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Phytochemical characterization, evaluation of protein content and bioprospection of lectins and trypsin inhibitors in extract of *Sesbania virgata* (Cav.) Poir Seeds

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Abstract: *Fabaceae* presents 145 subclades and 737 genera. Among them, the genus *Sesbania*, with about of 500 species, such as *Sesbania virgata*, whose seeds present high protein content. The present study aimed to characterize phytochemically, to evaluate the protein content and to bioprospect lectins and trypsin inhibitors in the protein extract of *Sesbania virgata* seeds. Seed meal was used to obtain Total Protein Extract (TPE) in Glycine-NaOH 0.1 mol.L⁻¹ pH 9.0 NaCl 0.15 mol.L⁻¹ (1:15, w/v). After 22 hours of magnetic stirring, the extract was centrifuged (7.076.3 x g, 30 min, and 4 °C) and the supernatant collected and named TPE. In this, it was determined the total protein content using the bovine serum albumin standard curve; bioprospecting lectins by hemagglutination assays in rabbit erythrocytes (CEUA/UFPB n° 178/2015), and trypsin inhibitors by enzyme assays using the enzyme bovine trypsin; and phytochemical characterization was given by different qualitative tests. TPE presented as protein content 7.13 mgP/mL and 106.96 mgP/gF; hemagglutination titers were 32 UH/mL, with 480 UH/gFa and 4.4887 UH/mgP; not precipitated trypsin inhibitors; and in phytochemical tests, reacted positively to saponins, catechins, flavones, flavonols, xanthonenes, anthocyanins, anthocyanidins

and flavonoids, but reacted negatively to pyrogallol and catechin tannins, leucoantocyanidins, steroids and triterpenes. In other assays performed by our group (data not shown) in which an extract was obtained from buffer Glycine-HCl 0.1 mol.L⁻¹ pH 2.6 NaCl 0.15 mol.L⁻¹, under the same conditions, it was found that this extraction method was not able of bioprospecting lectins, but favored the precipitation of protease inhibitors. It is understood, thus, that the extraction method influences the obtaining of TPE, the demarcation of considerable protein content and numerous phytochemical compounds, besides the hemagglutination assays suggest the presence of lectins. Together, all these constituents make TPE a versatile biotechnological tool, to check various biological activities.

Keywords: biologically active proteins; hemagglutinating activity; phytochemical constituents; *sesbania virgata*; lectins; trypsin inhibitors.

1. Introduction

The legume seeds are able to provide a diet rich in several nutrients (Torres, 2013), such as *Sesbania virgata*, a legume (Silva, 2013) with shrub habit (Lisboa et al., 2006), popularly known as black acacia (Branzini, González & Zubillaga, 2012), saranzinho, mãe-josé and feijãozinho (Araújo et al., 2004), whose seeds contain numerous biologically active proteins (Hossain & Becker, 2001).

Commonly, legume seeds exhibit biological activities by the mechanism of action of proteins, as the *lectins*, an antinutritional factor of protein origin with ability to agglutinate erythrocytes and/or to precipitate glycoconjugates (Rüdiger, 1998), mediated by the structure or similarity between the binding sites present on the surface of their membranes (Santana et al., 2008). As well as, *protease inhibitors*, protein factors with skillful ability to inhibit the catalytic activity of numerous proteolytic enzymes (Neurath, 1989; Ryan, 1990; Brzin & Kidric, 1995).

The properties displayed by its legume seeds encourage the development of natural or synthetic bioactive products capable of being employed as biotechnological tools. Thus, the present study aimed to characterize phytochemically, to evaluate the protein content and to bioprospect lectins and trypsin inhibitors in the protein extract of *Sesbania virgata* seeds.

2. Results and Discussion

2.1 Phytochemical analysis

When present, phytochemicals compounds to express in low intensity, saponins, catechins,

flavones, flavonols, xanthenes, anthocyanins, anthocyanidins and flavonoids. However, pyrogallol or catechic tannins did not observed (Table 1), similar to that seen in *Coriandrum sativum* for catheter tannins (Téllez-López et al., 2017).

Thus, it is possible to infer that the biological activities and bioprospection of biologically active proteins, as they occur without the interference of tannins, in particular, will be free of the possibility of false-positive results, as commonly occurs in lectin activity tests.

Leucoantocyanidins, steroids and triterpenes also were not detected (Table 1), suggesting that their non-precipitation is a result of insoluble complexes that form between proteins or carbohydrates present in TPE, as well as by the action of the solvents used.

2.2 Soluble protein content and bioprospection of lectin and trypsin inhibitors

The soluble protein content present in TPE was 7.13 mgP/mL and 106.96 mgP/mgF. Hemagglutination titers were 32 UH/mL, with 480 UH/gFa and 4.4887 UH/mgP and the lectin activity is seen with 60 minutes of starting the test and remaining for up to 24 hours after. TPE do not have apparent antitryptic activity.

In other assays performed by our group (data not shown) in which an extract was obtained from buffer Glycine-HCl 0.1 mol.L⁻¹ pH 2.6 NaCl 0.15 mol.L⁻¹, under the same conditions, it was found that this extraction method was not able of bioprospecting lectins, but favored the precipitation of protease inhibitors.

This buffer showed values ranging from 62.99% to 74.16% inhibition, and 0.71 and 6.96 mg of inhibited trypsin/g sample, proving that for *Sesbania virgata* seeds, it is necessary that this protease inhibitor be extracted in alkaline pH.

While the extract obtained of the Glycine-NaOH (0.1 mol.L⁻¹, pH 9.0) containing NaCl 0.15 mol.L⁻¹, was chosen as the most effective for to bioprospect lectins.

Table 1. Bioprospection of phytochemical components present in Total Protein Extract obtained from *Sesbania virgata* seeds. Legend: (-) Negative reaction; (+) Positive reaction.

Compound classes	Reactions	Result
Pyrogallic and cathectic tannins	Ferric chloride	-
Saponins	Chloroform	+
Catechins	Colorimetric	+
Flavones, flavonols and xanthones	Colorimetric	+
Anthocyanins, anthocyanins and flavonoids	Colorimetric	+
Leucoantocianidins	Colorimetric	-
Steroids	Colorimetric	-
Triterpenes	Colorimetric	-

3. Materials and Methods

3.1 Total Protein Extract (TPE) extraction

S. virgata (Cav.) Pers. was collected in João Pessoa, Paraíba, Brazil (7°09'51.8"S 34°54'01.1"W) and deposited in Professor Lauro Pires Xavier Herbarium (JPB n° 63198). Healthy and mature seeds were pulverized until a fine flour (FF) was obtained. 1g of the FF was homogenized in 15mL of Glycine-NaOH (0.1 mol.L⁻¹, pH 9.0), containing NaCl 0.15 mol.L⁻¹. The extract remained for 22 hours of constant magnetic stirring, and centrifuged at 7.076.3 x g for 30 minutes, 4 °C (Eppendorf Centrifuge 5430R). The precipitate was discarded, the supernatant collected and named TPE.

3.2 Phytochemical partial characterization

The phytochemical partial characterization was determined by colorimetric reactions, according to methodologies prescribed by Barbosa et al. (2001), Matos (1997) and Robbers et al. (1997).

3.3 Determination of soluble protein content

The soluble protein content was determined by colorimetric reactions, using bovine serum albumin as standard and

Coomassie Brilliant Blue G-250 as chromogenic, according Bradford (1976).

3.4 Lectin and trypsin inhibitors detection assays

Detection of lectin activity was performed using haemagglutination assays against 3% rabbit erythrocytes (Debray et al., 1981). The entire procedure was approved by the Ethics Committee for the Use of Animals (CEUA/UFPB, n° 178/2015).

Antitryptic activity detection assays, in the presence or absence of trypsin inhibitors, were performed according by Xavier-Filho et al. (1989), using bovine trypsin and DL-BApNA as its chromogenic substrate.

4. Conclusions

It is understood, thus, that the extraction method influences the obtaining of TPE, the demarcation of considerable protein content and numerous phytochemical compounds, besides the hemagglutination assays suggest the presence of lectins. Together, all these constituents make TPE a versatile biotechnological tool, to check various biological activities.

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Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Araújo, E.C. et al. Caracterização morfológica de frutos, sementes e plântulas de *Sesbania virgata* (Cav.) Pers. *Revista Brasileira de Sementes* **2004**, 26, 105-110.
2. Barbosa, W.L.R. et al. *Manual para análise fitoquímica e cromatográfica de extratos vegetais*. Universidade Federal do Pará: Editora UFPA, Brasil, 2001.
3. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal Biochemistry* **1976**, 72, 248-254.
4. Branzini A., González R.S., Zubillaga M. Absorption and translocation of copper, zinc and chromium by *Sesbania virgata*. *Journal of Environmental Management* **2012**, 102, 50-54.
5. Brzin, J.; Kidric, M. Proteinases and their inhibitors in plants: role in normal growth and in response to various stress conditions. *Biotechnology and Genetic Engineering Reviews* **1995**, 13, 421-467.
6. Debray, H. et al. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *European Journal of Biochemistry* **1981**, 117, 41-55.
7. Hossain, M.A.; Becker, K. Nutritive value and antinutritional factors in different varieties of *Sesbania* seeds and their morphological fractions. *Food Chemistry* **2001**, 73, 421-431.
8. Lisboa, C.G.S. et al. Endo- β -mannanase from the endosperm of seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae): purification, characterisation and its dual role in germination and early seedling growth. *Brazilian Journal of Plant Physiology* **2006**, 18, 269-280.
9. Matos, F.J.A. *Introdução à fitoquímica experimental*. 2. ed. Fortaleza: EUFC, Brasil, 1997.
10. Neurath, H. Proteolytic processing and physiological regulation. *Trends in Biochemical Sciences* **1989**, 14, 268-271.
11. Robbers, J.E.; Speediem, M.K.; Tyler, V.E. *Farmacognosia: biotecnologia*. São Paulo: Premier, Brasil, 1997.
12. Rüdiger, H. Plant lectins – more than just tools for glycoscientists: occurrence, structure, and possible functions of plant lectins. *Acta Anatomica* **1998**, 161, 130-152.
13. Ryan, C.A. Protease inhibitor in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology* **1990**, 28, 425-449.
14. Santana, M.A. Isolamento, propriedades bioquímicas e estudos biológicos da lectina de sementes da *Macrotyloma axillare* (E. Meyer). Mestrado em Ciências Biológicas, Universidade Federal de Ouro Preto, 2004.
15. Silva, K.B. Características químicas, físicas e biológicas de solos sob *Sesbania virgata* (Cav.) Pers. Mestrado em Ciência do Solo, Universidade Federal de Lavras, Lavras, 2013.
16. M.Á. Téllez-López, et al. Evaluation of the chelating effect of methanolic extract of *Coriandrum sativum* and its fractions on wistar rats poisoned with lead acetate. *African Journal of Traditional, Complementary and Alternative Medicines* **2017**, 14, 92-102.
17. Torres, J. Characterization of the nutritive value of tropical legume grains as alternative ingredients for small-scale pork producers using in vitro enzymatic hydrolysis and fermentation. *Journal of Animal Physiology and Animal Nutrition* **2013**, 97, 1066-1074.
18. Xavier-Filho, J et al. 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*. *Journal of Agriculture and Food Chemistry* **1989**, 37, 1139-1143.