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ORIGINAL ARTICLE

Antioxidant activity and bioactive compounds in ora-pro-nóbis flour (*Pereskia aculeata* Miller)

*Atividade antioxidante e compostos bioativos na farinha de ora-pro-nóbis (*Pereskia aculeata *Miller)*

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Abstract

The ora-pro-nóbis (*Pereskia aculeata* Miller) is a cactus popularly known as "poor man's meat" due to its popular use related to the protein content found in its leaves and its low cost, thus being classified as an Unconventional Food Plant (UFP). The plant is recognized for the high nutritional value of their leaves, and it can be used in cooking and folk medicine. However, studies on the chemical characteristics and antioxidant capacity of ora-pro-nóbis fruits are scarce. This study aimed to evaluate the antioxidant potential and phenolic compounds of the stem and leaf flours and fruit pulp of *P. aculeata* Miller. The stem and leaves were washed and dried in a circulation oven at 60° ± 2 °C, and crushed in an industrial blender. The fruit was washed and macerated *in natura*, then hydroethanolic extracts were prepared. The content of phenolic compounds was evaluated using the Folin-Ciocalteau reagent and the results showed a total phenol content in the stem, leaves and fruits of 0.25; 1.01 and 118.2 mg EAG g^{-1} of extract, respectively. Antioxidants were evaluated using the DPPH method, obtaining values for stem, leaf and fruit of 1.20; 1.40 and 1.50 mg of fruit g^{-1} of DPPH respectively, and also by the ABTS method of stem, leaf and fruit of:13.82; 6.30; 3.20 µmol of Trolox g^{-1}, respectively. Thus, *P. aculeata* Miller presented in its stem, leaves and fruits an expressive amount of phenolic and antioxidant compounds, being a potential resource to contribute to the prevention of several disorders associated with the production of free radicals as well as an alternative in the enrichment of foods and being a useful source for a food additive.

Keywords: Phenolic compounds; Unconventional food plant (UFPs); Principal component analysis (PCA).

Resumo

A ora-pro-nóbis (*Pereskia aculeata* Miller) é uma cactácea popularmente conhecida como "carne de pobre" devido ao seu uso popular pelo teor de proteína encontrado em suas folhas e seu baixo custo, sendo classificada como uma planta alimentícia não convencional (PANC). A planta é reconhecida pelo alto valor nutritivo das folhas, a parte mais utilizada na culinária e na medicina popular. Entretanto, trabalhos sobre as características químicas e a capacidade antioxidante dos frutos

de ora-pro-nóbis são escassos. Objetivou-se com este estudo avaliar o potencial antioxidante e os compostos fenólicos das farinhas do caule e da folha, e da polpa do fruto da *P. aculeata* Miller. O caule e as folhas foram lavados e secos em estufa de circulação a 60°± 2 °C, e triturados em liquidificador industrial. O fruto foi lavado e macerado *in natura* e, em seguida, foram feitos extratos hidroetanólicos. O teor de compostos fenólicos foi avaliado utilizando o reagente de Folin- Ciocalteau e os resultados demonstraram um teor de fenóis totais no caule, nas folhas e nos frutos de 0,25, 1,01 e 118,2 mg EAG g^{-1} de extrato, respectivamente. Os antioxidantes foram avaliados através do método de DPPH, obtendo-se os valores para caule, folha e fruto de 1,20, 1,40 e 1,50 mg de fruta g^{-1} de DPPH, respectivamente, e pelo método ABTS, para caule, folha e fruto, os valores de 13,82, 6,30 e 3,20 µmol de Trolox g^{-1}, respectivamente. Desta forma, a *P. aculeata* Miller apresenta, em seu caule, suas folhas e seus frutos, uma expressiva quantidade de compostos fenólico e antioxidante, sendo um recurso potencial para contribuir para a prevenção de diversos distúrbios associados com a produção de radicais livres e uma alternativa no enriquecimento de alimentos e na alimentação saudável, podendo ser um potencial aditivo alimentar.

Palavras-chave: Compostos fenólicos; Planta alimentícia não convencional (PANC); Análise de componentes principais (ACP).

Highlights

- *Pereskia aculeata* Miller presented an expressive amount of phenolic compounds
- *Pereskia aculeata* Miller has the potential to be used as a source of natural antioxidant for food enrichment

1 Introduction

Among the diversity of species of Unconventional Food Plants (UFPs), *Pereskia aculeata* Miller, popularly known in Brazil as ora-pro-nóbis (OPN), is native to the American continent (Silva et al., 2018) and consumed increasingly more in southeastern Brazil (Dias et al., 2005). The bioactive compounds present in *P. aculeata* Miller are dispersed in all parts of the plant (Moraes et al., 2018). Substances belonging to the group of phenolic acids (Moraes et al., 2020), alkaloids (Pinto et al., 2015) and flavonoids (Moraes et al., 2020; Agostini-Costa et al., 2012) described in the species have been shown antioxidant, antimicrobial (Garcia et al., 2019), anti-inflammatory, emollient, antitumor and expectorant activities (Pinto et al., 2015; Maisuthisakul et al., 2008).

Several studies have reported the mucilage importance of OPN in the preparation of different types of food such as bread, petit suisse cheese, and ice cream, among others (Magalhães et al., 2019; Silva et al., 2021; Santos et al., 2022) in order to increase the protein content of the products. However, it could be noted that few studies of the bioactive compounds found in this species have been undertaken in Brazil (Guimarães et al., 2020). Under these circumstances, it is essential to investigate the chemical composition and bioactivity of our flora. Bioactive compounds can be useful, for example, to combat oxidative stress that is often associated with premature aging and the emergence of several chronic diseases (Oliveira et al., 2017). Garcia et al. (2019) studied the phytochemical profile and some biological activities of OPN leaves (*P. aculeata* Miller), *i.e.*, when detecting the compounds of the hydroethanolic extract (70%, v/v), and observed that caftaric acid represented more than 49% of the phenolic content. In addition, the extract showed antioxidant capacity, antimicrobial activity against Gram-positive and/or Gram-negative bacteria, and no toxicity against the liver primary culture.

Owing to these potential activities, plants rich in phenolic compounds with high antioxidant potential will definitely open up interesting possibilities for the domestic and foreign markets. When having an insight into the antioxidant potential of UFP, such as *P. aculeata* Miller, it can be noted that the dissemination of conscious consumption *in natura* or the use as a component of new food and pharmaceutical products may be improved (Moraes et al., 2021).

Considering the scarcity of works on the different parts of OPN, this study aimed to perform an inedited research on the physicochemical profile and some antioxidant activities of *P. aculeata* Miller with respect to its stem, leaf flours and fruit pulp, in order to expand the current knowledge on the potentialities of this nutrient-rich food (superfood). For this purpose, the hydroethanolic extract of OPN was characterized in terms of phenolic composition and antioxidant capacity.

2 Material and methods

2.1 Experimental conditions and plant materials

The experiment was conducted at the Federal Institute of Goiás, Campus Morrinhos, Goiás, in the food analysis laboratory. The samples of OPN (*P. aculeata* Miller) were collected in the municipality of Morrinhos, at the Federal Institute of Goiás – Campus Morrinhos, from May 2020 to July 2021. Random sampling was used for collecting different samples. Samples were taken from the middle third of the plant (stem, leaves and fruits).

2.2 Obtaining and preparing samples

All parts of the plant were washed in running water and rinsed in distilled water. The stem and leaves were dried at a temperature of 60 °C ± 2 °C, for 12 hours, using an oven with air circulation (Quimis-Q314M Diadema, Brazil) to reduce humidity (< 10%), subsequently they were crushed in a blender (Philco PH 900, Jundiaí, Brazil) to obtain a homogeneous powder. The entire fruit was macerated *in natura* using a mortar and pestle. The fruit pulp and flours obtained were submitted to polyethylene plastic packaging and covered with aluminum foil, covered with aluminum foil, and frozen at -18 °C. Figure 1 shows the steps in this process.

Figure 1. General diagram of the experiment.

2.3 Hydroethanolic extract

The extract was prepared according to Rufino et al. (2007), with modifications. Firstly, 1g of stem and leaf flour was weighed, then diluted to a volume of 40mL of hydroethanolic solvent (70% ethanol and 30% distilled water). For the fruit pulp, 5 g of sample were weighed and put into 40mL of hydroethanolic solvent. These mixtures were homogenized for 15 minutes on an orbital shaker table (SL-180/A) at 100 rpm and then centrifuged (Macro Coleman 80/2) for 15 minutes at 1500 rpm. The supernatant was removed and filtered by using no. 4 filter paper placed in a 100mL flask, and distilled water was added to complete the volume. The extracts were stored in an amber glass until the time of analysis.

2.4 Chemical analysis

2.4.1 Physicochemical characteristics of the flours

Physicochemical characteristics of the OPN flours were determined according to the American Association of Cereal Chemists (2018) methods as follows: moisture (method44-15.02); protein (method 46-13.01); ash (method 08-01.0); Total Titratable Acidity (TTA) (method 02-31.01); and pH (method 02-52.01). Lipids were determined according to the Association of Official Analytical Chemistry (2012) and the carbohydrates by difference were calculated.

2.4.2 Total soluble solids

The Total Soluble Solids (TSS) content was determined by reading the Brix degree in a portable digital refractometer (Atago PAL-1 3810, Brazil), according to the method proposed by the Association of Official Analytical Chemistry (2012) as follows: 1g sample was diluted in 20 mL of distilled water and filtered by using no. 04 filter paper, a few drops were put in the refractometer, the refractive index was read, and the values were expressed in °Brix.

2.4.3 Determination of total phenolic compounds

The content of phenolic compounds in the hydroethanolic extract was determined using a spectrophotometer (Bel UV-M 51) at 750 nm, and a Folin-Ciocalteau reagent, according to Waterhouse (2002). The quantification was based on the establishment of the standard curve of gallic acid, in the range of 5 to 50 mg L^{-1}. Analyses were performed in triplicate and results were expressed in milligrams of Gallic Acid Equivalent (GAE) per gram of sample.

2.4.4 Antioxidant activities

Antioxidant activity was evaluated using two methods:

DPPH: the antioxidant potential was determined by the DPPH method (2,2 diphenyl-1-picrylhydrazyl), according to Rufino et al. (2007). The degree of discoloration of the DPPH radical was obtained using an aliquot of 0.1 mL of the hydroethanolic extract that was transferred into test tubes containing 3.9 mL of the DPPH radical, thus allowing for homogenization in a tube shaker. The reading was made in a dark room and then taken on a spectrophotometer (Bel UV-M 51) at 517 nm.

ABTS: the ability to scavenge the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical was determined according to the method described by Rufino et al. (2007). An aliquot of 30 µL of the hydroethanolic extract was placed into test tubes containing 3.0 mL of the ABTS radical, was subsequently homogenized, left to rest for 6 minutes and then, read in a spectrophotometer (Bel UV-M 51) at 734 nm.

2.5 Statistical analysis

The experiment was carried out according to a completely randomized design. All analytical determinations were performed in the three different tissues (leaf, stem, fruit) in each assay performed. Results were subjected to Analysis of Variance (ANOVA) followed by Tukey's test at 5% probability ($p < 0.05$) using Statistica 7.0 software (STATSOFT, England/Wales). Principal Component Analysis (PCA) was undertaken to identify correlations between the bioactive compounds, and the physicochemical properties of the different parts of the plant (leaf, stem and fruit pulp). The average values for bioactive compounds and physicochemical properties were placed in columns (variables), and the different parts of the plant were placed across the rows (cases). The data were standardized before analysis. The PCA analysis was performed with a correlation matrix and without factor rotation.

3 Results and discussion

Table 1 shows results obtained in the analysis of OPN flour of the moisture and ash, however, it could be noted that there was no significant difference, which was also observed in other studies, when having insight into the contents of moisture varying from 7.38 to 12.46, and contents of ash varying from 12.57 to 25.69 (Almeida et al., 2014; Vargas et al., 2017; Candido et al., 2022; Santos et al. 2022).

The protein levels of OPN were 21.08% for the leaf and 9.56% for the stem (Table 1). These levels were higher than those found by Candido et al. (2022) and Santos et al. (2022), who reported protein contents of 17.7% and 18.7%, respectively. These same authors observed varying levels of protein in OPN flours that ranged between 28.4 and 28.99%. No protein was found in the fruit. Considering the high protein content found in *P. aculeata* Miller, it is noteworthy to mention that leaves can be used in the preparation of salads, soups or its mucilage can substitute eggs in food preparations, which is especially interesting for consumers with food allergies or diet restrictions (Garcia et al., 2019).

The lipids results showed contents varying from 0.38% to 3.04% (Table 1) with significant differences between the results ($p < 0.05$). These levels were lower than those found by Almeida et al. (2014) and similar to those reported by Vargas et al. (2017) and Santos et al. (2022). This parameter may be influenced by seasonality, seeing that low lipid content is important for a low-carb diet or fat-restricted diet (Almeida et al., 2014).

Carbohydrates levels ranged from 62.1% to 9.53% (Table 1). The result for the OPN leaf flour was in line with that reported by Vargas et al. (2017). No results were found for the stem and fruit of OPN.

Table 1. Results obtained from physicochemical characteristics of OPN.

Parameters	Stem Flours[1]*	Leaf Flours[1]*	Fruit Pulp[2]*
Moisture (%)	15.74 ± 0.04^{b}	15.46 ± 0.02^{b}	89.37 ± 0.08^{b}
Ash (%)	11.02 ± 0.01^{a}	12.38 ± 0.12^{a}	0.72 ± 0.03^{b}
Proteins (%)	9.56 ± 0.42^{b}	21.08 ± 0.56^{a}	0.00 ± 0.00^{c}
Lipids (%)	1.58 ± 0.03^{b}	3.04 ± 0.21^{a}	0.38 ± 0.42^{c}
Carbohydrates (%)	62.1 ± 0.24^{a}	48.04 ± 0.34^{b}	9.53 ± 0.27^{c}
Total Acidity (g mL^{-1})	0.74 ± 0.01^{b}	2.04 ± 0.11^{a}	0.11 ± 0.08^{b}
°Brix	0.02 ± 0.00^{c}	0.3 ± 0.04^{b}	0.8 ± 0.02^{a}
pH	6.73 ± 0.01^{a}	5.66 ± 0.03^{b}	6.92 ± 0.02^{a}

*Values presented means ± standard deviation (n=3). Lowercase letters indicate significant differences according to Tukey's test ($p < 0.05$).
[1]Results based on the dry weight. [2]Results based on fresh weight.

As for the TTA, these results showed higher acidity in the leaf flour than in the other parts of OPN. According to Chim et al. (2013), acidity is an important parameter for product quality, in which reactions involved in decomposition, such as hydrolysis, oxidation and fermentation, generate acidic compounds that, consequently, increase the acidity of the medium.

The values of TSS represent acids, salts, vitamins, amino acids, some pectins and sugars present in vegetables (Lima et al., 2001). It was observed that the variable concentration of soluble solids presented results ranging from 0.02 to 0.8 (Table 1), with a higher value in the fruit, which was expected since ripe fruit was harvested (Taiz et al., 2017).

The results of the leaves showed more acid with pH values of 5.66, which fits with the TTA that also showed higher values. This acidity in vegetable results from the presence of organic acids such as citric, malic, lactic and tartaric acids, among others, and this characteristic is related to the acidity of vegetables (Chitarra & Chitarra, 2005; Santos et al., 2017).

The values obtained for phenolic compounds content and antioxidant capacity by the DPPH and ABTS methods are presented in Table 2.

Table 2. Total phenolic compounds (TPC) and antioxidant capacity by DPPH and ABTS methods in hydroethanolic extract (HE) of *Pereskia aculeata Miller*.

SAMPLES	TPC (mg GAE g^{-1})**	DPPH (mg of fruit g of $DPPH^{-1}$)	ABTS (µmol de Trolox g^{-1})
Stem[1]	0.5 ± 0.00^{b}	1.2 ± 0.00^{b}	13.82 ± 0.00^{a}
Leaf Flour[1]	1.01 ± 0.00^{b}	1.4 ± 0.00^{a}	6.30 ± 0.00^{b}
Fruit Pulp[2]	118.2 ± 0.20^{a}	1.5 ± 0.00^{a}	3.20 ± 0.00^{c}

Values constitute mean ± standard deviation (n=3). Lowercase letters indicate significant differences according to Tukey's test ($p < 0.05$). **GAE: gallic acid equivalent.[1]Results based on dry weight. [2]Results based on fresh weight.

According to Table 2, the phenolic compounds content in the stems flour and leaves was significantly lower than in the fruit pulp. In this study, the stem flour obtained values of 0.5 mg GAE, in which the G^{-1} results were lower than those obtained by (Moraes et al., 2020) who obtained average levels of 80.06 mg GAE.g^{-1}. However, these researchers used an aqueous solvent at a high temperature which must have positively influenced greater TPC extraction. The values found in this work for leaf flour were 1.01mg GAE g^{-1} while Freitas et al., (2021) reported 13.84 mg GAE g^{-1} for leaves, nevertheless, a solvent of different polarity was used, as well as time and proportions. Several studies showed a variety of methodologies (Pinto et al., 2015), however, different methods and solvents were used for extraction. Garcia et al. (2019) used the same solvent to accomplish the separation of the compounds used in this work, but not the extraction method. This difference can be explained by the different chemical composition between the species (*P. aculeata* Miller *vs P. bleo*), as well as by differences in the preparation of samples and extracts, and the influence of environmental factors (luminosity, water availability, soil chemical composition and temperature), that can affect the profile of secondary metabolites of these matrices (Moraes et al., 2020).

The fruit showed relevant proportions of TPC in relation to the stem and leaves. The results obtained in this work for fruit pulp were higher than those found by Silva et al. (2018) when studying ripe fruits, they could obtain values of 113.42 mg GAE g^{-1}, *i.e.*, values that corroborate with those found in this assay. Moraes et al. (2021) obtained values of 9.81 mg GAE g^{-1}. It should be noted that the analysis of phenolic compounds can be influenced by the extract used. However, it is known that other types of compounds that may be present in abundance in plant extracts, such as reducing sugars and ascorbic acid, can also reduce the Folin Ciocalteu reagent, therefore resulting in overestimated values of TPC by this method (Sánchez-Rangel et al., 2016).

With respect to the DPPH reduction test, no significant differences were seen between the *P. aculeata* Miller leaves flour and fruit pulp ($p < 0.05$), regardless of the findings of the stems flour. Although, our values obtained quantifications higher than those found by Moraes et al. (2020) for stem tea, which was 0.503 mg/mL, or for other species, such as the Chilean boldo, which obtained 0.430 mg/mL (Souza et al., 2019). Using the same methodology of this assay and different extract, it could be observed that Silva et al. (2018) was unable to detect the antioxidant capacity of OPN. Jardim et al. (2021), when working with a similar extract and a different drying method, obtained average values of 0.032 mg of fruit/g of DPPH, a result lower than that found in this test, which used a more effective method of extraction, thus ensuring better performance of the activity of OPN.

The antioxidant capacity by ABTS showed that the best sample was in the stem flour, followed by the leaf flour and fruit pulp. Freitas et al. (2021), when ONP leaves were used using a solution of glycerin and cereal alcohol, found values of 11.93 µmol of Trolox g^{-1}, and these values were closer to those found in this study. Jardim et al. (2021), by working with ONP and using a similar extract, also obtained similar average values of 12.27 µmol of Trolox.g^{-1}. The literature shows diverging results when different solvents are used in the extraction step, indicating that the most appropriate solvent must be carefully chosen for the selective extraction of natural antioxidants (Queiroz et al., 2014). With relevant levels of antioxidant activity, parts of OPN may be added to food products, possessing them nutraceutical properties.

Principal component analysis revealed that the OPN leaf was characterized by the contents of TTA, proteins, ash and lipids. The stem was characterized by carbohydrate content and antioxidant activity by the ABTS method, while the fruit pulp was characterized by moisture, °Brix, TPC and antioxidant activity by the DPPH method (Figure 2).

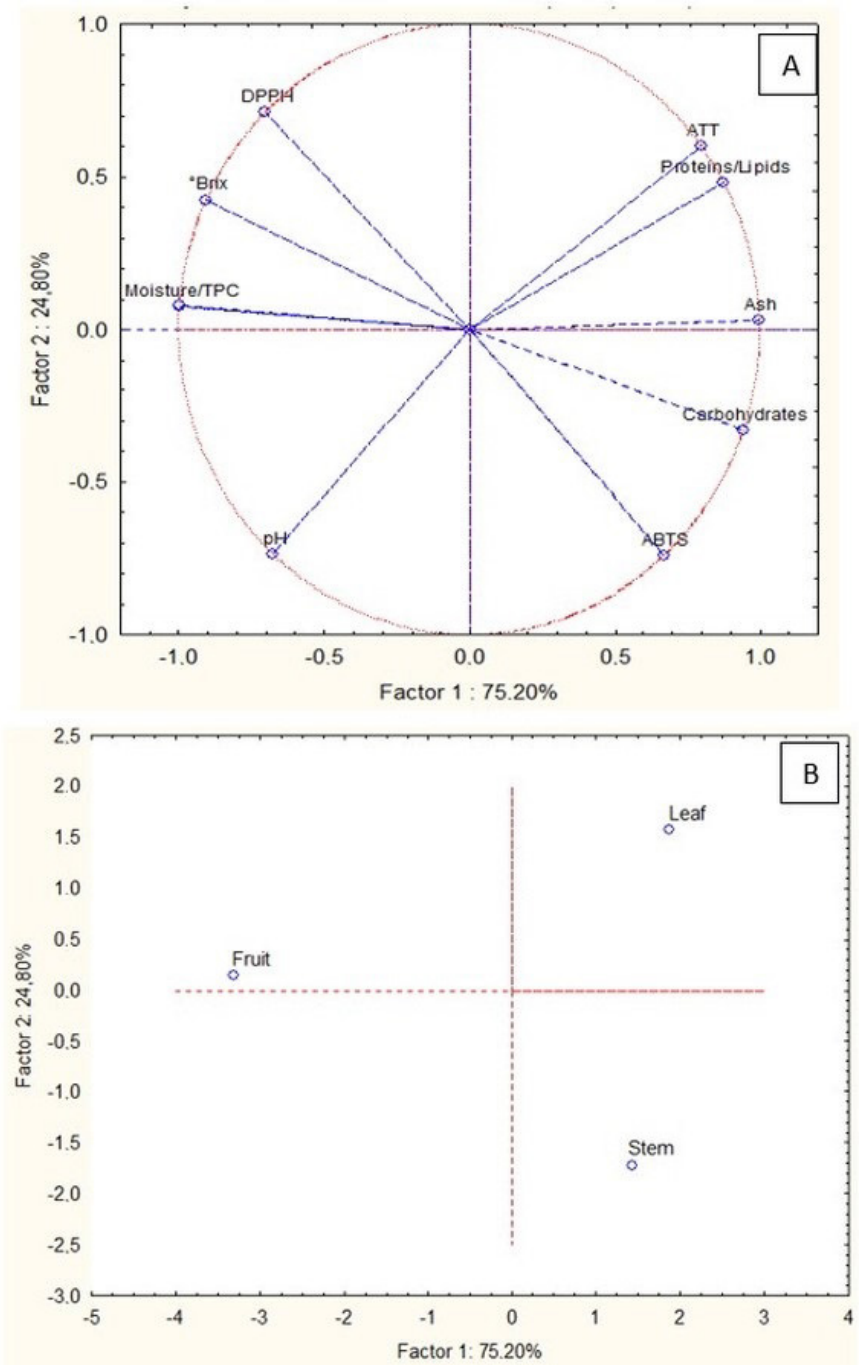

Figure 2. Principal Component Analysis for the ora-pro-nóbis (OPN). (A) physicochemical properties; (B) Samples.

The analyses indicated that TPC was positively correlated with DPPH and °Brix, while protein content was positively correlated with lipids and ash, and negatively with carbohydrate content and ABTS. The pH content did not characterize any part of the plant and was negatively correlated with the moisture content, °Brix, DPPH and TPC.

4 Conclusion

Under the conditions described in this study, the content of phenolic compounds presented a relevant natural source and high antioxidant content was found by the DPPH and ABTS methods when compared to other extracts and methodologies.

Pereskia aculeata Miller (OPN) can contribute to a high potential for human food enrichment and to a source of natural antioxidants. It can be added to school meals, for children with malnutrition and as an extra source of natural antioxidants being added to industrial food products.

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