

Nutritional composition and biological activities (antioxidant and antifungal) of *Sesbania virgata* (Cav.) Pers. seeds

ABSTRACT

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Sesbania virgata (Cav.) Pers. is a Pantropical legume (Fabaceae) able to colonize riverbanks in Brazilian semiarid regions, commonly used to recover degraded soils. Although its seeds have high nutritional value, are little explored for biotechnological and biological applications. In order to begin to fill the void existing in this theme, the present research described the nutritional composition of the *S. virgata* seeds, in addition to its antioxidant and antimicrobial activities. The energy value of *S. virgata* seeds was 366.6 kcal 100g⁻¹, and among the investigated macronutrients, the protein content stands out (60.8%). However, the carbohydrate and crude fat contents are also promising, highlighting the abundance in polyunsaturated fatty acids, especially linoleic acid and linolenic acid. *S. virgata* seeds are an excellent source of essential (leucine, lysine and valine) and non-essential amino acids (glutamic acid, aspartic acid and arginine). Under the assay conditions, lectins and trypsin inhibitors were not demarcated. Additionally, *S. virgata* seeds confer antifungal activity against *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus* and *Penicillium citrinum*, and antioxidant activity, for ABTS^{••} and DPPH[•] scavenging methods. In view of these findings, the nutritional composition of *S. virgata* seeds encourages its use as natural source of functional products, and its biological activities stimulate its biotechnological and pharmacological application as exogenous antifungal and antioxidant agent.

KEYWORDS: *Sesbania virgata*. Proximate Composition. Antifungal Activity. Antioxidant Activity. Biological Relevance.

INTRODUCTION

For many years, animal-derived nutrients, especially proteins, have been used as functional ingredients in food products. However, this practice is environmentally unsustainable due to the high consumption of natural resources, mainly water, associated with intensive livestock (BURGOS-DÍAZ et al., 2020). Thus, new strategies to provide food sustenance are needed, highlighting legume seeds are used as effective substitutes for animal protein (RUIZ-LÓPEZ et al., 2019). In addition, the progression of diseases caused by oxidative and microbial infections associated with the absence of completely effective treatments encourages the development of research involving the biological applications of legume seeds.

The Fabaceae family (aka Leguminosae) is a widely distributed, economically important group of crops and a staple human food. In the Caatinga, the Brazilian Savanna, Fabaceae is family most diverse (MOHAMMED and QORONFLEH, 2020). Among the numerous legume species described, *Sesbania virgata* (Cav.) Pers., a fast-growing shrub popularly known as “saranzinho”, “mãe-josé” and “feijãozinho”, stands out for colonizing riparian forests and riverbanks from Brazil, Argentina, Uruguay and Paraguay (MIGNONI et al., 2018).

For many years *S. virgata* is used as green manure specie to improve production of food crops and recover degraded areas (EVANS and ROTAR, 2020). It is also used as a protein supplement for ruminants, in the tropical regions of Africa and Australia (GUTTERIDGE, 1995), and are edible plants in Argentina, Bangladesh and India (HOSSAIN and BECKER, 2001). However, other uses for *S. virgata* have not been identified, and to the best of our knowledge, the nutritional and biotechnological potentials of its seeds have been little investigated yet. Therefore, in order to start filling the existing void about the biological applications of *S. virgata* seeds, this research focusing to investigate on its nutritional composition, and antioxidant and antimicrobial activities.

MATERIAL AND METHODS

EQUIPMENT, STANDARDS AND REAGENTS

Analog Pachymeter 150 Mm (Professional (Pro), Western), Milli-Q system (Millipore®, USA), Centrifuge model 5430 R (Eppendorf, Germany), UV-Vis

Spectrophotometer model UV-1800 (Shimadzu Corp., Japan) and Spectrophotometer Leitz-Photometer 340-800 (Ernst Leitz, Germany). The Sigma-Aldrich® (USA) reagents were: bovine trypsin, DL-BApNA (DL-benzoyl-arginine-p-nitroanilide), bovine serum albumin (BSA), Coomassie Brilliant Blue (G-250 and R-250), polyethylene glycol 400, sodium dodecyl sulfate (SDS), thioglycol, DL-2-aminobutyric acid, ABTS** (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid), Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH* (2,2-diphenyl-1-picrylhydrazyl), RPMI-1640 medium, nystatin, fluconazole and chloramphenicol. Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) and Broth Brain Heart Infusion (BHI) were acquired from Difco Laboratories (France). Resazurin dye and molecular mass markers (225 – 12 kDa) were acquired from INLAB (Brazil) and GE Healthcare (Amersham™, USA), respectively. All reagents were of analytical grade.

BIOLOGICAL MATERIALS AND SAMPLE HANDLING/PREPARATION

S. virgata was collected in João Pessoa-PB, Brazil (7°09'51.8"S 34°54'01.1"W) and deposited in Professor Lauro Pires Xavier Herbarium (JPB), João Pessoa-PB, Brazil (JPB n. 63198). The material collection occurred under authorization of SisGen (Brazilian National Management System Genetic Heritage and Associated Traditional Knowledge, loose translation) – SisGen n. A1C2041. *S. virgata* seeds were dried in an environment with air circulation (± 27 °C) and pulverized in an electric mill. The obtained seed meal was homogenized (100 mg) in distilled water (1.0 mL), stirred in vortex-type tube shaker at room temperature and centrifuged at 5000 x g, for 5 minutes. The sediment was discarded and the supernatant, named ASSv, was used in further analyses.

Staphylococcus aureus (ATCC-13150 and LM-117), *Staphylococcus epidermidis* (ATCC-12228), *Pseudomonas aeruginosa* (ATCC-25853 and P-03), *Bacillus subtilis* (ATCC- 6633), *Escherichia coli* (ATCC-10436 and EC-12), *Candida albicans* (ATCC-76645 and LM-122), *Candida tropicalis* (ATCC-13803, LM-64 and LM-7), *Aspergillus flavus* (LM-714 and LM-247) and *Penicillium citrinum* (LM-9 and LM-60) were donated by Dr. Edeltrudes de Oliveira Lima, from Universidade Federal da Paraíba, João Pessoa-PB, Brazil.

MORPHO-PHYSICO-CHEMICAL ANALYSES

Density, hydration (HC) and absorption (AC) capacities, and hydration index (HI) of seeds were determined according to Chavan et al. (1999). The water absorption capacity of seeds (WAC) was determined according to Plhak et al. (1989). Water absorption indexes by seeds (IWAs) and seed flour (IWAf), and seed flour solubility index in water (IFS) were determined according to Okezie; Bello (1988). The morphological standards (length/width) and the form and flattening seeds (thickness/width) were determined according to Silva et al. (2016). The proportions of seed coat + endosperm and cotyledons in the seed were determined by reference to the weight of 100 seeds.

PROXIMATE COMPOSITION AND ENERGY VALUE

The moisture, ash, crude fat and crude proteins contents from *S. virgata* seeds were determined according to AOAC (2000). Seed flour was dried at 105 °C for 24 hours, with subsequent rest and additional heat treatment. At the end, the moisture content (%) was recorded. Seed flour was placed in a Muffle, 550 °C, until ash content was obtained, using this equation: % Ash = $[(W3-W1) / W2] \times 100\%$, where W1 = weight of oven-dried empty crucible, W2 = weight of seed flour, and W3 = weight of ash and crucible. Crude fat analysis was done according to Soxhlet method. The percentage of crude fat content was calculated using the equation: % Crude fat = $[(\text{Weight before extraction} - \text{Weight after extraction}) / (\text{Seed flour weight})] \times 100\%$. Analysis of protein was done by the Kjeldahl method and the results were obtained using a conversion factor of 6.25. Digestible carbohydrates content was calculated by difference, using this equation: % Carbohydrates = $100\% - [(\text{Moisture} (\%) + \text{Ash} (\%) + \text{Crude fat} (\%) + \text{Crude protein} (\%))]$. The energy value was calculated according this equation: Energy value (Kcal 100g⁻¹) = $[(\text{Crude fat} (\%) \times 9 \text{ Kcal}) + (\text{Crude protein} (\%) \times 4 \text{ Kcal}) + (\text{Carbohydrates} (\%) \times 4 \text{ Kcal})]$.

FATTY ACIDS ANALYSIS

The lipid profile and fatty acids methylation were obtained by Folch et al. (1957) and determined according to Hartman; Lago (1973). The identification and quantification of fatty acid esters was carried out by gas chromatography (Varied 430-GC, USA), coupled with flame ionization detector, fused silica capillary column

(SPTM-2560, SUPELCO, USA) with dimensions of 100 m x 0.25 mm and 0.20 µm film thickness. Helium was used as drag gas (flow rate of 1.0 mL/min). The chromatograms were registered in software type Galaxie Chromatography Data System. The fatty acids were identified by comparison of the retention times of the methyl esters from seeds with standards Supelco Kit ME19 (Fatty Acid Methyl esters C4-C24).

TOTAL AMINO ACID PROFILE

Total amino acids were determined by acid hydrolysis in aqueous solution of 6N hydrochloric acid double distilled, at 104 °C, containing 0.1% phenol (w/v). After drying and concentration of the hydrolyzed material, it was suspended in 170 mM sodium citrate buffer, pH 2.2, containing 15.0% polyethylene glycol 400 and 0.4% thioglycol (MOORE et al., 1958). Amino acid analysis was performed in a 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). The amino acids were quantified by comparison to standard (Thermo Scientific, USA). DL-2-aminobutyric acid was used as an internal standard. The contents of different amino acids are presented as g amino acid per 100g of protein and compared with the FAO/WHO (2007) reference for individuals aged >18 years. The essential amino acid (EAA) score was calculated as: $EAA\ score = (g\ of\ EAA\ in\ 100\ g\ of\ protein\ of\ S.\ virgata\ seed / g\ of\ EAA\ in\ 100\ g\ of\ protein\ in\ FAO/WHO\ standard) \times 100$.

SOLUBLE PROTEIN CONTENT AND SDS-PAGE

Total soluble protein content was measured using BSA as standard and Coomassie Brilliant Blue G-250 as chromogenic reagent (BRADFORD, 1976). The estimation of the relative molecular weight of the proteins was conducted by electrophoresis (SDS-PAGE) in the presence of 1.0% SDS and β-mercaptoethanol (LAEMMLI, 1970). The application gel was prepared in the concentration of 3.5% and the separation gel, 12.5%. The gel was fixed in 12.5% trichloroacetic acid and stained with 0.005% Coomassie Brilliant Blue R-250. The weight estimation was obtained by comparison to the relative electrophoretic mobility of the molecular weight standard.

ANTINUTRITIONAL COMPOUNDS

Lectins were detected by hemagglutination assays (DEBRAY et al., 1981), using *Oryctolagus cuniculus* 3.0% erythrocyte (Ethics Committee for the Use of Animals, CEUA/UFPB, n. 178/2015). The presence of hemagglutination was determined in triplicate, by serial dilution and direct visualization of clots. The results were expressed as the inverse of the title of the highest dilution that still showed visible hemagglutination. 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-BAPNA, 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.

BIOLOGICAL ACTIVITIES

Antioxidant activity

The antioxidant activity was determined by ABTS (RE et al., 1999) and DPPH (MORALES and JIMENEZ-PEREZ, 2001) scavengers' methods. ABTS^{•+} (7.0 mM) was prepared in aqueous solution and DPPH[•] (0.06 mM) in methanolic solution. The determination of the antioxidant activity occurred in a light protected environment, with reading on Spectrophotometer-Vis, at wavelength of 734 nm for ABTS^{•+} and 515 nm, for DPPH[•]. In triplicate, 500, 1000 and 2000 µL of ASSv (100 mg mL⁻¹) were transferred to test tubes containing 3.0 mL of the ABTS^{•+} or DPPH[•] radicals and the percentage of radical scavenging was determined, using Trolox 1.0 mM as positive control and ultrapure water as negative control.

Antimicrobial activity

Bacterial strains were housed in NA, stored at 4 °C and used to determine antibacterial activity. The assays were performed in BHI broth, with chloramphenicol (100 µg mL⁻¹) as negative control. Yeast and filamentous fungi were housed in SDA, stored at 37 – 35 °C and used to determine antifungal activity. The assays were performed in RPMI 1640 medium, with nystatin (100 µg mL⁻¹) as a negative control for yeast and fluconazole (50 µg mL⁻¹) for filamentous fungi. The

microbial suspensions were prepared according to the 0.5 McFarland Scale tube, adjusted to 10^5 CFU mL⁻¹ (HADACEK and GREGER, 2000; NCCLS, 2000). The minimum inhibitory concentration (MIC) was determined by the microdilution technique ($1024 - 32 \mu\text{g mL}^{-1}$) (ELOFF, 1978) and the bacterial growth was accompanied by the colorimetric change of 0.01% resazurin dye.

STATISTICAL ANALYSES

Data are expressed as mean \pm standard deviation of three repetitions. Analysis of variance (ANOVA) was performed for data analyses using GraphPad Prism® version 6.01 (GraphPad Software, USA), with Tukey's post-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

MORPHO-PHYSCOCHEMICAL CHARACTERIZATION

S. virgata seeds showed coloring (light brown tones) and density ($0.9 \pm 0.01 \text{ g mL}^{-1}$), similar to other *Sesbania* species (HOSSAIN and BECKER, 2001). Its individual weight and weight of 100 seeds were $0.1 \pm 0.01 \text{ g seed}^{-1}$ and $7.6 \pm 0.01 \text{ g 100 seeds}^{-1}$, respectively. The seeds dimensions were $0.4 \pm 0.00 \text{ cm}$ thick, $0.4 \pm 0.00 \text{ cm}$ width and $0.6 \pm 0.01 \text{ cm}$ long, thus being the full and elliptical type, according to Silva et al. (2016). The dry mass of *S. virgata* pods was $0.9 \pm 0.00 \text{ g pod}^{-1}$ and each pod houses 6 seeds. The pods showed $0.6 \pm 0.00 \text{ cm}$ thick, $0.9 \pm 0.01 \text{ cm}$ wide and $6.1 \pm 0.00 \text{ cm}$ long. The small size of pods with few seeds is interesting because *S. virgata* can firmly fix its fruitlessness on peduncle, reducing the chances of the peduncle bending and breaking. In addition, the light weight of pods prevents the branches from touching the soil.

The HC ($0.0 \pm 0.00 \text{ g seed}^{-1}$ and $15.0\% \pm 0.00$), AC ($0.1 \pm 0.01 \text{ mL seed}^{-1}$ and $38.0\% \pm 0.02$), HI (0.3 ± 0.01), WAC ($0.1 \pm 0.01 \text{ mL seed}^{-1}$ and $38.0\% \pm 0.00$) and IWAs (0.2 ± 0.01) indexes of *S. virgata* seeds were also similar to other *Sesbania* species (HOSSAIN and BECKER, 2001). *S. virgata* seeds still present IWAf and IFS of $3.6 \pm 0.00 \text{ g H}_2\text{O g flour}^{-1}$. Water is a limiting factor in the vegetable nutritional quality, and these indexes reflects the balance of the aqueous stream with the loss of compounds soluble solids from seeds; the hardness and impermeability of the

seed shells, with consequent low hydration and water absorption; the amount of water that is absorbed by the starch granules from flour; and the degree of degradation that water causes in the fibers. Thus, it stands out that the morpho-physicochemical characteristics of *S. virgata* seeds express its quality in different processing, in addition to corresponding to a proper percent of hydrophilic molecules, according to Hodge; Osman (1976) criteria.

NUTRITIONAL COMPOSITION

The high protein content ($60.8\% \pm 0.18$) stands out in relation to the other macronutrients from *S. virgata* seeds, and in relation to the protein contents recorded for other legumes (RUIZ-LÓPEZ et al., 2019), including other *Sesbania* species (HOSSAIN and BECKER, 2001). This promising value encourages the use of *S. virgata* seeds as a surprising protein nutritional matrix. In the food industry, especially, the use of *S. virgata* seeds can serve as a food base to overcome protein deficiency. If applied for that purpose, the daily intake of protein be quantified according to the age group.

The moisture content of *S. virgata* seeds ($8.4\% \pm 0.00$) is in accordance with the Brazilian legislation (BRASIL, 2005). This content was even higher than the contents presented by other *Sesbania* species (HOSSAIN and BECKER, 2001). The ash content of *S. virgata* seeds ($4.8\% \pm 0.02$) is slightly at odds with the maximum content (4.0%) allowed by Brazilian legislation (BRASIL, 1978). However, the ash index does not always characterize the real inorganic richness present in the seed, because some chemical properties can be altered in the determination (AOAC, 2005). Also, this value may still be correlated with the *S. virgata* seed collection site, a soil abundant in wet matter and constant visitation of cattle, which may have interfered in the ash content of *S. virgata* seeds.

The crude fat content of *S. virgata* seeds ($7.6\% \pm 0.19$) exceeds the contents of other *Sesbania* species (HOSSAIN and BECKER, 2001) and can play an important role in the nutritional status of the world population, recognizing its importance as renewable sources of fatty acids. Thus, to monitor lipid bioavailability, Table 1 presents the total fatty acids from *S. virgata* seeds. Eight fatty acids were detected, four saturated and four unsaturated. The sum of all the unsaturated fatty acids detected in the chromatogram was 68.3%. Among them, the polyunsaturated

were the most abundant (45.8%), with two important essential fatty acids, linoleic acid and linolenic acid. This data was promising because polyunsaturated fatty acids are essential to the architecture of cell membranes and play fundamental roles in many cellular processes. The remainder of the chromatogram (31.7%) contemplate saturated fatty acids. Among them, palmitic acid was the one that contributed to the total fatty acid profile, followed by stearic acid.

Table 1 - Fatty acid composition of *Sesbania virgata* seeds (as a percentage of total fatty acids). Data are expressed as mean \pm standard deviation of three repetitions (n = 3).

Variable	Common Name	Fatty Acid	%
Saturated	Palmitic acid	C16:0	26.0 \pm 2.95
	Stearic acid	C18:0	4.8 \pm 0.55
	Behenic acid	C22:0	0.0 \pm 0.00
	Lignoceric acid	C24:0	0.8 \pm 0.00
Monounsaturated	Palmitoleic acid	C16:1	9.6 \pm 0.50
	Oleic acid	C18:1	13.0 \pm 0.95
Unsaturated	Linoleic acid	C18:2n6c	27.8 \pm 3.15
	Linolenic acid	C18:3n3	17.9 \pm 0.40

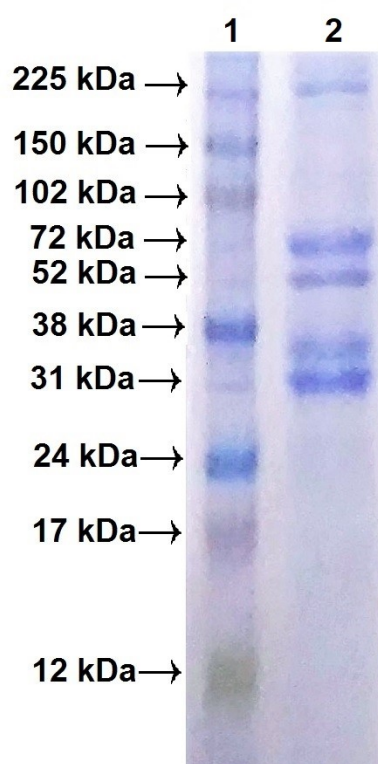
Similar to our results, a study (HOSSAIN and BECKER, 2001) on the lipid profile of *Sesbania aculeata*, *Sesbania rostrata* and *Sesbania sesban* seeds indicates that the content of unsaturated fatty acids exceeds the content of saturated. Our results also corroborate the indication of palmitic acid, oleic acid and linoleic acid as the most abundant fatty acids. Although the bioavailability of these fatty acids varies among the species studied, Mohammed; Qoronfleh (2020) provided evidence that the abundance of unsaturated fatty acids in seeds, especially oleic and linoleic acids, is associated with numerous potentialities.

The total carbohydrate content of *S. virgata* seeds (13.7% \pm 0.03) is promising, because genetic engineers and breeders can be developed research on the use of carbohydrates in the food industry, overcoming historical interest in foods rich exclusively in oils and/or proteins. Thus, the *S. virgata* seeds are a matrix conducive to obtaining different types of macronutrients. So, we suggest *S. virgata* seeds as natural food additives (energy value = 366.6 kcal 100g⁻¹) or substrate for biotechnological applications due to the benefits that its macronutrients can provide, especially proteins.

PROTEIN ANALYSES

The high protein content ($60.8\% \pm 0.18$) stimulated us to deepen the analyses on the protein profile of *S. virgata* seeds. The soluble protein content was $0.2 \pm 0.04 \text{ mg mL}^{-1}$, and at least five bands with molecular weights can be observed in the range of 225 to 31 kDa, according to Fig 1.

Figure 1 - Electrophoretic profile (SDS-PAGE, 12.5%) of *Sesbania virgata* seeds. Caption: (1) molecular weight markers, 225 – 12 kDa; (2) aqueous solution of *Sesbania virgata* seeds (ASSv, 30 μ l).



The main protein bands (75 – 31 kDa, Fig. 1) are slightly aggregated in a range similar to the molecular masses of globulins, one of the four classes of proteins storage legume seeds. The expression of this range of molecular masses (75 – 31 kDa) was similar to molecular masses of protein fractions from *Phaseolus vulgaris* seeds, a bean that occupies a prominent position in the national agricultural scenario (KENMOE et al., 2020). This finding was interesting because these protein fractions exhibited potential performance as anti-aggregating agents for deoxyhemoglobin S, anti-sickle cell, besides preventing the adherence of sickle cells to endothelial cells. In addition to the nutritional importance already demarcated to *S. virgata* seeds, our results stimulate the development of new

studies, aiming the detailed characterization of proteins from *S. virgata* seeds and expansion of its therapeutic, technological and nutritional possibilities.

TOTAL AMINO ACID PROFILE

Amino acid composition and essential amino acid (EAAs) score are shown in Table 2. The most abundant non-essential amino acids from *S. virgata* seeds are glutamic acid ($6.8 \pm 0.00 \text{ g } 100\text{g}^{-1}$), aspartic acid ($5.0 \pm 0.00 \text{ g } 100\text{g}^{-1}$) and arginine ($3.8 \pm 0.00 \text{ g } 100\text{g}^{-1}$). However, alanine and proline are deficient in *S. virgata* seeds. Among essential amino acids, leucine ($4.3 \pm 0.01 \text{ g } 100\text{g}^{-1}$), lysine ($3.2 \pm 0.00 \text{ g } 100\text{g}^{-1}$) and valine ($2.8 \pm 0.01 \text{ g } 100\text{g}^{-1}$) are abundant in *S. virgata* seeds, while methionine and cysteine are deficient. Our results corroborate to Hossain and Becker (2001), a study that points out that *S. aculeata*, *S. rostrata* and *S. sesban* seeds are also deficient and abundant in the same amino acids. Compared to WHO/FAO (2007), four of the eight EAAs, such as histidine, threonine, isoleucine and phenylalanine + tyrosine, are adequate or higher. The remaining four were limiting amino acids. The methionine + cysteine content and EAAs score are below that recommended to the WHO/FAO (2007). This result was already expected, because according to Mubarak (2005), most legume seeds are deficient in sulphur-containing amino acids.

One of the most attractive features of *S. virgata* seeds is the high protein content and the good balance of amino acids. Both were shown to maintain the balance of nitrogen in biological system. The deficiency of *S. virgata* seeds in EAAs could be balanced in association with a diet complemented with cereals. According to Anitha et al. (2020), cereals are naturally rich in methionine and cysteine, and the right combination of legumes seeds and cereals has prospects of enhancing the nutritional value of foods and contributing to a balanced dietary system. Additionally, *S. virgata* seeds may be a potential source of dietary protein in monogastrics, as demonstrated in recent studies (ZHAO et al., 2020) on animal supplementation with free proteins and amino acids.

Table 2 - Total amino acids profile (g amino acid 100g protein⁻¹) present in *Sesbania virgata* seeds. Caption: Data are expressed as mean \pm standard deviation of three repetitions (n = 3). (Met) Methionine; (Cys) Cysteine; (Phe) Phenylalanine; (Tyr) Tyrosine; (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. Tryptophan not determined.

Amino acids	Amino acid content (g 100g ⁻¹)	EA*	EAE**
<i>Essential amino acids</i>			
Histidine	1.4 \pm 0.00	1.5	93.2
Threonine	2.4 \pm 0.00	2.3	103.2
Tyrosine	2.1 \pm 0.00	-	-
Valine	2.8 \pm 0.01	3.9	70.8
Methionine	0.7 \pm 0.00	-	-
Cysteine	0.1 \pm 0.00	-	-
Isoleucine	2.4 \pm 0.00	3.0	78.6
Leucine	4.3 \pm 0.01	5.9	72.7
Phenylalanine	2.5 \pm 0.00	-	-
Lysine	3.2 \pm 0.00	4.5	70.0
Met + Cys ¹	0.8	2.2	38.6
Phe + Tyr ²	4.6	3.8	121.2
<i>Non-essential amino acids</i>			
Aspartic acid	5.0 \pm 0.00	-	-
Glutamic acid	6.8 \pm 0.00	-	-
Serine	2.7 \pm 0.00	-	-
Glycine	2.7 \pm 0.01	-	-
Arginine	3.8 \pm 0.00	-	-
Alanine	2.4 \pm 0.01	-	-
Proline	2.1 \pm 0.00	-	-

ANTINUTRITIONAL FACTORS

The conditions used in the assay were not sufficient to detect the presence of lectins and trypsin inhibitors in *S. virgata* seeds. Hossain; Becker (2001) report the presence of these antinutritional factors in *S. aculeata*, *S. rostrata* and *S. sesban* seeds, but its contents are very low. Although, when isolated, these antinutritional factors have biological relevance, its absence in *S. virgata* seed is an interesting finding, because lectins and trypsin inhibitors generally do not degrade easily in the gastrointestinal tract and can be complexed with digestive enzymes, interfering in the nutrient absorption (SILVESTRINI et al., 2017). Additionally, the

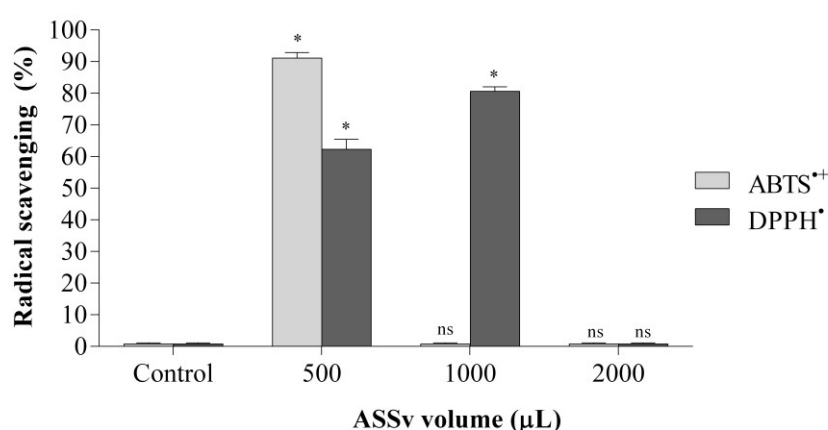
acceptability and use of legumes seeds have been affected by the presence of these antinutritional factors, which reduce the bioavailability nutrients (SHARAN et al., 2021). The non-detection may have occurred due to the use of water as the only extraction agent and the absence of vigorous mechanical agitation processes, capable of breaking the cell walls of *S. virgata* seeds and solubilizing these proteins.

BIOLOGICAL APPLICATIONS

Antioxidant activity

Recognizing the importance of neutralizing the deleterious effects of reactive species and offering new therapeutic possibilities from natural compounds, the Fig. 2 illustrates the antioxidant activity of *S. virgata* seeds. In the ABTS scavenging activity, only 500 μL was effective ($91.1\% \pm 0.02$) and no antioxidant activity was perceived in the other tested doses. However, for DPPH scavenging activity, *S. virgata* seeds present $62.3\% (\pm 0.05)$ and $80.6\% (\pm 0.02)$ activity in 500 and 1000 μL , respectively, without activity in 2000 μL . Statistical significance was found in all concentrations tested.

Figure 2 - Antioxidant activity of *Sesbania virgata* seeds. Caption: Negative control (ultrapure water, Milli-Q). Data are expressed as mean \pm standard deviation of three repetitions ($n = 3$); (ASSv) aqueous solution of *Sesbania virgata* seeds; (ns) no statistical significance; (*) statistical significance, $p < 0.05$.



Although *S. virgata* seeds are effective as antioxidant agents for both methods investigated, a difference is observed between the manifestation of antioxidant activity. According to Martysiak-Żurowska; Wenta (2012), the DPPH scavenging method is characterized by a lower sensitivity than the ABTS scavenging method,

because the ABTS^{•+} radical reacts rapidly with lipophilic and hydrophilic antioxidants in several matrices, including food extracts. Here, the ABTS scavenging activity is interrupted at doses above 500 μL probably because, when the concentration of antioxidant compounds from *S. virgata* seeds exceeds this range, stereochemical reactions begin and antioxidant reactions tend to saturate the antioxidant system. Schaich et al. (2015) suggest that this phenomenon occurs due to the formation of additional ducts from antioxidant compounds, interrupting electrons from accessing the active site of the antioxidant system.

However, oxidation reactions are also present in many food products, and supplemental natural antioxidants are also needed to increase product stability and prevent oxidation deterioration during processing and storage (LOURENÇO et al., 2019). Recent research (LI et al., 2020; MORENO-VALDESPINO et al., 2019) have been exploring the use of natural antioxidants in the food and pharmaceutical industry, aiming to increase shelf time, the appearance of numerous products and development of new plant-based therapeutic options. Thus, encouraged by its nutritional value and antioxidant potential, we suggest the use of *S. virgata* seeds as an exogenous source of biomolecules for industrial and therapeutic purposes.

Antifungal activity

The genera *Candida*, *Aspergillus* and *Penicillium* include a huge variety of medically, agriculturally and industrially important species capable of promoting infections or contaminate plant-based foods and feeds. Due to its pathogenicity and the need to provide new therapeutic options for the management of fungal infections, we present the *S. virgata* seeds as a potential antifungal agent (Table 3).

According to our analyses, the MIC required to promote inhibition of *C. albicans* and *C. tropicalis* was 256 $\mu\text{g mL}^{-1}$, which represents an optimal activity, according to Houghton et al. (2007) criteria. Although *S. sesban* (MAREGESI et al., 2008) also presents anti-*Candida albicans* activity, our results are more promising, because the MIC of *S. virgata* is four times lower. However, not all *Sesbania* species exhibit activity against *C. albicans*, such as *S. grandiflora* (SRINIVASAN et al., 2001). In turn, the MIC required to promote inhibition of *A. flavus* and *P. citrinum* is 1024 $\mu\text{g mL}^{-1}$, representing a moderate activity, according to Houghton et al. (2007)

criteria. Another record of antifungal activity was found for protein from *S. virgata* seeds (PRAXEDES et al., 2011). This antifungal protein is toxic to *Aspergillus niger*, *Cladosporium cladosporioides* and *Colletotrichum gloesporioides*, reinforcing that *S. virgata* seeds are excellent sources of antifungal biomolecules.

Table 3 - Determination of minimum inhibitory concentration (MIC) of *Sesbania virgata* seeds. Caption: (+) fungal growth occurred; (-) did not occur fungal growth; (-----) not applied.

Drugs	Yeasts					Filamentous fungi			
	<i>C. albicans</i> (ATCC-76645)	<i>C. albicans</i> (LM-122)	<i>C. tropicalis</i> (ATCC-13803)	<i>C. tropicalis</i> (LM-64)	<i>C. tropicalis</i> (LM-7)	<i>A. flavus</i> (LM-714)	<i>A. flavus</i> (LM-247)	<i>P. citrinum</i> (LM-9)	<i>P. citrinum</i> (LM-60)
<i>S. virgata</i> seeds	512	512	512	1024	1024	1024	1024	1024	1024
MIC ($\mu\text{g mL}^{-1}$)									
Means control	-	-	-	-	-	-	-	-	-
Fungus control	+	+	+	+	+	+	+	+	+
Nystatin ($100 \mu\text{g mL}^{-1}$)	-	-	-	-	-	-----	-----	-----	-----
Fluconazole ($50 \mu\text{g mL}^{-1}$)	-----	-----	-----	-----	-----	+	+	+	+

The human mortality index by fungal infection increases exponentially due to the development of resistance mechanisms to chemical antifungals (HOUSŤ et al., 2020). Additionally, fungi cause significant damage to the food industry, demanding the use of chemical additives to increase food life and prevent fungal proliferation (RITOTA and MANZI, 2020). Thus, recent global challenges require ecologically sustainable strategies to prevent and treat fungal infections, such as the production of natural antimicrobials, especially plant derivatives. Therefore, studies such as this, aiming at screening of plants for fungicide agents, are of great importance and the *S. virgata* seeds deserve to be highlighted due to the promising results presented here.

Antibacterial activity

Although *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli* cause important infections in humans, animals and vegetables, and inhibition of its development is necessary, the antimicrobial biomolecules from *S. virgata* seeds were not toxic to these strains, under the conditions tested. Antibacterial activity depends on the interactions of bioactive agents with bacterial cell wall components (COELHO et al., 2018). Thus, in the range of concentrations tested, the antimicrobial biomolecules from *S. virgata* seeds may not have been able to recognize the components of bacterial cell wall, making it impossible to inhibit the growth of the investigated strains.

To the best of our knowledge, the antibacterial potential of *S. virgata* seeds has not yet been characterized and the studies available for the *Sesbania* genus point to *S. grandiflora* as the most promising species. However, according to our analyses, the manifestation of antibacterial activity depends on the tested bacterium, the method of analysis, the plant part and the dose used. Although *S. grandiflora* is the most promising species, when antibacterial activity was investigated by plate diffusion assays (SRINIVASAN et al., 2001), *S. grandiflora* inhibited only the growth of *S. aureus*, among all the 10 strains tested. Maregesi et al. (2008) report that although *Sesbania sesban* inhibits the growth of *S. aureus* and *P. aeruginosa*, the required MIC is higher than the range of concentrations tested in our study. The tropism of antimicrobial components by the vegetable also deserves to be highlighted, because each *S. sesban* plant tissue presented a MIC for *Bacillus cereus* inhibition, i.e.: roots (1000 µg mL⁻¹), twigs (500 µg mL⁻¹) and leaves (250 µg mL⁻¹).

The antimicrobial biomolecules from *S. virgata* seeds also did not inhibit the development of *Bacillus subtilis*, a non-pathogenic bacterium of great importance to the medical and food industry. This result makes it possible to expand the repertoire of available studies on the symbiotic associations between *Sesbania* and bacteria in sustainable agriculture (LIN et al., 2018). It is also possible to idealize studies on combinations of *S. virgata* and *B. subtilis*, and its application in agriculture, based on the promising results obtained by Rao et al. (2017). These authors developed a successful combination of *B. subtilis* with organic additives for the effective management of soil-borne pathogens and obtaining maximum yield

of carrot. Thus, once not inhibiting the *B. subtilis* development, *S. virgata* seed may provide the soil a variety of functional nutrients, while *B. subtilis* exerts the biocontrol of field pests. Together, the results of this research with other studies on *Sesbania* potentialities, expand the nutritional and biotechnological repertoire of *S. virgata* seeds.

CONCLUSIONS

This study presents the nutritional characterization of *Sesbania virgata* seeds, with important highlights for abundance in protein (60.8% of total protein; 75 – 31 kDa, the main protein bands), and essential and non-essential amino acids. However, the carbohydrate and crude fat contents are also promising, highlighting the abundance in polyunsaturated fatty acids, especially linoleic acid and linolenic acid. These results are also associated with a positive health impact, because *S. virgata* seeds exhibit antioxidant and antifungal activities. By introducing studies on the discovery of ecologically friendly therapeutic possibilities, we believe that *S. virgata* seeds can be explored as sources of biomolecules with antibiotic and antioxidant properties, besides highlighting its economic and nutritional potential. The identification and isolation of these biomolecules are being employed by our group. We suggest investigating the toxic effects of *S. virgata* seeds flour on humans, animals and the environment to confirm the safety of its application in various sectors of the industry.

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AUTHORS' CONTRIBUTIONS

C.A.A.G. and G.C.S.S.: Conceptualization, Methodology, Writing - original draft. S.N.F. and M.S.M.: Nutritional Characterization Assays. C.A.A.G., E.S.N.,

G.C.S.S., M.A.T.S., M.T.B.P. and T.S.G.: Protein, Amino Acid and Antioxidant Assays.

D.F.S. and E.O.L.: Microbiological Assays.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Composição nutricional e atividades biológicas (antioxidante e antifúngica) das sementes de *Sesbania virgata* (Cav.) Pers.

RESUMO

Sesbania virgata (Cav.) Pers. é uma leguminosa (Fabaceae) Pantropical capaz de colonizar margens de rios da região semiárida brasileira, sendo comumente utilizada para recuperação de solos degradados. Embora contemplem alto valor nutricional, suas sementes são pouco exploradas para aplicações biotecnológicas e biológicas. Propondo iniciar o preenchimento dessa lacuna, a presente pesquisa descreveu a composição nutricional das sementes de *S. virgata*, além de suas atividades antioxidante e antimicrobiana. O valor energético das sementes de *S. virgata* foi de 366,6 kcal 100g⁻¹ e, dentre os macronutrientes investigados, destaca-se o teor de proteínas (60,8%). No entanto, os teores de carboidratos e lipídeos totais também são promissores, destacando-se a abundância em ácidos graxos poli-insaturados, principalmente ácido linoleico e ácido linolênico. As sementes de *S. virgata* também são excelentes fontes de aminoácidos essenciais (leucina, lisina e valina) e não essenciais (ácido glutâmico, ácido aspártico e arginina). Nas condições de ensaio, lectinas e inibidores de tripsina não foram detectados. Além disso, as sementes de *S. virgata* conferem atividade antifúngica contra *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus* e *Penicillium citrinum*, e atividade antioxidante, em ensaios de eliminação dos radicais ABTS•+ e DPPH•. Diante desses achados, a composição nutricional das sementes de *S. virgata* impulsiona seu uso como fonte natural de produtos funcionais e suas atividades biológicas estimulam sua aplicação biotecnológica e farmacológica como agente antifúngico e antioxidante exógeno.

PALAVRAS-CHAVE: *Sesbania virgata*. Composição proximal. Atividade antifúngica. Atividade antioxidante. Relevância Biológica.

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