SciForum
MOL2NET

PHYTOCHEMICAL CHARACTERIZATION OF THE PROTEIN FRACTION DIOSCORINE, OBTAINED FROM TUBERS OF *Dioscorea cayennensis*

Aleson de Sousa [1,*], Francielly de Araújo [2], Giulian da Silva Sá [2], Nathália Galvão Silva [3], Kamila Bezerra [4] and Carlos Gadelha [5]

[1] Biomedical, specialist in Cytology, Master in Cellular and Molecular Biology, Federal University of Paraiba - UFPB; [2] Biologist, Master in Cellular and Molecular Biology Federal University of Paraiba - UFPB; E-Mails: franciellyng@hotmail.com/giuliancesarsa@gmail.com; [3] Biologist, Master's in Biological Sciences, Federal University of Pernambuco – UFPE; E-Mail: nathaliargalvao@gmail.com; [4] Graduate's in Biological Sciences, Federal University of Paraiba – UFPB; E-Mail: kamiilasantoss13@gmail.com; [5] Agronomist, Doctor in Biochemistry, Federal University of Paraiba – UFPB; E-Mail: calbgadelha@gmail.com

***** Author to whom correspondence should be addressed; E-Mail: aleson_155@hotmail.com; Tel.: +55-31 83 – 99632 7961;

Received: / Accepted: / Published:

Abstract: Studies relating the consumption of food and their nutritional properties have a great contribution in the field of health. Intake of bioactive compounds, such as proteins, vitamins, minerals and other molecules present in foods can also present substances considered antinutritives or toxic, such as cyanide, polyphenols, nitrate, saponins, protease inhibitors and lectins. The tubers are rich in carbohydrates, proteins, vitamins, organic acids, anthocyanins, phytosterols, glycolipids, antioxidants, besides presenting low lipid content. Experimental analyzes of dioscorine, the main protein present in several varieties of yams (*Dioscorea spp.*) has been performed over the years, demonstrating several biological activities. The present study aims to isolate the protein fraction dioscorine present in tubers of *Dioscorea cayennensis* and to perform its phytochemical characterization by determining the presence of secondary compounds. The tubers underwent the process of separating the mucilage and obtaining the ultrafine flour, the protein extraction was performed in buffer solution: Tris HCl 0.05M pH 8.3, the total extract was submitted to protein precipitation with ammonium sulfate in the fraction 45-75% (F_{45-75}). The material underwent dialysis with molecular exclusion cutoff of 8 kDa, and lyophilization for sample concentration. Phytochemical determination was performed using specific techniques such as: saponins (foam-stirring test); tannins (reaction with ferric chloride/lead acetate/gelatin); carbohydrates (Molisch test); starch (iodine test); anthocyanins, anthocyanidins and flavonoids (pH variation test/heating); catechins (reaction with hydrochloric acid/heating), flavonols and xanthones (reaction with granulated magnesium/hydrochloric acid/heating). The chemical compounds identified in the total extract were: tannins,

carbohydrates, saponins, flavones, flavonols and xanthones, the protein fraction did not show any phytochemical compound. Therefore, the isolated protein fraction of tubers of *D. cayennensis* showed no influence of secondary metabolites on their biological activities, being a target for the synthesis of natural products, considering their biotechnological and pharmaceutical importance.

Keywords: dioscorine, *Dioscorea cayennensis*, proteins, yam, phytochemical

1. Introduction

The chemical substances present in food, the nutrients, have crucial functions for living things such as growth, maintenance and cell repair (STIPANUK; CAUDILL, 2013). Currently, studies relate the consumption of foods to the nutritional and functional properties they offer to human and animal health, through the ingestion/absorption of biomolecules, such as proteins, carbohydrates, lipids, vitamins and minerals. However, some biomolecules present in these foods may also present substances considered antinutritives and/or toxic, such as cyanide, polyphenols, nitrate, oxalic acid, saponins, protease inhibitors and lectins (SILVA et al., 2010; ARMOUR et al., 1998). Among these foods, rhizomes and tubers are usually rich in carbohydrates, proteins, vitamins, minerals and organic acids. Among the vitamins, is cited mainly thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6) and ascorbic acid (C). They also have anthocyanins, phytosterols, glycolipids and antioxidants, besides having low lipid content (RAMOS; RAMOS; HIANE, 1997). Experimental analyzes of the dioscorine, the main protein present in several species of the genus Dioscorea, have been made over the years by several researchers; in these, immunolocalization tests, showed that dioscorine accumulates mainly in the vacuoles of plant cells of tubers (CONLAN et al., 1997). Therefore, the objective of the present study was to isolate the protein fraction dioscorine (PFD) present in *Dioscorea cayennensis* tubers and to perform their phytochemical characterization by determining the presence of secondary compounds in the protein fraction.

2. Results and Discussion

Protein Extraction and PFD Obtainment

Due to the presence of high carbohydrate contents, the sample required complex processing, with separation/precipitation steps to obtain a protein rich material, according to Guerreiro (2002). The interferers present in yam mucilage represented about 60% of the tuber composition. The initial mass of the UF had a yield of 40%, used in the accomplishment of protein extraction to obtain the PFD.

The isolation steps for obtaining PFD are summarized in Table 1. In this, it is found that the steps used allowed a yield of 16% compared to the initial product UF. In practice, to obtain 1.0 mg of lyophilized PFD it was necessary to repeat the method about 6 times. This result is in line with those previously used for PFD isolation by Conlan et al. (1997), Lu et al. (2012) and Liao et al. (2006), which obtained the isolation of the same protein fraction in other species of the same genus. Although conducted in different species of the genus Dioscorea, the process of isolation/purification share similarities between them, since all use protein extraction with buffer Tris 0.05M pH 8.3, followed by precipitation with ammonium sulfate, to obtain a specific fraction (F_{45-75}). It is well known in the literature that tubers of amylaceous species, such as *D. cayennensis*, as reserve structures, can take advantage of edaphoclimatic variations, to survive and transform their organic content by modifying the structures of their molecules (ZANONLL, 2009; DIOP; CALVERLEY, 1998).

.

Phytochemical Characterization of PFD

The application of qualitative methods in phytochemical prospecting is relevant because it allows the initial screening with a lower cost (AYOOLA et al., 2008). Some constituents of plant extracts may present specific biological activities, as is the case of saponins (hemolytic activity, anti-inflammatory, antifungal, antibacterial, antimicrobial, antiparasitic, cytotoxic and antitumor) (SPARG; LIGHAT; VAN STADEN, 2004); Catechins, flavones,

flavonols, xanthones, anthocyanins, anthocyanidins and flavonoids (antioxidant, antitumor and anti-inflammatory) (ROCHA et al., 2011); Tannins (antioxidant, anti-inflammatory, antibacterial, antifungal, tissue regeneration and activation of immune response) (MAGALHÃES, 2004).

Table 2 shows the results obtained for the phytochemical profile determined from TE and PFD. In this, it was possible to verify groups of chemical compounds from TE, as well as the absence of these biological interferers in PFD, demonstrating that the fractionation was efficient to eliminate the compounds evaluated.

Among the evaluated chemical compounds, it was observed in TE the presence of tannins, carbohydrates, saponins, flavones, flavonols and xanthones. This result is consistent with those reported by Magalhães (2004), that in extracts of leaves of *D. alata* found a large amount of saponins and free steroids. This study also highlighted the presence of antibacterial activity against *Staphylococcus aureus*, although its relation with the presence of phytochemicals was not established.

Table 1. Isolation steps of PFD

Steps	Total Proteins (mg/mL)[A]	Isolation Times[B]	Recovery (%)[C]
UF	2.6	-	100
TE	0.694	3.8	27
PFD	0.413	6.5	16

[A] Milligrams of soluble proteins per milliliter;
[B] Repetition of the method for the isolate to reach 1mg of lyophilized sample;
[C] Yield (related to the proteins isolation mg/mL).

Table 3. Phytochemical Characterization of TE and PFD

Classes	Reactios	Presence	
		TE	PFD
Tannins	*Gelatine*	+	-
	Iron salts	-	-
	Lead Acetate	-	-
Starch	*Iodine*	-	-
Carbohydrates	*Molisch*	+	-
Saponins	*Foam*	+	-
Catechins	*Color (yellow-brown)*	-	-
Flavones, Flavonols and Xanthones	*Color (yellow-orange)*	+	-
Anthocyanins, Anthocyanidins and Flavonoids	*Color (red-purple)*	-	-

(+) Positive reaction, (-) Negative reaction

3. Materials and Methods

Extraction and Quantification of Soluble Proteins

The tubers, previously sanitized, underwent the process of separating the mucilage and obtaining the ultrafine flour (UF), as described by Guerreiro (2002) with adaptations. The obtained UF was subjected to protein extraction in buffer solution Tris-HCl 0.05M pH 8.3, in the proportion of 1:10 (w/v), with subsequent centrifugation at 10,000 rpm. The supernatant obtained (total soluble extract - TE) was filtered on qualitative filter paper and placed on protein precipitation in ammonium sulfate, according to Lin et al. (2009), aiming to obtain the fraction 45-75% (F_{45-75}). This material was dialyzed against distilled water in a cellulose membrane with limit of molecular exclusion of 8 kDa. After dialysis, the sample was lyophilized (freeze dryer Terroni LS 3000) and stored for further analysis. The lyophilized material was denominated Protein Fraction Dioscorine (PFD). The concentration of soluble proteins present in TE was determined according to the method described by BRADFORD (1976), using bovine serum albumin (BSA) as standard.

Phytochemical Analysis

TE and PFD were phytochemically characterized for the purpose of determining the classes of compounds in the biological material under study, according to Mattos (1997) with some adaptations. For each of the secondary metabolites and other constituents analyzed, specific techniques were used, such as: Saponins (foam-stirring test); Tannins (reaction with ferric chloride/lead acetate/gelatin); Carbohydrates (Molisch test); Starch (iodine test); anthocyanins, anthocyanidins and flavonoids (pH variation test/heating); Catechins (reaction with hydrochloric acid/heating), flavonols and xanthones (reaction with granulated magnesium/hydrochloric acid/heating). The presence or absence of these phytochemicals was verified qualitatively from the observation of the expected characteristic reaction.

4. Conclusions

The phytochemical compounds identified in the total extract were: (tannins, carbohydrates, saponins, flavones, flavonols and xanthones); the Protein Fraction Dioscorine is free of such phytochemical compounds. Therefore, the protein fraction Dioscorine had no influence of secondary metabolites on its biological activities, future tests should be carried out to elucidate the bioactive properties of this protein fraction.

Author Contributions

Therefore, the isolated protein fraction of tubers of *D. cayennensis* showed no influence of secondary metabolites on their biological activities, being a target for the synthesis of natural products, considering their biotechnological and pharmaceutical importance.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

STIPANUK, MARTHA H.; CAUDILL, MARIE A. **Biochemical, Physiological, and Molecular Aspects of Human Nutrition-E-Book**. Elsevier health sciences, 2013.

SILVA, MARIA CRISTINA et al. Extração da lectina da folha de mandioca (Manihot esculenta Crantz) eo efeito de cátions divalentes na atividade hemaglutinante. **Ciência e Tecnologia de Alimentos**, v. 30, n. 1, p. 103-107, 2010.

ARMOUR, JULIA C. et al. Protease inhibitors and lectins in soya beans and effects of aqueous heat-treatment. **Journal of the Science of Food and Agriculture**, v. 78, n. 2, p. 225-231, 1998.

ZANONIII, LIDIANE WALTERIII ALENCAR JUNIOR. Desenvolvimento vegetativo em diferentes hastes da planta de mandioca em função da época de plantio. **Ciência Rural**, v. 39, n. 3, p. 657-663, 2009.

DIOP, A.; CALVERLEY, D. J. B. Storage and processing of roots and tubers in tropics. Rome: **Food and Agricultural Organization of United Nations**; Agro-Industries and Postharvest Management Service Agro Supports System Division. 1998. Disponível em: http://www.fao.org/docrep/X5444E/E/x5414e00.htm#contents/ Acesso em: 26 jul. 2016.

RAMOS FILHO, MANOEL MENDES; RAMOS, MARIA ISABEL LIMA; HIANE, PRISCILA AIKO. CHEMICAL EVALUATION OF TARO (Colocasia esculenta L. Schott) CULTIVATED IN THE FLOOD PLAINS OF MATO GROSSO DO SUL PANTANAL REGION (BRAZIL). **Boletim do Centro de Pesquisa de Processamento de Alimentos**, v. 15, n. 2, 1997.

CONLAN, STEVEN et al. Characterisation of the yam tuber storage protein dioscorin. **Journal of plant physiology**, v. 153, n. 1-2, p. 25-31, 1998.

GUERREIRO, LIZIELLE MARIA RICARDO. Avaliação de amidos nativos em condições de estresse adaptados ao processamento de alimentos. 2002. xv, 181 f. Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, 2002.

LIN, PEI-LAN et al. Yam storage protein dioscorins from Dioscorea alata and Dioscorea japonica exhibit distinct immunomodulatory activities in mice. **Journal of agricultural and food chemistry**, v. 57, n. 11, p. 4606-4613, 2009.

BRADFORD, MARION M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry**, v. 72, n. 1-2, p. 248-254, 1976.
AYOOLA, G. A. et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. **Tropical Journal of Pharmaceutical Research**, v. 7, n. 3, p. 1019-1024, 2008.
SPARG, SG; LIGHT, M. E.; VAN STADEN, J. Biological activities and distribution of plant saponins. **Journal of ethnopharmacology**, v. 94, n. 2-3, p. 219-243, 2004.
ROCHA, WESLEY SILVEIRA et al. Compostos fenólicos totais e taninos condensados em frutas nativas do cerrado. **Revista Brasileira de Fruticultura**, v. 33, n. 4, p. 1215-1221, 2011.
PAULINO MACHADO MAGALHÃES, LUCIMÉRI; FERREIRA CAVALCANTI DE ALBUQUERQUE, JULIANNA. Estudo Fitoquímico e Biológico das folhas da dioscorea alata (DIOSCOREACEAE). 2004. Dissertação (Mestrado). Programa de Pós-Graduação em Biotecnologia de Produtos Bioativos, Universidade Federal de Pernambuco, Recife, 2004.