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Properties of Galactose Specific Lectin from *Bosqueia Angolensis* Pulp

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ABSTRACT

Lectins are heterogeneous class of proteins (glycoproteins) that do not alter their covalent structure even after binding. They are found in a wide range of vegetables and some fruits. They are known to have biological activities including anti-proliferative, anti-tumour, mitogenic, vasorelaxing, immune-potentiating, insecticidal, antifungal, antiviral, antibacterial and hypotensive. The sugar specificity of a lectin which can be obtained from inhibition experiments using simple sugars and crude lectin preparations, permits the design of a suitable purification procedure. The aims of this study are to determine the beneficial properties of partially purified *B. angolensis* pulp lectin. This study was carried out to investigate the properties of protein extract obtained from *Bosqueia angolensis* pulp. The crude extract was partially purified using 70% $(NH_4)_2SO_4$ saturation. The precipitate was dialysed. The hemaglutinating activity of the lectin was investigated using blood group O, A, B and Rabbit blood. The sugar that inhibited the agglutination of the blood most was galactose which was employed in the minimum inhibition concentration (MIC) assay. The minimum concentrations at which galactose exhibits its inhibition capacity to agglutination of blood using crude and purified lectin are 0.05mM and 0.8mM respectively. The lectin has positive hemagglutination activity for both rabbit and human erythrocytes with preference to rabbit erythrocytes and it does not agglutinate blood group O of the human red blood cells. Conclusively, *Bosqueia angolensis* pulp extract contains protein (lectin) that has hemaglutinating activity which is galactose specific and can be effectively exploited for pharmaceutical and various industrial applications.

KEYWORDS: Lectin; *Bosqueia angolensis*, Hemaglutinating activity; Galactose

1. Introduction

Lectins are heterogeneous class of proteins (glycoproteins) that do not alter their covalent structure even after binding. They lack enzymatic activity on their ligand and they are of non-immune origin. They are multimeric consisting of non-covalently associated subunits which gives them the ability to agglutinate cells or form precipitates with glyco-conjugates in a manner similar to antigen-antibody reactions (Lin *et al*., 2020). They also agglutinate certain animal cells possessing specific binding sites for

particular sugars (Chettri *et al*., 2021). Their specificity is defined by the mono or oligosaccharides that inhibit the agglutination competitively. They are found in a wide range of vegetables and some fruits. They are known to have biological activities useful in clinical therapeutics, including anti-proliferative, anti-tumour, mitogenic (Chettri *et al*., 2021), vasorelaxing, immune-potentiating, insecticidal, antifungal, antiviral, antibacterial and hypotensive (Cagliari *et al.,* 2018). Lectins vary in composition, molecular weight, subunit structure and number of sugar binding sites per molecule. Although they are found ubiquitously in plant species, they have variable structures and specific activities according to the plants they originate from (Santos *et al.,* 2014). Their functions, uses and applications are immense and varied. They are similar to antibodies in their ability to agglutinate red blood cells, also play important roles in the immune system by recognizing carbohydrates that are found exclusively on pathogens (Oliveira *et al.,* 2020).

Bosqueia angolensis is a tropical rain forest tree that grows as high as 40 meters. It is called "koko eran" in the Yoruba speaking states of South Western states of Nigeria and called "oze" in Ibo speaking states (Odekanyin and Akeredolu, 2016). It has abundant latex flow when cut at the node. It contains many essential amino acids non-essential amino acids. It is referred to as "hospitality tree" in the cultural Igbo Community is a member of the botanical family, Moracea (Akinsoji et al., 2020). Its green glossy leaves resemble those of 'Ogbono' (*Irvingia gabonensis*). Hence, the study seek to investigate the properties of lectin obtained from *B. angolensis* pulp.

Knowledge of the sugar specificity of a lectin which can be obtained from inhibition experiments using simple sugars and crude lectin preparations, permits the design of a suitable purification procedure (Odiegwu *et al.,* 2020). The aims of this study are to determine the beneficial properties of partially purified *B. angolensis* pulp.

2. Materials and Methods

2.1 Materials

Phosphate buffer, sodium chloride, sodium hydroxide, ethylenediaminetetraacetate (EDTA), glutaraldehyde, sodium azide, sodium dihydrogen phosphate, Copper (11) tetraososulphate (vi) pentahydrate, sodium-potassium tartarate, sodium carbonate, folin reagent and all other reagents were of analytical grade.

2.2 Methods

2.2.1 Plant Collection and Extract Preparation

Intact fruit of *B. angolensis* were picked from the Botanical garden of Obafemi Awolowo University, Ile-Ife. The fruits were identified at Ife herbarium, Botany Department, Obafemi Awolowo University, Ile-Ife Nigeria. The pulp was removed from the seed by mashing the fruit gently and homogenized manually in five (5) volumes of 0.0125M phosphate buffer, pH 7.2 containing 0.1M (9%) NaCl and then centrifuged at 4000 rpm for 15mins. The supernatant obtained constitute the crude extract.

2.2.2 Isolation of the Lectin from the Pulp

The extract was precipitated at 70 % ammonium sulphate saturation and left overnight at room temperature. The precipitate was collected by centrifugation at 3000 rpm for 30 min. The precipitate was re-dissolved in phosphate buffered saline, pH 7.2 and dialysed. The dialysate was centrifuged at 3000 rpm for 10 min to remove debris and other undissolved materials.

2.2.3 Blood Collection

Human blood groups A, B and O were donated by willing Achievers University postgraduate students and rabbit red blood cells were obtained from rabbit obtained from Animal house facility of Achievers University, Owo, Nigeria.

2.2.4 Glutaraldehyde Fixation of RBCs

The rabbit and human red blood cells (RBCs) were fixed with glurataldehyde according the method described by Pattanapanyasat *et al.* (2010). Blood samples were collected into heparinized bottles and centrifuged at 3000 rpm for 15 mins at room temperature using bench centrifuge. The RBCs were collected and washed five (5) times with phosphate buffered saline (PBS, pH 7.2). Glutaraldehyde (25%) was diluted to 1% (v/v) with PBS and kept at 0 °C. The chilled glutaraldehyde-PBS solution was used to dilute the RBCs to 4% (v/v). The suspension of cells and glurataldehyde was incubated for an hour at 40 ˚C with occasional mixing. The fixed cells were collected by centrifugation at 3000 rpm for 5 mins and washed five times with PBS. The cells were suspended in PBS containing 0.02% (w/v) sodium azide, to a final concentration of 2% (v/v) and stored at 4 ˚C until required.

2.2.5 Hemaglutination Assay

The pulp crude extract was tested for the presence of lectin by hemagglutinating activity assay performed in U-shaped microtitre plates using glutaraldehyde-fixed erythrocytes following the method of Odekanyin and Akeredolu (2016). Briefly, 100 µl PBS was pipette sequentially into wells arranged in rows of 12 wells. The crude extract (100 µl) was added into the first well to obtain a 1: 2 dilution. A serial dilution was then performed and made up with (75 µl) of the 4% of glutaraldehyde-fixed erythrocyte suspension undisturbed for 1 hrs in order to allow agglutination to take place. The titre value of the lectin was taken as the reciprocal of the highest dilution of the extract exhibiting visible hemagglutination and this will be equivalent of one hemagglutination. Blood group specificity of the *Bosquiea angolensis* pulp crude extract was established by using erythrocytes from different blood groups of the human ABO system and that of the rabbit.

2..2.6 Hapten inhibition Test

The sugar specificity of the lectin was investigated by comparing sugars on the basis of minimum concentration required to inhibit the agglutination of erythrocyte by the lectin (Goldstein and Hayes, 1978). The extract was diluted serially until the end-point dilution causing heamagglutination. Fifty (50 µl) of the sugar solution (0.2 M) was added to each well while the control well contained PBS instead of sugar solution. Fifty (50 µl) of RBC suspension was added to each well, and the titre of lectin activity was determined. Inhibitory sugars caused a reduction in the titre of the lectin activity shown by the PBS-control experiment. The sugars tested are N-acetylglucosamine, 2-deoxy-D-glucose, sorbose, sucrose, glucosamine, mannitol, lactose, glucose, maltose arabinose and galactose.

2.2.7 Protein Determination

Protein concentrations were determined according to Lowry *et al.* (1951) using Bovine serum albumin as standard.

2.2.8 Partial Purification of Pulp Lectin

The pulp extract was brought to 70 % ammonium sulphate saturation and was left overnight at room temperature. The precipitate was collected by centrifugation at 3000 rpm for 30 min. The precipitate was redissolved in PBS, pH 7.2 and dialyzed exhaustively against distilled water. The dialyzate was centrifuged at 3000 rpm for 10 min to remove debris and other undissolved materials.

3. Results and Discussions

3.1 Results

3.1.1 Protein Concentration

The result of investigation of the protein concentration in the pulp extraction of *Bosquiea angolensis* is 3.286 mg/ml. The data suggested that *Bosquiea angolensis* has some level of protein which can be directly related to its radical scavenging activity as proposed by Odekanyin and Akeredolu (2016).

Heamagglutination Activity of Crude Extract of *Bosquiea angolensis* Pulp

The presence lectin can be determined by its ability to agglutinate various blood group erythrocytes. This is a specific property of lectins. Therefore, phopshate buffer saline crude extract of *Bosquiea angolensis* pulp agglutinates specifically with human red blood cell of the A, B, and rabbit erythrocyte but does not agglutinate blood group O. This result is shown in table 1. Table 2 shows the partially purified extract of pulp of *Bosquiea angolensis* pulp, it agglutinates human red blood cells of the group A, B, O and rabbit erythrocyte with greater preference for rabbit red blood cells.

Table 1: Heamagglutination Test for Blood Group Specificity of the Crude Extract of *B. angolensis* Pulp

Blood groups	Titre value
A	2^8
B	2^8
O	2^0
Rabbit	2^9
Negative	2^0
Positive	2^{12}

Key: A: Blood group A
B: Blood group B
O: Blood group O
Rabbit: Rabbit erythrocytes

3.1.2 Sugar Specificity

The results of sugar inhibition studies to define the sugar specificity of the crude extract of *Bosquiea angolensis pulp* are as presented in table 3. The summary of the result shows that the B. *angolensis* extract hemaglutining activity was completely inhibited by galactose. Hence, it is classified as galactose-specific lectin.

The summary of MIC experiment is shown on table 4. The minimum concentrations at which galactose exhibits its inhibition capacity in agglutination of blood using crude and partially purified lectin are 0.05mM and 0.8mM respectively. This is an indication that the lectin is very active even in crude preparations.

3.2 Discussions

Lectins are types of protein also known as antinutrient that have ability to binds to certain carbohydrates. They are ubiquitous. Some types of lectins are completely safe, while others may pose health risks. The result of investigation of the protein concentration in the pulp extraction of *Bosquiea angolensis* is 2.2869 mg/ ml according to biuret method. This result was corroborated by Lowry method which gave protein concentration of 3.286 mg/ ml. This informs that *Bosquiea angolensis* pulp has some level of protein.

Table 2: Heamagglutination Test for Blood Group Specificity of the Partially Purified Extract of *B. angolensis* Pulp

Blood Groups	Titre Value
A	2^7
B	2^8
O	2^2
Rabbit	2^{12}
Negative	2^0
Positive	2^{12}

Key: A: Blood group A
B: Blood group B
O: Blood group O
Rabbit: Rabbit erythrocytes

Lectins are carbohydrate-binding proteins that are highly specific for sugar groups that are part of other molecules, so cause agglutination of particular cells or precipitation of glycoconjugates and polysaccharides The reactions between lectins and cell membrane is believed to result in the alteration of cell; the changes which may influence cellular properties such as aggregation (agglutination) and deformity of erythrocytes (Chen *et al.,* 2021). Lectins are present in most plant foods but especially high in: legumes, such as beans, lentils, peas, soybeans, and peanuts.

The antigens of the ABO system are carbohydrate antigens. The determinant carbohydrate structures are synthesized stepwise by the action of glycosyltransferases which transfer single monosaccharide residues onto an appropriate precursor substance. In blood group O individuals several inactive alleles of the ABO locus have been detected, which encode inactive transferases. These mutilated enzymes are not able to synthesize a defined blood group specificity. Blood group O is therefore characterized by the presence of unchanged A and B precursor (Pendu *et al.,* 2021). The result obtained for the different blood groups showed that the crude extract of *Bosquiea angolensis* showed positive hemagglutination activity except blood group O. This is an indication of the presence of lectin protein that is blood group specific. In contrast to protein-based blood groups, the antigens of the ABO system are carbohydrate antigens. The determinant carbohydrate structures are synthesized stepwise by the action of glycosyltransferases which transfer single monosaccharide residues onto an appropriate precursor substance. Blood group O has no antigens, but contains both antibodies-A and antibodies-B in the plasma. Blood group AB has both A and B antigens, but no antibodies. Studies have shown that people with type O tend to have a greater resistance to many serious diseases. In this work, The highest agglutination activity of B. angolensis was found with glutaraldehyde-treated rabbit erythrocytes; fresh and glutaraldehyde-treated human erythrocytes of blood groups A, B and rabbit erythrocyte were also agglutinated, whereas those from groups O was not. This result was consistent with the work of Coelho and da Silva (2000) on simple method to purify milligram quantities of the galactose-specific lectin fron the leaves of *Bauhinia monandra.*

Lectins have been found to bind free sugars or sugar residues of polysaccharides, glycoproteins or glycolipids (Hamid *et al*., 2013). Lectin interacts with sugar or glycan residues with a unique binding domain known as the carbohydrate recognition domain (CRD) comprised of about 120–160 amino acids. They

can hold more than one simple sugar in their binding sites because of their structural similarity (Gabba *et al.,* 2021). Lectins of *Bosquiea angolensis* pulp have been found to be inhibited by galactose. Various sugars were tested at 0.2 M to see if inhibition of hemagglutination occurred as shown in table 3.

Table 3: Sugar Specificity

Sugars	Titre Value
Negative	2^0
Positive	2^{12}
N-acetylglusamine	2^{10}
2-deoxy-D-glucose	2^{11}
Sorbose	2^{10}
Sucrose	2^{10}
Glucosamine	2^9
Mannitol	2^{10}
Lactose	2^{11}
Glucose	2^{10}
Maltose	2^9
Arabinose	2^{11}
Galactose	$\mathbf{2^0}$

Each experiment consisted of 100µl of serially diluted sample in U-shaped microtitre wells. To each was added 50µl of 0.2M sugar solution in PBS and 75 µl of 4 % erythrocyte suspension of rabbit.

Galactose gave a strong inhibitory effect among the sugars tested. Therefore, it is galactose specific binding lectin protein. They possess two or more binding sites that recognize specific sugar groupings and bind to cell surface glycoproteins and glycolipids.

Table 4. Minimum Inhibition Concentration (MIC) of Galactose using Rabbit Erythrocytes for Crude and Dialyzed Extracts of *Bosquiea angolensis*

Concentration	Crude Extract	Dialyzed Extract
Positive	2^7	2^{11}
0.2M	2^2	2^2
0.1M	2^3	2^3
0.05M	2^5	2^5
0.025M	2^6	2^7
0.0125M	2^7	2^8
0.00625M (6.25mM)	2^{12}	2^8
0.003125M (3.125mM)	2^{12}	2^8
0.001565M (1.565mM)	2^8	2^9
0.0007815M (0.7815mM)	2^8	2^9
0.0003907M (0.391mM)	2^9	2^0
0.0001954M (0.195mM)	2^9	2^0
0.0000977M (0.098mM)	2^9	2^0
0.0000489M (0.049mM)	2^8	2^0

Each experiment consisted of 100µl of serially diluted sample in U-shaped microtitre wells. To each was added 50µl of serially diluted galactose solution of specified concentration and 75µl of 4 % erythrocyte suspension of rabbit.

The sugar specificity of lectin enables its analytical application in detection, typing and control of bacteria and fungi (Hendrickson and Zherdev, 2018). The sugar specificity test of crude extract of lectin from B. *angolensis* was carried out and it was found that galactose had the highest inhibitions, while the minimum inhibition concentration of partially purified and crude extract of lectin from *B. angolensis* was also carried out and it was found that the minimum inhibition concentration of galactose was at 0.8 mM and 0.05 mM of galactose. This is an indication that the crude lectin is quite inhibited at lower concentration of galactose than the partially purified lectin obtained from B angolensis.

Lectins manifest a diversity of activities including antitumor, immunomodulatory, antifungal, HIV-1 reverse transcriptase inhibitory, and anti-insect activities, which may find practical applications in many therapeutic areas. Lectin proteins may be characterized with respect to glyco-forms and carbohydrate structure by means of affinity chromatography, blotting, affinity electrophoresis, and affinity immunoelectrophoresis with lectins, as well as in microarrays, as in evanescent-field fluorescence-assisted lectin microarray (Kale and Deshmukh, 2017).

4. Conclusions

Bosqueia angolensis pulp extract contains protein (lectin) that has hemaglutinating activity which is galactose specific and can be effectively exploited for pharmaceutical and various industrial applications that can be a source of national economic empowerment even at its unpurified state.

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