



Leaf and stem of Ora-pro-Nóbis (*Pereskia aculeata* Mill): Physicochemical composition, biological activities, and gastrointestinal bioaccessibility of protein and antioxidants

Folha e caule de Ora-pro-Nóbis (*Pereskia aculeata* Mill): composição físico-química, atividades biológicas e bioaccessibilidade gastrointestinal de proteínas e antioxidantes

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Pereskia aculeata Mill commonly known as Ora-pro-nóbis (OPN), is an unconventional food plant that thrives in tropical and subtropical climates and is resistant to pests. Despite these advantages, its stems are underutilized due to their thorns and fibrous texture, which limit their consumption and exploration of bioactive compounds. This study aimed to evaluate the physicochemical composition and biological activities of dehydrated OPN stems and leaves, as well as the impact of *in vitro* gastrointestinal digestion on proteins, phenolic compounds (TPC), flavonoids (TFC), and antioxidant activity. Leaf and stem flours exhibited high granulometric uniformity, low water activity (<0.6), and low moisture content (<9%). The flours were also rich in proteins (20% in leaves, 10% in stems), lipids (10-5%), and essential minerals such as potassium (30-20 mg/g), sodium (3 mg/g), and calcium (1-2.4 mg/g). They also demonstrated antibacterial activity (MIC 0.021 mg/mL) for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Stem flour had a high fiber content (70 %) and showed greater inhibition for α -amylase (37.25%) and α -glucosidase (21.53%) enzymes. During digestion retained significant protein amount (leaf/gastric phase (FG): 22%; leaf/intestinal phase (FI): 61%; stem/FG/FI: ~35%), TPC (leaf-stem/FG: 88%-75%; leaf-stem/FI: 60%-75%) and TFC (leaf-stem/FG: 35%-50%; leaf-stem/FI: 22%-16%). Antioxidant activity remained stable, exceeding 100% after the intestinal phase. These findings highlight the strong potential of OPN leaf and stem flours as sources of nutrients and bioactive compounds, offering promising applications in the food and pharmaceutical industries.

Keywords: phenolic compounds, antiglycemic, *in vitro* digestion.

Pereskia aculeata Mill, comumente conhecida como Ora-pro-Nóbis (OPN), é uma planta alimentícia não convencional que prospera em climas tropicais e subtropicais e apresenta resistência a pragas. Apesar dessas vantagens, seus caules são subutilizados devido à presença de espinhos e à textura fibrosa, limitando seu consumo e exploração de compostos bioativos. Este estudo teve como objetivo avaliar a composição físico-química e as atividades biológicas de caules e folhas desidratados de OPN, bem como o impacto da digestão gastrointestinal *in vitro* sobre proteínas, compostos fenólicos totais (TPC), flavonoides totais (TFC) e atividade antioxidante. As farinhas de folhas e caules apresentaram alta uniformidade granulométrica, baixa atividade de água (<0,6) e baixo teor de umidade (<9%). Mostraram-se também ricas em proteínas (20% nas folhas, 10% nos caules), lipídios (10-5%) e minerais, como potássio (30-20 mg/g), sódio (3 mg/g) e cálcio (1-2,4 mg/g). Também apresentaram atividade antibacteriana (CIM 0,021 mg/mL) contra *Pseudomonas aeruginosa*, *Staphylococcus aureus* e *Escherichia coli*. A farinha do caule apresentou elevado teor de fibras (70%) e maior capacidade de inibição das enzimas α -amilase (37,25%) e α -glicosidase (21,53%). Durante a digestão, manteve-se quantidades significativas de proteínas (folha/fase gástrica (FG): 22%; folha/fase intestinal (FI): 61%; caule/FG/FI: ~35%), TPC (folha-caule/FG: 88%-75%; folha-caule/FI: 60%-75%) e TFC (folha-caule/FG 35%-50%; folha-caule/FI: 22%-16%). A atividade antioxidante manteve-se estável, ultrapassando 100% após a fase intestinal. Esses achados destacam o potencial das farinhas de folhas e caules de OPN como fontes de nutrientes e compostos bioativos, com aplicações promissoras nas indústrias alimentícia e farmacêutica.

Palavras-chave: compostos fenólicos, antiglicêmico, digestão *in vitro*.

1. INTRODUCTION

The increasing global demand for functional and nutritious foods reflects growing interest in promoting healthy eating habits and preventing diet-related diseases. In this context, unconventional vegetables present promising natural sources of bioactive compounds and nutrients [1], with low or no toxicity, offering potential health benefits [2].

Among the vegetables, *Pereskia aculeata* Mill., commonly known as ora-pro-nóbis (OPN), stands out as an easy-to-grow option, adaptable to tropical and subtropical climates, and resistant to pests. Its broad, smooth, lanceolate, and succulent leaves are rich in protein, iron, and folic acid [3, 4]. The OPN stem has short, curved spines (false spines) and contains fiber [1]. Both the leaves and stem exhibit antimicrobial, anti-inflammatory, antitumor, antioxidant, and healing properties [1, 4-6].

A clinical study found that the consumption of OPN flour by overweight men resulted in significant improvements in gastrointestinal symptoms and feelings of satiety [7]. Bioactive compounds present in plants like OPN have also shown beneficial effects on regulating glucose metabolism and improving insulin sensitivity [8]. Furthermore, due to its high protein content, OPN has become an attractive option for individuals seeking alternatives to animal-derived proteins [9]. However, not all proteins in food are absorbed and utilized efficiently to meet the body's nutritional needs.

Silva et al. (2021) [10], found that approximately 90% of the protein content in the leaves, seeds, and inflorescences of *Amaranthus viridis* was potentially absorbable. In contrast, lower protein digestibility (55%) was observed in commercially available soy and pea burgers, compared to 63% for beef burgers [11]. Several factors, such as protein structure and the presence of antinutritional components like fiber and phytates in plant-based foods, can affect nutrient digestibility. Despite these advancements, OPN stems remain underutilized due to their spines and fibrous consistency, limiting their consumption and exploration for phytochemical compounds and bioactive properties [2, 5, 6]. Furthermore, to date, no study has reported the potential of OPN in inhibiting digestive enzymes.

Therefore, the objective of this study was to evaluate the physico-chemical composition and biological activities of the dehydrated stem and leaf of OPN and the impact of *in vitro* gastrointestinal digestion on proteins, total phenolic compounds, total flavonoids and antioxidant activity.

2. MATERIAL AND METHODS

2.1 Materials

Ora-pro-nóbis (*Pereskia aculeata* Mill.) was collected in the Diamantina region (18°14'17"S, 43°36'40"W), Brazil, in August 2022. The leaves and stems were separated and washed under running water. After drying for approximately 48 h until constant weight was achieved in an oven with air circulation at 50 ± 5 °C, the samples were ground using a Willy Macro knife mill (Sv-1) equipped with a 20-mesh sieve. The leaf and stem flours were then separately packaged and stored at -15 ± 2 °C until analysis.

The plant material was registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number AFB3E80. A voucher specimen was prepared and deposited at the Herbarium of the Municipal Botanical Museum of Curitiba under accession number 190589.

2.2 Physicochemical Composition

The color of ora-pro-nóbis flours (leaf and stem) was evaluated according to the CIELab* parameters using a colorimeter (Brand: HunterLab, Model: MiniScan EZ 4000, Virginia, USA). The L* value represents lightness (ranging from 0 for black to 100 for white), and the a* and b* values represent chromaticity (where +a* is red, -a* is green, +b* is yellow, and -b* is blue). The

following parameters were calculated: Hue angle $h^\circ = \tan^{-1}(b^*/a^*) + 180^\circ$ for $a^* < 0$, or $h^\circ = \tan^{-1}(b^*/a^*)$ for $a^* > 0$, and Chroma $C^* = (a^2 + b^2)^{1/2}$ [12].

The particle size profile was determined using a set of vibrating sieves with progressively decreasing mesh openings (100, 80, 60, 32, 20, 9). Samples (50 g) were placed on the sieve with the largest opening and subjected to constant agitation for 10 minutes in an electromagnetic shaker (Bertel, Brazil, 110 V). The fractions retained on each sieve were weighed, and the percentage of material (%) was calculated [13].

Water activity (Aw) was measured using an AquaLab 3TE (Decagon, Washington). Moisture (AOAC 925.09), ash (AOAC 923.03), protein (AOAC 960.52), and total fiber (AOAC 991.43) were analyzed according to standardized official methods [14, 15]. Lipid content was determined by the Bligh and Dyer method [16]. Reducing and total sugars were quantified by the Somogyi-Nelson method [17]. Carbohydrates were calculated by difference.

Mineral analysis was performed using an X-ray fluorescence spectrometer (Model EDX 720, Shimadzu®; Kyoto, Japan), equipped with an X-ray tube, liquid nitrogen for cooling, and PCEDX software (version 1.11 Shimadzu®). The samples, previously dried for 24 hours in a muffle furnace at 150 °C, were placed in polypropylene film sample holders. The analysis conditions included a 10 mm collimator, scanning voltages of 0 - 40 keV (Ti-U) and 0 - 20 keV (Na-Sc), with a 100-second scan for each sample.

2.3 Preparation of Extracts

Aqueous (SW and LW) and hydroethanolic (S12, S18, L12 and L80; v/v) extracts of the vegetative parts (stem and leaf, respectively) of ora-pro-nóbis were prepared following the method described by Andrade et al. (2022) [18]. For each extraction, 1 g of the sample, 6 mL of solvent (distilled water or ethanol at 12% and 80%, v/v), was added, followed by vortexing for 30 seconds and incubation in an ultrasonic bath (Unique, model USC-2850A, Campinas, São Paulo, Brazil) for 15 minutes at 25 ± 2 °C. The extracts were centrifuged at 2500 rpm for 10 minutes at 25 °C, and the supernatants were collected. The solid phase underwent a second extraction, and the supernatants were combined, filtered, and stored at -10 ± 5 °C for later analysis.

2.4 Total phenolic compounds, total flavonoids and biological activity

Total phenolic compounds (TPC) were determined following the methodology of Singleton and Rossi [19] and total flavonoids (TFC) were quantified according to Zhishen et al. (1999) [20]. Antioxidant activity was assessed using the reduced iron scavenging assay (FRAP) as described by Benzie and Strain [21], and the DPPH radical capture method as outlined by Brand-Williams et al. (1995) [22]. The extracts were evaluated in 96-well microplates following the modifications proposed by Ávila et al. (2022) [23], and readings were taken using a microplate reader (Multiskan™ Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan) at wavelengths of 690 nm (TPC), 540 nm (TFC), 620 nm (FRAP), and 540 nm (DPPH). Results were expressed as mg of gallic acid equivalent (GAE)/100 g for TPC, mg of catechin equivalent (CE)/100 g for TFC, and antioxidant activity was expressed as mmol of Trolox equivalent (TE)/100 g of OPN flour.

The *in vitro* antibacterial activity was evaluated following the methodology described by Ávila et al. (2019) [24]. Standard strains of three bacterial species were included: two Gram-negative strains (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 8739) and one Gram-positive strain (*Staphylococcus aureus* ATCC 25923). Serial dilutions of the extracts were prepared in Müller-Hinton Broth, ranging from 0.1666 to 0.0013 mg/mL.

Gentamicin was used as a positive control. The extracts were used without prior solvent removal. To control for the potential antimicrobial effect of the extraction solvents, water and ethanol solutions (12% and 80%, v/v) were included as negative controls. The ethanol used was analytical grade (96 °GL) and diluted to the required concentrations. Dilutions were prepared to maintain equivalent final solvent concentrations between samples and controls. Extract concentrations showing inhibitory effects comparable to those of the solvent controls were not considered indicative of antibacterial activity. A 5% (v/v) methanolic solution of

2,3,5-triphenyltetrazolium chloride (TTC) was added to visually confirm bacterial growth. The Minimum Inhibitory Concentrations (MIC) were expressed in $\mu\text{g/mL}$ of the extract.

The α -amylase inhibitory activity was measured using the starch-iodine method, as described by Van Quan et al. (2019) [25], with modifications. In a 96-well microplate, 20 μL of each leaf and stem flour extract (LW, L12, L80, SW, S12 and S80) at concentrations ranging from 0.02 to 0.5 mg/mL (0.02, 0.05, 0.26, 0.4, and 0.5 mg/mL), were pre-incubated with 20 μL of α -amylase solution (1 U/mL) at 37 °C for 10 minutes. Then, 30 μL of starch solution (0.5%) was added. After 6 minutes of incubation at 37 °C, 20 μL of hydrochloric acid (1 M) and 120 μL of 0.25 mM aqueous iodine solution were added. The absorbance was measured at 570 nm.

The α -glucosidase inhibitory activity was evaluated in a microplate as described by Wang et al. (2021) [26]. A mixture containing 50 μL of the extracts, at concentrations ranging from 0.029 to 0.111 mg/mL (0.029, 0.036, 0.046, 0.057, and 0.111 mg/mL), 50 μL of potassium phosphate buffer (0.1 M, pH 7.0), and 100 μL of α -glucosidase (1 U/mL) was incubated for 15 minutes at 37 °C. Then, 50 μL of p-nitrophenyl- α -D-glucopyranoside (pNPG – 3 mg/mL) substrate was added, and the mixture was incubated for 45 minutes at 37 °C. The absorbance was measured at 405 nm. The percentage of enzyme inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{((\text{Abscontrol}) - (\text{Abs sample} - \text{Absblank}))}{\text{Abscontrol}} \times 100$$

Where: Abscontrol represents the absorbance of the phosphate buffer + enzyme; Abs sample represents the absorbance of the extract + enzyme; and Absblank represents the absorbance of the extract + phosphate buffer. Acarbose was used as the positive control, while water and ethanol (12% and 80%, v/v) were used as the negative controls.

2.5 In Vitro Gastrointestinal

The *in vitro* gastrointestinal digestion of the flours was carried out according to the Infogest protocol [27], with adaptations proposed by Silva et al. (2021) [28]. The process consisted of three phases: oral, gastric, and intestinal. In each experiment, 0.5 g of flour was mixed with 2.5 mL of salivary fluid and 3 mL of extract. During the oral phase, the pH was adjusted to 7.0 using 1 M NaOH, and water was added to bring the total volume to 6 mL. The mixture was incubated in a water bath at 37 °C with agitation for 2 minutes. In the gastric phase, the pH of the mixture was adjusted to 3.0 using 1 M HCl, and water was added to bring the total volume to 12 mL. The mixture was incubated at 37 °C for 2 hours to simulate gastric digestion. The intestinal phase began with the adjustment of the pH to 7.0 using 1 M NaOH, followed by the addition of water to achieve a total volume of 24 mL. The mixture was incubated again at 37 °C for 2 hours. Both the gastric and intestinal phases were terminated by immersion in an ice bath.

The solutions were centrifuged at 2500 rpm for 10 minutes, and the supernatants were collected, frozen, and lyophilized for subsequent analysis of proteins, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using the FRAP and ABTS assays. Bioaccessibility (%) was defined as the proportion of a compound released during the simulated digestion process relative to its initial content in the undigested sample.

2.6 Statistical Analysis

The normality of data was verified using the Shapiro-Wilk test, while homogeneity of variance was assessed using the Brown-Forsythe test. Results were presented as the mean $\pm$ standard deviation. A one-way ANOVA was applied to determine significant differences for each dependent variable. For data with non-homogeneous variances ($p < 0.05$), the Kruskal-Wallis test was used. When significant differences were observed between the leaf and stem, a t-test was conducted to compare the means. The Tukey test was employed to evaluate differences among treatments ($p < 0.05$). Pearson's correlation analysis was used to assess associations between pairs of variables.

3. RESULTS AND DISCUSSION

The color of ora-pro-nóbis flour varied depending on the plant's vegetative part. The luminosity (L^*) values and the chromaticity parameters a^* and b^* indicated that the leaf flour exhibited a color closer to black and green with a less intense yellow hue. In contrast, the stem flour showed a tendency toward white, green, and yellow tones, respectively. The chroma (C^*) values were near zero, reflecting a neutral coloration characteristic of white or gray. The hue angle (h°) results pointed to a yellowish tint, with angles close to 90° (Table 1). The natural green color of OPN leaves offers a potentially healthy alternative to artificial colorants commonly used in food and beverages. Meanwhile, flour from stems can impart a subtle green tint to food products. Furthermore, the dehydrated leaves and stems present a gluten-free option for replacing traditional flours, such as wheat, in food preparation [29].

Table 1. Physicochemical characterization of dehydrated ora-pro-nóbis leaf and stem flours (*Pereskia aculeata* Mill.).

Parameters	Leaf	Stem
L^*	32.96 ± 0.36^b	65.18 ± 3.29^a
a^*	0.10 ± 0.13^b	0.72 ± 0.12^a
b^*	16.21 ± 0.29^b	27.22 ± 1.15^a
C^*	16.22	27.74
h°	89.65	88.48
Fineness Modulus	0.03 ± 0.01^a	0.02 ± 0.01^b
Mean Geometric Diameter	106.62 ± 0.04^a	105.39 ± 0.02^b
Water activity	0.57 ± 0.01^a	0.47 ± 0.01^b
g/100g		
Moisture	8.39 ± 0.10^a	5.94 ± 0.13^b
Ash	22.45 ± 0.07^a	7.21 ± 0.07^b
Protein	21.26 ± 0.53^a	10.70 ± 0.22^b
Lipids	10.14 ± 0.96^a	4.47 ± 0.06^b
Total fiber	17.52 ± 0.01^b	69.72 ± 0.87^a
Reducing sugar	0.09 ± 0.01^b	0.16 ± 0.01^a
Total sugar	0.71 ± 0.03^b	1.21 ± 0.03^a
Carbohydrate	19.44	0.59
mg/g		
Potassium (K)	29.27 ± 0.01^a	19.96 ± 0.01^b
Sodium (Na)	3.22 ± 0.01^a	3.14 ± 0.01^b
Calcium (Ca)	1.07 ± 0.01^b	2.40 ± 0.01^a
Phosphorus (P)	0.79 ± 0.01^b	1.93 ± 0.01^a
Iron (Fe)	0.03 ± 0.01^b	0.06 ± 0.01^a
Magnesium (Mg)	0.03 ± 0.01^b	0.06 ± 0.01^a

Different letters in each row indicate significant differences according to the t-test ($p < 0.001$). Proximate composition values are expressed as g/100 g of flour and mineral contents as mg/g. Results are based on analytical determinations and expressed on a flour basis. Values reported in the discussion for 30 g servings were calculated proportionally from these data. Values are presented as mean $\pm$ standard deviation ($n = 3$). L^* represents lightness, while a^* and b^* denote chromatic parameters, with a^* spanning from red ($+a^*$) to green ($-a^*$) and b^* ranging from yellow ($+b^*$) to blue ($-b^*$). C^* represents color saturation, and h° corresponds to the hue angle.

An additional noteworthy aspect is the high particle size uniformity observed in both the leaf and stem samples, which enhances the sensory quality of the product. This characteristic is highly valued in the food and pharmaceutical industries, as it influences texture, bioavailability, dissolution, and product consistency. Consequently, particle size uniformity is often considered a critical quality control attribute and plays a key role in the development of new products [13, 30]. The stem flour showed a lower proportion of fine particles, resulting in a reduced fineness modulus and mean geometric diameter.

The fresh leaves and stems of ora-pro-nóbis had moisture contents of $76.94 \pm 1.78\%$ and $76.77 \pm 3.24\%$, respectively. After dehydration, moisture decreased to 8.39% in leaf flour and

5.94% in stem flour (Table 1), corresponding to reductions of approximately 9.2-fold and 12.9-fold, respectively. The low water activity (<0.6) and low moisture content ($<9\%$ for leaves and $<6\%$ for stems) in the resulting flours (Table 1), are advantageous for inhibiting microbial growth and improving the stability and shelf life of these by-products [31]. Additionally, the leaves demonstrated a significantly higher ash content (22.45 g/100 g) compared to the stems (7.21 g/100 g), indicating a greater mineral concentration [28].

Proteins accounted for over 20% of the leaf mass and more than 10% of the stem mass in OPN. The leaves also contained approximately twice the lipid content of the stems. Conversely, the stems exhibited four times the fiber content and higher quantities of total and reducing sugars. These findings align with Silva et al. (2023) [3], who reviewed 241 scientific articles on *Pereskia*. The protein content in OPN leaves ranged from 14% to 29%, lipids from 4% to 16%, carbohydrates from 29% to 32%, soluble fibers from 2% to 5%, and insoluble fibers from 19% to 34%.

Fiber from the leaves provides 20% of the recommended dietary reference intake (DRI) for adult women and 13% for men, while fiber from the stem meets 84% of the DRI for women and 55% for men. In this context, leaf flour serves as an excellent source of protein for individuals pursuing plant-based diets. Additionally, the dehydrated leaves and stems are rich in fiber and low in fat, with 3.04 g of lipids per 30 g serving of leaf flour and 1.34 g per 30 g serving of stem flour.

According to the guidelines for healthy adults aged 19 to 50, daily protein requirements are approximately 1 g per kg of body weight, while fiber needs are 25 g for women and 38 g for men. A 30 g serving of leaf flour provides 6.4 g of protein and 5 g of fiber, whereas the same serving of stem flour offers 3.0 g of protein and 21 g of fiber. These values show that 30 g of leaf flour provides 10% of the protein DRI for an adult weighing 65 kg, while the same amount of stem flour contributes 5%.

Potassium emerged as the most abundant nutrient, with 29 mg/g in the leaves and 20 mg/g in the stems, Table 1. Potassium plays a vital role in nervous system function, muscle contractions (including the heart), and blood pressure regulation [32]. Calcium, crucial for bone and tooth health, blood clotting, muscle function, and nerve impulse transmission [33], was present in higher quantities in the stems (2 mg/g). Sodium levels were similar in both plant parts, while iron and magnesium were found in smaller amounts.

Analysis of OPN leaf flour extracts, using water, 12% ethanol, and 80% ethanol as extraction agents, revealed concentrations of TPC, TFC, and antioxidant activity in leaf extracts that were approximately twice as high as those in stem extracts (Figures 1g and h). These findings for the leaves align with previously reported values for Soxhlet and supercritical fluid extractions [34].

Prior studies have identified over eleven phenolic compounds, organic acids, flavonoids, and terpenes in OPN leaves. These compounds have been linked to several biological activities beneficial to human health, including antioxidant, antihemolytic, and antimicrobial effects, as well as the inhibition of lipid peroxidation, recovery of inflammatory markers, and anticholinergic properties [4, 34, 35]. In contrast, the stem of the plant serves primarily structural and transport functions. Its resources are focused on forming conductive tissues such as xylem and phloem, along with supportive cells. These structures are specialized for substance conduction rather than the storage or production of bioactive compounds [36].

As the ethanol concentration in the extraction increased, higher TPC, TFC, DPPH, and FRAP values were observed for both the leaves and stems of the OPN plant. The 80% ethanolic extracts proved most effective in recovering antioxidant compounds (Figure 1h). However, the aqueous extract (LW) was more efficient than the 12% ethanolic extract (L12) for extracting TPC from the leaves.

For the stems, no significant difference in TPC was observed between the aqueous extract (SW) and the 12% ethanolic extract (S12). Similarly, for both leaves and stems, TFC values showed no significant differences between the aqueous and 12% ethanolic extracts (LW and L12; SW and S12). These variations among extracts can be attributed to water's solubilizing properties for certain phenolic compounds and the distinct chemical structures and characteristics of the compounds present in different parts of the plant. These factors influence the extraction efficiency of phenolic and flavonoid compounds [36].

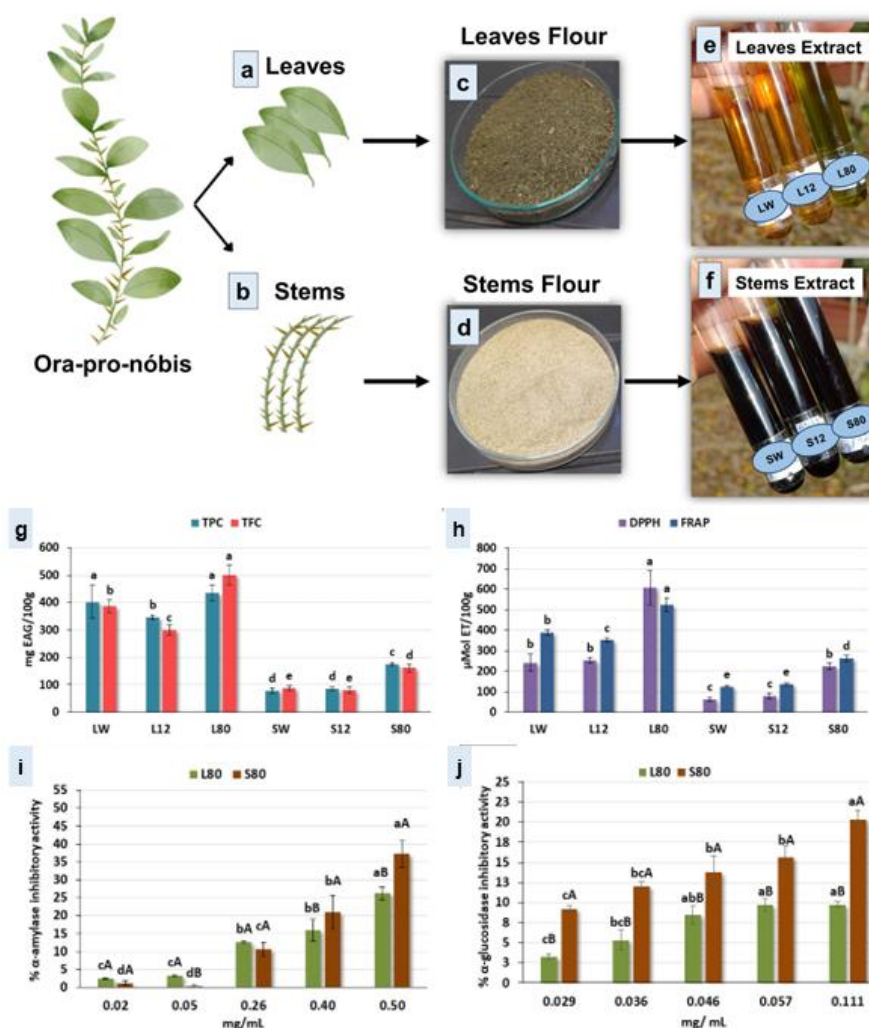


Figure 1: Overview of Ora-pro-nobis: a) Fresh leaves, b) Fresh stems, c) Leaf flour and d) Stem flour; e) Leaf extracts and f) Stem extracts with water LW, ethanol 12% L12, and L80 ethanol 80% L80; g) Total phenolic content (TPC), total flavonoid content (TFC), and h) Antioxidant activity by DPPH and FRAP assays; i) α-amylase and j) α-glucosidase inhibitory activity. Columns with different lowercase letters are significantly different between each sample or concentration according to Tukey's test ($p < 0.05$). Columns with equal capital letters in the columns are statistically equal (L80 and S80) according to the t-test ($p < 0.05$).

Only the 80% ethanol extracts from both the leaves and stems demonstrated efficacy in inhibiting all three tested bacterial strains (*P. aeruginosa*, *S. aureus*, and *E. coli*) with a minimum inhibitory concentration (MIC) of 0.021 mg/mL. This performance was comparable to that of the antibiotic gentamicin, which showed MICs of 0.004 mg/mL for *P. aeruginosa*, 0.0009 mg/mL for *S. aureus*, and 0.002 mg/mL for *E. coli*.

Similarly, Bussmann et al. (2010) [37] reported MIC values ranging from 0.008 to 256 mg/mL for ethanolic and aqueous extracts of 141 medicinal plant species, highlighting the superior antibacterial activity and broader spectrum of ethanolic extracts compared to aqueous ones. These findings are consistent with studies evaluating the antibacterial activity of ethanolic extracts from OPN leaves, which demonstrated effectiveness against *P. aeruginosa*, *S. aureus*, and *E. coli* [35, 38]. Notably, this is the first study to document antibacterial activity in ethanolic extracts of OPN stems.

The antibacterial and antioxidant activities of these extracts are associated with the presence and concentration of total phenolic and flavonoid compounds. A significant positive Pearson correlation ($p < 0.05$) was found between TPC and TFC values ($r = 0.98$) and between TPC and antioxidant activity as measured by DPPH and FRAP assays ($r = 0.81$ and 0.96 , respectively).

Additionally, DPPH and FRAP results were strongly correlated ($r = 0.93$), confirming the consistency of these complementary methods for evaluating antioxidant activity.

Beyond antibacterial and antioxidant properties, the ethanolic extracts also inhibited the enzymes α -amylase (Figure 1i) and α -glucosidase (Figure 1j). The stem extract (S80) demonstrated significantly higher inhibition of α -amylase (37.25%) and α -glucosidase (21.53%) at concentrations of 0.50 mg/mL and 0.11 mg/mL, respectively, compared to the leaf extract (L80). This enzymatic inhibition delays carbohydrate digestion and absorption in the digestive tract, promoting more stable postprandial blood glucose levels. These findings align with Thengyai et al. (2020) [39], who highlighted the potential of plant-based compounds, similar to OPN, in regulating postprandial glycemia, mitigating side effects, and preventing type 2 diabetes.

Protein content is influenced not only by its initial concentration in the source material but also by the extent to which the body can utilize this protein following digestion, referred to as protein bioaccessibility. During the gastric phase of digestion, both leaf and stem flours exhibited a reduction in their initial protein content. However, the bioaccessibility of proteins was lower for the leaf flour compared to the stem flour. Only about 22% of the total proteins in the leaf were bioaccessible during this phase, whereas the stem flour achieved a higher value of approximately 36% (Figure 2a).

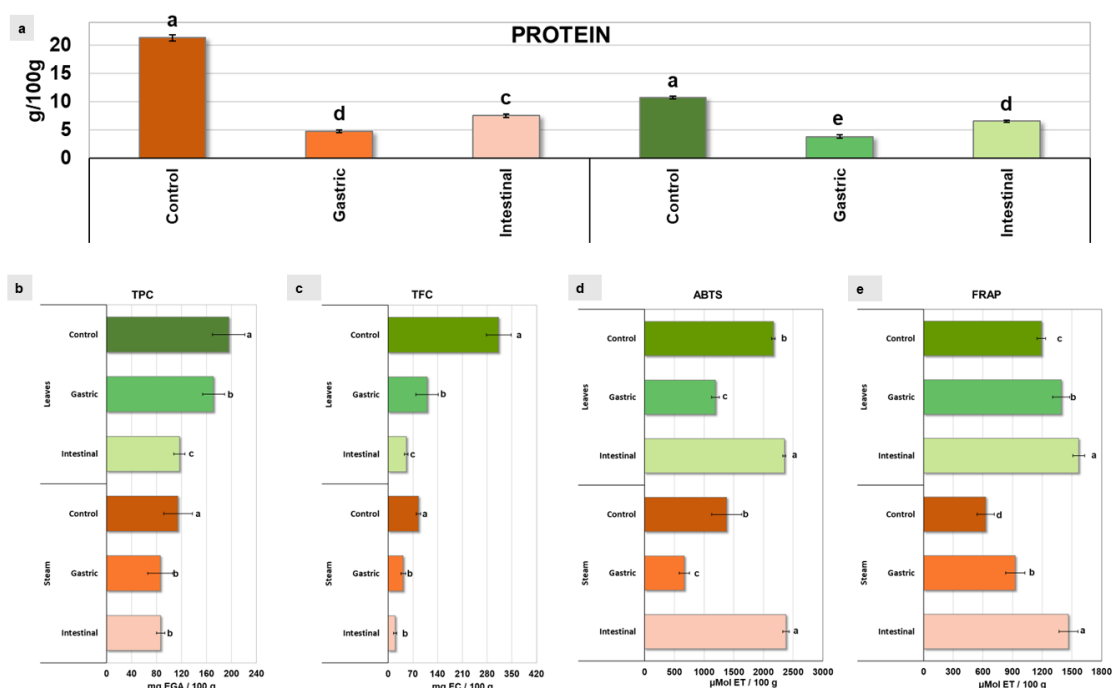


Figure 2: a) Protein content in dehydrated leaf and stem flours before and after the gastric and intestinal digestion phases. Letters b, c, d and e represent the total phenolic compounds (TPC), total flavonoid content (TFC), and antioxidant activity (measured by ABTS and FRAP assays) of leaf and stem flour before and after the gastric and intestinal phases. *Data are presented as mean values $\pm$ standard deviation ($n = 3$). For individual bars, mean values with different letters indicate a significant difference ($p \leq 0.05$) between undigested samples (control) and those subjected to the gastric and intestinal digestion phases for leaf and stem flours.

A similar pattern was observed in a comparative study evaluating the nutritional quality of proteins from *Moringa oleifera* leaves and seeds. After enzymatic hydrolysis using pepsin-pancreatin, the seed flour demonstrated significantly higher protein digestibility (61%) compared to the leaf flour (57%). This suggests that factors such as the structural composition and presence of anti-nutritional compounds might influence protein bioaccessibility across different plant parts [40].

During the intestinal phase, the amount of protein released and digested was significantly higher for the leaf, indicating that the protein structure in the leaf is more accessible and easier

for digestive enzymes to break down. Approximately 61% of the proteins in the leaf flour were accessible and digestible during this phase. In contrast, the stem exhibited a pattern like the gastric phase, with only 35% protein bioaccessibility in the intestinal phase (Figure 2a).

These findings suggest that the proteins in the leaves are more likely to be utilized by the body during digestion compared to those in the stem. This difference may be attributed to variations in the structure or composition of the proteins in each part of the plant, as well as potential interactions with other components in the food matrix (e.g., higher fiber content in the stem) that may inhibit the breakdown and absorption of proteins during digestion [41].

The bioaccessibility of phenolic compounds in the leaf reached 88% after the gastric phase (171 mg EGA/100g) and 60% after the intestinal phase (117 mg EGA/100g) (Figure 2b). In the stem, the TPC content did not show significant differences between the phases, but it was higher than in the leaf during the intestinal phase, with 75% bioaccessibility (85 mg EGA/100g). Similarly, flavonoids showed greater bioaccessibility in the gastric phase, with 35% for the leaf (110 mg EC/100g) and 50% for the stem (43 mg EC/100g), which is double the amount observed in the intestinal phase (22% or 51 mg EC/100g for the leaf and 16% or 19 mg EC/100g for the stem) (Figure 2c). The phenolic compounds in the leaves may be more complex or bound to other molecules like fibers or proteins, making them less accessible to digestive enzymes in the stomach and reducing their hydrolysis, release, and availability during the gastric phase.

Antioxidant activity did not follow the same pattern as TPC and TFC. Initially, the control sample antioxidant activity was 2167 $\mu\text{mol ET}/100\text{g}$ (ABTS) and 1191 $\mu\text{mol ET}/100\text{g}$ (FRAP) for the leaf, while the stem showed values of 1381 $\mu\text{mol ET}/100\text{g}$ (ABTS) and 628 $\mu\text{mol ET}/100\text{g}$ (FRAP). During the gastric phase, antioxidant activity remained relatively stable, with about 50% retention in ABTS for both parts, and an increase of 17% for the leaf and 48% for the stem in FRAP activity. After the intestinal digestion phase, a significant increase in antioxidant activity was observed for both the leaf and stem, with the stem demonstrating the highest bioaccessibility. The ABTS values in the intestinal phase increased to 2351 $\mu\text{mol ET}/100\text{g}$ (an 8% increase) for the leaf and 2379 $\mu\text{mol ET}/100\text{g}$ (a 72% increase) for the stem. Similarly, FRAP values increased to 1570 $\mu\text{mol ET}/100\text{g}$ (a 32% increase) for the leaf and 1465 $\mu\text{mol ET}/100\text{g}$ (a 133% increase) for the stem (Figure 2d and 2e).

This enhancement corresponds to an increase of more than 100%, highlighting a marked improvement in antioxidant bioaccessibility. The more alkaline pH of the intestine compared to the stomach may facilitate the release of antioxidants, as some molecules are more stable in less acidic environments. These results suggest that while the bioaccessibility of bioactive compounds can vary throughout the digestive process, antioxidant activity does not follow a linear trend relative to bioaccessibility. Additionally, during digestion, some molecules may be biotransformed by intestinal digestive enzymes (pancreatin and protease activity) on the complex food matrix, facilitating the release of compounds with antioxidant activity bound to the matrix [42-44].

4. CONCLUSION

The flours demonstrated a high concentration of proteins, essential minerals, and phenolic compounds with antioxidant activity. Stems flour is rich in fiber and showed greater enzymatic inhibitory activity against α -amylase and α -glucosidase. Both flours exhibited significant antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, and retained a significant proportion of proteins, phenolic compounds, and antioxidant activity after *in vitro* digestion. These findings highlight the potential of *Pereskia aculeata* leaf and stem flour for applications in the food and pharmaceutical industries as functional ingredient, as flour or additive into food development. The study also reinforces the value of exploring non-conventional plants from Brazilian biodiversity. However, further research is needed to characterize the bioactive compounds of *Pereskia aculeata*, their interactions with other components, and to validate their safety and efficacy through *in vivo* studies.

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