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Antimicrobial potential of *Alpinia purpurata* lectin (ApuL): Growth inhibitory action, synergistic effects in combination with antibiotics, and antibiofilm activity

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ABSTRACT

The *Alpinia purpurata* inflorescence contains a lectin (ApuL), which has immunomodulatory activities on human cells. In the present work, it was evaluated the antibacterial and antifungal effects of ApuL against human pathogens. ApuL showed bacteriostatic activity against non-resistant (UFPEDA-02) and an oxacillin-resistant isolate (UFPEDA-672) of *Staphylococcus aureus* with minimal inhibitory concentrations (MIC_{50}) of 50 and 400 μg/mL, respectively. In addition, it showed bactericidal effect on the non-resistant isolate (minimal bactericidal concentration: 200 μg/mL). For *Candida albicans* and *Candida parapsilosis*, ApuL showed fungistatic effect (MIC_{50}: 200 and 400 μg/mL, respectively). The lectin was able to impair the viability of the microorganism cells, as indicated by propidium iodide (PI) staining. Analysis of growth curves, protein leakage, and ultrastructural changes supported that ApuL acts through distinct mechanisms on *S. aureus* isolates. Ultrastructural analysis of ApuL-treated *Candida* cells revealed malformations with elongations and bulges. ApuL-oxacillin combination showed synergistic effect on the oxacillin-resistant isolates UFPEDA-670 and 671, which were not sensitive to lectin alone. Synergism was also detected for ApuL-ceftazidime against a multidrug-resistant isolate of *Pseudomonas aeruginosa*. Synergistic action of ApuL-fluconazole was detected for *C. parapsilosis*, which was insensitive to the drug alone. Biofilm formation by *S. aureus* non-resistant isolate and *C. albicans* was remarkably inhibited by ApuL at sub-inhibitory concentrations. In conclusion, ApuL showed differential effects on non-resistant and resistant bacterial isolates, was active against *Candida* species, and showed synergistic action in combination with antibiotics.

1. Introduction

Pathogenic microorganisms represent a growing concern in public health since the overuse and misuse of antibiotics have led to the rapid emergence of resistant isolates [1]. This situation has stimulated the search for new molecules for controlling pathogens to be used alone or as additive or synergistic agents in combined therapies together with the currently available antibiotics [2].

Abbreviations: ApuL, *Alpinia purpurata* lectin; CasuL, *Calliandra surinamensis* lectin; CFU, colony-forming units; ΣFIC, sum of fractional inhibitory concentration index; HA, hemagglutinating activity; ICMBio, *Instituto Chico Mendes de Conservação da Biodiversidade*; MHA, Mueller Hinton Agar; MHB, Mueller Hinton Broth; MBC, minimal bactericidal concentration; MFC, minimal fungicidal concentration; MIC, minimal inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; NC, negative control; OD, optical density; ORSA, oxacillin-resistant *S. aureus*; PC, positive control; PI, propidium iodide; SDA, Sabouraud Dextrose Agar; SDB, Sabouraud Dextrose Broth; SEM, scanning electron microscopy; TO, thiazol orange; UFPE, *Universidade Federal de Pernambuco*; UFPEDA, culture collection from the *Departamento de Antibióticos* of UFPE; URM, University Recife Mycologia; WDCM, World Data Centre of Microorganisms

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Resistance to antimicrobials is an important survival mechanism that favors microorganisms in the infection process. In bacteria, some of the molecular and biochemical mechanisms of resistance involve inactivation or modification of drugs by enzymes, development of efflux pumps, alterations in membrane permeability, and structural changes to barriers and antibiotic targets [3,4]. The tolerance that some pathogens develop against antimicrobial drugs leads to acute, persistent, chronic, and relapsing human infections [5].

The degree of pathogenicity of a microorganism depends directly on its virulence factors, which correspond to mechanisms that increase the ability to cause an infection [6]. These mechanisms include structures, strategies, or production of substances that aid the microorganism in tissue invasion and in evading the immune system defenses. For example, the formation of biofilms is a notorious strategy by which microorganisms improve their resistance [7]. Biofilms are complex microbial communities with higher resistance to adverse conditions and commercial drugs than planktonic forms. In addition, this lifestyle facilitates host colonization [8,9].

The pathogenic Gram-negative bacterium *Pseudomonas aeruginosa* has an intrinsic resistance to penicillin and cephalosporin, conferred mainly by the overproduction of β-lactamases [4,10]. This species commonly causes nosocomial infections, being the most frequent cause of respiratory infections in immunocompromised patients [11]. *Staphylococcus aureus* is a Gram-positive bacterium that causes several types of infections such as bacteremia; endocarditis; and infections in bone, respiratory, and endovascular tissue [12]. Currently, there are reports on the occurrence of isolates resistant to β-lactams such as methicillin and oxacillin (MRSA/ORSA isolates, respectively) [13], fluoroquinolone [14], and vancomycin [15]. In addition, biofilms have direct importance in the pathogenicity of *P. aeruginosa* and *S. aureus* [16].

The *Candida* genus is a heterogeneous group of yeasts that cause opportunistic infections in immunocompromised hosts; among the infections involving this genus, approximately 90% are caused by *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida glabrata*, and *Candida krusei* [17,18]. *Candida* strains resistant to diverse antifungals, such as azoles, echinocandins, and polyenes, have been reported [19], and the mechanisms involved include the decrease of intracellular drug concentration by the action of efflux pumps, drug target alterations and metabolic bypasses [20]. The relevance of biofilm formation in *Candida* infections has also been described [21].

Natural products comprise a diversity of bioactive molecules that have been evaluated as potential innovative therapeutic agents, playing an extremely important role in the discovery and development of new drugs [22]. Lectins are carbohydrate-binding proteins widely distributed in plants and exhibiting diverse biological activities, including antimicrobial action [23–25]. A lectin termed ApuL was isolated from the inflorescence bracts of *Alpinia purpurata* (Viell.) K. Schum. [26], which is an ornamental plant of the Zingiberaceae family, native to the Pacific islands, but currently cultivated on a large scale in several countries owing to its ability to bloom continuously throughout the year and the exuberance and durability of the flowers [27].

ApuL is an acidic and oligomeric protein of 34 kDa whose hemagglutinating activity (HA) is resistant to heat and is stimulated in the presence of calcium and magnesium ions. Its HA is not inhibited by monosaccharides, but decreases after incubation with the glycoproteins fetuin and ovalbumin [26]. The authors also reported that this lectin showed an interesting immunomodulatory effect, with an ability to induce the release of nitric oxide and cytokines belonging to the Th1 (IFN-γ, TNF-α, and IL-6) and Th17 (IL-17A) profiles; in addition, ApuL stimulated the differentiation and activation of both $CD8^+$ and $CD4^+$ T lymphocytes.

This work aimed to evaluate the antimicrobial and antibiofilm activities of ApuL against *S. aureus* and *P. aeruginosa* (standard and antibiotic-resistant isolates) as well as *Candida* species. Cell viability, growth curves, protein leakage, and changes in cell ultrastructure were evaluated. In addition, we determined the effects of lectin-antibiotic combinations on microbial growth.

2. Materials and methods

2.1. Lectin isolation

Inflorescences were collected at the campus of the *Universidade Federal de Pernambuco* (UFPE), with authorization (number 36301) of the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio) from the Brazilian Ministry of Environment. A voucher specimen was deposited under number 53,376 at the herbarium UFP Geraldo Mariz from the *Centro de Biociências* of the UFPE. The access was recorded (AEF1C1C) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen).

ApuL was purified according to the protocol previously described by Brito et al. [26]. The bracts were washed with distilled water, dried (28 °C, 2 days) and powdered. The powder was homogenized in 0.15 M NaCl, in a 10% (w/v) proportion, for 16 h at 28 °C. Next, the suspension was passed through a filter paper and gauze and centrifuged (10 min, 3000 g). The extract was treated (4 h, 28 °C) with ammonium sulphate (40% saturation), and the supernatant fraction was collected and dialyzed (6 h, two liquid changes) against distilled water. The dialyzed fraction was loaded (2 mL; 2.5 mg protein) onto a Sephadex G-75 (GE Healthcare Life Sciences, Sweden) column (30.0 × 1.5 cm) previously equilibrated with 0.15 M NaCl for gel-filtration chromatography. The column was irrigated with 0.15 M NaCl at a flow rate of 20 mL/h. The fractions were monitored for absorbance at 280 nm, and the first peak corresponded to ApuL.

2.2. Protein concentration and hemagglutination assay

Protein concentration was determined according to Lowry et al. [28] using bovine serum albumin as standard (31.25–500 μg/mL). The hemagglutinating activity (HA) was evaluated using a 2.5% (v/v) suspension of glutaraldehyde-treated rabbit erythrocytes [29]. The erythrocytes were collected through a protocol approved by the Ethics Committee on Animal Experimentation from UFPE (process 23076.033782/2015-70). In the HA assay, the sample (50 μL) was diluted two-fold in 0.15 M NaCl in 96-well microplates and then 50 μL of the erythrocyte suspension was added to each well. Erythrocytes incubated with 0.15 M NaCl were used as controls. After 45 min, the HA was recorded as the reciprocal of the highest sample dilution that promoted hemagglutination. Specific HA corresponded to the ratio between the HA and the concentration of protein (mg/mL). A HA inhibitory assay was performed by incubating (15 min, 28 °C) the lectin with 0.2 M fetuin or 0.2 M ovalbumin solution prior to the addition of the erythrocyte suspension.

2.3. Preparation of microbial inocula

Standard isolates of *Pseudomonas aeruginosa* (ATCC-27853, named as UFPEDA-416 in the collection) and *Staphylococcus aureus* (ATCC-6538, named as UFPEDA-02 in the collection) were provided by the UFPEDA culture collection (WDCM 114) of the *Departamento de Antibióticos* from UFPE. Multidrug-resistant isolates of *P. aeruginosa* (UFPEDA-261 and UFPEDA-262) and oxacillin-resistant *S. aureus* isolates (UFPEDA-670, UFPEDA-671 and UFPEDA-672) were also obtained from this collection. The isolation sites and resistance profiles of these isolates are shown in Table 1. Clinical isolates of the yeasts *Candida albicans* (URM 5901) and *Candida parapsilosis* (URM 6345) were obtained from the 'University Recife Mycologia' (URM) culture collection from the *Departamento de Micologia* of UFPE.

The stock cultures were maintained at −20 °C in sterile nonfat milk powder with 10% (v/v) glycerol. Bacteria were cultured in Mueller Hinton Agar (MHA) overnight at 37 °C and yeasts were cultured in