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BIOACTIVE PROPERTIES OF PROTEIN EXTRACT FROM LEAVES OF PSIDIUM BAHIANUM LANDRUM & FUNCH (MYRTACEAE)

PROPRIEDADES BIOATIVAS DE EXTRATO PROTEICO DE FOLHAS DE PSIDIUM BAHIANUM LANDRUM & FUNCH (MYRTACEAE)

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ABSTRACT

The Myrtaceae family is notable for its global diversity and abundance most notably the genus Psidium, which comprises approximately 100 species with economic significance and therapeutic potential. Accordingly, this study investigated the biological functions of proteins found in the leaves of *Psidium bahianum* Landrum & Funch, collected from an Atlantic Forest fragment located on the *Campus II* grounds of UNEB in Alagoinhas, Bahia. Proteins were extracted using Phosphate-Buffered Saline (PBS), and their electrophoretic profile was analyzed via SDS-PAGE. The study also evaluated antimicrobial activity (using disk diffusion assays and minimum inhibitory concentration—MIC), cytotoxicity (against sheep red blood cells and *Artemia salina* Leach), as well as hemagglutinating and antioxidant properties. A protein concentration of 34 mg/mL was obtained, with molecular weights ranging from 16 to 184 kDa. The disk diffusion assay demonstrated inhibitory activity against Gram-positive bacteria (*B. cereus, B. subtilis, M. luteus, and S. aureus*) and Gram-negative bacteria (*S. choleraesuis*), with inhibition zones ranging from 7.7±1.2 to 16±0.6 mm; the MIC was 0.34 mg/mL for all bacteria that showed positive results in the disk diffusion assay. No cytotoxicity was observed in sheep erythrocytes, while low toxicity was recorded in *A. salina* [with a median lethal concentration (LC50) of 758 µg/mL over a 72-hour period]. At the concentrations tested, the presence of hemagglutinating lectins was observed, along with a free-radical scavenging capacity of 90.85±0.49. Promising results were obtained for the proteins present in the *P. bahianum* leaf extracts, which warrants further investigation into their biological potential.

Keywords: Protein extract; Lectins; Hemoagglutination; Pathogenesis-related proteins; Antimicrobials; Bioprospecting.

RESUMO

A família Myrtaceae destaca-se pela diversidade e abundância mundial, especialmente o gênero Psidium, que reúne cerca de 100 espécies, com importância econômica e potencial terapêutico. Portanto, o presente trabalho investigou as funções biológicas das proteínas de folhas de *Psidium bahianum* Landrum & Funch de um fragmento de Mata Atlântica do *campus* II – UNEB, Alagoinhas-Bahia. Foi realizado extração de proteínas em Tampão Salina Fosfato (PBS) e avaliado o perfil eletroforético através da técnica SDS-PAGE. Foi avaliada a atividade antimicrobiana (teste difusão em disco e concentração mínima inibitória- MIC), citotóxica em hemácias de carneiro e em *Artemia salina* Leach, hemoaglutinante e antioxidante. Obteve-se uma concentração de 34mg/mL de proteínas, com pesos moleculares variados (de 16 a 184 kDa). O teste de difusão em disco evidenciou ação inibitória frente a bactérias Gram-Positiva (*B. cereus, B. subtilis, M. luteus e S. aureus*) e Gram-Negativa (*S. choleraesuis*) com halos de inibição variando de 7.7±1.2 a 16±0.6 e MIC de 0.34 mg mL^{-1} para todas as bactérias com resultados positivos na difusão em disco. Não foi observada citotoxicidade em eritrócitos de ovelha, tendo sido registrada baixa toxicidade em *Artemia salina* [concentração letal para 50% dos indivíduos (CL50) de 758 µg mL^{-1} em um período de 72 horas]. Nas concentrações testadas, observou-se a presença de lectinas hemaglutinantes, e a capacidade de sequestro de radicais livres de 90.85±0.49. Foram obtidos resultados promissores para as proteínas presentes nos extratos das folhas de *P. bahianum*, o que demanda investigações adicionais sobre o seu potencial biológico.

Palavras-chave: Extrato proteico; Lectinas; Proteínas relacionadas a patogênese; bioprospecção; hemoaglutinação; antimicrobianos.

1. INTRODUCTION

Plants have several defense mechanisms against predators and pathogens. Proteins and peptides with low molecular weights, known as pathogenesis-related proteins (PR proteins), are among these mechanisms. PR proteins are constitutively expressed at low levels under normal conditions; however, stress conditions induce their increase [1]. They are classified into 17 groups (PRP-1 to PRP-17) based on common physicochemical and immunological characteristics [2].

Lectins are a unique class of heterogeneous proteins that can recognize and reversibly bind to a variety of sugar structures on the cell surface. They are found in several plant groups and are abundant in plant seeds and various plant tissues, such as leaves [3].

Lectins compose specific protein groups widely used due to their applicability, including for carbohydrate and glycoconjugate identification, hormone isolation, identification of tumor cells, and inhibition of microbial growth [4]. Additionally, they are involved in defense mechanisms against pathogenic microorganisms, viruses, insects, nematodes, and herbivorous predators, interacting and interfering with glycoconjugates in the growth, development, and physiology of these organisms [5].

Antimicrobial activities have been discovered for several plant proteins in recent decades. Therefore, the use of antimicrobial proteins and peptides is among the strategies for combating infections caused by microorganisms [1].

The increasing and indiscriminate use of antibiotics has accelerated the selection process of microorganisms more resistant to the currently used chemotherapeutics, raising global concern about this

problem [6]. *Pseudomonas* spp. and *Salmonella* spp. are among these microorganisms, with a focus on some strains of *Escherichia coli*, which are already responsible for 50% of hospital infections [7]. Fungi are also disease-causing agents; the fungal species that most frequently cause infections in humans are *Candida* spp. and *Aspergillus spp*. [8].

Plants have a significant economic function, mainly for medicinal purposes in the treatment of several pathologies [9]. Brazil has a widely rich plant diversity and is considered the country with the greatest biodiversity in the world, home to more than 50,000 plant species. Angiosperms are among these plant groups, exhibiting a diversity of 76% of samples compared to other groups [10].

The genus *Psidium* (family Myrtaceae) is one of the most representative in Brazil, with 60 species of the 130 cataloged species worldwide [11]. Species of this genus are found in all biomes of Brazil; however, the Atlantic Forest biome is known for having high biodiversity, with many endemic species yet to be thoroughly studied regarding their biotechnological potentials [12].

Studies on extracts obtained in organic solvents, predominantly containing secondary metabolites, have revealed potential antimicrobial, antioxidant, and antitumor properties for species of this genus [13-14].

However, few studies on extracts of protein components (primary metabolites) are found.

Considering the importance of the genus Psidium, the aim of this study was to assess the antimicrobial, antioxidant,

hemagglutinating, and toxic potentials of proteins extracted from *Psidium bahianum* leaves.

2. MATERIALS AND METHODS

Botanical material collection

Young leaves of *Psidium bahianum* were collected from an Atlantic Forest remnant at the *Campus* II of the State University of Bahia (UNEB), in Alagoinhas, Bahia, Brazil (12°10'42.62"S and 38°24'39.52"W). The plant was identified through taxonomic comparison with a specimen deposited in the herbarium collection of the State University of Bahia (HUNEB), where the material was also incorporated and cataloged under registration number 14.918.

Protein extraction and concentration determination

The collected leaves were placed in a cooler with ice and transported to the Immunology and Molecular Biology Laboratory at the Institute of Health Sciences of the Federal University of Bahia.

The leaves were washed with distilled water, cut into small pieces, and macerated using a mortar and pestle in the presence of liquid nitrogen, employing sterilized and cooled materials. A two-gram sample of the obtained powder was transferred to a falcon tube and dissolved in 10 mL of chilled phosphate buffered saline (PBS) at pH 7.2. The sample was vigorously agitated on a vortex at maximum speed at 4 °C for 30 minutes, then centrifuged at 1,400 rpm and 4° C for 20 minutes. The supernatant was transferred to an Eppendorf tube and stored at -20 °C until used in the assays [15]. The protein concentration in the sample was determined using a protein quantification kit (Bio-Rad; modified LOWRY method).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The evaluation of the obtained protein bands (50µg/mL) involved subjecting the sample to vertical electrophoresis in polyacrylamide gel. The discontinuous SDS-PAGE electrophoretic system was used, composed of a stacking gel consisting of 4% acrylamide-bisacrylamide (29.2%-0.8%), 10% sodium dodecyl sulfate (SDS), 0.05% ammonium persulfate, 0.05% TEMED, and 0.5 M tris-HCl, at pH6.8. The running gel consisted of 12% acrylamide-bisacrylamide (29.2%-0.8%), 1.5 M tris-HCl pH 8.8, 10% SDS, 0.05% ammonium persulfate, 0.05% TEMED, and 1.5 M tris-HCl, at pH 8.8. Electrophoresis was performed in a migration buffer containing 0.124 M tris, 0.96 M $_{glycine}$, 0.5% SDS, at pH 8.3 for 1 hour and 30 minutes at a current of 30 mA.

Assessment of protein extract antimicrobial activity

Microorganisms

The protein extract was tested on bacterial and fungal strains from the Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC® 6538 ™), *Bacillus subtilis* (ATCC® 6633 ™), *Bacillus cereus* (ATCC® 9592™), *Micrococcus luteus* (ATCC® 10240 ™), *Escherichia coli* (ATCC® 94863 ™), *Salmonella choleraesuis* (ATCC® 14028™), *Aspergillus niger* (ATCC® 16404 ™), *Candida albicans* (ATCC® 18804 ™), and *Nakaseomyces glabrata* (ATCC® 728 ™).

Disk diffusion

The protein extract antimicrobial activity was evaluated using the disk diffusion methodology, following the recommendations of the

Clinical and Laboratory Standards Institute (16). Bacteria were cultured on Müeller-Hinton Agar at 37 °C for 24 hours, while fungi were cultured on Sabouraud Dextrose Agar at 37 °C for 48 hours. Subsequently, 5 mm diameter filter paper discs impregnated with 10 mg mL-1 of protein extract (protein concentration of 34 mg mL-1) were applied to the agar inoculated with the test microorganisms. Discs impregnated with 30 µg of tetracycline (Laborclin®) and discs with ciclopirox olamine (0.1%) were used as positive controls in bacterial and fungal assays, respectively. The antimicrobial activity was determined by measuring the inhibition zones immediately after the incubation period.

Minimum inhibitory concentration (MIC)

The MIC was evaluated according to the protocol described in the Clinical and Laboratory Standards Institute [16]. A 100 µL aliquot of the culture medium was distributed in 96-well plates, and then 100 µL of proteins were added in triplicate and serially diluted from the first (A) to the last row (H) (1:2 dilution). Microbial inoculum (100 µL) was added, resulting in a volume of 200 µL per well, with concentrations ranging from 84 to 10500 µg mL-1. Kanamycin (30 µg) and ciclopirox olamine (0.1%) were used as positive controls, while pure culture medium and saline solution were used as negative controls. The plates were incubated at 37 °C for 24 and 48 hours for bacteria and fungi, respectively.

The inhibitory activity was classified as bactericidal or bacteriostatic by seeding 10 µL of the inoculum from wells that did not show microbial growth in the MIC assay on Petri dishes containing sterile and antibiotic-free Mueller Hinton medium for 24 hours at 37 °C [17].

Toxicity tests

Cytotoxicity test on sheep erythrocytes

The hemolytic assay was performed by adding protein extract concentrations (10, 100, 500, and 1,000 µg mL-1) to a 2% suspension of defibrinated sheep blood (Laborclin®). The suspensions were slowly mixed for one minute in two steps: they were left to rest for 30 minutes after the first agitation and, then, homogenized again and left to rest for 150 minutes at room temperature. Subsequently, the samples were centrifuged at 3,000 rpm for 5 minutes. Hemolysis was considered when the plasma fraction exhibited a reddish hue. Distilled water was used as the positive control, while saline solution was used as the negative control.

Toxicity test on *Artemia salina* Leach

The protein extract toxicity was evaluated in nauplii of *Artemia salina* Leach [18]. *A. salina* nauplii were deposited in an interconnected compartment containing sterile seawater. The assays were conducted in triplicates and consisted of adding seawater and protein extract concentrations (10, 100, and 1,000 µg mL-1) in 24-well plates, where, subsequently, 10 A. Salinas nauplii were added in each well. Seawater was used as the negative control. Survivors were counted within 24, 48, and 72 hours of exposure to the protein extract concentrations. The protein extract toxicity was categorized based on its lethal concentration for 50% of individuals (LC50), as follows: non-toxicity (LC50>1,000 µg mL^{-1}); low toxicity (LC50>500 µg mL^{-1}); moderate toxicity (LC50 between 100 and 500 µg mL^{-1}); and high toxicity (LC50<100 µg mL^{-1}) [19].

Assessment of hemagglutinating activity (HA)

The hemagglutinating activity (HA) assay was conducted following the methodology described by Correia and Coelho [20], with modifications. A 100 µL aliquot of saline solution was added into 5 tubes, followed by addition of 100 µL of protein extract concentrations (10; 100; 1,000;5,000; and 10,000 µg mL-1) and 100 µL of 3% diluted sheep erythrocytes. These tubes were incubated at 37 °C for 30 minutes. Subsequently, the presence of hemagglutination was macroscopically evaluated. Blood in solution (without protein extract) and blood in distilled water were used as negative controls.

Assessment of antioxidant activity

The protein extract antioxidant activity was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [21]. A 100 µL aliquot of protein extract (protein concentration of 34 mg mL-1) was added to 96-well titration plates. Subsequent serial dilution was performed, and then 100 µL of DPPH solution (0.5 mM) was added to each well. The plates were stored protected from light and readings were taken after one hour using a UV-Vis spectrophotometer at 620 nm. The percentage of DPPH free radical scavenging (% DPPH-RS) was determined using the following equation [21]:

$$\%DPPH\text{-}RS = \{[(Abscontrol - Abssample) \times 100] / Abscontrol\}$$

Where: *Abscontrol* is the absorbance of the control (DPPH and ethanol); and *Abssample* is the absorbance of the sample.

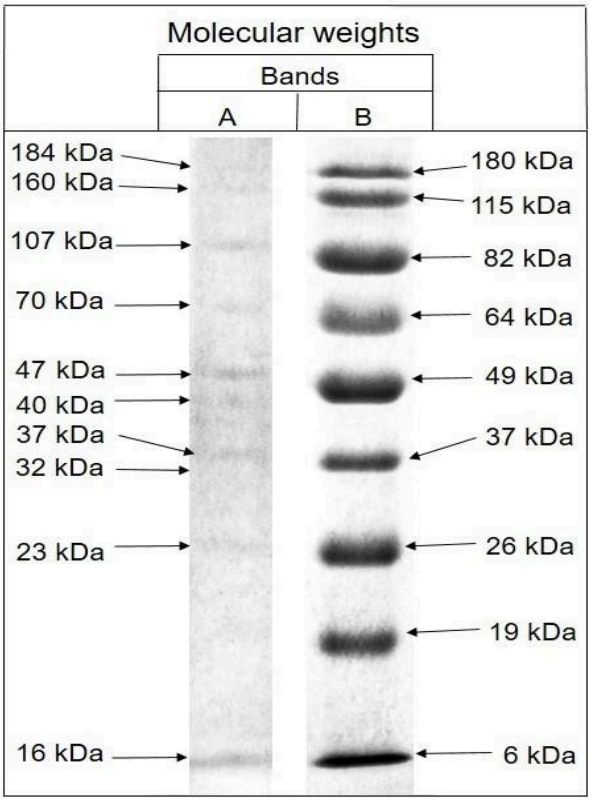
Statistical analysis

The data evaluated were subjected to analysis of variance, comparing the means with the TUKEY test at 5% probability, both performed with the aid of the R program, using the DPLYR package and GGPLOT2 for data analysis.

3. RESULTS

3.1. Molecular Weight Of Proteins

Figure 1. Electrophoretic pattern of proteins found in young *Psidium bahianum* leaves. A = Bio-Rad protein standard; B = Intensity of protein bands. Curve with R^2 = 0.988, analyzed using the software Gel Analyzer 2010.



The final protein concentration was expressed in mg of protein per mL, yielding a concentration of 34 mg mL-1. The electrophoretic analysis of the protein extract from *Psidium bahianum* leaves showed a total of 10 protein bands with molecular weights

approximately at 184, 160, 107, 70, 47, 40, 37, 32, 23, and 16 kDa (Figure 1).

3.2. Evaluation Of Antimicrobial Activity

3.2.1. Disk Diffusion

The disk diffusion test revealed antimicrobial action of the protein extract (at a concentration of 34 mg mL-1) against gram-positive bacteria (*Bacillus cereus, B. subtilis, Micrococcus luteus, and Staphylococcus aureus*) and gram-negative bacteria (*Salmonella choleraesuis*) (Table 1). The disk diffusion analysis revealed that there was a statistically significant difference between the inhibition zone of the control and the inhibition zone caused by the protein extract for the bacteria *B. cereus, B. subtilis, M. luteus, S. aureus and Salmonella choleraesuis*. However, there was a statistically significant difference ($p<0.005$) between the inhibition zone of *M. luteus* and the other microorganisms, as well as the inhibition zone of *S. choleraesuis*, which was statistically significant ($p<0.005$) when compared the inibition zone of *B. cereus, B. subtilis* and *S. aureus*.

Table 1. Sensitivity of microorganisms to the protein extract from young *Psidium bahianum* leaves based on the disk diffusion test.

Inhibition halo diameter (mm)			
Microorganism	**ATCC® ***	**Classification**	**(Mean±standard deviation)**
Bacillus cereus	9592	Gram-positive	(9±1)
Bacillus subtilis	6633	Gram-positive	(7.7±1.2)

Micrococcus luteus	10240	Gram-positive	(16±0.6)
Staphylococcus aureus	6538	Gram-positive	(8±2.3)
Escherichia coli	94863	Gram-negative	–
Salmonella choleraesuis	14028	Gram-negative	(11±0.4)
Aspergillus niger	16404	Filamentous	–
Candida albicans	18804	Yeast	–
Nakaseomyces glabrata	728	Yeast	–
* ATCC: American Type Culture Collection Bacterial control: 30 µg of tetracycline (Laborclin®) Fungal control: Ciclopirox olamine (0.1%)			

3.2.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the *P. bahianum* leaf protein extract was 0.34 mg mL-1, inhibiting the growth of the same microorganisms inhibited in the previous test (Table 2).

Table 2. Minimum Inhibitory Concentration (MIC) of protein extract from young *Psidium bahianum* leaves.

					MIC
					Microorg
	Protein concentration	*B. cereus*	*B. subtilis*	*M. luteus*	*S. aur*

A	10.75 mg mL^{-1}	+	+	+	+

⚠ Esta tabela possui muitas colunas e foi cortada para impressão. Para visualizá-la completa, acesse o artigo original em: https://revistatopicos.com.br/artigos/bioactive-properties-of-protein-extract-from-leaves-of-psidium-bahianum-landrum-e-funch-myrtaceae?noblockage

3.3. Toxicity Tests

Regarding the tests carried out to evaluate the toxicity of the protein extract, it was found that in the hemolytic test with sheep erythrocytes the extract was non-toxic in all concentrations tested, however, the evaluation of toxicity in *Artemia salina*, the extract showed low toxicity, with a lethal concentration for 50% of individuals (LC50) of 758µg mL-1 within 72 hours. When analyzing mortality as a function of concentration, we found that 1000µg/mL presents a statistically significant difference ($p<0.005$) when compared to the other concentrations, while in relation to the time of 72, 48 and 24h 1000µg/mL is different and has a statistically significant ($p<0.005$) difference between itself and in relation to the other times in all concentrations.

3.4. Assessment Of Hemagglutinating Activity (HA)

Regarding the hemagglutinating activity test to determine the presence or absence of lectins in the protein extract from *P. bahianum* leaves, hemagglutination was found at all tested protein extract concentrations.

3.5. Assessment Of Antioxidant Activity

The evaluation of antioxidant activity showed a percentage of free radical scavenging of 90.85±0.49 (percentage plus standard deviation), which is a significantly important result).

4. DISCUSSION

Several studies have reported varied molecular weights of plant lectins, such as 12.2, 13, and 13.8 kDa [22], 32 and 34 kDa [23], 200 KDa [24] and 204 KDa [25], which typically associate into dimers and trimers. Protein fractions suggestive of lectins were found in the present study (Figure 1). However, pathogenesis-related proteins (PR proteins) exhibiting in vitro antimicrobial action may also be present [2], as they also have relatively low molecular weights (5, 8, 9, 15, 17, 20, 25, 27, 30, 35.40, and 75 kDa) [25, 26].

The protein fractions identified in this study exhibit molecular weights similar to those described by other authors for pathogenesis-related proteins (PRPs), making it possible to establish potential correspondences with different families of PR proteins: 16 kDa (PR-1 e PR-5); 23 kDa (PR-5 e PR-6); 32 kDa (PR-2 e PR-8); 37 kDa (PR-2 e PR-3); 40 kDa (PR-2 e PR-3); 47 kDa (PR-3 ou PR-9, PR11); e 70 kDa (PR-7 ou PR-9) [27, 28].

Studies evaluating essential oils or extracts from *Psidium* spp. in different solvents have shown antimicrobial activity of these materials [29, 13]. However, studies evaluating the antimicrobial activity of protein extracts were not found.

Most of the bacteria inhibited by the evacuated protein extract are gram-positive bacteria. These bacteria are more sensitive to drugs due to their bacterial wall structure. However, the gram-negative bacterium *S. choleraesuis* showed sensitivity to the protein extract at

the same concentrations that inhibited the gram-positive bacteria. *Salmonella* spp. are the most common agents causing diseases transmitted by contaminated food to humans [30].

The tested fungal strains did not show sensitivity to the protein extract in both tests. Lectins can exhibit several biological activities, as they are proteins that bind to several molecular structures. A study using the MIC test to assess the antimicrobial activity of two plant lectins with molecular weights of 14 and 25.5 kDa, respectively, against *Candida albicans* and *C. tropicalis* did not find inhibitory activity of the evaluated lectins [31].

An article published using the concentration of 60 mg mL-1 of a purified lectin (31 kDa) from *Phaseolus vulgaris* showed antifungal activity against strains of *Fusarium oxysporum*, *Coprinus comatus*, and *Rhizoctonia solani* [32]. Although the lectins and PR proteins from *P. bahianum* leaves did not exhibit antifungal activity against the tested strains in the present study, other strains may be tested and show inhibitory results. However, in the present study, a set of proteins was tested at a concentration (34 mg mL^{-1}) approximately half that of the concentration used for the purified lectin from *Phaseolus vulgaris* (60 mg mL^{-1}).

The antimicrobial activity of lectins is attributed to their potential for agglutinating microorganisms, while PR proteins have other mechanisms, such as permeabilization of the cell plasma membrane, hydrolysis of the cell wall, and direct toxicity [33].

Species of the family Myrtaceae present antimicrobial activity in different plant parts, from which secondary metabolite components are extracted using various solvents [13]. However, the findings of the

present study are unprecedented due to the use of a protein extract, making it difficult to compare these results with data from the scientific literature. Nonetheless, these results highlight the importance of the present study, as they showed antimicrobial activity of the tested protein extract.

The results of the cytotoxicity test did not show effect of the protein extract from *P. bahianum* leaves on sheep erythrocytes. A similar result was found in a study testing extracts from leaves and stems of *P. bahianum* obtained with different solvents [13]. A study reported low toxicity in human erythrocytes when testing methanolic extract of *P. guineense* Swartz at different concentrations [34]. Understanding the possible cytotoxic effects of plant extracts with medicinal potential is important, as hemolysis induces deleterious effects, mainly on the cardiovascular system and the kidneys through the increase of plasma hemoglobin [35].

Conducting toxicity studies with *A. salina* is important, as the toxic action of extracts on this microcrustacean is correlated with antitumor activities. The lethality assay in *A. salina* is the most appropriate to easily monitor the biological activity of different plant species, as well as an excellent predictive tool for the toxic potential of plant extracts in humans [36]. A study conducted with *P. bahianum* demonstrated that leaf extracts in ethyl acetate and ethanol had moderate toxicity in *A. salina*, with LC50 of 489.80 and 323.60 µg mL^{-1}, respectively [13].

A study evaluating the toxicity of extracts from the bark and leaves of *P. guajava* found LC50 of 480.14 (bark extract) and 949.13 µg mL^{-1} (leaf extract), with higher mortality rates for the bark extract [37]. A study conducted with *P. myrsinites* found a high toxicity for the

essential oil (LC50 of 95.30 µg mL^{-1}), moderate toxicity for hexane extract fractions (LC50 of 205.70 (g mL^{-1}), and no toxicity for the other tested fractions [38]. The essential oil of *P. Bahianum* demonstrated in 24 and 48 hours the LD50 is 371.48 and 302µg/mL of essential oil, being considered moderately toxic, and in 72 hours the LD50 is 33.11µg/mL, considered highly toxic [39].

These studies were conducted using secondary metabolites. However, they allowed for the assessment of the toxic potential of species of the *Psidium genus*, including *P. bahianum*, whose different extracts (protein and secondary metabolites) showed low toxicity. The toxicity in *A. salina* found in the present study was low; however, complementary tests could be conducted on different tumor cell lines, considering the absence of cytotoxicity in sheep erythrocyte membranes. The scientific literature has indicated that the presence of hemagglutination suggests nontoxicity, i.e., the absence of cell membrane rupture [5].

A plant lectin with an estimated molecular weight of 128 kDa was tested on human erythrocytes and showed no toxicity at a concentration of 1,000 µg mL-1. Thus, the proteins detected in the polyacrylamide gel electrophoresis test, with molecular weights close to those of the found plant lectins, indicated the presence of nontoxic lectins in the evaluated protein extract [33].

A galactose-specific lectin found in *P. guajava* has hemagglutinating properties for red blood cells and *E. coli*, inhibiting the growth of this microorganism [40], however, the molecular weight of the lectin was not informed in this study. Moreover, few studies evaluating lectins from *Psidium* spp. are available.

Lectin isolated from *Phaseolus vulgaris* showed high hemagglutination in a 2% suspension of sheep erythrocytes [33]. Similar results were found in the present study, using a similar test protocol. The results of the present study showed presence of lectins with potential hemagglutinating activity in the protein extract from *P. bahianum* leaves; however, further studies should be conducted to better characterize this lectin and other proteins present in this extract.

The result of the antioxidant assay demonstrated that the protein extract is capable of eliminating free radicals. Similar percentages were found for the same plant species using the solvents hexane, ethyl acetate, and ethanol, with 69.51±1.520, 82.71±1.270, and 82.97±1.360, respectively (stem) and 75.40±2.130, 80.79±1.530, and 81.07±1.280, respectively (leaves) [13].

A review study on the antioxidant activity of *P. guajava* reported that plant extracts could be a new therapeutic tool against several diseases and their complications, as cellular damage to DNA, proteins, and lipids caused by free radicals leads to several conditions, including rheumatoid arthritis, cirrhosis, diabetes, cancer, cardiovascular diseases, and cellular aging; antioxidant substances prevent oxidative damage by eliminating radicals and chelating metal ions [41].

Furthermore, other studies have shown the antioxidant activity of extracts from fruits, stems, and leaves of *Psidium* spp., but these studies have focused mainly on secondary metabolites rather than proteins.

5. CONCLUSIONS

The study reveals that the extract contains lectins that play important roles, such as antimicrobial effects against Gram-positive and Gram-negative bacteria. In addition, these proteins are non-toxic to sheep erythrocytes, have low toxicity to *Artemia salina*, and demonstrate the ability to scavenge free radicals. These promising results pave the way for future investigations, which may further explore the therapeutic and biotechnological potential of the proteins present in the leaves of *P. bahianum.*

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Abbreviations

The following abbreviations are used in this manuscript:

ATCC Type Culture Collection

MIC Minimum Inhibitory Concentration

PR pathogenesis-related proteins

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