar-inhibition assays indicate that VaL belongs to the group of GalNAc/Gal-specific group of *Dalbergieae* lectins since it demonstrated binding to all of the tested galactosides, with additional binding to L-rhamnose. For glycoproteins, porcine stomach mucin strongly inhibited the lectin-induced agglutination (MIC: 0.016 mg/mL), which can be explained by the fact that mucins are composed of > 80% (based on mass) of *O*-

glycans, known to be rich in galactosides [37]. This specificity profile is shared with other *Dalbergieae* lectins from the same group such as the lectin from peanut nodules, PNA [38, 39], VML and VGL [21, 22].

The purification of legume lectins can be a complicated process and there is no standard protocol. Lectins from the *Dalbergieae* tribe have usually been purified with methodologies that include saline precipitation and liquid chromatography, either affinity chromatography, ion-exchange chromatography or a combination of both. VaL purification revealed itself to be a relatively straightforward process, the lectin was purified to a high degree in a single affinity chromatography step using a guar gum matrix. The applied steps are similar to those applied to purify the lectins from *Vatairea macrocarpa, Vatairea guianensis, Arachis hypogaea* and *Lonchocarpus capassa*, all of which involved a galactoside affinity chromatography [21, 22, 38, 40, 41]. This group of lectins usually presents glycosylated polypeptides with a molecular weight close to 36 kDa. Maximal activities are observed at the temperature range of 30 to 70 ºC and a pH range between 6 and 8 [21, 22], which correlates well with the activity observed for VaL [12]. Although there are very few data in the literature, two *Vatairea* lectins are known to be composed of three polypeptide chains (α, β and γ) generated through proteolytic processing, which VaL does not appear to undergo, even considering the high similarity between these lectins [42]. VaL, like most *Dalbergieae* lectins, is a metalloprotein dependent on Ca^{2+} and Mn^{2+} ions. Metal-binding residues are conserved in most of these lectins and are essential for the correct folding of the carbohydrate recognition domain. BLASTp searches of the peptides against the non-redundant database indicated a high sequence similarity with the galactose-specific lectin sequences from PNA [43, 44], VML[45] , VGL [22] and RPbAI [46] (Fig 3). Sequence alignments of VaL and VML revealed identical residues that make up the carbohydrate-binding domain (Asp87, Gly105, Phe127, and Asn129) [47]. The metal-binding residues and *N*-glycosylation sites are also present [45, 48]. More studies on the primary and 3D-structures are needed for a better structural characterization of the lectin.

Glycan array trials are a powerful technique to unravel the fine specificity of lectins and are readily applicable nowadays considering that the synthesis of oligosaccharides, *N*-glycans, *O*-glycans, *N*-glycan core oligosaccharides, glycolipid type glycans, and other glycans is possible following chemoenzymatic synthetic routes [49, 50]. In the current work, a collection of 144 chemo enzymatically prepared glycan structures were assayed in the form of glycan microarrays. The synthetic glycan collection containing both mammalian and non-mammalian structures was robotically arrayed on activated glass slides and tested against fluorescently labeled VaL. The selectivity towards Gal and GalNAc deciphered from the hemagglutination inhibition assays was corroborated with the glycan array data and additionally, more defined information about the binding preferences of the lectin was obtained. In the glycan microarray, VaL demonstrated specificity for glycans with terminal β1-4Gal, β1-4GalNAc, and glycans containing LDNF epitopes, all of these containing terminal galactosides. The glycans with a terminal LDNF motif revealed reduced binding and binding was completely inhibited for LewisX-containing glycans, which is most likely due to steric hindrance caused by the fucosyl residue. This result represents a leap in the knowledge about the specificity of galactoside-specific *Dalbergieae* lectins since there are no data regarding glycan array profiles for previously purified lectins.

Resistance development by insects caused by the continuous usage of insecticides has increased the need for the discovery of novel molecules with insecticidal potential. Lectins are shown to be promising in this area with several literature reports demonstrating that plant lectins can be interesting insecticidal agents [13]. Previous reports indicate that lectins can induce aggregation of insect cells both in carbohydrate-dependent or carbohydrate-independent ways. *Sclerotinia sclerotiorum* agglutinin and *Rhizoctonia solani* agglutinin bind to the insect cell surface via the carbohydrate-binding domain [51, 52] and, in contrast, *Orysata* induces cell-aggregation in a glycan-independent manner [53]. It was not possible to observe morphological changes or cell debris in CF1 cells treated with VaL, indicating that the effect is caused by the induced aggregation. In contrast, no toxicity or any evidence of interaction of VaL with S2 and GutAW cells was observed. To date, there are few data of *Dalbergieae* lectins as insecticidal agents, one of which was published by Law and Kfir [54] who tested the effect of *Arachis hypogaea* lectin against *Chilo partellus* (*Lepidoptera*) and observed about 46% of mortality for larvae that consumed the lectin. The authors also reported lectin binding on the microvilli of the midgut cells in agreement with what was seen in the current work. In literature, there are a large number of reports showing the toxicity of legume lectins against insects of different orders, including *Lepidoptera*, *Coleoptera*, and *Hemiptera*. The mechanism behind these activities is not fully described, but it is likely that interactions with different glycoproteins or glycan structures present on the insect cell's surface can interfere with several physiological processes [53, 55]. Previously insecticidal activity was shown for the lectins from *Bauhinia monandra*, *Canavalia ensiformis*, *Cratylia floribunda*, *Dioclea grandiflora*, *D. rostrata*, *Griffonia simplicifolia*, *Phaseolus vulgaris*, and *Pisum sativum* [13, 56]. The assay presented here is the first indication for the potential insecticidal activity of VaL. Further research is needed to investigate the insecticidal effects of this lectin as well as possible applications.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY

Data available on request from the authors.

REFERENCES

1. Ambrosi, M., Cameron, N.R., Davis, B.G.: Lectins: tools for the molecular understanding of the glycocode. Org. Biomol. Chem. 3, 1593–1608 (2005). https://doi.org/10.1039/b414350g

2. Peumans, W.J., Van Damme, E.J.M.: Lectins as Plant Defense Proteins. Plant Physiol. 109, 347–352 (1995). http://dx.doi.org/10.1104/pp.109.2.347

3. Tsaneva, M., Van Damme, E.J.M.: 130 years of Plant Lectin Research. Glycoconj. J. 37, 533–551 (2020). https://doi.org/10.1007/s10719-020-09942-y

4. Komath, S.S., Kavitha, M., Swamy, M.J.: Beyond carbohydrate binding: new directions in plant lectin research. Org. Biomol. Chem. 4, 973–988 (2006). https://doi.org/10.1039/b515446d

5. De Coninck, T., Van Damme, E.J.M.: The multiple roles of plant lectins. Plant Sci. 313, 111096 (2021). https://doi.org/10.1016/j.plantsci.2021.111096

6. Araripe, D.A., Pinto-Junior, V.R., Neco, A.H.B., Santiago, M.Q., Osterne, V.J.S., Pires, A.F., Lossio, C.F., Martins, M.G.Q., Correia, J.L.A., Benevides, R.G., Leal, R.B., Assreuy, A.M.S., Nascimento, K.S., Cavada, B.S.: Partial characterization and immobilization in CNBr-activated Sepharose of a native lectin from *Platypodium elegans* seeds (PELa) and comparative study of edematogenic effect with the recombinant form. Int. J. Biol. Macromol. 102, 323–330 (2017). https://doi.org/10.1016/j.ijbiomac.2017.03.193

7. Alencar, N.M.N., Assreuy, A.M.S., Alencar, V.B.M., Melo, S.C., Ramos, M.V., Cavada, B.S., Cunha, F.Q., Ribeiro, R.A.: The galactose-binding lectin from *Vatairea macrocarpa* seeds induces *in vivo* neutrophil migration by indirect mechanism. Int. J. Biochem. Cell Biol. 35, 1674–1681 (2003). https://doi.org/10.1016/s1357-2725(03)00138-9

8. Marques, G.F.O., Pires, A.F., Osterne, V.J.S., Pinto-Junior, V.R., Silva, I.B., Martins, M.G.Q., Oliveira, M.V., Gomes, A.M., de Souza, L.A.G., Pavão, M.S.G., Cavada, B.S., Assreuy, A.M.S., Nascimento, K.S.: *Vatairea guianensis* lectin stimulates changes in gene expression and release of TNF-α from rat peritoneal macrophages via glycoconjugate binding. J. Mol. Recognit. 34, 1-10 (2021). https://doi.org/10.1002/jmr.2922

9. De Freitas Pires, A., Bezerra, M.M., Amorim, R.M.F., do Nascimento, F.L.F., Marinho, M.M., Moura, R.M., Silva, M.T.L., Correia, J.L.A., Cavada, B.S., Assreuy, A.M.S., Nascimento, K.S.: Lectin purified from *Lonchocarpus campestris* seeds inhibits inflammatory nociception. Int. J. Biol. Macromol. 125, 53–60 (2019). https://doi.org/10.1016/j.ijbiomac.2018.11.233

10. Avichezer, D., Arnon, R.: Differential reactivities of the *Arachis hypogaea* (peanut) and *Vicia villosa* B4 lectins with human ovarian carcinoma cells, grown either in vitro or *in vivo* xenograft model . FEBS Lett. 395, 103–108 (1996). http://dx.doi.org/10.1016/0014-5793(96)01010-1

11. Li, X.-T., He, M.-L., Zhou, Z.-Y., Jiang, Y., Cheng, L.: The antitumor activity of PNA modified vinblastine cationic liposomes on Lewis lung tumor cells: In vitro and in vivo evaluation. Int. J. Pharm. 487, 223–233 (2015). https://doi.org/10.1016/j.ijpharm.2015.04.035

12. Nascimento, K.S., Silva, M.T.L., Oliveira, M.V., Lossio, C.F., Pinto-Junior, V.R., Osterne, V.J.S., Cavada, B.S.: *Dalbergieae* lectins: A review of lectins from species of a primitive *Papilionoideae* (leguminous) tribe. Int. J. Biol. Macromol. 144, 509–526 (2020). https://doi.org/10.1016/j.ijbiomac.2019.12.117

13. Reyes-Montaño, E.A., Vega-Castro, N.A.: Plant Lectins with Insecticidal and Insectistatic Activities. Insecticides - Agriculture and Toxicology, pp. 17-41 (2018). http://dx.doi.org/10.5772/intechopen.74962

14. Walski, T., De Schutter, K., Cappelle, K., Van Damme, E.J.M., Smagghe, G.: Distribution of Glycan Motifs at the Surface of Midgut Cells in the Cotton Leafworm Demonstrated by Lectin Binding. Front. Physiol. 8, 1020 (2017). https://doi.org/10.3389/fphys.2017.01020

15. De Lima, H.C.: Revisão taxonômica do gênero Vataireopsis Ducke (Leg. Fab.). Ducke (Leg. Fab.). Rodriguésia. 32, 21-40 (1980). http://dx.doi.org/10.1590/2175-78601980325404

16. Valle, M.L.A., de Sousa Aleluia Santos, B., Jardim, J.G.: A xiloteca do Centro de Pesquisas do Cacau e as madeiras da Mata Atlântica. Mata Atlântica. Paubrasilia. 2(2), 7-13 (2019). http://dx.doi.org/10.33447/paubrasilia.v2i2.28

17. Christoforo, A.L., de Moura Aquino, V.B., Govone, J.S., Dias, A.M.P., Panzera, T.H., Lahr, F.A.R.: Alternative model to determine the characteristic strength value of wood in the compression parallel to the grain. Maderas. Ciencia y tecnología. 22(3), 281-290 (2020). http://dx.doi.org/10.4067/s0718-221x2020005000303

18. Kerkhof, P.C.M.V.D., Van De Kerkhof, P.C.M., Van Der Valk, P.G.M., Swinkels, O.Q.J., Kucharekova, M., De Rie, M.A., De Vries, H.J.C., Damstra, R., Oranje, A.P., De Waard-van der Spek, F.B., Van Neer, P., Lijnen, R.L.P., Kunkeler, A.C.M., Van Hees, C., Haertlein, N.G.J., Hol, C.W.: A comparison of twice-daily calcipotriol ointment with once-daily short-contact dithranol cream therapy: a randomized controlled trial of supervised treatment of psoriasis vulgaris in a day-care setting. British Journal of Dermatology. 155, 800–807 (2006). http://dx.doi.org/10.1111/j.1365-2133.2006.07393.x

19. Hoffmann, J., Gendrisch, F., Schempp, C.M., Wölfle, U.: New Herbal Biomedicines for the Topical Treatment of Dermatological Disorders. Biomedicines. 8, (2020). https://doi.org/10.3390/biomedicines8020027

20. Cavada, B.S., Bari, A.U., Pinto-Junior, V.R., Lossio, C.F., Silva, M.T.L., Souza, L.A.G., Oliveira, M.V., Souza-Filho, C.H.D., Correia, S.E.G., Ana Paula Moreira, Lima, L.D., Osterne, V.J.S., Nascimento, K.S.: Purification and partial characterization of a new lectin from *Parkia panurensis* Benth. ex H.C. Hopkins seeds (*Leguminosae* family; *Mimosoideae* subfamily) and evaluation of its biological effects. Int. J. Biol. Macromol. 145, 845–855 (2020). http://dx.doi.org/10.1016/j.ijbiomac.2019.10.102

21. Cavada, B.S., Santos, C.F., Grangeiro, T.B., Nunes, E.P., Sales, P.V., Ramos, R.L., De Sousa, F.A., Crisostomo, C.V., Calvete, J.J.: Purification and characterization of a lectin from seeds of *Vatairea macrocarpa* Duke. Phytochemistry. 49, 675–680 (1998). https://doi.org/10.1016/s0031-9422(98)00144-7

22. Silva, H.C., Nagano, C.S., Souza, L.A.G., Nascimento, K.S., Isídro, R., Delatorre, P., Rocha, B.A.M., Sampaio, A.H., Assreuy, A.M.S., Pires, A.F., Damasceno, L.E.A., Marques-Domingos, G.F.O., Cavada, B.S.: Purification and primary structure determination of a galactose-specific lectin from *Vatairea guianensis* Aublet seeds that exhibits vasorelaxant effect. Process Biochem. 47, 2347–2355 (2012). https://doi.org/10.1016/j.procbio.2012.09.014

23. Sultan, N.A.M., Kenoth, R., Swamy, M.J.: Purification, physicochemical characterization, saccharide specificity, and chemical modification of a Gal/GalNAc specific lectin from the seeds of *Trichosanthes dioica*. Arch. Biochem. Biophys. 432, 212–221 (2004). https://doi.org/10.1016/j.abb.2004.09.016

24. Datta, D., Pohlentz, G., Schulte, M., Kaiser, M., Goycoolea, F.M., Müthing, J., Mormann, M., Swamy, M.J.: Physico-chemical characteristics and primary structure of an affinity-purified α-D-galactose-specific, jacalin-related lectin from the latex of mulberry (*Morus indica*). Arch. Biochem. Biophys. 609, 59–68 (2016). https://doi.org/10.1016/j.abb.2016.09.009

25. Appukuttan, P.S., Surolia, A., Bachawat, B.K.: Isolation of two galactose-binding proteins from *Ricinus communis* by affinity chromatography. Indian J. Biochem. Biophys. 14, 382–384 (1977)

26. Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227, 680–685 (1970). https://doi.org/10.1038/227680a0

27. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254 (1976). https://doi.org/10.1006/abio.1976.9999

28. Zacharius, R.M., Zell, T.E., Morrison, J.H., Woodlock, J.J.: Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. 30, 148–152 (1969). https://doi.org/10.1016/0003-2697(69)90383-2

29. DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28(3) 350–356 (1956). http://dx.doi.org/10.1021/ac60111a017

30. Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V., Mann, M.: In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. 1, 2856–2860 (2006). https://doi.org/10.1038/nprot.2006.468

31. Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J.: Basic local alignment search tool. J. Mol. Biol. 215, 403-410 (1990). http://dx.doi.org/10.1016/s0022-2836(05)80360-2

32. Robert, X., Gouet, P.: Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Research. 42, 320-324 (2014). http://dx.doi.org/10.1093/nar/gku316

33. Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G.: Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539 (2011). https://doi.org/10.1038/msb.2011.75

34. Brzezicka, K., Echeverria, B., Serna, S., van Diepen, A., Hokke, C.H., Reichardt, N.-C.: Synthesis and microarray-assisted binding studies of core xylose and fucose containing N-glycans. ACS Chem. Biol. 10, 1290–1302 (2015). https://doi.org/10.1021/cb501023u

35. Smagghe, G.J., Elsen, K., Loeb, M.J., Gelman, D.B., Blackburn, M.: Effects of a fat body extract on larval midgut cells and growth of *Lepidoptera*. *In Vitro* Cell. Dev. Biol. Anim. 39, 8–12 (2003). https://doi.org/10.1290/1543-706X(2003)039<0008:EOAFBE>2.0.CO;2

36. Smagghe, G., Goodman, C.L., Stanley, D.: Insect cell culture and applications to research and pest management. *In Vitro* Cellular & Developmental Biology - Animal. 45, 93–105 (2009). http://dx.doi.org/10.1007/s11626-009-9181-x

37. Fu, C., Zhao, H., Wang, Y., Cai, H., Xiao, Y., Zeng, Y., Chen, H.: Tumor-associated antigens: Tn antigen, sTn antigen, and T antigen. Hladnikia . 88, 275–286 (2016). https://doi.org/10.1111/tan.12900

38. Law, I.J., Haylett, T., Strijdom, B.W.: Differences in properties of peanut seed lectin and purified galactose- and mannose-binding lectins from nodules of peanut. Planta. 176, 19-27 (1988). http://dx.doi.org/10.1007/bf00392475

39. Lotan, R., Skutelsky, E., Danon, D., Sharon, N.: The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). J. Biol. Chem. 250, 8518–8523 (1975). https://doi.org/10.1016/s0021-9258(19)40790-4

40. Singh, R., Das, H.R.: Purification of lectins from the stems of peanut plants. Glycoconj. J. 11, 282–285 (1994). https://doi.org/10.1007/BF00731199

41. Joubert, F.J., Sharon, N., Merrifield, E.H.: Purification and properties of a lectin from *Lonchocarpus capassa* (apple-leaf) seed. Phytochemistry. 25, 323–327 (1986). https://doi.org/10.1016/S0031-9422(00)85474-6

42. Alves Filho, J.G., do Nascimento, A.S.F., Gondim, A.C.S., Pereira, R.H., da Cunha, R.M.S., Nagano,

C.S., Teixeira, E.H., Nascimento, K.S., Cavada, B.S.: Isoform characterisation, heterologous expression and functional analysis of two lectins from *Vatairea macrocarpa*. Protein Pept. Lett. 20, 1204–1210 (2013).

43. Lauwereys, M., Foriers, A., Sharor, N., Strosberg, A.D.: Sequence studies of peanut agglutinin. FEBS Lett. 181, 241–244 (1985). https://doi.org/10.1016/0014-5793(85)80267-2

44. Young, N.M., Johnston, R.A., Watson, D.C.: The amino acid sequence of peanut agglutinin. Eur. J. Biochem. 196, 631–637 (1991). https://doi.org/10.1111/j.1432-1033.1991.tb15859.x

45. Calvete, J.J., Santos, C.F., Mann, K., Grangeiro, T.B., Nimtz, M., Urbanke, C., Sousa-Cavada, B.: Amino acid sequence, glycan structure, and proteolytic processing of the lectin of *Vatairea macrocarpa* seeds. FEBS Lett. 425, 286–292 (1998). https://doi.org/10.1016/s0014-5793(98)00243-9

46. Van Damme, E.J., Barre, A., Rougé, P., Van Leuven, F., Peumans, W.J.: The seed lectins of black locust (*Robinia pseudoacacia*) are encoded by two genes which differ from the bark lectin genes. Plant Mol. Biol. 29, 1197–1210 (1995). https://doi.org/10.1007/BF00020462

47. Sousa, B.L., Filho, J.C.S., Kumar, P., Pereira, R.I., Łyskowski, A., Rocha, B.A.M., Delatorre, P., Bezerra, G.A., Nagano, C.S., Gruber, K., Cavada, B.S.: High-resolution structure of a new Tn antigen-binding lectin from *Vatairea macrocarpa* and a comparative analysis of Tn-binding legume lectins. The International Journal of Biochemistry & Cell Biology 59,103–110 (2015). http://dx.doi.org/10.1016/j.biocel.2014.12.002

48. Calvete, J.J., Santos, C.F., Mann, K., Grangeiro, T.B., Nimtz, M., Sousa-Cavada, B.: Primary structure and posttranslational processing of *Vatairea macrocarpa* seed lectin. J. Protein Chem. 17, 545–547 (1998). https://doi.org/10.1016/S0014-5793(98)00243-9

49. Serna, S., Etxebarria, J., Ruiz, N., Martin-Lomas, M., Reichardt, N.-C.: Construction of N-glycan microarrays by using modular synthesis and on-chip nanoscale enzymatic glycosylation. Chemistry. 16, 13163–13175 (2010). https://doi.org/10.1002/chem.201001295

50. Echeverria, B., Serna, S., Achilli, S., Vivès, C., Pham, J., Thépaut, M., Hokke, C.H., Fieschi, F., Reichardt, N.-C.: Chemoenzymatic Synthesis of N-glycan Positional Isomers and Evidence for Branch Selective Binding by Monoclonal Antibodies and Human C-type Lectin Receptors. ACS Chem. Biol. 13, 2269–2279 (2018). https://doi.org/10.1021/acschembio.8b00431

51. Hamshou, M., Smagghe, G., Shahidi-Noghabi, S., De Geyter, E., Lannoo, N., Van Damme, E.J.M.: Insecticidal properties of *Sclerotinia sclerotiorum* agglutinin and its interaction with insect tissues and cells. Insect Biochem. Mol. Biol. 40, 883–890 (2010). https://doi.org/10.1016/j.ibmb.2010.08.008

52. Hamshou, M., Van Damme, E.J.M., Caccia, S., Cappelle, K., Vandenborre, G., Ghesquière, B., Gevaert, K., Smagghe, G.: High entomotoxicity and mechanism of the fungal GalNAc/Gal-specific Rhizoctonia solani lectin in pest insects. J. Insect Physiol. 59, 295–305 (2013). https://doi.org/10.1016/j.jinsphys.2012.12.003

53. Chen, P., De Schutter, K., Serna, S., Chen, S., Yang, Q., Reichardt, N.-C., Van Damme, E.J.M., Smagghe, G.: Glycosylation reduces the glycan-independent immunomodulatory effect of recombinant Orysata lectin in *Drosophila* S2 cells. Sci. Rep. 11, 17958 (2021). https://doi.org/10.1038/s41598-021-97161-2

54. Law, I.J., Kfir, R.: Effect of mannose-binding lectin from peanut and pea on the stem borer Chilo partellus. Entomologia Experimentalis et Applicata 82, 261–265 (1997). http://dx.doi.org/10.1046/j.1570-7458.1997.00139.x

55. Chen, P., De Schutter, K., Pauwels, J., Gevaert, K., Van Damme, E.J.M., Smagghe, G.: Binding of Orysata lectin induces an immune response in insect cells. Insect Sci. 29, 717-729 (2021). https://doi.org/10.1111/1744-7917.12968

56. Macedo, M.L.R., Oliveira, C.F.R., Oliveira, C.T.: Insecticidal activity of plant lectins and potential application in crop protection. Molecules. 20, 2014–2033 (2015). https://doi.org/10.3390/molecules20022014

FIGURE LEGENDS

Figure 1. Affinity chromatography on a guar gum polysaccharide matrix (2 cm x 15 cm). The crude extract was applied and kept in contact for 4 h. Unbound proteins were washed with 150 mM NaCl containing 5 mM $CaCl_2$ and 5 mM $MnCl_2$, and the lectin was eluted with 100 mM glycine-HCl, pH 2.6, containing 150 mM NaCl. Fractions were collected in a volume of approximately 3 mL at a flow rate of 1 mL/min and were detected by spectrophotometry at 280 nm. SDS-PAGE of purified lectin from *V. araroba*. 1. Prestained Protein Molecular Weight Marker – bands: 120 kDa, 85 kDa, 50 kDa, 35 kDa, 25 kDa, and 20 kDa. 2. Purified Val and 3. VaL labeled with Periodic acid-Schiff reagent. Standard curve to measure the apparent molecular weight of the lectin.

Figure 2. Glycan microarray screening of VAL lectin. Each histogram represents the average of RFU for four replicate spots with the standard deviation of the mean. Green histograms represent structures with terminal β1-4Gal epitope, blue histograms represent structures with terminal β1-4GalNAc epitopes and orange histograms represent structures with LDNF epitopes.

Figure 3. Alignment of the partial sequence of VaL with the sequences from the lectins of *Arachis hypogaea* (UNIPROT ID: P02872), *Robinia pseudoacacia* (UNIPROT ID: Q41161), *Vatairea macrocarpa* (UNIPROT ID: P81371) and *Vatairea guianensis* (UNIPROT ID: P86893). Amino acids highlighted in red represent similar residues.

Figure 4. Effect of VaL on CF1 cells. a) Graph depicting the effect of VaL on CF1 cell numbers at different concentrations and time points b) Cell agglutination induced by the lectin c) Control cells. *** $p<0.001$, **$p<0.01$ and *$p<0.05$ in relation to the control, Kruskal-Wallis test.

Figure 1

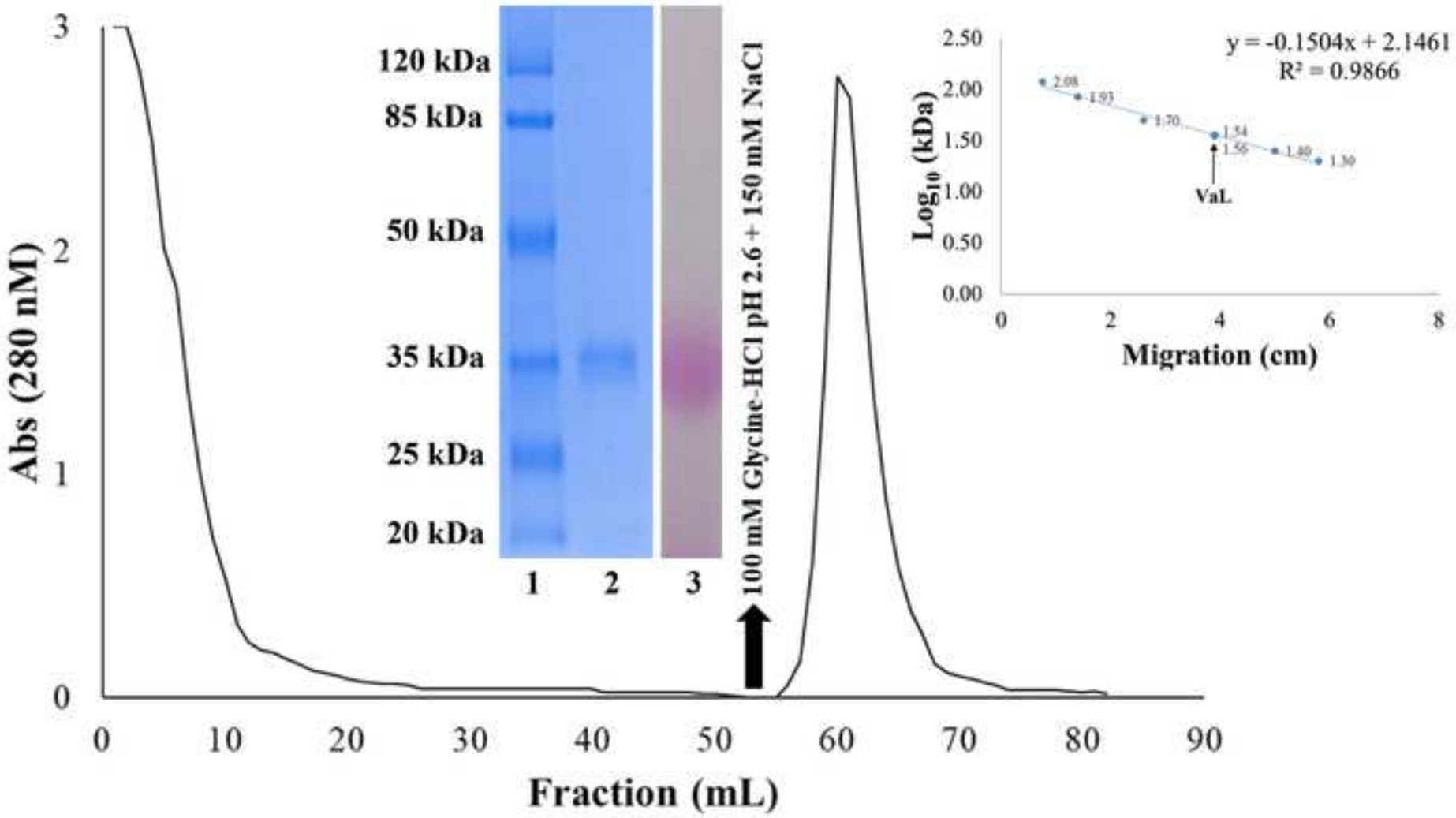


Figure 2

Click here to access/download;Figure;Fig.2.tif

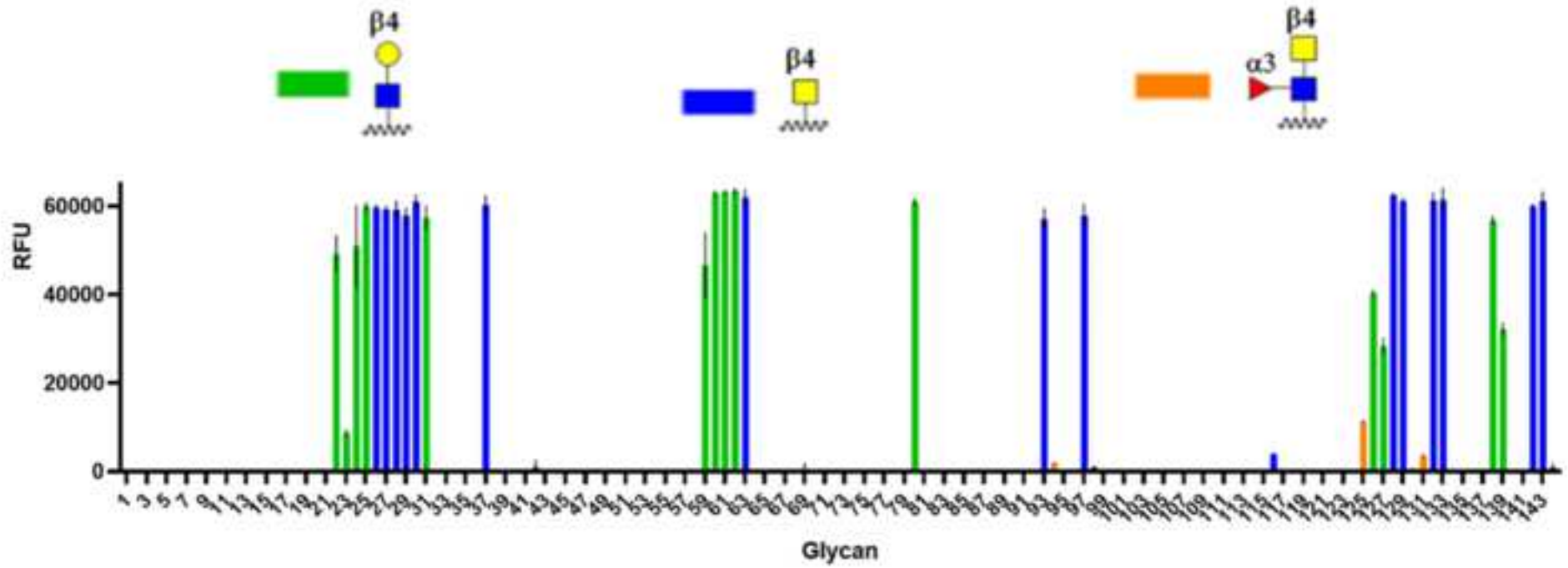


Figure 3

Click here to access/download;Figure;Fig.3.tif

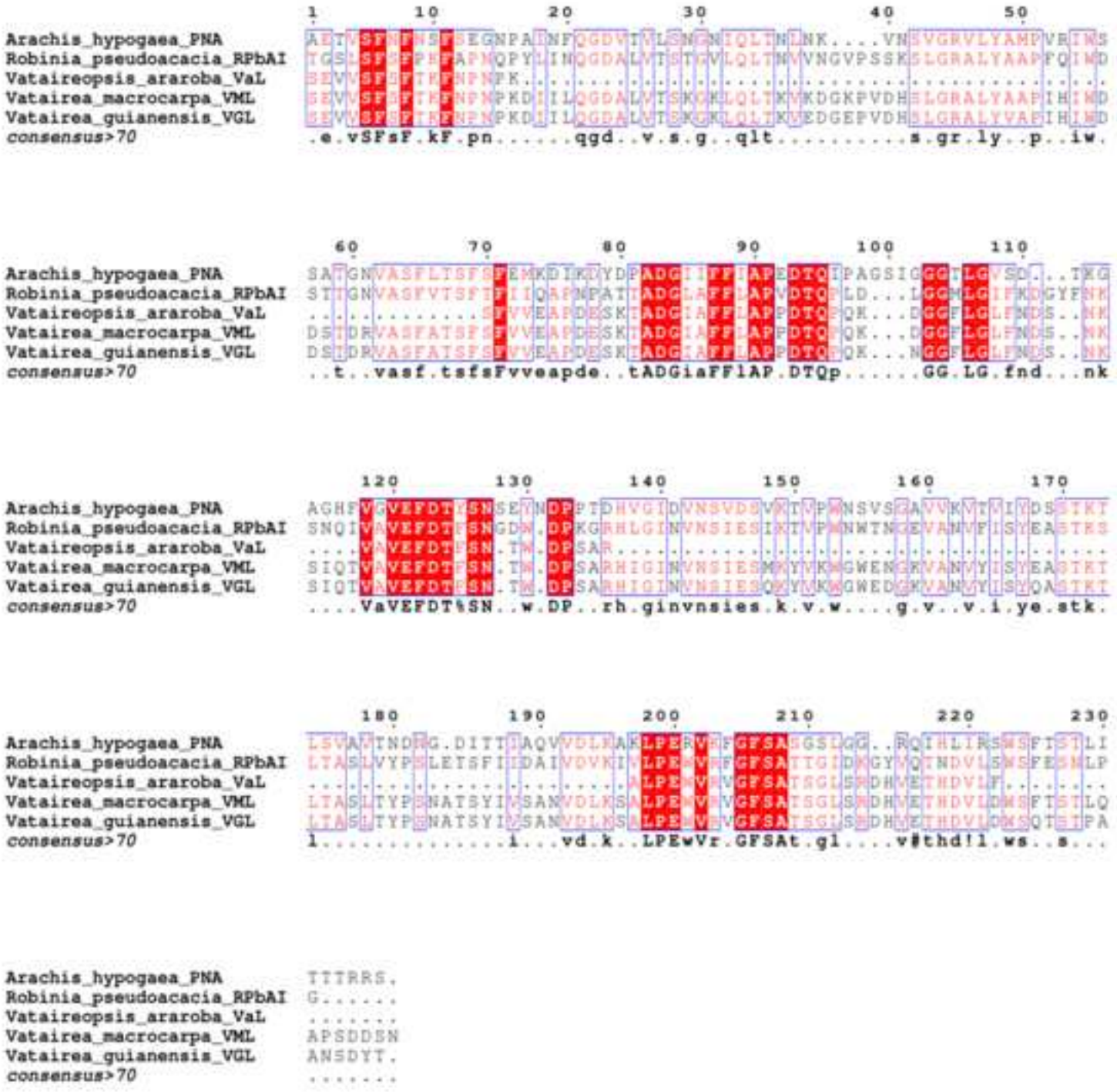
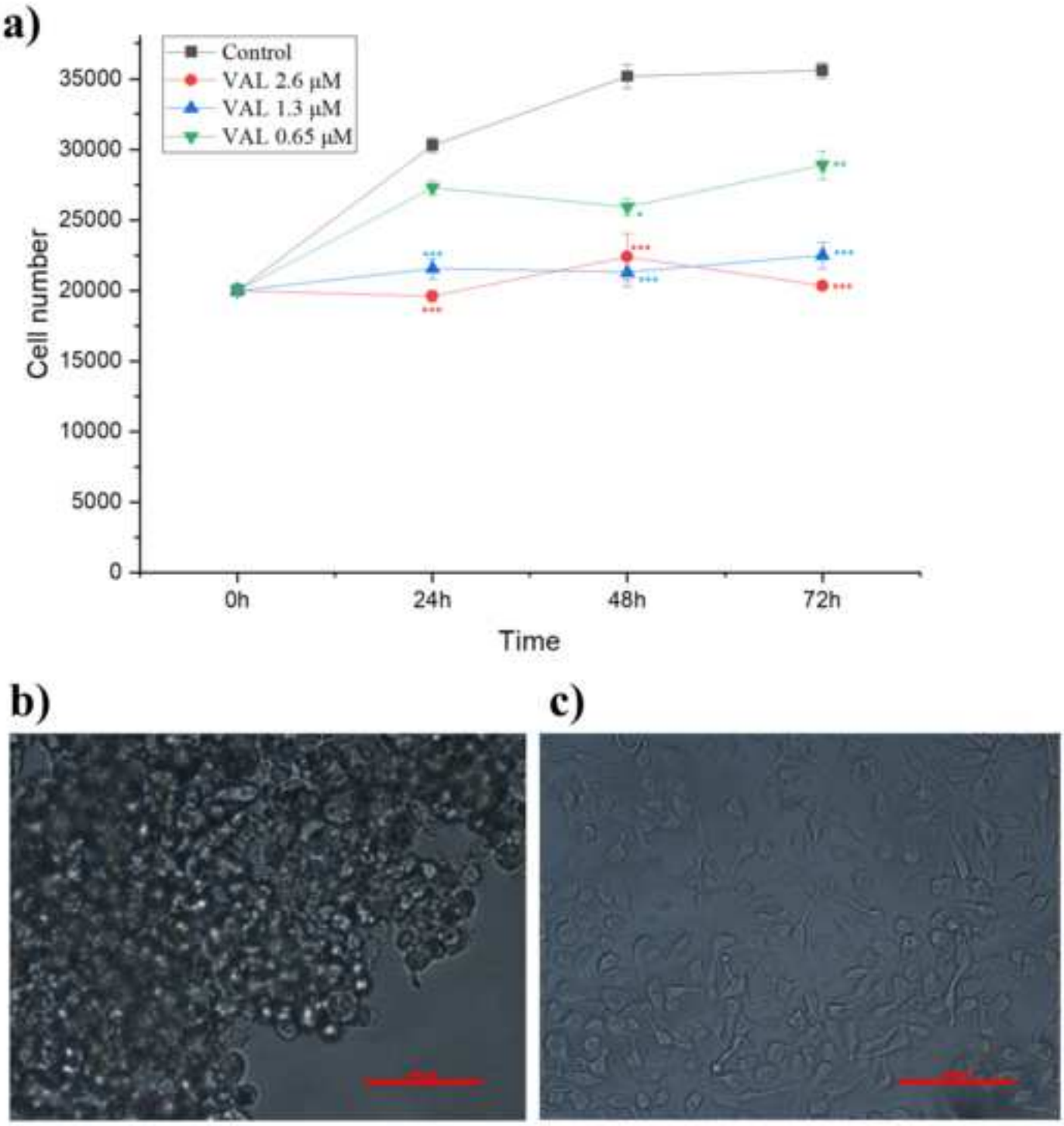


Figure 4



Online resources

Table 1. Purification of the lectin from *Vataireopsis araroba* seeds

Fractions	**[a] Total protein (mg/mL)**	**[b] Total A.H. (U.H./mL)**	**[c] Specific activity (U.H./mg)**	**[d]Purification (fold)**	**Yield (%)**
Crude extract	0.06	16,384	273,067	1	100
Guar gum affinity chromatography	0.04	131,072	3,276,800	12	66,66

[a] Soluble proteins content. [b] Hemagglutinating activity expressed in hemagglutinating units (H.U.). [c] Specific activity calculated as the ratio between the hemagglutinating activity and the protein content. [d] Purification, calculated as the ratio between the Specific Hemagglutinating Activity of the fractions with the crude extract.

Table 2. Inhibitory effect of saccharides and glycoprotein on the hemagglutinating activity of *Vataireopsis araroba* lectin

Carbohydrates/Glycoproteins	**Minimum Inhibitory Concentration**
D-glucose	NI
D-mannose	NI
α-methyl-D-glucopyranoside	NI
α-methyl-D-mannoside	NI
D-galactose	25 mM
N-acetylgalactosamine	0.4 mM
α-lactose	1.6 mM
N-acetylglucosamine	NI
L-rhamnose	50 mM
L-fucose	NI
Ovalbumin	0.25 mg/mL
Mucin	0.016 mg/mL

NI, sugar not inhibitory.