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BIOMEDICAL SCIENCES

Protein fraction from *Sesbania virgata* (Cav.) Pers. seeds exhibit antioxidant and antifungal activities

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Abstract: *Sesbania virgata* (Cav.) Pers. seeds are protein sources with health and environmental benefits. In this research, proteins with lectin activity were identified in a protein fraction from *S. virgata* seeds (PFLA), as well its antioxidant and antimicrobial potentials, in addition to cytotoxic effects. To obtain PFLA, seed flour was homogenized in Glycine-NaOH (100 mM; pH 9.0; NaCl 150 mM) and precipitated in ammonium sulfate. PFLA concentrates bioactive lectins (32 HU/mL, 480 HU/gFa, 18.862 HU/mgP) and essential amino acids (13.36 g/100g protein). PFLA exerts antioxidant activity, acting as a promising metal chelating agent (~77% of activity). Analyzes of cell culture assay results suggest that antioxidant activity of PFLA may be associated with the recruitment of essential molecules to prevent the metabolic impairment of cells exposed to oxidative stress. PFLA (256 – 512 µg/mL) also exhibits antifungal activity, inhibiting the growth of *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Penicillium citrinum*. Cytotoxic analysis indicates a tendency of low interference in the proliferation of 3T3 and HepG2 cells in the range of PFLA concentrations with biological activity. These findings support the notion that PFLA is a promising adjuvant to be applied in current policies on the management of metal ion chelation and fungal infections.

Key words: adjuvants, antifungal, antioxidant, lectins, safety, seed proteins.

INTRODUCTION

The Fabaceae family (aka Leguminosae) is a widely distributed and economically important human food staple (Mohammed & Qoronfleh 2020). Legume have been part of human nutrition for centuries and are used in folk medicine as products with multidirectional medicinal effects. Beans, peas, peanuts, chickpeas, lentils, broad beans and soybeans are edible legume species (Grdeń & Jakubczyk 2023). In particular, the increasing legume seed consumption as a strategy for enhancing food security, reducing malnutrition, and improving health outcomes on a global scale remains an ongoing subject of profound research interest (Ohanenye et al. 2022).

Sesbania virgata (Cav.) Pers. (Fabaceae) is a fast-growing shrub popularly known as "saranzinho", "mãe-josé'' and "feijãozinho" widely distributed in riparian forests of South, Southeast and Midwest regions of Brazil, Argentina, Uruguay, and Paraguay (Dutra et al. 2019). In the last decade, *S. virgata* also began to colonize riverbanks in the Brazilian Northeast (Teixeira et al. 2018). This adaptive plasticity has been encouraging farmers to use *S. virgata* to improve food production and recover degraded areas (Evans & Rotar 2020). In India, numerous species of *Sesbania* are used in folk medicine for

the treatment of epileptic seizures (Kasture et al. 2002), dysentery, fever, headaches, smallpox, and stomatitis (Hasan et al. 2012). In Africa and Australia, *S. virgata* is widely used as a protein source for ruminants (Gutteridge et al. 1995, Gutteridge 1994), and in Argentina, Bangladesh and India, *S. virgata* is an edible plant (Hossain & Becker 2001, Siddhuraju et al. 1995).

In the last 10 years, a promising growth has been observed in the awareness of nutraceuticals, and its use as therapeutic supplements is now recognized as part of Complementary and Alternative Medicine (Puri et al. 2022). Nutraceuticals are compounds present in foods that have beneficial effects on human health and wellness. These compounds include vitamins, flavonoids, polyunsaturated fatty acids, dietary fibers, minerals, peptides, and proteins (Chandra et al. 2022). Protein plants, especially, have been the subject of growing interest from researchers and consumers due to its potential health benefits, as well as its positive environmental impact, in addition to offering unique advantages in the production of pharmaceuticals for humans and animals (Liu & Timko 2022, Sá et al. 2022, Ahnen et al. 2019). In previous analyses (Sá et al. 2021), we revealed that *S. virgata* seeds are promising sources of proteins (among the investigated macronutrients, 60.8% were proteins) capable of being functionalized in formulations for numerous purposes.

The biological properties of plant protein-derived formulations may be associated with lectins, a special protein class available in all tissues and organs plant that have at least one non-catalytic domain capable of bringing reversible and specifically to mono- or oligosaccharides (Van Damme 2022). Despite their strong similarity on the level of their amino acid sequences and tertiary structures, their carbohydrate specificities and quaternary structures vary widely (Loris et al. 1998). Legume lectins, in special, exhibit considerable variation in their quaternary structure arising out of small changes in their sequence, enabling numerous interactions and biological activities (Srinivas et al. 2001). Legume lectins can serve as potential target molecules for developing practical applications in the fields of food; agriculture; health and pharmaceutical research (Lagarda-Diaz et al. 2017).

Although some studies on obtaining and isolation of lectins from *Sesbania* species are available (Sultana et al. 2019, Biswas et al. 2009a, Hossain et al. 2001), none discuss its prospection on *S. virgata* seeds. Therefore, in order to start filling the existing void about knowledge regarding the biotechnological potential of proteins from *S. virgata* seeds, we identified the presence of proteins with lectin activity in *S. virgata* seeds, as well its antioxidant and antimicrobial potentials, in addition to cytotoxic effects. Our analyzes will contribute to the development of several industry sectors by offering insights on alternative adjuvants based on a plant ineffectively exploited in Brazil.

MATERIALS AND METHODS

The project was approved by the Research Ethics Committee of Universidade Federal da Paraíba (CEUA/UFPB n. 178/2015), and registered on Plataforma Brasil (CAAE: 51873021.0.0000.5537), a unified national database of research records (Brazil).

Equipment, chemicals and drugs

Milli-Q system (Millipore®, USA), Incubator Themoforma Serie II Water CO_2 Model 3110 (Thermo Scientific™ Forma™, USA), Centrifuge model 5430 R (Eppendorf, Germany), High-Performance Liquid Chromatograph (VARIAN, Waters 2690, USA) with C18 LUNA 100 Å column

(4.6 mm x 250 mm; 5.0 µm particle; Phenomenex, USA), and UV-Vis Spectrophotometer model UV-1800 (Shimadzu Corp., Japan). Bovine serum albumin, bovine trypsin, Coomassie Brilliant Blue (G-250 and R-250), carbohydrates, DL-2-aminobutyric acid, DL-BAρNA (DL-benzoyl-arginine-ρ-nitroanilide), ferrozine, L-glutamine, polyethylene glycol 400, pyrocatechol violet, regenerated cellulose membrane (14 kDa), RPMI-1640 medium, sodium dodecyl sulfate (SDS), synthetic antimicrobials (nystatin, fluconazole and chloramphenicol) and thioglycol were acquired from Sigma-Aldrich (USA). Colorimetric reagent Folin-Ciocalteu, copper II sulfate pentahydrate, iron chloride, iron III chloride, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and trichloroacetic acid (TCA) were acquired from Merck (Germany). Ethylenediamine tetra acetic acid (EDTA) and potassium ferricyanide were acquired from Vetec Quimica Fina Ltda (Brazil). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were acquired from Cultilab (Brazil). The Brain Heart Infusion broth (BHI), Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) were acquired from Difco Laboratories (France). Ethyl alcohol and hydrogen peroxide were acquired from CRQ (Brazil). Gallic acid, iron II sulfate, metal ions (Ca, Mg and Mn), resazurin dye, sodium phosphate and sodium salicylate were acquired from Casa da Química Ind. E Com. (Brazil), CRB – Cromato Produtos Químicos Ltda (Brazil), Dinâmica Química Contemporânea (Brazil), INLAB (Brazil), Diadma (Brazil) and FLUKA (Germany), respectively. The high molecular mass markers (225 – 12 kDa) were acquired from GE Healthcare (Amersham™ ECL™ Rainbow™, USA) and all reagents were of analytical grade.

Plant collection, protein extraction and obtaining PFLA

Sesbania virgata was collected in João Pessoa-PB, Brazil (7°09'51.8"S 34°54'01.1"W), in October 2016, under SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) regulations (SisGen n. A1C2041). A specimen (voucher JPB n. 6319) was deposited at the Professor Lauro Pires Xavier Herbarium, João Pessoa-PB, Brazil.

Healthy and mature *S. virgata* seeds were set to dry for five days, pulverized in an electric mill and delipidated in n-hexane. After evaporation of n-hexane, the resulting material was homogenized (1:15, w/v) in different extractor systems: Glycine-HCl (100 mM; pH 2.6; NaCl 150 mM), Glycine-NaOH (100 mM; pH 9.0; NaCl 150 mM), Tris-HCl (100 mM; pH 7.4; NaCl 150 mM), Tris-NaOH (100 mM; pH 7.2; NaCl 150 mM), NaCl 150 mM, and distilled water. The extracts remained for 1, 4, 19, 22 and 24 h on magnetic stirring, at 25 °C, with subsequent centrifugation (7.000 x g, 20 min, 4 °C). The supernatants were collected and used in the detection of lectin activity, according to Lectin activity assay.

The crude extract (CE) that presented more potent lectin activity (based on HU values) was fractionated by the Osborne (1924) method to obtain albumin, globulin, prolamin, and glutelin fractions; and precipitated in ammonium sulfate (Scopes 1994), under five saturation intervals (w/v): 0-20%, 20-40%, 40-60%, 60-80%, and 80-100%. Each fraction was exhaustively dialyzed against distilled water in a regenerated cellulose membrane (14 kDa), lyophilized, and its lectin activity tested. The fraction that presented more potent lectin activity (based on HU values) was named Protein Fraction with Lectin Activity (PFLA).

Lectin activity assay

The lectin activity was macroscopically detected by hemagglutination assays (Debray et al. 1981), using 3% native erythrocyte of *Oryctolagus cuniculus* (CEUA/UFPB n. 178/2015). The tests were carried out in triplicate, by serial dilution. The negative control was performed with a NaCl 150 mM. The presence of hemagglutination was determined by direct visualization at different times (30, 60, 120, 1080, and 1440 minutes). The results were expressed as inverse of the titration from highest dilution with visible hemagglutination (HU/mL), as well as the number of hemagglutinating units per milligram of protein (HU/mgP) and per gram of flour (HU/gF).

Antioxidant activity

Total antioxidant activity of PFLA (500, 100, 10 and 1 µg/mL) was evaluated by the reducing power (Yen & Chen 1995), iron-chelating (Dinis et al. 1994), copper-chelating (Anton 1960), and hydroxyl-scavenging (Dasgupta & De 2007) assays. The results of reducing power assay were expressed as the percentage of activity for 0.1 mg/mL (highest activity) of ascorbic acid. The results of the copper- and iron-chelating assays were expressed as the percentage of chelating effect, using the following Equation: Chelating Effect (%) = [(Ac – A_{PFLA}) / Ac] x 100, where Ac: absorbance of control tube, and A_{PFLA}: absorbance of PFLA. The results of hydroxyl-scavenging assay were expressed according to the following Equation: Radical Scavenging (%) = [(Ac – A_{PFLA}) / (Ac – Ab)] x 100, where Ac: absorbance of the control tube, A_{PFLA}: absorbance of PFLA, and Ab: absorbance of the blank tube.

Antioxidant activity of PFLA in cell culture was evaluated by the MTT reduction method (Mosmann 1983). After determining the injury condition (H_2O_2-induced oxidative stress), according to Ouyang et al. (2011) and Sá et al. (2023), murine fibroblast cell lines (3T3, ATCC® CRL-1658™) were exposed to different concentrations of H_2O_2 (5.0 to 0.5 mM). As a result, at a concentration of 4 mM the cells suffered enough damage to decrease MTT expression by up to 45% (positive control). Concentrations greater than 4 mM caused damage well over 45%, while the negative control (without H_2O_2) showed no damage. The 3T3 cells were initially exposed to H_2O_2 (4 mM) for 1 h, and later treated with the PFLA (1000, 100 and 1 µg/mL), for 24 h. The absorbance (570 nm) of the control without H_2O_2 was considered to be a 100% reduction in MTT assay, and the values of the treated cells were calculated as a percentage of the negative control, without H_2O_2. Results were expressed as the percentage of MTT reduction, according to the following Equation: MTT Reduction (%) = [(A_{PFLA} / Ac) x 100], where A_{PFLA}: absorbance of cells subjected to treatment with PFLA, and Ac: absorbance of cells from the negative control.

Antimicrobial activity

Bacterial (*Staphylococcus aureus* ATCC-13150, *S. aureus* LM-117, *Staphylococcus epidermidis* ATCC-12228, *Pseudomonas aeruginosa* ATCC-25853, *P. aeruginosa* P-03, *Bacillus subtilis* ATCC-6633, *Escherichia coli* ATCC-10436, and *E. coli* EC-12), yeast (*Candida albicans* ATCC-76645, *C. albicans* LM-122, *Candida tropicalis* ATCC-13803, *C. tropicalis* LM-64, and *C. tropicalis* LM-7) and filamentous fungi (*Aspergillus flavus* LM-714, *A. flavus* LM-247, *Penicillium citrinum* LM- 9, and *P. citrinum* LM-60) were maintained at 4 °C in SDA and BHI, and incubated at 35 ± 2 °C for 24-48 h, respectively. The microorganism suspension was prepared according to the 0.5 McFarland scale tube and adjusted to 90% T (CLSI 2015, 2008, Hadacek & Greger 2000, Cleeland & Squires 1991). The minimum inhibitory concentration (MIC) was determined by the micro dilution technique (1024 to 32 µg/mL) (Cleeland &

Squires 1991, Eloff 1998). The bacterial growth was accompanied by the colorimetric change of the 0.01% resazurin dye. The MIC was defined as the lowest concentration of PFLA capable of visually inhibiting microbial growth with no dye color change. Chloramphenicol (100 µg/mL) was the negative control for bacterial assays, and nystatin (100 µg/mL) and fluconazole (50 µg/mL) were the negative controls for yeast and filamentous fungi assays, respectively. In our investigations, we analyzed the antimicrobial potential of PFLA against ATCC strains, because they are well characterized and very popular for this purpose, in addition to clinical isolates characterized by antibiograms.

Cytotoxicity assay

The cytotoxicity of PFLA (1000, 500, 100 and 1 µg/mL) was evaluated using Mosmann's (1983) method. Hepatocellular carcinoma cells (HepG2, ATCC® HB8065™) and murine fibroblast cells (3T3, ATCC® CRL-1658™) were donated by Dr. Viviane Souza do Amaral and Dr. Silvia Regina Batistuzzo de Medeiros, respectively, from Universidade Federal Do Rio Grande Do Norte - UFRN, Natal-RN, Brazil. HepG2 and 3T3 cells (5 x 10^4 cells) were cultivated in DMEM and supplemented with 10% FBS, 2% L-glutamine and 1% streptomycin/penicillin, at 37 °C and 5% CO_2. The control included cells cultivated in DMEM and 10% FBS, and was able to show 100% reduction of MTT (1.0 mg/mL), considered as 100% proliferation. Results were expressed as the percentage of MTT reduction, using the following equation: MTT Reduction (%) = (A_{PFLA} – Ac) x 100, where A_{PFLA}: absorbance of cells subjected to treatment with PFLA, and Ac: absorbance of cells from the negative control. Concentrations that promoted cell viability below 80% were considered antiproliferative and potentially toxic.

Initial characterization of PFLA

Protein content and electrophoretic analysis

Total soluble protein content was measured according to Bradford (1976) method, using bovine serum albumin (1.0 mg/mL) as standard, and Coomassie Brilliant Blue G-250 as chromogenic reagent. Analysis of total protein was done by the Kjeldahl method involving three steps (i.e., digestion, distillation, and titration) (AOAC 2000). The results of the total protein content were obtained using a conversion factor of 6.25 to convert the nitrogen values to protein. The estimation of the relative molecular weight of the proteins was conducted by electrophoresis (SDS-PAGE) in the presence of SDS 1% and β-mercaptoethanol, according to Laemmli (1970). The application gel was prepared in the concentration of 3.5% and the separation gel, 12.5%. The molecular weight estimation was obtained by comparison to the relative electrophoretic mobility of the molecular weight standard (225 to 12 kDa).

Detection of interferents

Total sugars were quantified with phenol-sulfuric acid method, using D-galactose (10 mg/mL) as the standard (Dubois et al. 1956). Total phenolic compounds were quantified by the Folin-Ciocalteu colorimetric method, using gallic acid (10 mg/mL) as the standard (Singleton et al. 1998). Pyrogallic and cathectic tannins were prospected according to Arnason et al. (1995). For the detection of trypsin inhibitors (Xavier-Filho et al. 1989), bovine trypsin (0.3 mg/mL) was used as the standard enzyme, and DL-BAρNA as its chromogenic substrate. The inhibitor unit (IU) was defined as the amount of inhibitor that can decrease by 0.01 nm the absorbance value in the trypsin inhibitor assay, and since specific activity was considered, the relationship between IU and amount of protein used in the assay.

Influence of divalent cations and sugar specificity

To determine the influence of divalent cations (40 mM), PFLA was dialyzed against EDTA 250 mM containing NaCl 150 mM, for 24 h; followed by a dialysis against NaCl 150 mM. The hemagglutination activity was determined before and after addition of Ca, Mg, Mn, and its combination (Ca/Mg, Ca/Mn, and Mg/Mn). Carbohydrate-binding specificity was determined by minimum inhibitory concentration of sugar that exhibits complete inhibition of hemagglutination (Ramos et al. 1996). An initial concentration (1.0 M) of the following sugars was prepared: D-Fructose, D-Glucose, D-Mannose, D-Xylose, L-Arabinose, and L-Sorbose.

Total amino acid profile

Initially, PFLA was subjected to acid hydrolysis by aqueous solution of 6N hydrochloric acid double distilled at 104 °C, containing 0.1% phenol. After drying and concentration of the hydrolyzed material, it was suspended in 170 mM sodium citrate buffer, pH 2.2, containing 15% polyethylene glycol 400 and 0.4% thioglycol (Moore & Spackman 1958). Amino acid analysis was performed in a High-Performance Liquid Chromatograph with C18 LUNA 100 Å column (4.6 mm x 250 mm; 5.0 µm particle). The amino acids were quantified by comparison to standard amino acids. DL-2-aminobutyric acid was used as an internal standard. The contents of different amino acids were presented as g amino acid/100g of protein and compared with the FAO/WHO (2007) reference pattern for individuals aged >18 years. The essential amino acid (EAA) score was calculated as: EAA score = (g of EAA in 100 g of protein of PFLA / g of EAA in 100 g of protein in FAO/WHO standard) x 100.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) of three repetitions (n=3). One-way ANOVA statistical analysis was performed for data analysis using GraphPad Prism® version 6.01 (GraphPad Software, USA), with Tukey's post-test. A P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Thirty extracts from *S. virgata* seeds were obtained, with lectin activity evaluated between 32 and 0 HU/mL, 480 and 0 HU/gF, and 273.613 and 1.4879 HU/mgP. Among them, the extract obtained in Glycine-NaOH (100 mM; pH 9.0; NaCl 150 mM), stirred for 22h, named CE, stands out for expressing visible hemagglutination after 60 min of incubation, maintaining activity for up to 24 h. Lectin activity of CE was quantified in 32 HU/mL, 480 HU/gFa, and 4.4878 HU/mgP. Our results corroborate with Bose et al. (2019) observations on the extraction buffer enriching different functional classes of proteins in seed protein extraction processes, as not all extracts were effective in solubilizing lectins in the assay. Feyzi et al. (2015) also state that protein extraction processes in seeds are optimized by controlling pH concentration and stirring time, which may justify the detection of lectins under specific extraction conditions.

The CE was fractionated by both Osborne and Scopes methods, aiming to concentrate the proteins with lectin activity, and the results are represented in Table I. The albumin, globulin, prolamin, acid glutelin, and basic glutelin fractions did not show visible lectin activity, similar to the protein fraction 80-100% by the ammonium sulfate precipitation method. However, the other protein fractions obtained from ammonium sulfate precipitation expressed lectin activity, evaluated between 32 and 2 HU/

Table I. Determination of lectin activity in extracts and protein fractions obtained from *Sesbania virgata* seeds. (HU/mL) inverse of the title of the highest dilution that still showed visible hemagglutination; (HU/mgP) number of hemagglutinating units per milligram of protein; (HU/gF) number of hemagglutinating units per gram of flour; (nd) hemagglutinating activity not detected.

Extract and Protein Fractions	UH/mL	UH/gFa	UH/mgP
Crude extract	32	480	4.4878
Albumin fractions	nd	nd	nd
Globulin fractions	nd	nd	nd
Prolamin fractions	nd	nd	nd
Acid glutelin fractions	nd	nd	nd
Basic glutelin fractions	nd	nd	nd
Fraction 0-20% (w/v)	2	30	17.9033
Fraction 20-40% (w/v)	8	120	15.0014
Fraction 40-60% (w/v)	32	480	18.8618
Fraction 60-80% (w/v)	8	120	5.1906
Fraction 80-100% (w/v)	nd	nd	nd

mL, 480 and 30 HU/gF, and 18.8618 and 5.1906 HU/mgP. The 40-60% (w/v) salt saturation range yielded a protein fraction with lectin activity (PFLA), whose hemagglutination titers are as follows: 32 HU/mL, 480 HU/gFa and 18.862 HU/mgP. Compared to EC, PFLA hemagglutination is more stable, similar to that described by Pires et al. (2019) for the *Lonchocarpus campestris* extract, regarding the improvement of lectin stability by extract fractionation with ammonium sulfate, probably due to a better solubilization of these proteins in a specific salt concentration range (i.e., 40-60%, w/v). The total protein content of PFLA (56.36% ± 0.63) and CE (49.59% ± 0.03) was higher than those described in cowpea (27 – 31%) (Gerrano et al. 2018). The saline precipitation process also favored the concentration of soluble proteins in PFLA (1.70 mg/mL ± 0.01), at levels higher than those described for CE (0.12 mg/mL ± 0.01), and in the protein fraction 30-60% (0.72 mg/mL) from *Calotropis gigantean* seeds (Chachadi 2019).

Among the proteins solubilized in PFLA, our analyses suggest the presence of lectins. However, components capable of interfering with lectin activity can also be extracted, such as polyphenols and sugars. The methods used to quantify polyphenols and sugars suggest that PFLA is free of these contaminants. The absence of tannins is a particularly important factor. There are papers that show the tannins occurrence can give false positive results to lectin detection, as tannins are also able to bind to proteins in solution (Fish & Thompson 1991). Furthermore, PFLA does not concentrate trypsin inhibitors. Thus, ammonium sulfate fractionation not only increased the protein concentration in PFLA, but may also have favored obtaining a protein fraction free of interference. Apparently, PFLA lectins are Ca^{2+} and Mn^{2+} dependent, because when erythrocytes were treated with these ions, the hemagglutination stability of PFLA was maintained for longer, and the erythrocyte networks were visibly more stable, similar to that expected for legume lectins (Itin et al. 1996). PFLA lectin activity was inhibited by D-Mannose and D-Glucose, at all concentrations tested, exhibiting a potent inhibition at higher carbohydrate concentrations (1.0 to 0.25 mM).

SDS-PAGE analyzes (Fig. 1a, b) suggest the presence of six protein bands in PFLA. The major bands are slightly aggregated at the middle of the gel, in the range of 55 – 31 kDa, an electrophoretic pattern similar to typical oligomerization of legume lectins (Tan-Wilson & Wilson 2012). Previous studies (Biswas et al. 2009a, b) describe similar molecular masses for *Sesbania* lectins, suggesting that the major proteins of PFLA may be lectins. In addition to SDS-PAGE analysis, the amino acid composition of PFLA was determined (Table II). The method

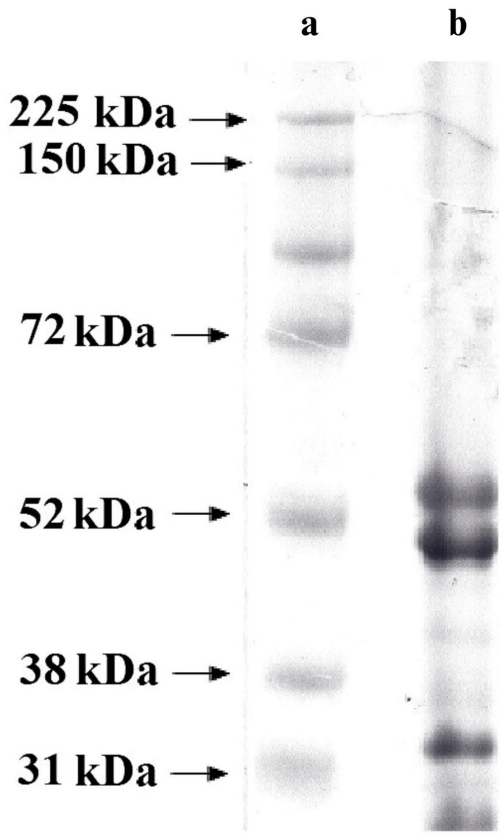

Figure 1. Electrophoretic profile (SDS-PAGE, 12.5%) of Protein Fraction with Lectin Activity (PFLA). (a) molecular weight markers, 225-12 kDa; (b) PFLA.

employed to obtain PFLA yielded an abundant protein fraction in the following essential amino acids residues: histidine (5.95 g/100g protein), leucine (2.46 g/100g protein), and lysine (2.11 g/100g protein), in addition to the sum of phenylalanine + tyrosine (2.84 g/100g protein). These amino acids represent a mass of 13.36 g/100g protein, equivalent to 63.49% of essential amino acids content of PFLA. However, methionine (0.31 g/100g protein) and cysteine (0.09 g/100g protein) are deficient in PFLA. This result was already expected, as most legume seeds (Mubarak 2005), including *Sesbania* seeds (Hossain & Becker 2001), are deficient in sulfur-containing amino acids. The most abundant non-essential amino acids from PFLA are glutamic acid (6.58 g/100g protein), arginine (4.76 g/100g protein), and aspartic acid (3.69 g/100g protein), equivalent to 70.99% of non-essential amino acids from PFLA. Compared to the FAO/WHO (2007) standards for essential amino acid values indispensable to the diet of individuals aged >18 years, histidine, threonine and phenylalanine + tyrosine scores are adequate or higher. The remaining amino acids in the chromatogram were limiting amino acids. Stødkilde et al. (2018) showed cysteine and methionine are also limiting in numerous protein fractions from

Table II. Total amino acids profile of Protein Fraction with Lectin Activity (PFLA). (1) sulphur-containing amino acids; (2) aromatic amino acids, except tryptophan; (*) Essential amino acid values indispensable to the human diet, for individuals aged >18 years, Standard FAO/WHO, 2007; (**) Essential amino acid score; (-) not applied. Results expressed in g amino acid/g protein^{-1} as mean ± standard error of the mean (SEM) (n=3). Tryptophan not determined.

Amino acids	Amino acid content	EA*	EAA**
Amino acids essentials			
Histidine	5.95 ± 0.00	1.5	396.78
Threonine	1.18 ± 0.00	2.3	51.41
Tyrosine	1.21 ± 0.00	-	-
Valine	1.50 ± 0.00	3.9	38.46
Methionine	0.31 ± 0.00	-	-
Cysteine	0.09 ± 0.00	-	-
Isoleucine	1.36 ± 0.00	3.0	45.37
Leucine	2.46 ± 0.00	5.9	41.69
Phenylalanine	1.64 ± 0.00	-	-
Lysine	2.11 ± 0.00	4.5	46.84
Methionine + Cysteine[1]	0.39	2.2	17.95
Phenylalanine + Tyrosine[2]	2.84	3.8	74.80
Non-essential amino acids			
Aspartic acid	3.69 ± 0.01	-	-
Glutamic acid	6.58 ± 0.00	-	-
Serine	1.92 ± 0.00	-	-
Glycine	1.58 ± 0.00	-	-
Arginine	4.76 ± 0.01	-	-
Alanine	1.22 ± 0.00	-	-
Proline	1.42 ± 0.00	-	-

legumes, a limitation overcome by combining protein fractions with other protein sources abundant in this limiting amino acids.

Plant seeds offer a perfect production platform for molecules of high nutritional, industrial and therapeutic value due to its notorious protein storage capacity (Jha et al. 2022, Khan et al. 2020). PFLA is an excellent source of protein to be exploited in programs to overcome protein-energy malnutrition, reinforcing previous studies on nutritional potentialities of *Sesbania* seeds in Africa, Australia and Bangladesh (Hossain & Becker 2001, Gutteridge et al. 1995, Gutteridge 1994). Furthermore, legumes are important components of the Mediterranean diet. Notably, Europe imports 70% of the plant protein consumed by the human population (Rubiales & Mikić 2015), and the results described in our research may enhance this scenario, considering that the increase in the global human population will require more alternative protein sources. Additional studies must be carried out for a complete indication of PFLA as a protein additive in human and animal food.

The antioxidant activity of PFLA was investigated by several assays, and analysis of results suggests PFLA as a promising metal chelating agent (Table III). At all investigated concentrations, PFLA exerted copper- and iron-chelating activity, with better results for copper-chelating assays. The copper chelating potential of PFLA (76.01%) is higher to those described by Carrasco-Castilla et al. (2012) for *Phaseolus vulgaris* lectin extracts (~15%), at the concentration of 100 µg/mL. In the best of our searches, no study was identified that evaluated the copper- and iron-chelating activity with protein fractions obtained from *Sesbania* genus, therefore, data comparison cannot be performed. However, Carrasco-Castilla et al. (2012) report that protein fractions from legume seeds are effective in metal chelation, especially copper-chelating, corroborating the results described in our research. These authors also suggest that negatively charged amino acids, mainly aspartic acid and glutamic acid, contribute to antioxidant activity. These amino acids are abundant in PFLA, and the protein fractions obtained by Carrasco-Castilla et al. (2012) share proteins with similar molecular weights to PFLA. Torres-Fuentes et al. (2012) and Gallegos-Tintoré et al. (2011) also suggest that the metal chelating activity is due to proteins and amino acids present in plant protein fractions obtained. So far, there is no cure for patients with diseases involving copper and iron overload; thus, it is necessary to control their levels by administration of compounds with chelating properties (Berger et al. 2019, Chen et al. 2019). Currently, great attention has been given to bioactive compounds from plants, and international agencies have encouraged the development and use of botanical formulations with antioxidant properties (Guo et al. 2020). These discussions strengthen the biological importance of PFLA as an antioxidant agent, especially for its metal chelating properties, whose promising activity can be attributed to its protein compounds (lectins and amino acids).

Hydroxyl-scavenging activity of PFLA is only observed at the concentration of 500 µg/mL (12.06%), and with decreasing concentrations of PFLA, activity is not observed (Table III). In

Table III. Antioxidant activity of Protein Fraction with Lectin Activity (PFLA). Results expressed in percentage (%) as mean ± standard error of the mean (SEM) (n=3).

PFLA (µg/mL)	Copper-chelating activity (%)	Iron-chelating activity (%)	Hydroxyl radical-scavenging activity (%)
500	73.16 ± 0.02	7.70 ± 0.05	12.06 ± 0.21
100	76.01 ± 0.07	15.78 ± 0.18	0.00 ± 0.07
10	76.67 ± 0.01	25.57 ± 0.06	0.00 ± 0.70
1	19.16 ± 0.03	28.69 ± 0.03	0.00 ± 0.64

the investigated range of concentrations, PFLA was not able to exert antioxidant activity by reducing power assay. Botanical formulations from other *Sesbania* species have already been characterized for hydroxyl-scavenging and reducing power assays (Siddhuraju et al. 2014, Shyamala & Vasantha 2010), and the studies suggest that the phenolic compounds were responsible for the biological activity. This fact would justify the absence of antioxidant activity for these assays, since phenolic compounds were not identified in PFLA.

The antioxidant effects of PFLA were investigated in models that mimic its behavior on cell metabolism. Under experimental conditions, PFLA was not able to regenerate the metabolic state of cells exposed to oxidative stress by H_2O_2, compared to the positive control (45% cell viability). Analyzes of the results suggest that the antioxidant mode of action of PFLA may be associated with the recruitment of essential molecules to prevent the impairment of the metabolic activity of cells exposed to oxidative stress. Because the regulation of the expression of genes that encode proteins involved in the inactivation of reactive species has not been followed, the exact mechanism underlying these events needs to be determined in further studies.

In addition to antioxidant activity, PFLA also exhibits antifungal activity, inhibiting the growth of *C. albicans* (MIC = 256 µg/mL), *C. tropicalis* (MIC = 256 µg/mL), *A. flavus* (MIC = 512 µg/mL), and *P. citrinum* (MIC = 512 µg/mL) (Table IV), which represents an optimal activity, according to Sartoratto et al. (2004) and Houghton et al. (2007) criteria. Previous studies (Ghosh 2009), including other *Sesbania* species (Ajitha et al. 2016, Maregesi et al. 2008), report the antifungal activity of proteins with lectin activity obtained from plants, with molecular masses similar to PFLA, showing antifungal activity against the pathogens investigated in this research. Praxedes et al. (2011) report the occurrence and concentration of antifungal proteins in a fraction with molecular mass similar to PFLA, obtained from *S. virgata* seeds. Other antifungal protein fractions are reported whose antifungal activity was attributed to its lectins (Silva et al. 2019, Gautam et al. 2018, Gupta et al. 2018). These results suggest that proteins with lectin activity from PFLA promoted the antifungal activity.

Table IV. Antifungal activity of Protein Fraction with Lectin Activity (PFLA). (MIC) minimum inhibitory concentration (expressed in µg/mL); (+) fungal growth occurred; (-) did not occur fungal growth; (-----) not applied.

	Microorganisms								
	Yeasts				**Filamentous fungi**				
PFLA MIC and controls	*C. albicans* (ATCC-76645)	*C. albicans* (LM-122)	*C. tropicalis* (ATCC-13803)	*C. tropicalis* (LM-64)	*C. tropicalis* (LM-7)	*A. flavus* (LM-714)	*A. flavus* (LM-247)	*P. citrinum* (LM-9)	*P. citrinum* (LM-60)
PFLA MIC (µg/mL)	256	256	256	256	256	512	512	512	512
Means control	-	-	-	-	-	-	-	-	-
Fungal control	+	+	+	+	+	+	+	+	+
Nystatin (100 µg/mL)	-	-	-	-	-	-----	-----	-----	-----
Fluconazole (50 µg/mL)	---	---	---	---	---	+	+	+	+

Fungal infections are very recurrent in hospitalized patients, resulting in 1.7 million deaths per year (Houšť et al. 2020), in addition to compromising several sectors of industry and agriculture (Fidel et al. 2020, Jiang & Xiang 2020, Toghueo & Boyom 2020). In particular, human mortality rates from *Candida* infections are found to be around 45%; the reasons involve inefficient diagnostic techniques and antifungal

drug resistance (Dahiya et al. 2022). Thus, there is an urgent need to offer adjuvant options to available therapies. The antifungal activity of PFLA against *Candida* strains (Table IV) raises interest for future studies on the synergism of PFLA and the synthetic antifungals, at even lower pharmacological doses. Recent studies (Ghaly et al. 2020) reinforce the relevance and efficacy of this practice, highlighting the importance of using natural formulations, alone or with synthetic antifungals, in the management of fungal infections. According to Cos et al. (2006) criteria, the antifungal activity of PFLA is not completely relevant, because it is greater than 100 µg/mL. However, Gertsch (2009) argues that ethnopharmacological research should enable new insights on plant pharmacology, aiming at obtaining new bioactive chemical entities and/or the development of botanical drugs.

At tested concentrations (1024 – 32 µg/mL), PFLA did not exhibit antibacterial activity against *B. subtillis*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*, similar to other studies that investigated the antibacterial activity of *Sesbania* formulations (Ajitha et al. 2016, Srinivasan et al. 2001, Valsaraj et al. 1997). In addition, these studies also emphasize that *Sesbania*-based formulations were unable to inhibit the growth of *Salmonella enterica*, *Salmonella paratyphi* and *Enterococcus faecalis*. Although some studies indicate antibacterial activity for *Sesbania* species (Guzman et al. 2018, Anantaworasakul et al. 2017, Maregesi et al. 2008), the antibacterial activity of the obtained formulations only occurs at concentrations higher than those investigated in this research (MIC > 100 mg/mL). In addition, the previously mentioned studies suggest that the antibacterial action in *Sesbania* is due to its phenolic compounds. As PFLA is free of phenolic compounds, we believe that lectins and other bioactive components expressed in PFLA may not have been sufficiently solubilized, at the tested concentrations, to exert antibacterial activity against the investigated bacteria.

An extremely important aspect in evaluating the safety and toxicity of plant formulations is their impact in cell metabolism. In this study, the cytotoxic effects of PFLA (Fig. 2) were evaluated in two cell lines (murine fibroblast and hepatocellular carcinoma cells, 3T3 and

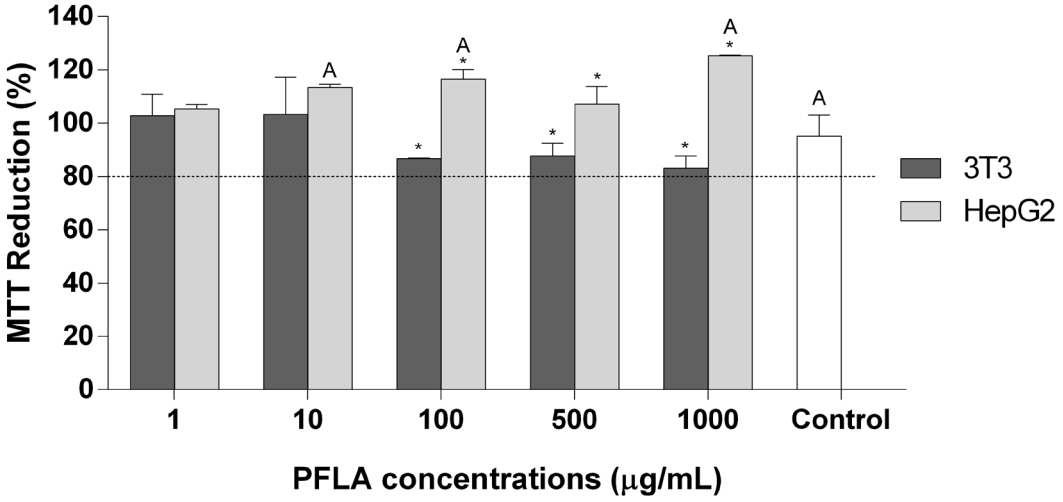

Figure 2. Cytotoxicity of Protein Fraction with Lectin Activity (PFLA) against 3T3 and HepG2 cells. Concentration of PFLA: 1 to 1000 µg/mL. On the x-axis, there are the PFLA concentrations (in µg/mL) and assay control, and on the y-axis, the percentage of cell proliferation. (*) statistical differences between the same concentrations; (A) statistical difference with assay control. Results expressed as mean ± standard error of the mean (SEM) (n=3).

HepG2, respectively) by the MTT assay, a simple and rapid colorimetric assay (Alley et al. 1988). Compared to the positive assay control (100% cell viability), no statistical significance (P = 0.0311) was found between all investigated PFLA concentrations, indicating a tendency of low interference in the proliferation of 3T3 and HepG2 cells. This finding is interesting as PFLA did not compromise the metabolic activity of these cell lines in the range of concentrations with biological activity, and suggest the cytotoxic safety of PFLA.

CONCLUSIONS

In previous studies, we showed that *S. virgata* seeds are promising sources of proteins. In this study, these proteins were concentrated in a safe protein fraction, rich in lectins, with antioxidant and antifungal activities. These findings support the notion that plant formulations are promising sources of biomolecules to be applied in current policies on the management of metal ion chelation and fungal infections. The implementation of natural plant formulations, such as PFLA, in public health and environment policies is already a viable possibility. Further studies on the toxic effects of PFLA are underway to expand understanding of the PFLA safety as a potential therapeutic adjuvant.

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REFERENCES

AHNEN RT, JONNALAGADDA SS & SLAVIN JL. 2019. Role of plant protein in nutrition, wellness, and health. Nutr Rev 77: 735-747.

AJITHA B, REDDY YAK, RAJESH KM & REDDY PS. 2016. *Sesbania grandiflora* leaf extract assisted green synthesis of silver nanoparticles: antimicrobial activity. Mater Today: Proceedings 3: 1977-1984.

ALLEY MC, SCUDIERO DA, MONKS A, HURSEY ML, CZERWINSKI MJ, FINE DL, ABBOTT BJ, MAYO JG, SHOEMAKER RH & BOYD MR. 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48: 589-601.

ANANTAWORASAKUL P, HAMAMOTO H, SEKIMIZU K & OKONOGI S. 2017. *In vitro* antibacterial activity and in vivo therapeutic effect of *Sesbania grandiflora* in bacterial infected silkworms. Pharm Biol 55: 1256-1262.

ANTON A. 1960. Colorimetric estimation of aluminum with pyrocatechol violet. Anal Chem 32: 725-726.

AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 2000. Official Methods Analysis of AOAC International. Washington, DC: Association of Official Analytical Chemists.

ARNASON JT, MATA R & ROMEO JT. 1995. Phytochemistry of Medicinal Plants. New York: Plenum Press, 364 p.

BERGER MM, PANTET O, SCHNEIDER A & BEN-HAMOUDA N. 2019. Micronutrient deficiencies in medical and surgical inpatients. J Clin Med 8: 1-17.

BISWAS S, AGRAWAL P, SAROHA A & DAS HR. 2009a. Purification and mass spectrometric characterization of *Sesbania aculeata* (Dhaincha) stem lectin. Protein J 28: 391-399.

BISWAS S, SAROHA A & DAS HR. 2009b. A lectin from *Sesbania aculeata* (Dhaincha) roots and its possible function. Biochemistry 74: 329-335.

BOSE U, BROADBENT JA, BYRNE K, HASAN S, HOWITT CA & COLGRAVE ML. 2019. Optimization of protein extraction for in-depth profiling of the cereal grain proteome. J Proteome Res 197: 23-33.

BRADFORD MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Chem 72: 248-254.

CARRASCO-CASTILLA J, HERNÁNDEZ-ÁLVAREZ AJ, JIMÉNEZ-MARTÍNEZ C, JACINTO-HERNÁNDEZ C, ALAIZ M, GIRÓN-CALLE J, VIOQUE J & DÁVILA-ORTIZ G. 2012. Antioxidant and metal chelating activities of peptide fractions from phaseolin and bean protein hydrolysates. Food Chem 135: 1789-1795.

CHACHADI VB. 2019. Isolation of blood group non-specific lectin from *Calotropis gigantean* seeds. Jordan J Biol Sci 12: 141-145.

CHANDRA S, SAKLANI S, KUMAR P, KIM B & COUTINHO HDM. 2022. Nutraceuticals: pharmacologically active potent dietary supplements. Biomed Res Int 2022: 1-10.

CHEN B, WEN X, JIANG H, WANG J, SONG N & XIE J. 2019. Interactions between iron and α-synuclein pathology in Parkinson's disease. Free Radic Biol Med 141: 253-260.

CLEELAND R & SQUIRES E. 1991. Evaluation of new antimicrobials "*in vitro*" and in experimental animal infections. In: LORIAN VMD (Ed), Antibiotics in Laboratory Medicine. Philadelphia: Lippincott Williams & Wilkins, p. 739-788.

CLSI – CLINICAL LABORATORY STANDARDS INSTITUTE. 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A3. Wayne, PA: Clinical Laboratory Standards Institute, 13 p.

CLSI – CLINICAL LABORATORY STANDARDS INSTITUTE. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A10. Wayne, PA: Clinical Laboratory Standards Institute, 13 p.

COS P, VLIETINCK AJ, BERGHE DV & MAES L. 2006. Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol 106: 290-302.

DAHIYA S, SHARMA N, PUNIA A, CHOUDHARY P, GULIA P, PARMAR VS & CHHILLAR AK. 2022. Antimycotic drugs and their mechanisms of resistance to *Candida* species. Curr Drug Targets 23: 116-125.

DASGUPTA N & DE B. 2007. Antioxidant activity of some leafy vegetables of India: a comparative study. Food Chem 101: 471-474.

DEBRAY H, DECOUT D, STRECKER G, SPIK G & MONTREUIL J. 1981. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. Eur J Org Chem 117: 41-55.

DINIS TCP, MADERIA VM & ALMEIDA LM. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. Arch Biochem Biophys 315: 161-169.

DUBOIS M, GILLES KA, HAMILTON JK, REBERS PA & SMITH F. 1956. Colorimetric method for determination of sugars, and related substances. Anal Chem 28: 350-356.

DUTRA FV, CARDOSO AD, BLESA TSF, SÃO JOSÉ AR & MELO TL. 2019. Biometric parameters of fruits and seeds of *Sesbania virgata* (cav.) Pers. Sci Electron Arch 12: 51-56.

ELOFF JN. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64: 711-713.

EVANS DO & ROTAR PP. 2020. *Sesbania* In Agriculture. Flórida: CRC Press, 206 p.

FAO/WHO – FOOD AND AGRICULTURE ORGANIZATION & WORLD HEALTH ORGANIZATION. 2007. Protein and amino acid requirements in human nutrition, Technical Report Series N. 935, Geneva, FL: World Health Organization, 284 p.

FEYZI S, VARIDI M, ZARE F & VARIDI MJ. 2015. Fenugreek (*Trigonella foenum graecum*) seed protein isolate: extraction optimization, amino acid composition, thermo and functional properties. J Sci Food Agric 95: 3165-3176.

FIDEL PL, YANO J, ESHER SK & NOVERR MC. 2020. Applying the host-microbe damage response framework to *Candida* pathogenesis: current and prospective strategies to reduce damage. J Fungi 6: 1-26.

FISH BC & THOMPSON LU. 1991. Lectin-tannin interactions and their influence on pancreatic amylase activity and starch digestibility. J Agric Food Chem 39: 727-731.

GALLEGOS-TINTORÉ S, TORRES-FUENTES C, MARTÍNEZ-AYALA AL, SOLORZA-FERIA J, ALAIZ M, GIRÓN-CALLE J & VIOQUE J. 2011. Antioxidant and chelating activity of *Jatropha curcas* L. protein hydrolysates. J Sci Food Agric 91: 1618-1624.

GAUTAM AK, GUPTA N, NARVEKAR DT, BHADKARIYA R & BHAGYAWANT SS. 2018. Characterization of chickpea (*Cicer arietinum* L.) lectin for biological activity. Physiol Mol Biol Plants 24: 389-397.

GERRANO AS, JANSEN VAN RENSBURG WS, VENTER SL, SHARGIE NG, AMELEWORK BA, SHIMELIS HA & LABUSCHAGNE MT. 2018. Selection of cowpea genotypes based on grain mineral and total protein content. Acta Agric Scand 69: 155-166.

GERTSCH J. 2009. How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. J Ethnopharmacol 122: 177-183.

GHALY MF, SHAHEEN AA, BOUHY AM & BENDARY MM. 2020. Alternative therapy to manage otitis media caused by multidrug-resistant fungi. Arch Microbiol 202: 1231-1240.

GHOSH M. 2009. Purification of a lectin-like antifungal protein from the medicinal herb, *Withania somnifera*. Fitoterapia 80: 91-95.

GRDEŃ P & JAKUBCZYK A. 2023. Health benefits of legume seeds. J Sci Food Agric 103: 5213-5220.

GUO Q, LI F, DUAN Y, WEN C, WANG W, ZHANG L, HUANG R & YIN Y. 2020. Oxidative stress, nutritional antioxidants and beyond. Sci China Life Sci 63: 866-874.

GUPTA N, GAUTAM AK & BHAGYAWANT SS. 2018. Biochemical characterization of lectin from wild chickpea (*Cicer reticulatum* L.) with potential inhibitory action against human cancer cells. J Food Biochem 43: 1-10.

GUTTERIDGE RC. 1994. The perennial *Sesbania* species. *In*: GUTTERIDGE RC & SHELTON HM (Eds), Forage tree legumes in tropical agriculture. Queensland: CAB International, p. 49-64.

GUTTERIDGE RC, SHELTON HM & ORAM RN. 1995. Register of Australian herbage plant cultivars. B. Legumes. 24. *Sesban* (A) *Sesbania sesban* (L.) Merril (*Sesban*) Cv. Mount Cotton Aust J Exp Agric 35: 561-561.

GUZMAN JPMD, CORTES AD, NERI KD, CORTEZ CE & DE LAS ALAS TPL. 2018. Antibacterial and antibiofilm activities of *Sesbania grandiflora* against foodborne pathogen *Vibrio cholerae*. J Appl Pharm Sci 8: 67-71.

HADACEK F & GREGER H. 2000. Testing of antifungal natural products: methodologies, comparatibility of results and assay choice. Phytochem Anal 11: 137-147.

HASAN N, OSMAN H, MOHAMAD S, CHONG WK, AWANG K & ZAHARILUDDIN ASM. 2012. The chemical components of *Sesbania grandiflora* root and their antituberculosis activity. Pharmaceuticals 5: 882-889.

HOSSAIN MA & BECKER K. 2001. Nutritive value and antinutritional factors in different varieties of *Sesbania* seeds and their morphological fractions. Food Chem 73: 421-431.

HOSSAIN MA, FOCKEN U & BECKER K. 2001. Evaluation of an unconventional legume seed, *Sesbania aculeata*, as a dietary protein source for common carp, *Cyprinus carpio* L. Aquaculture 198: 129-140.

HOUGHTON PJ, HOWES MJ, LEE CC & STEVENTON G. 2007. Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. J Ethnopharmacol 110: 391-400.

HOUŠŤ J, SPÍŽEK J & HAVLÍČEK V. 2020. Antifungal Drugs. Metabolites 10: 1-16.

ITIN C, ROCHE AC, MONSIGNY M & HAURI HP. 1996. ERGIC-53 is a functional mannose-selective and calcium-dependent human homologue of leguminous lectins. Mol Biol Cell 7: 483-493.

JHA UC, NAYYAR H, PARIDA SK, DESHMUKH R, VON WETTBERG EJB & SIDDIQUE KHM. 2022. Ensuring global food security by improving protein content in major grain legumes using breeding and 'omics' tools. Int J Mol Sci 23: 1-27.

JIANG N & XIANG L. 2020. Allergic bronchopulmonary aspergillosis misdiagnosed as recurrent pneumonia. Asia Pac Allergy 10: 1-6.

KASTURE VS, DESHMUKH VK & CHOPDE CT. 2002. Anxiolytic and anticonvulsive activity of *Sesbania grandiflora* leaves in experimental animals. Phytother Res 16: 455-460.

KHAN MS, JOYIA FA & MUSTAFA G. 2020. Seeds as economical production platform for recombinant proteins. Protein Pept Lett 27: 89-104.

LAEMMLI UK. 1970. Cleavage of structural proteins during assembly of head of bacteriophage-T4. Nature 227: 659-680.

LAGARDA-DIAZ I, GUZMAN-PARTIDA AM & VAZQUEZ-MORENO L. 2017. Legume Lectins: Proteins with Diverse Applications. Int J Mol Sci 18: 1-18.

LIU H & TIMKO MP. 2022. Improving protein quantity and quality-the next level of plant molecular farming. Int J Mol Sci 23: 1-35.

LORIS R, HAMELRYCK T, BOUCKAERT J & WYNS L. 1998. Legume lectin structure. Biochim Biophys Acta 1383: 9-36.

MAREGESI SM, PIETERS L, NGASSAPA OD, APERS S, VINGERHOETS R, COS P, BERGHE DA & VLIETINCK AJ. 2008. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. J Ethnopharmacol 119: 58-66.

MOHAMMED SG & QORONFLEH MW. 2020. Seeds. In: ESSA MM & QORONFLEH MV (Eds), Personalized Food Intervention and Therapy for Autism Spectrum Disorder Management. Denmark: Springer, p. 421-468.

MOORE S, SPACKMAN DH & STEIN WH. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Fed Proc 17: 1107-1115.

MOSMANN T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

MUBARAK AE. 2005. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chem 89: 489-495.

OHANENYE IC, EKEZIE FC, SARTESHNIZI RA, BOACHIE RT, EMENIKE CU, SUN X, NWACHUKWU ID & UDENIGWE CC. 2022. Legume Seed Protein Digestibility as Influenced by Traditional and Emerging Physical Processing Technologies. Foods 11: 1-21.

OSBORNE TB. 1924. The Vegetable Proteins. (Monographs on Biochemistry) 2 ed. London: Longmans, Green and Co., 154 p.

OUYANG JN, YAO XQ, TAN J & WANG FX. 2011. Renal epithelial cell injury and its promoting role in formation of calcium oxalate monohydrate. J Biol Inorg Chem 16: 405-416.

PIRES AF ET AL. 2019. Lectin purified from *Lonchocarpus campestris* seeds inhibits inflammatory nociception. Int J Biol Macromol 125: 53-60.

PRAXEDES PG, ZERLIN JK, DIAS LO & PESSONI RAB. 2011. A novel antifungal protein from seeds of *Sesbania virgata* (Cav.) Pers. (*Leguminosae-Faboideae*). Braz J Biol 71: 687-692.

PURI V, NAGPAL M, SINGH I, SINGH M, DHINGRA GA, HUANBUTTA K, DHEER D, SHARMA A & SANGNIM T. 2022. A Comprehensive Review on Nutraceuticals: therapy support and formulation challenges. Nutrients 14: 1-22.

RAMOS MV, MOREIRA RA, CAVADA BS, OLIVEIRA JTA & ROUGE P. 1996. Interaction of lectins from the sub tribe Diocleinae with specific ligands. R Bras Fisiol Veg 8: 193-199.

RUBIALES D & MIKIĆ A. 2015. Introduction: legumes in sustainable agriculture. Crit Rev Plant Sci 34: 2-3.

SÁ GCS ET AL. 2023. *Tephrosia toxicaria* (Sw.) Pers. extracts: Screening by examining aedicidal action under laboratory and field conditions along with its antioxidant, antileishmanial, and antimicrobial activities. PLoS ONE 18: e0275835.

SÁ GCS, BEZERRA PVV, SILVA MFA, SILVA LB, BARRA PB & UCHÔA AF. 2022. Arbovirus vectors insects: are botanical insecticides an alternative for its management? J Pest Sci 95: 1-20.

SÁ GCS, SILVA MAT, FIGUEREDO SILVA D, SANTI-GADELHA T, FRAGOSO SP & MADRUGA MS, PACHECO MTB, LIMA EO, UCHÔA AF & GADELHA CAA. 2021. Nutritional composition and biological activities (antioxidant and antifungal) of *Sesbania virgata* (Cav.) Pers. seeds. Rev Bras Tec Agroind 15: 3648-3672.

SARTORATTO A, MACHADO ALM, DELARMELINA C, FIGUEIRA GM, DUARTE MCT & REHDER VLG. 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol 35: 275-280.

SCOPES RK. 1994. Protein purification: Principles and practice. 3 ed. New York: Springer Verlag, 380 p.

SHYAMALA GOWRI S & VASANTHA K. 2010. Antioxidant activity of *Sesbania grandiflora* (pink variety) L. Pers. Int J Eng Sci Technol 2: 4350-4356.

SIDDHURAJU P, ABIRAMI A, NAGARANI G & SANGEETHAPRIYA M. 2014. Antioxidant capacity and total phenolic content of aqueous acetone and ethanol extract of edible parts of *Moringa oleifera* and *Sesbania grandiflora*. Inter J Biol Biomol Agri Food Biotech Eng 8: 1090-1098.

SIDDHURAJU P, VIJAYAKUMARI K & JANARDHANAN K. 1995. Studies on the underexploited legumes, *Indigofera linifolia* and *Sesbania bispinosa*: Nutrient composition and antinutritional factors. Int J Food Sci Nutr 46: 195-203.

SILVA HDF ET AL. 2019. *Portulaca elatior* root contains a trehalose-binding lectin with antibacterial and antifungal activities. Int J Biol Macromol 126: 291-297.

SINGLETON VL, ORTHOFER R & LAMUELA-RAVENTÓS RM. 1998. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol 299: 152-178.

SRINIVASAN D, NATHAN S, SURESH T & LAKSHMANA PP. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol 74: 217-220.

SRINIVAS VR, REDDY GB, AHMAD N, SWAMINATHAN CP, MITRA N & SUROLIA A. 2001. Legume lectin family, the 'natural mutants of the quaternary state', provide insights into the relationship between protein stability and oligomerization. Biochim Biophys Acta 1527: 102-111.

STØDKILDE L, DAMBORG VK, JØRGENSEN H, LÆRKE HN & JENSEN SK. 2018. White clover fractions as protein source for monogastric: dry matter digestibility and protein digestibility-corrected amino acid scores. J Sci Food Agric 98: 2557-2563.

SULTANA M, SHAKIL AHMED FR & ALAM MT. 2019. Identification of lectins from the seeds of Bangladeshi plants *Sesbania bispinosa* and *Senna occidentalis* by hemagglutination assay. Asian J Green Chem 3: 518-524.

TAN-WILSON AL & WILSON KA. 2012. Mobilization of seed protein reserves. Physiol Plant 145: 140-153.

TEIXEIRA FP, FARIA JMR, PEREIRA WVS & JOSÉ AC. 2018. Maturation and desiccation tolerance in seeds of *Sesbania virgata* (Cav.) Pers. Floresta e Ambient 25: 1-9.

TOGHUEO RMK & BOYOM FF. 2020. Endophytic *Penicillium* species and their agricultural, biotechnological, and pharmaceutical applications. 3 Biotech 10: 1-35.

TORRES-FUENTES C, ALAIZ M & VIOQUE J. 2012. Iron-chelating activity of chickpea protein hydrolysate peptides. Food Chem 134: 1585-1588.

VALSARAJ R, PUSHPANGADAN P, SMITT UW, ADSERSEN A & NYMAN U. 1997. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol 58: 75-83.

VAN DAMME EJM. 2022. 35 years in plant lectin research: a journey from basic science to applications in agriculture and medicine. Glycoconj J 39: 83-97.

XAVIER-FILHO J, CAMPOS FAP, ARY MB, SILVA CP, CARVALHO MMM, MACEDO MLR, LEMOS FJA & GRANT G. 1989. Poor

correlation between the levels of proteinase inhibitors found in seeds of different cultivars of cowpea (*Vigna unguiculata*) and the resistance susceptibility to predation by *Callosobruchus maculatus*. J Agric Food Chem 37: 1139-1143.

YEN G-C & CHEN H-Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 43: 27-32.

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