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CasuL: A new lectin isolated from *Calliandra surinamensis* leaf pinnulae with cytotoxicity to cancer cells, antimicrobial activity and antibiofilm effect

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ABSTRACT

This work describes the isolation of a lectin (CasuL) from the leaf pinnulae of *Calliandra surinamensis* and the evaluation of its cytotoxic, antimicrobial and antibiofilm properties. Proteins from pinnulae extract were precipitated with ammonium sulphate (60% saturation) and submitted to Sephadex G-75 chromatography, which yielded isolated CasuL (purification factor: 113). Native CasuL is an acidic protein (pI 5.82) with a relative molecular mass of 48 kDa. This lectin is also an oligomeric protein composed of three subunits and mass spectrometry revealed similarities with a *Sorghum bicolor* protein. CasuL did not undergo unfolding when heated but changes in conformation and hemagglutinating activity were detected at basic pH. CasuL did not reduce the viability of human peripheral blood mononuclear cells but was toxic to leukemic K562 cells (IC_{50} 67.04 ± 5.78 μg/mL) and breast cancer T47D cells (IC_{50}: 58.75 ± 2.5 μg/mL). CasuL (6.25–800 μg/mL) only showed bacteriostatic effect but was able to reduce biofilm formation by *Staphylococcus saprophyticcus* and *Staphylococcus aureus* (non-resistant and oxacillin-resistant isolates). CasuL showed antifungal activity against *Candida krusei* causing alterations in cell morphology and damage to cell wall. In conclusion, the pinnulae of *C. surinamensis* leaves contain a thermo-stable lectin with biotechnological potential as cytotoxic, antibiofilm, and antifungal agent.

1. Introduction

Lectins are proteins that bind specifically to both free and cell surface attached carbohydrates. This property may trigger various cellular responses and confers to these proteins several biological activities [1,2]. In plants, lectins are reported to be involved in defense against pathogens, herbivores and predators. This physiological role has stimulated the investigation of their biotechnological potential in the control of microbial infections and pest insects [3–6]. In addition, vasorelaxant effect [7], immunomodulatory action [8] as well as cytotoxic and antitumor activities [9–11] are some properties among the wide range of biological activities described for plant lectins.

Lectins are found in many plants of the family Fabaceae (Leguminosae), to which belongs the *Calliandra* genus (subfamily Mimosoideae). This genus comprises plants widely used in the tra-

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ditional medicine for treatment of infectious diseases as malaria and leishmaniasis [12]. Leaves and branches of *Calliandra calotyrsus* and *Calliandra porturicensis* are described as sources of compounds with activity against intestinal nematodes of ruminants [13] besides analgesic, anticonvulsant, and anti-ulcer properties [14,15]. *Calliandra surinamensis* (popularly known as "pink powder puff", "pompon du marin" or "esponjinha-rosa", among other names) is a perennial, tropical and low-branching shrub widely used as ornamental species in Brazil. However, few studies are available about their biological properties and biotechnological potential. The use of *C. surinamensis* as an ornamental plant, resistant to pathogens and insects, stimulates the evaluation of this plant as source of bioactive molecules such as lectins.

Plant lectins have shown remarkable anticancer properties *in vivo* and *in vitro* and clinical studies have shown that they can function as an alternative in cancer therapy [16–18]. In addition, drug delivery systems strategies have been investigated to increase the bioavailability of antitumor lectins [19–21]. Lectins can be toxic to cancer cells via different mechanisms, which are generally initiated by interaction with specific receptors, glycosylated or not, on the membrane of cancer cells. After this step, the lectins can be internalized through endocytosis and addressed to different compartments leading to activation of signaling pathways related to cell death [18,22].

Lectins with antibacterial activity can interact with peptidoglycans, lipopolysaccharides and other molecules present in the cell wall of Gram-positive and Gram-negative species, interfering with cell growth and viability as well as blocking interaction sites with host cells preventing infection [6,23,24]. Lectins also exert antifungal effect, which may be linked to interaction with chitin, cellulose, glucans and mannoproteins present in the cell wall, interfering with fungal growth, cell homeostasis and spore germination [25,26].

Bacterial biofilms are multicellular communities enclosed in a self-produced polymeric matrix and able to attach to abiotic and biotic surfaces. Biofilms are involved in the most of bacterial infections in humans. An additional difficulty to control the biofilm formation is that several antibacterial molecules are unable to penetrate deeply into the biofilm being often entrapped by the extracellular polymeric matrix. Furthermore, under antibiotic pressure, bacterial cells in the biofilm can express resistance mechanisms making them recalcitrant to a considerable number of antibiotics [27]. In this sense, natural compounds, including lectins, have been evaluated for their ability to inhibit biofilm formation by pathogenic bacteria [28–30].

This work reports the isolation and characterization of a lectin from *C. surinamensis* leaf pinnulae (*C. surinamensis* lectin: CasuL) as well as the evaluation of its cytotoxic activity against human normal and cancer cells, antibacterial and antibiofilm activities against Gram-positive and Gram-negative pathogens, and antifungal activity against *Candida* species.

2. Materials and methods

2.1. Lectin purification

Leaves of *C. surinamensis* were collected in Recife (Pernambuco, Brazil) and put to dry at 28 °C during 15 days. Next, the pinnulae were detached and powdered using a blender. This powder was suspended in 0.15 M NaCl (10%, w/v) and homogenized during 16 h using a magnetic stirrer. Next, the suspension was filtered through gauze and centrifuged (12,000*g*, 15 min, 4 °C) to obtain the crude extract. The extract was treated with ammonium sulphate at 60% saturation [31] during 4 h under magnetic stirring. After this period, the material was centrifuged (3000*g*, 15 min) and the precipitated (PF) and supernatant (SF) fractions were collected and dialyzed against distilled water (4 h) followed by 0.15 M NaCl (4 h). The extract and fractions were evaluated for protein concentration and hemagglutinating activity (HA) as described in the next section.

PF (3.0 mg of protein) was loaded onto a Sephadex G-75 column (30 × 1 cm) equilibrated with 0.15 M NaCl. After sample loading, the column was irrigated with 0.15 M NaCl, 0.5 M glucose, 0.5 M mannose, and 1.0 M NaCl, in this order. The elution of proteins was monitored by measuring the absorbance at 280 nm. The protein peak eluted with 0.15 M NaCl corresponded to CasuL, which was then evaluated for protein concentration and HA.

2.2. Protein concentration and hemagglutination assay

Protein concentration was determined according to Lowry et al. [32] using bovine serum albumin (31.25–500 μg/mL) as standard. The hemagglutinating activity (HA) was determined using a suspension of rabbit erythrocytes (2.5% v/v) previously treated with glutaraldehyde [33]. Ethics Committee on Animal Experimentation of the *Universidade Federal de Pernambuco* approved the erythrocytes collection method (process 23076.033782/2015-70). The sample (50 μL) was serially two-fold diluted in 0.15 M NaCl in a row of a 96-well microplate and then 50 μL of the erythrocyte suspension were added to each well. Erythrocytes incubated only in 0.15 M NaCl were used as control. Each assay was performed in duplicate. The number of HA units (HAU) was determined as the reciprocal of the highest dilution of the lectin that promoted full agglutination of rabbit erythrocytes. The specific HA was calculated as the ratio between the HAU and the protein concentration (mg/mL).

For HA inhibition assay, the lectin samples were incubated for 15 min with monosaccharides (glucose, mannose methyl-α-D-glucopyranoside, *N*-acetylglucosamine or galactose at 0.2 M), the disaccharide maltose (0.2 M) or glycoproteins (fetuin, ovalbumin or bovine serum albumin at 0.5 mg/mL) prior to the addition of erythrocyte suspension.

2.3. Evaluation of protein homogeneity and estimation of native molecular mass

CasuL was submitted to PAGE for native basic proteins (12% acrylamide gel, w/v) according to Reisfeld et al. [34] and 1% (w/v) Amido Black in 10% (v/v) acetic acid was used as staining solution. The lectin was also submitted to PAGE for acidic proteins in 12% acrylamide gel (w/v) prepared according to Davis [35] and the polypeptide bands were detected with 0.02% (v/v) Coomassie Blue in 10% (v/v) acetic acid.

To determine the lectin native molecular mass, CasuL (2.0 mL, 2.0 mg of protein) was chromatographed onto a Hiprep 16/60 Sephacryl S-100HR column (16 mm × 60 cm) coupled to the AKTAprime plus system (GE Healthcare Life Sciences, Uppsala, Sweden) and equilibrated with 0.15 M NaCl. A flow rate of 0.5 mL/min was maintained and fractions of 3.0 mL were collected. A mixture of bovine serum albumin (66 kDa), ovalbumin (45 kDa) and lysozyme (14 kDa) from Sigma-Aldrich (USA) was chromatographed at the same conditions. The relative molecular mass of CasuL was calculated by comparison with migration of these molecular mass markers.

2.4. Determination of isoelectric point and subunit composition

To determine the isoelectric point of CasuL, the protein (150 μg) was solubilized in a rehydration solution [2% (w/v) CHAPS, 1% (v/v) IPG buffer pH 3–10, 0.002% (w/v) bromophenol blue] for 20 min at 28 °C. The sample was taken up into the strip (linear pH gradient 3–10; 7 cm) passively during rehydration for 16 h at 25 °C. The isoelectric focusing was performed using the IPGphor III system (GE