CHAPTER
23

Prospection of lectins present in the seeds of *Bauhinia subclavata* Benth and *Crotalaria spectabilis* Roth

https://doi.org/10.56238/alookdevelopv1-023

Alana Alves Farias
Laboratory of Genetics, Department of Exact and Earth Sciences II (DCET-II), Universidade do Estado da Bahia (UNEB). Bahia, Brazil
Neuroin Institute for Human Research and Development (NEURO-IN). Bahia, Brazil
E-mail: farias_aa@hotmail.com

Elda Nunes da Silva
Laboratory of Genetics, Department of Exact and Earth Sciences II (DCET-II), Universidade do Estado da Bahia (UNEB). Bahia, Brazil
E-mail: elda.biologia@gmail.com

Wilian Rosário de Oliveira
Laboratory of Genetics, Department of Exact and Earth Sciences II (DCET-II), Universidade do Estado da Bahia (UNEB). Bahia, Brazil
E-mail: mestrewilian@hotmail.com

Evandro José Lima Rego
Laboratory of Genetics, Department of Exact and Earth Sciences II (DCET-II), Universidade do Estado da Bahia (UNEB). Bahia, Brazil
E-mail: ejlrego@gmail.com

ABSTRACT
A signaling cell is the basis for the triggering of many cellular processes. Among the molecules that mediate these mechanisms are simple carbohydrates or conjugates making up the cell surface membrane receptors and the other proteins, particularly lectins, which would act by binding to these receptors to mediate different activities of cellular metabolism. In this study, we verified the ability of protein extracts obtained from seeds of Bauhinia subclavian, Benth, and Crotalaria spectabilis, Roth to recognize receptors present on human erythrocytes, promote the agglutination of these cells.

Keywords: lectin, hemagglutination, *Bauhinia subclavata, Crotalaria spectabilis*

1 INTRODUCTION

Lectins are proteins of non-immune origin, capable of binding to carbohydrates and agglutinating cells or precipitating polysaccharides and glycoconjugates (GOLDSTEIN *et al.*, 1980). This heterogeneous group of proteins differs from antibodies since it does not originate in the immune system and also differs from carbohydrate-binding enzymes because they do not modify the structure of the carbohydrate to which they bind, which occurs in enzymatic processes. However, there are lectins that in addition to the electronic binding site, have an enzymatic site (LORIS, 2002; Singh *et. al*, 1999; Cummings, 1997). But they differ from carbohydrate-binding enzymes such as glycosidases, glucanases, and chitinases in that they do not modify the structure of the carbohydrate to which they bind, which occurs in enzymatic processes.

Initially isolated from plants, they have a universal distribution, ranging from viruses to mammals. In enterobacteria they would be essential in adhesion to the intestinal epithelium; in fungi and some yeasts, they would have acted in the aggregation and subsequent flocculation of cells; in invertebrates, they are found in hemolymph and would play a role in resistance to infections (SINGH *et. al*, 1999). In more complex animals, lectins are produced in soluble form or are bound to the membrane (ZATTA and CUMMINGS, 1991). In snakebite venoms, they could supposedly be involved in the metabolic processes of the snake itself, not acting on the animal exposed to the venom (OGILVIE *et al.*, 1989). ACa2+ dependent and galactose-specific lectin was purified from the sea cucumber, *Cucumaria echinata*; exhibiting hemolytic and hemagglutinating activities (TOMOMITSU, 2020) In plants they are distributed everywhere; but they have a higher concentration in seeds, especially those of the species of the Leguminosae family. However, its real role is unknown, although many hypotheses about its physiological functions have been suggested.

Evidence suggests that lectins from seeds, tissues, and rhizomes (GREENWOOD et al., 1986, NSIMBA-LUBAKIE and PEUMANS, 1986) would be a specialized form of storage proteins that may occasionally have other functions (ETZLER, 1985, PUSZTAI *et al*., 1983, PUSZTAI, 1991). The presence of lectins in the seeds would be related to their role as an organ responsible for the perpetuation of the species, thus requiring substances that promote the protection of the genetic information necessary for the formation of a new individual.

Some experiments suggest that lectins may play a role in the cellular recognition system in higher plants, such as that involving the interaction of pollen with appropriate stigma. This process is part of a system of self-incompatibility because it prevents fertilization by self-fertilization or crossing between plants with the same alleles, which helps in the maintenance of heterozygosity by favoring cross-pollination (FERRARI *et al.*, 1981; SANTOS SOUSA *et al*, 2013).

The functions attributed to plant lectins as agents in the defense mechanism are mainly based on the ability of some lectins to bind to the cell wall of bacteria. The defensive role of lectins would not be restricted to bacterial action but would prevent the development of fungi and also the attack of predatory insects (TOMS, 1981; LEACH *et al*., 1982; PUSZTAI, 1991; LIMA, 2017). The association of bacteria with the root surface occurs in a specific way and the first to suggest the role of lectins in this process was Saint–Paul (1961), without, however, obtaining conclusive results. Other studies suggest that lectins would act in cellular organization, embryo morphogenesis, storage, or transport of carbohydrates (MOREIRA *et al*., 1991; Rüdiger and GABIUS, 2001).

Certainly, the presence of the various tissues points to different physiological roles, and it is more sensible to consider them as multifunctional, perhaps having different functions in a plant or different functions in different species.

According to Sharon and Lis (1989), lectins play a fundamental role in the control of several normal and pathological processes of living beings. Nowell (1960) observed that the lectin present in *Phaseolus vulgaris* could stimulate *in vitro* the morphological transformation of lymphocytes and their proliferation. This mitogenic action enabled its use in morphological and functional studies, particularly of lymphocytes, as well as for the diagnosis of immunodeficiencies and monitoring of the effects of immunosuppressants and immunotherapeutics (SHARON, 1993, FARIAS**,** 2015, OLIVEIRA, 2018).

There is no consensus as to the mechanism of action of these proteins in cell activation. One hypothesis considers that binding to receptors produces a modification of membrane structure; triggering a series of biochemical events inside the cell that lead to cellular activation. On the other hand, lectins could act indirectly, that is, cells modified by binding to lectin would stimulate the proliferation of other lymphocytes, in a mechanism analogous to what occurs in a lymphocyte mixing reaction, where cells of one genotype cause stimulation of cells of another genotype (LIS and SHARON, 1986).

Understanding the mechanisms of action by which receptors and signaling molecules regulate processes such as metabolism, adhesion, proliferation, and cell differentiation opens up numerous possibilities in the areas of immunology and oncology since cell signaling is directly involved in the control and survival of cells.

Recent studies related to SARS-CoV-2 and the establishment of COVID-19 show the participation of lectins in different ways in the establishment of infection allowing a better understanding of the mechanisms involved in the interaction of the virus with cell membrane receptors (ERIKSSON *et al.* 2020; LENZA *et al.* 2020; Rahimi, 2020; RAMBALDI *et al*., 2020).

The best-known property of lectins is the binder activity, and its detection is carried out mainly through the hemagglutination assay. (ERSSON *et al.* , 1973, REGO *et al.* , 2002). However, lectins may present an absence of agglutinating activity in human erythrocytes or lack specificity for a certain blood group, may hemagglutinate erythrocytes of different species, and sometimes present variation in the capacity of hemagglutinate depending on the geographical origin of the plant species (MACHUKA, 2000).

In this work we demonstrated the presence of lectins in the seeds of two legume species, *Bauhinia subclavata* Benth and *Crotalaria spectabilis* Roth, capable of binding specifically to glycidic receptors present on the surface of human erythrocytes.

2 METHODOLOGY

2.1 BIOLOGICAL MATERIAL

The seeds of *Bauhinia subclavata* Benth (Figure 1) were collected at *Campus II* of the Universidade do Estado da Bahia – UNEB, located in the municipality of Alagoinhas – BA, referenced at 11º 55' 51" and 12º 15' 23" South latitude and 38º15'00" and 38º 35'00" West longitude. The seeds of *Crotalaria spectabilis* Roth (Figure 2) were obtained from the Agronomic Institute of Campinas – IAC, Campinas – SP.

Figure 1 – *Bauhinia subclavata* Benth. Tree, leaf, flower, pods, and seeds.

Photo: Alana Farias

Figure 2 - *Crotalaria spectabilis* Roth: Fruits, Seeds.

Photo: Wilian Oliveira

2.2 PROTEIN EXTRACTION

Approximately 40 g of whole seeds of each species were crushed separately and a solution of 0.15 M NaCl was added to each sample until the final concentration of 1/10 (m/v), remaining under light agitation for 1 hour at room temperature. The suspension was centrifuged at 5000 x g for 20 minutes, and the precipitate was discarded and the supernatant reserved.

Each of the supernatants was added, under agitation and slowly, acetone at a temperature of 4ºC until a final concentration of 80% (v/v). After 30 minutes of decantation, the material was centrifuged at 5000 x g for 20 minutes. The supernatant was discarded and the protein precipitate spread in petri dishes and dried at room temperature. After drying, the material was sprayed and diluted in saline.

2.3 COLLECTION, PREPARATION OF ERYTHROCYTES AND BIOLOGICAL ASSAYS

About 4 ml of blood A, B, AB, and O were collected aseptically, and the erythrocytes were washed four times with saline solution (0.15M NaCl), at 1000 x g for 10 minutes at room temperature for each 1 ml of blood added 4 ml of saline, until the red blood cells were free of plasma and leukocytes. The precipitate was resuspended in the same solution to obtain a final suspension of erythrocytes at 2% (v/v). Hemagglutination tests were performed with saline and ketone extracts.

The assays were performed on microtitration plates of 96 wells (Sigma Chemical Co USA) (12 columns and 8 rows). The first wells of the rows were filled with 50 l of the sample this was then diluted serially, with agitation and transfer of 50 l to the next well to the penultimate well of the row. After the dilutions, 50 l of 2% erythrocyte suspension (v/v) was added to the wells. The final volume of the assay was 100 µl, containing 50 µl of saline solution, diluted with the extracts under study and 50 µl of erythrocyte suspension, except for the wells of the last column which contained only 50 µl of saline solution and 50 µl of erythrocyte suspension, as these corresponded to the controls. The readings were made visually after incubation in the periods of 1h, 6h, 12h, 18h, and 24h at room temperature, comparing with the respective negative controls (only the suspension of erythrocytes in saline solution).

3 RESULTS AND DISCUSSION

The results of the binding capacity of the crude extract and the protein precipitate obtained from the seeds of *Crotalaria spectabilis* Roth and *Bauhinia subclavia*n Benth are summarized in Table 1, indicating the reactivity of the proteins for the different blood types tested.

Red blood cells are among the best cells for agglutination tests. The lectin when adsorbing the red blood cells through receptors existing on the cell surface promotes agglutination, a phenomenon called hemagglutination. Suspended red blood cells are put in contact with a suspension of the lectin. The action of gravity, in the absence of lectin or lectin, does not specify the red blood cells sediment in the form of a compact button at the bottom of the well and the agglutinated red blood cells sediment in a diffuse way forming a network of cells that covers the bottom of the well.

The positive results were detected visually after 1 hour of incubation through the formation of

a network of red blood cells that remained to cover the entire wall from the sides to the bottom (Figure 3, Figure 4).

Tests with the crude extract of *Bauhinia subclavian Benth* revealed lower specificity of this material (Table 2 and 3) about the protein extract, which is explained by the fact that the crude extract has other components that cause interference in the process of recognition of cell receptors. The protein precipitate showed greater agglutination capacity of human erythrocytes, precisely because it contains only proteins.

Figure 3 – Preparation of the plates for the cell agglutination assay

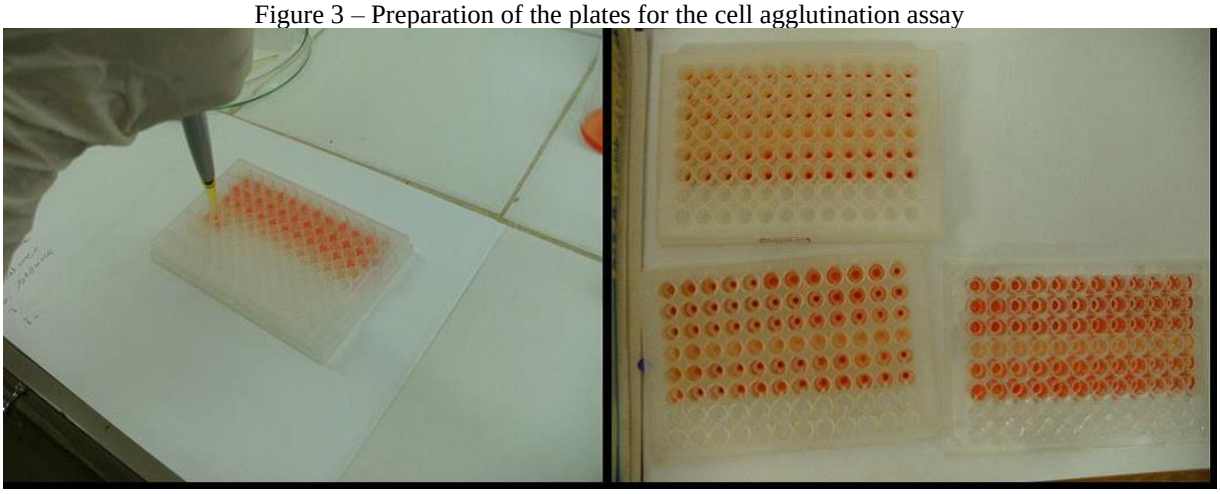

Photo: Wilian Oliveira

Figure 4 – A no activity. B binder activity

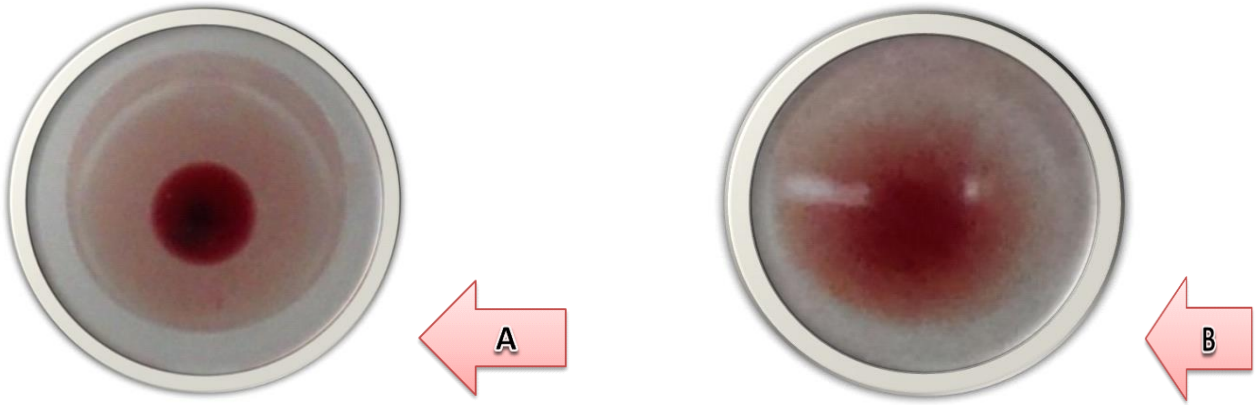

Photo: Alana Farias

Table 1 – Hemaguitinating activity

Types Blood	*Crotalaria spectabilis*	*Bauhinia subclavata*
A	+	+
B	-	+
AB	-	+
O	+	-

The material of *Crotalaria spectabilis* Roth showed specificity only for blood types A and O, not reacting positively to types B and AB, while the assays performed from the samples of Bauhinia subclavian Benth, that the protein present can interact with blood types A, B, and AB, and in the same way as *Bauhinia subclav*ian Benth is non-reactive for type O, according to the data presented in tables 2 and 3, respectively.

The absence of reactivity to erythrocytes type B and AB (*Crotalaria spectabilis* Roth) and type O (*Bauhinia subclavia*n Benth) was verified both for crude and protein extracts, which shows that it was no elements present in the crude extract that inhibited hemagglutination, but point out a real inability of the structure of the proteins of these species to recognize the glycidic portions present on the surfaces of these different erythrocytes.

Tests with the crude extract of *Bauhinia subclavia*n Benth revealed lower specificity of this material (Table 3), about the protein extract, which is explained by the fact that the crude extract presents other components that cause interference in the process of recognition of cell receptors. On the other hand, the tests performed with the protein precipitate showed a greater capacity of agglutination of human erythrocytes, precisely because it presents only proteins. Hemagglutination is only observed about erythrocytes type B and AB when the assays are performed with the protein extract.

The binding of lectin to membrane receptors can be influenced or inhibited by sialic acids, heterocyclic amines, carbohydrates, and some metal ions or if the receptors present in the cells are not sufficient or are inaccessible. Therefore, the removal of sialic acid from the cell surface increases agglutination due to decreased electrostatic repulsion between cells and lectins, as well as allowing sensitivity of membrane receptors. Thus, certain lectins do not agglutinate erythrocytes unless they are pretreated with enzymes (REGO *et al*., 2002). The trypsinization of red blood cells alters their surface by removing sialic acid from the cell surface and as a result decreasing the electrostatic repulsion between cells and lectins; in addition to allowing interaction with potential membrane receptors that are obstructed, making the sensitivity of the system greater. The use of purified lectins also offers greater sensitivity and specificity to the assay.

The molecular region of lectin that recognizes and interacts with a specific sugar sequence is called the carbohydrate recognition domain (CDR) and is present in all subunits of lectin

(DRICKAMER, 1993). The binding site described in detail for concanavalin A (HARDMAN et al., 1982) and is well preserved in all other legume lectins (LORIS *et al*., 1998).

Plant lectins can be classified according to the number of CRDs they present. Merolectins have at least one CRD, holo lectins comprise lectins with two or more identical or very similar domains, chimerolectins or hemilectins are those consisting of one or more CRDs plus one distinct domain with catalytic activity or with other biological activity, super lectins also have at least two CRDs but differ from holo lectins in the ability to recognize structurally unrelated sugars.

Experimental evidence points to the role of lectins and carbohydrates in many of the events involving cellular recognition, a result of the interactions between these molecules, providing the selectivity of reactions (NILCOLSON, 1974; SHARON and LIS, 1989), which would influence or control physiological processes such as cancer, fertilization, embryogenesis, migration, cell proliferation and differentiation, immune defense, infection by bacteria and viruses.

The type of carbohydrate present on the cell surface can be determined by adding different sugars to the assay and checking which of them is capable of inhibiting agglutination. By binding to lectin, sugar prevents the recognition of cellular receptors. This is the principle of the hemagglutination inhibition reaction. Blood types "A" and "O" are present on the cell surface α-N-acetyl-D-galactosamine and α-L-fucose, respectively. Thus, theseto sugars if added to the assays would be able to block the action of lectin. This evidence opens the perspective of control of pathophysiological processes that are mediated by the binding of lectins to the glycidic portions of the cell surface.

Table 2: Agglutinating Activity of *Crotalaria spectabilis* Roth.

Types Blood	**Extract Saline**	**Extract Ketonic**
A	+	+
B	-	-
AB	-	-
O	+	+

Table 3 – Agglutinating Activity of *Bauhinia subclavata* Benth

Types Blood	**Extract Saline**	**Extract Ketonic**
A	+	+
B	-	+
AB	-	+
O	-	-

4 FINAL CONSIDERATIONS

The molecular characteristics of lectins allow their use as a remarkable biotechnological tool in areas that require understanding the biological processes involved in cell recognition, such as cancer research, immunology, characterization, and differentiation of cells. In addition, it allows its use as a molecular marker in studies of phylogenetic evolution and speciation, since molecular data help when there are different hypotheses as to classification. Research involving lectins also enables a better understanding of the role of these proteins in vegetables. Thus, the presence of lectins in the seeds of *Crotalaria spectabilis* Roth and *Bauhinia subclavata* Benth brings perspectives of new studies involving these molecules

REFERENCES

CUMMINGS, R. D. Lectins as tool for glycoconjugate purification and characterizations. Glyco-science, status e perspectives. Capitulo 10. Ed. Gabius. Germany, 1997

DRICKAMER, K.; TAYLOR, M. E. Biology of animal lectins. *Annu. Rev. Cell Biol*, n. 9, p. 237-264. 1993.

ERSSON, B.; ASPBERG, K.; PORATH, J. The Phytohemagglutinin from sunn hemp seeds (*Crotalaria juncea*). Purification by biospecific affinity chromatography. Journal Biochimica et Biophysica Acta, v. 310, n. 2, p. 446-452, 1973.

ERIKSSON, O. *et al*Mannose-Binding Lectin is Associated with Thrombosis and Coagulopathy in Critically Ill COVID-19 PatientsThromb Haemost. Dec;120(12):1720-1724, 2020

ETZLER, M. E. Plant lectins: Molecular and biological aspects. *Annual Review of Plant Physiology*, n. 36, p.209-234, 1985.

FERRARI, T. E.; BRUNS, D.; WALLECE, D. H. Isolation of plant glycoprotein involved with the control on intercelular recongnition. *Plant. Physiol*, n. 67, p. 270-277. 1981.

FARIAS, A. A. Análise da marcação de células da linhagem C6 de glioma com as lectinas vegetais CpL, WGA e Con A. Dissertação de Mestrado- UFBA/FIOCRUZ-BA, Salvador, 2015.

GOLDSTEIN, I. J.*et al*. What should be called a lectin. *Nature*, 285: 66, 1980.

GREENWOOD, J.S. *et al*. *Sambucus nigra* agglutinin is located in protein bodies in the phloem parenchyma of the bark. *Plant*, n. 167, p. 257-258, 1986.

HARDMAN, K. D.; AGARWAL, R. C.; FREISER, M. J. Manganese and calcium binding sites of concanavalina A. *J. Mol. Biol*, n.157, p. 69-89. 1982.

LEACH, J. E.; CANTRELL, M. A.; SERQUEIRA, L**.** Hydroxproline-rich bacterial agglutinin from the potato. Extraction, purification and characterization. *Plant Physiology*, n.70, p. 1358-1358, 1982.

LENZA, M. *et al.* Structural Characterization of N-Linked Glycans in the Receptor Binding Domain of the SARS-CoV-2 Spike Protein and their Interactions with Human Lectins. Angew Chem Int Ed Engl Dec 21;59(52):23763-23771, 2020.

LIMA. T. E, Sartori, A. L. B, RODRIGUES, M. L. M. Plant antiherbivore defenses: *in* Fabaceae species of the Chaco. Braz J Biol. Apr-Jun;77(2):299-303, 2017.

LIS, H.; SHARON, N. Lectins as molecules and as tools. *Journal Annual Review Biochemistry*, v. 55, p. 35-67, 1986.

LORIS, R. *et al*. Legume lectin structure. *Journal Biochim Biophys Acta*, v. 1383, p. 9-36, 1998.

LORIS, R. *et al*. Legume lectin structure. *Journal Biochim Biophys Acta*, v. 1383, p. 9-36. 2002.

MACHUKA, J. Characterization of seed proteins of velvet bean (*Macuna pruriens*) from Nigeria. *Journal Food Chemistry*, mar, v. 68, n. 4, p. 421-427, 2000.

MOREIRA, R. A. *et al*. Plant lectin, chemical and biological aspects. *Mem. Inst. Osvaldo Cruz*, n. 86, p. 211-218, 1991.

NICOLSON, G. L. The interations of lectins with animal cell surfaces. *Int. Rev. Cytol.* v. 39, p. 89-190, 1974.

NOWELL, P. C. Phytohemaglglutinin: in initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* v. 20, p. 462-466, 1960.

NSIMBA-LUBAKI, M.; PEUMANS, W. J. Seasonal fluctuation of lectins in barks of Elderberry (*Sambucus nigra*) and Black locust (*Robinia pseudoacacia*). *Plant Physiology*, n. 80, p. 747-751, 1986.

OGILVIE, M. L.; BYL, J. A.W.; GARTNER, T. K. Platelet aggregation is stimulated by lactose-inhibitable snake venom lectins. *Thromb. Haemostasis*, n. 62, p. 704-707, 1989.

OLIVEIRA, W. R. de ; REGO, E. J. L. *et al.* . Isolation, characterization and analysis of the agglutinative activity of a lectin from Crotalaria spectabilis. *Journal of Plant Biochemistry and Biotechnology*, v. 1, p. 1-5, 2018.

PUSZTAI, A. Plant lectin. Cambrige University Press, Cambridge, 1991.

PUSZTAI, A. *et al*. Seed lectins: Distribution, location and biological role. In Seed Proteins (Daussant, J., Mosse, J. & Vauganh, J. eds.). Academic Press, New York, p.53-82, 1983.

REGO, E. J. L. *et al*. Lectins from seeds of Crotalaria pallida (smoth rattlexbox). *Journal Phytochemistry*, jul, v. 60, n. 5, p. 441-446, 2002.

RAHIMI, N. RAHIMI, N. C-type Lectin CD209L/L-SIGN and CD209/DC-SIGN: Cell Adhesion Molecules Turned to Pathogen Recognition Receptors *Biology* (Basel). Dec 22;10(1):1, 2020.

RAMBALDI, A.*et al*. Endothelial injury and thrombotic microangiopathy in COVID-19: Treatment with the lectin-pathway inhibitor narsoplimab. *Immunobiology.* Nov;225(6):152001, 2020

RÜDIGER, H.; GABIUS, H. J. Plant lectins: Occurrence, biochemistry, functions and applications. *Journal Glycoconjugate*, aug, v. 18, p. 589-613, 2001.

SANTOS SOUSA, A. dos; REGO, E. J. L.; RIBEIRO , F. de A. R. Viability and Action of CPL Lectin on in Vitro Germinability of Pollen Grains of *Malpighia emarginata* DC.-(Malpighiaceae). *American Journal of Plant Sciences*, v. 04, p. 53-58, 2013.

SAINT-PAUL, M. Les hemaglutinines végetables. *Transfusion*, n. 4, p. 3-37, 1961.

SHARON, N.; LIS, H. Lectins as cell recognition molecules. *Science*, n. 246, p. 227-234, 1989.

SHARON, N. Lectin-carbohydrate complexes of plants and animals: an atomic view. *Trends in Biochemical Science*, v. 18, p. 221-226, 1993.

SINGH R, S.; TIWARY, A. K.; KENNEDY, J. F. Lectins: Sources, Activities and Applications. *Journal Critical Reviews in Biotechnology*, mar, v.19, n. 2, p. 145-178, 1999.

TOMOMITSU, H. Galactose-Specific, Hemolytic Lectin CEL-III from Cucumaria echinata. *Methods Mol Biol* 2132:159-164, 2020.

TOMS, G. C. A. Lectins in Leguminosae. *In*: *Advances in Legumes Systematic* (Polhill, R. M. & Raven, P. H., eds.). Royal Botanic Garden, Kew. 1981.

ZATTA, P. F.; CUMMINGS, R. D. Lectins and their uses as biotechnological tools. *Journal Biochemical education*, jan, v. 20, n. 1, p. 2-9, 1991.