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Purification, characterization and antibacterial potential of a lectin isolated from *Apuleia leiocarpa* seeds

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ABSTRACT

Apuleia leiocarpa is a tree found in Caatinga that has great value in the timber industry. Lectins are carbohydrate-binding proteins with several biotechnological applications. This study shows the isolation, characterization, and antibacterial activity of *A. leiocarpa* seed lectin (ApulSL). The lectin was chromatographically isolated from a crude extract (in 150 mM NaCl) by using a chitin column. ApulSL adsorbed to the matrix and was eluted using 1.0 M acetic acid. Native ApulSL was characterized as a 55.8-kDa acidic protein. SDS-PAGE showed three polypeptide bands, whereas two-dimensional electrophoresis revealed four spots. The peptides detected by MALDI TOF/TOF did not show sufficient homology (<30%) with the database proteins. Circular dichroism spectroscopy suggested a disordered conformational structure, and fluorescence spectrum showed the presence of tyrosine residues in the hydrophobic core. The hemagglutinating activity of ApulSL was present even after heating to 100 °C, was Mn^{2+}-dependent, and inhibited by *N*-acetylglucosamine, D(−)-arabinose, and azocasein. ApulSL demonstrated bacteriostatic and bactericide effects on gram-positive and gram-negative species, being more effective against three varieties of *Xanthomonas campestris* (MIC ranging from 11.2 to 22.5 μg/mL and MBC of 22.5 μg/mL). The results of this study reinforce the importance of biochemical prospecting of Caatinga by revealing the antibacterial potential of ApulSL.

1. Introduction

The Caatinga is a type of vegetation exclusive to Brazil and has been recognized as one of the most important natural regions of the world [1]. Ethnobotanical surveys are important in bioprospecting, to find herbal medicines and other biotechnologically relevant compounds [2]. Although Caatinga is one of the most threatened biomes on the planet, few ethnobotanical studies have been conducted in the Brazilian semi-arid region [3].

Plants from Fabaceae family (leguminous plants) are well known because of the many species used for human consumption, such as soy, beans, and peas. However, Fabaceae plants that are not used in the diet have been poorly studied as sources of compounds with biotechnological potential [4]. *Apuleia leiocarpa* (Vogel) J. F. Macbride is a tree belonging to the sub-family Caesalpinioideae of Fabaceae. In Brazil, it is commonly known as "grápia" and "jataí," among other names [5]. It has a wide distribution, occurring from northeastern Brazil to Uruguay and Argentina, and it prefers mountain slopes and well-drained soils [6]. The tree is used in the tanning, timber, and construction industries [6–8]. It also has potential for use in agroforestry systems and has ornamental and reforestation uses [6,9].

Lectins are proteins from non-immune origin, which bind specifically and reversibly to free sugars or to the subterminal or terminal residues of glycoconjugates [10]. These proteins have been the most studied in leguminous species, because they are frequently very abundant in the seeds of these plants and may constitute up to 10% of the total protein [11,12]. Many lectins have been also isolated from other plant tissues and families [13].

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Lectins have been used in biorecognition technology to investigate the structure and function of complex carbohydrates and to map changes in cell surface during physiological and pathological processes [14]. They also have shown immunomodulatory, anti-inflammatory, antitumor, hypotensive, insecticidal, antiviral, antifungal, and antibacterial activities [13,15–21]. The antimicrobial activity of lectins may result from their ability to interact with carbohydrates on the cell surface of microbes. Antibacterial lectins can interact with bacterial cell wall components such as *N*-acetylglucosamine, *N*-acetylmuramic acid (MurNAc), tetrapeptides linked to MurNAc, and lipopolysaccharides [19].

The phytopathogenic bacteria *Xanthomonas campestris* pv. *campestris* causes black rot and produces extracellular enzymes that promote plant cell wall degradation, which contributes to its pathogenicity [22]. *X. campestris* pv. *viticola* is the causal agent of bacterial canker of grapevine, causing necrotic spots in inflorescences and dark, roughly rounded lesions in the rachis and berries. It is considered a pest in the northeastern states of Bahia, Pernambuco, and Piauí [23,24]. *X. campestris* pv. *malvacearum* attacks cotton crops causing angular lesions in the leaves that are initially green and oily, and later brown and necrotic [25].

An initial screening of 36 extracts from 27 Caatinga plants, including *A. leiocarpa*, revealed that 77.7% of the samples had hemagglutinating activity, which is indicative of the presence of lectin [26]. The present study describes the purification, characterization and antibacterial activity of a lectin extracted from the seeds of *A. leiocarpa* (ApulSL), as part of an effort to expand the knowledge of bioactive compounds found in Caatinga plants.

2. Materials and methods

2.1. Plant material and extract preparation

A. leiocarpa seeds were collected from the National Park of Catimbau (PARNA Catimbau), Pernambuco, Brazil, from January 2011 to July 2011. The taxonomic identification was performed in the Herbarium Dárdano de Andrade Lima at the *Instituto Agronômico de Pernambuco* (IPA) and the testimonial material was archived under the number 84886.

For preparation of the extract, the seeds were dried at 45 °C and processed by grinding. The seed flour was homogenized under agitation for 4 h at 28 °C with 150 mM NaCl to yield a final concentration of 10% (w/v). The homogenate was filtered through filter paper and centrifuged at 3600 rpm for 15 min. The supernatant was the crude extract (CE).

2.2. Protein content and hemagglutinating activity (HA)

Protein concentration was determined according to Lowry et al. [27] using a standard bovine serum albumin curve with values between 31.25 and 500 μg/ml. Protein concentration was also estimated using the absorbance at 280 nm.

The lectin activity was measured by determining hemagglutinating activity (HA) according to Paiva and Coelho [28] in 96-well microtiter plates (Kartell SPA, Italy). The HA assay was started by adding 50 μL of 150 mM NaCl to all wells and 50 μL of the sample in the second well of the horizontal row. Successive dilutions were performed until a ratio of 1:2048 was achieved. Next, 50 μL of a 2.5% (v/v) suspension of glutaraldehyde-treated erythrocytes [29] from humans (A, B or O-types) or rabbits was added. The number of units of hemagglutinating activity was defined as the reciprocal of the highest dilution of sample that promoted full agglutination of erythrocytes. The specific HA was defined as the ratio between the units and protein concentration (mg/mL).

2.3. Purification of ApulSL

The CE (1.0 mg of protein) was loaded onto a chitin (7.5 cm × 1.5 cm) column equilibrated with 150 mM NaCl. The column was washed with the equilibrating solution until absorbance at 280 nm was lower than 0.030. Elution was performed with 1.0 M acetic acid. Fractions of 2 ml were collected every 6 minutes and monitored by absorbance at 280 nm. The fractions with absorbance ≥0.100 were pooled (ApulSL) and dialyzed in aqueous 150 mM NaCl to remove the acetic acid.

2.4. Characterization of ApulSL HA

Inhibition of HA was evaluated using carbohydrates and glycoproteins. HA assays were performed as described above but replacing the solution of 150 mM NaCl with a solution of the carbohydrate prepared in 150 mM NaCl. In addition, there was an interval of 45 min between the end of sample dilution in carbohydrate solution and the addition of erythrocyte suspension. The concentrations of inhibitor solutions were 100 and 200 mM for carbohydrates [D(−) arabinose, L(+)-arabinose, fructose, fucose, glucose, galactose, D-lactose, D(+)-maltose, mannose, methyl-α-D-mannopyranoside, *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, raffinose, rhamnose, and D(−)-ribose] and 250 and 500 μg/mL for glycoproteins (azocasein, casein, fetuin, and thyroglobulin).

The effect of divalent ions (Ca^{2+}, Mg^{2+}, and Mn^{2+}) on the ApulSL HA was evaluated. The lectin was dialyzed with 5 mM EDTA (16 h at 4 °C) and then with 150 mM NaCl (6 h at 4 °C) to remove the EDTA. Next, the dialyzed ApulSL was incubated for 45 min with 10 mM Ca^{2+}, Mg^{2+}, or Mn^{2} and then HA was evaluated. The effect of these cations on HA of ApulSL non-treated with EDTA was also determined.

The effect of temperature on the ApulSL HA was evaluated by heating an aliquot of ApulSL (1.5 ml) during 30 min at 30, 40, 50, 60, 70, 80, or 100 °C and for 2 h at 100 °C prior to HA assay. HA of ApulSL was also determined after heating for 30 min at 121 °C in an autoclave.

The ApulSL HA was also evaluated after it was boiled for 5 min at 100 °C with electrophoresis buffer (1.0 M Tris–HCl pH 6.8; 0.2 g sodium dodecyl sulphate; 1.0 ml glycerol; 2 mg bromophenol blue). Control assays were also performed by incubating erythrocytes only with buffer.

2.5. Gel filtration chromatography

A sample of ApulSL (500 μg) in 150 mM NaCl was loaded onto a Hiprep Sephacryl™ 16/60 S100 HR column (GE Healthcare, Sweden) coupled to an ÄKTAprime system to determine the molecular mass of the native protein. The chromatography was performed at a flow rate of 0.5 mL/min in 150 mM NaCl. Fractions of 2 ml were collected and protein elution was monitored by absorbance at 280 nm. Molecular mass standards, phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa), were similarly chromatographed.

2.6. Polyacrylamide gel electrophoresis (PAGE)

PAGE for native acidic proteins [15% gel (w/v)] was performed according to Davis [30] and PAGE for native basic proteins was performed according to Reisfeld et al. [31]. Polypeptide bands were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 10% acetic acid (for acidic proteins) or 1% Amido Black in 10% acetic acid (for basic proteins).

Electrophoresis on a 15% (w/v) polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed