CARACTERIZAÇÃO FÍSICO-QUÍMICA DE FOLHAS E ATIVIDADE ANTIOXIDANTE DE EXTRATOS DE *PERESKIA ACULEATA* MILL. OBTIDOS POR ULTRASSOM

Physico-chemical characterization of leaves and antioxidant activity of *Pereskia aculeata* mill. Extracts obtained by ultrasound

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RESUMO: *Pereskia aculeata* Mill., popularmente conhecida como ora-pro-nobis e "carne-de-pobre", devido ao teor de proteína das folhas, é considerada planta alimentícia não-convencional (PANC). O trabalho teve como objetivo a caracterização físico-química das folhas de *P. aculeata* e a avaliação da atividade antioxidante e conteúdo de compostos fenólicos em seu extrato. As folhas foram liofilizadas e realizada a caracterização físico-química determinando umidade, proteínas, cinzas, lipídeos e minerais. Para avaliação do potencial antioxidante e dos compostos fenólicos totais foi realizada a extração assistida por ultrassom durante 1 hora a 40, 60 e 80ºC, em água. Nas folhas de *P. aculeata* foram encontradas quantidades significativas de proteínas (18,56%) e minerais (15,17%), principalmente cálcio (8.012,83 mg/100g) e potássio (10.693,80 mg/100g) e baixas concentrações de lipídeos (4,28%) e umidade (1,32%). A extração de 1 hora em ultrassom (40 kHz, 132 W) a 60ºC, foi suficiente para obter extratos com potencial antioxidante (0,291 mg/mL) e alta concentração de compostos fenólicos totais (4,209 mgEAG/100g). Os resultados referentes ao teor de proteína e compostos com propriedades antioxidantes indicam a utilização das folhas de *P. aculeata,* tanto para a alimentação *in natura*, como para uso de seus extratos em formulações de alimentos industrializados para aumento de seu valor nutricional.

Palavras-chave: Ora-pro-nobis. Liofilização. Proteína. Antioxidante. Fenólicos.

ABSTRACT: *Pereskia aculeata* Mill., popularly known as ora-pro-nobis and "poor meat", due to the protein content of the leaves, is considered a non-conventional food plant (UFPs). The objective of this work was the physicochemical characterization of *P. aculeata* leaves and the evaluation of the antioxidant activity and content of phenolic compounds in its extract. The leaves were lyophilized, and physical-chemical characterization was performed, determining moisture, proteins, ash, lipids and minerals. In order to evaluate the antioxidant potential and total phenolic compounds, ultrasound-assisted extraction in water was performed for 1 hour at 40, 60 and 80ºC. In *P. aculeata* leaves, significant amounts of proteins (18.56%) and minerals (15.17%) were found, mainly calcium (8,012.83 mg/100g) and potassium (10,693.80 mg/100g) and low concentrations of lipids (4.28%) and moisture (1.32%). The 1- hour ultrasound extraction (40 kHz, 132 W) at 60ºC was sufficient to obtain extracts with antioxidant potential (0.291 mg/mL) and high concentration of total phenolic compounds (4.209 mgGAE/100g). The results regarding the protein content and compounds with antioxidant properties indicate the use of *P. aculeata* leaves both as *in natura* food and the use of its extracts in industrialized food formulations to increase their nutritional value.

Keywords: ora-pro-nobis. Lyophilization. Protein. Antioxidant. Phenolic.

Introduction

Pereskia aculeata Mill. (Cactaceae), popularly known as ora-pro-nobis, is a fleshy plant, dark green in color, consisting of high levels of protein compared to other unconventional vegetables, and can be consumed *in natura* or in the form of flour. It is considered an "Unconventional Food Plant" (UFPs), that is, it is part of a group of plants that have one or more edible parts, being spontaneous or cultivated, native or exotic and are not frequently used in cooking (SANTOS et al., 2012; SOUZA, 2014; CUNHA et al., 2021).

UFPs are rich in nutrients and can play an important role in the adoption of healthier eating habits. An important nutritional characteristic of ora-pro-nobis leaves is their protein content, with about 15 to 28%, which is higher compared to other vegetables (MERCÊ et al., 2001; ALMEIDA; CORREA, 2012; SOUZA, 2014). *P. aculeata* is considered a nutritional supplement due to its protein, fiber, iron, calcium content, as it also has high mucilage content and presence of arabinogalactan biopolymer, arousing the interest of the food and pharmaceutical industries (DUARTE; HAYASHI, 2005; SOUSA et al., 2014).

In some species of the Cactaceae family, the presence of phenolic compounds and antioxidant activity were identified. Among these species, *P. aculeata* has benefits associated with antioxidant potential, which are correlated with the presence of phenolic acids (SOUSA et al., 2014, MORAES et al., 2020).

Antioxidants are substances that have the ability to protect cells against the effects of free radicals produced by the body; they may favor immunity increase and prevention of diseases such as rheumatoid arthritis, some types of cancer, cardiovascular diseases and those related to aging, such as Alzheimer's, among others. Classes of natural compounds with high antioxidant potential are phenolic acids, flavonoids and polyphenolic compounds. The presence of phenolic groups in these compounds promotes the stabilization of free radicals due to the resonance structures that can be formed, which is why they have high antioxidant activity (GHASEMZADEH; GHASEMZADEH, 2011).

Extraction methods that facilitate the process of obtaining bioactive compounds from leaves of *P. aculeata* were investigated by Torres et al. (2022), where extraction methods were evaluated using Soxhlet, pressurized liquid and supercritical fluid, demonstrating that the use of water as solvent becomes an alternative to avoid the degradation of bioactive compounds.

The use of ultrasound-assisted extraction has been studied by Lago et al. (2019) for the preparation of oil-in-water nanoemulsions from samples produced with mucilage extracted from leaves of *P. aculeata*, which were subjected to boiling, grinding, heating and centrifugation processes. Therefore, the application of ultrasound-assisted extraction has been considered a "green" technology, as it offers advantages in terms of selectivity, better quality, less use of chemicals and energy efficiency. It is considered suitable for the extraction of bioactive compounds from plant materials, which generally requires large amounts of organic solvents (PERERA; ALZAHRANI, 2021). This technique is based on extraction from high shear forces (cavitation), causing fragmentation of macromolecules and permeabilization of cell walls, presenting good results in the extraction of bioactive molecules (ALVES, 2020; PERERA; ALZAHRANI, 2021).

Although this species is studied, there are few works in the literature gathering the evaluation of the physicochemical characteristics of the leaves and the chemical composition and biological properties of extracts obtained by ultrasound. Thus, the present work aimed at the physical-chemical characterization of the leaves of ora-pro-nobis (*P. aculeata* Mill.) and the protein fractions of the extracts obtained as well as the ultrasound-assisted extraction, aiming at the evaluation of the antioxidant potential and total phenolic compounds.

Material and methods

Plant material

The leaves of ora-pro-nobis were collected in Erechim-RS, Brazil, under coordinates 27.656426 S and 52.307774 W, in January 2020 and recorded in the Herbarium Padre Balduíno Rambo HPBR 12.647 confirming the species *Pereskia aculeat*a Mill. (Cactaceae). The leaves were manually pre-selected and taken to the freezer (Brastemp) at -18ºC. After 24 h, the samples were submitted to lyophilization process (Edwards lyophilizer), for 72 h and then they were crushed and stored in amber bottles at room temperature.

Physical-chemical characterization of the leaves of *P. aculeata*

The following physicochemical parameters of ora-pro-nobis leaves were determined: moisture, proteins, lipids, mineral residue (ash), quantification of minerals and determination of protein fractions.

Moisture

Moisture was determined by the gravimetric method, where 2g of sample were

subjected to direct drying in oven with air recirculation (Fanem, model 320-SE) at 105 ºC until constant weight (AOAC, 2000).

Total Protein

The total nitrogen in the samples was determined by the Kjeldahl method, according to methodology n° 920.123 of the AOAC (2005). The samples were digested in a Kjeldahl digester system (Scientifica model DK20), using sulfuric acid and a catalyst pellet (3.5 g of K_2SO_4 and 3.5 mg of Se, FOSS). Then, distillation was carried out in a Kjeldahl still system (Tecnal TE-0364), using 4% boric acid solution with mixed indicator as the distilled ammonia receiving solution. Afterwards, the titration of the ammonium borate formed with 0.1 N HCl solution was performed. To quantify the protein, the nitrogen content was multiplied by the nitrogen--to-protein conversion factor of 6.25.

Lipids

The ether extract was determined according to the Soxlet method (New Technique model NT 340), using ±2 g of sample and petroleum ether as organic solvent (AOAC, 1995).

Mineral Residue (Ash)

The total mineral content (ash content) was determined according to the methodology described by AOAC (2005). Initially, the samples (±2 g) were evaporated, pre--carbonized on an electric plate, later incinerated in a muffle furnace (Fornos Lavoisier model 400C) at 550°C for approximately 8 h (ash appearance) and then quantified by the gravimetric method.

Quantification of minerals

The mineral residue obtained was diluted in 1M nitric acid solution for subsequent quantification of minerals. For mineral components Sodium (Na) and Potassium (K) (with standard concentration of 100 mg/mL) a microprocessed digital flame photometer (Analyser model 910) was used. For the components Zinc (Zn), Manganese (Mn), Magnesium (Mg), Copper (Cu), Calcium (Ca), and Cadmium (Cd), the Sanvantaa flame atomic absorption spectrometry method was used (model 6BC 3.11 A), according to the methodology described by AOAC (1995).

Determination of protein fractions

Protein fractions were evaluated by electrophoresis following the methodology of Laemmli (1970) with modifications, using 15% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and molar mass standard from 3.4 to 100 kDa (PageRuler, 4-20% Trisglycine SDS-PAGE). The extracts obtained from the ultrasound-assisted extraction technique were diluted in water and subjected to constant current of 300 mA and voltage of 250V for approximately 60 min. The protein bands present were visualized using bright blue dye solution (Blue R-250).

Ultrasound-assisted extraction

Two grams of sample and 150 mL of distilled water in Erlenmeyer were added and taken to the ultrasonic bath (UNIQUE®, model USC 1800A), operating at a frequency of 40 kHz, power 132 W for one hour, at temperatures of 40, 60 and 80 °C, using maximum power (100%), in triplicate. Subsequently, the samples were filtered with qualitative filter paper 60 x 60, 80g Whatman and the extracts obtained were placed in amber flasks and kept in a refrigerator (eight degrees).

Determination of phenolic compounds content

The determination of total phenols was performed using the Folin-Ciocalteau me-

thod, using gallic acid as reference standard, as described by Singleton, Othofer & Lamuela-Raventos (1999) with modifications. One mL of extract, 0.5 mL of Folin-Ciocalteau reagent, 2.5 mL of distilled water and 1 mL of 20% sodium carbonate (w/v) were added to the test tubes. The tubes were shaken and kept at room temperature, in the absence of light, for 10 minutes. Absorbance was measured using a spectrophotometer (Agilent Technologies, model 8453E) at 760 nm. The results were calculated from the standard curve of gallic acid (0.78 to 200 μg.mL-1), and were expressed in milligrams gallic acid equivalent (mgEAG)/100g of dry residue. Analyses were performed in triplicate.

Antioxidant activity (DPPH radical scavenging capacity)

The technique for determining the antioxidant activity consisted of measuring the absorption of the 2.2 diphenyl-1-picryl hydrazyl (DPPH) radical at 515 nm using a spectrophotometer (Logen LS-7052), through incubation for 30 min of 500 μL of solute containing increasing concentrations of the sample (0.1; 0.25; 0.5; 0.75; 1; 1.25; 5; 7.5; 10 mg/mL) in ethanol (scientific exodus 96% purity). For control, 500 μL of DPPH with 500 μL of ethanol were used. The percentage of DPPH radical uptake was calculated in terms of percentage of antioxidant activity (AA%) according to Equation 1 (MIRANDA; FRAGA, 2006):

$$AA\% = (100-(\text{Sample Abs}-\text{Blank Abs}))/(\text{Control Abs}) \times 100 \quad (1)$$

Where: Abs is the absorbance given in nanometers; AA: Antioxidant activity.

After evaluating the ideal concentration range, the concentration necessary to capture 50% of the DPPH free radical (IC_{50}) was calculated by linear regression analysis (SILVESTRI et al., 2010).

Statistical analysis

Data on antioxidant activity and total phenolic content of extracts obtained by ultrasound-assisted extraction at different temperatures were subjected to analysis of variance (ANOVA) followed by Tukey's test with 95% confidence, using the software Past version 2.17c.

Results and discussion

The results obtained for the physicochemical composition of the leaves of *P. aculeata* are shown in Tables I and II.

Table I - Physicochemical characterization of *P. aculeata* leaves

Component	Concentration[1] (%)
Moisture	1,32 ± 0,06
Protein	18,56 ± 0,58
Lipids	4,28 ± 0,19
Ash	15,71 ± 0,04

[1] Data show mean of three repetitions ± standard deviation.

The moisture found for leaves of *P. aculeata* in our study was four times lower than that found in the work by Alves (2020). It should be noted that high humidity values can influence both storage and packaging and food processing (CECCHI, 2007). Thus, the moisture content is related to its tendency to deterioration, and its partial elimination is important to reduce or inhibit growth of microorganisms, as well as to reduce the occurrence of enzymatic reactions (DALA PAULA, 2021).

The protein content found (18.56%) is within the limits reported in the literature, which indicates 15 to 28% variation, depending on cultivation conditions, climate, soil and region (MAZIA; SARTOR, 2012, GIRÃO et al., 2003; ALVES, 2020).

In our study, the lipid content was (~4%) lower than that found by Alves (2020) (6.49%) and Almeida et al. (2014) (5.07%). Thus, for the protein extraction process, the amount of lipids is a relevant parameter, since their high concentration in a food matrix can inhibit the access of water to proteins and cause reduction in the amount of extracted proteins (FEYZI et al., 2015; ALVES, 2020).

As for the ash content (15.71%) it is also within the limits reported in the literature (ALVES, 20120; ALMEIDA et al. 2014) showing ora-pro-nobis leaves as a substantial and important source of minerals, with calcium (8,012.83 mg/100g) and potassium (10,693.80 mg/100g) as the minerals found in greater amounts (Table II).

The concentration of calcium and potassium in analyzed samples was much higher when compared to values found by Almeida et al. (2014) (1,346.67 and 3,910.00 mg/100g) and Rodrigues (2016) (3,883 mg/100g and 2,683 mg/100g) respectively, although variations in mineral content may also be related to growing conditions and collection site (MAZIA; SARTOR, 2012).

Table II - Mineral composition of *P. aculeata* leaves

Mineral	mg/100g [1]
Zinc	5.37 ± 0.20
Manganese	162.06 ± 1.07
Calcium	8,012.83 ± 46.59
Magnesium	4,837.36 ± 0.23
Copper	1.70 ± 0.36
Cadmium	*
Sodium	497.39 ± 0.62
Potassium	10,693.80 ± 1.74

[1] Data show mean of three repetitions ± standard deviation.
*Not identified in the samples.

The levels of manganese, magnesium and copper were also higher than those reported by Oliveira et al. (2013) where 5.9, 2.8 and 0.9 mg/100g and lower zinc content (5.9 mg/100g) were obtained. Variations in nutrient contents and differences in the botanical structure of native plants are attributed, as they are collected in natural environments with no control over environmental factors such as water, light and temperature (SUBRAMANIAN et al., 2012; BARREIRA et al., 2021).

As they are calcium source plants, ora-pro-nobis leaves can improve nutritional quality, becoming a fundamental food species for food strategies and nutritional security of family groups whose eating habits are directly related to the consumption of this species (BARREIRA et al., 2021).

Based on medical advice, an adult, regardless of gender, needs to consume 4,700 mg/day of potassium, 420 mg/day of magnesium, 1,300 mg/day of calcium, 0.9 mg/day of copper and 2.3 mg /day of manganese (NRC, 1989; DRIs, 2001). Through our study it was possible to verify that 100 g portions of fresh ora-pro-nobis leaves supply 100% of these daily needs and the inclusion of these species in the menu can help to reduce the nutritional deficiencies of the population.

Figure 1 shows the results of the SDS PAGE analysis of the leaves and the extract obtained by ultrasound-assisted extraction.

The presence of 5 kDa proteins was observed in all extracts obtained (Figure 1). The results are close to those obtained by Takeiti et al. (2009), where peptides smaller than 6.5 kDa were found. Low molecular weight proteins can have different properties, and can be classified into proteases, peptones and peptides (FOOD INGREDIENTS BRASIL, 2012).

Table III shows the results of antioxidant activity expressed in IC_{50} values (necessary concentration of extract to inhibit 50% of the DPPH radical) and the content of total phenolic compounds (expressed in milligrams

of gallic acid equivalent (mg EAG) per 100 g of plant material) of the extracts obtained by the ultrasound maceration method at temperatures of 40, 60 and 80 ºC.

Figure 1 - SDS-PAGE analysis of the ultra-sound extract obtained at 40°C (A), 60°C (B), and 80°C (C), from ora-pro--nobis (OPN) leaves and standard (S) 3.4-100 kDa.

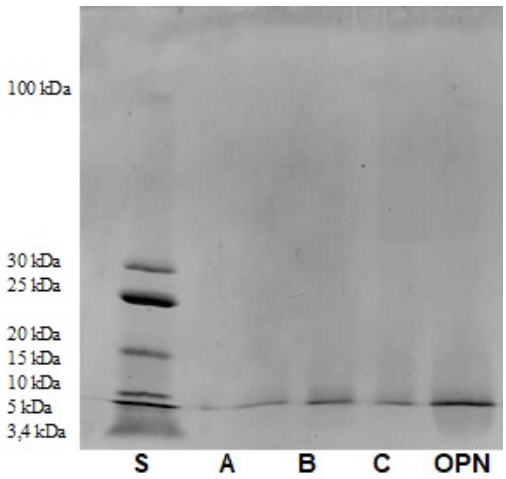

Table III - Antioxidant activity and total phenolic content of extracts obtained by ultrasound-assisted extraction at different temperatures

Temperature (ºC)	mgEAG/100g	IC_{50} (mg/mL)
40	$2.188^{b} \pm 1.865$	$0.491^{a} \pm 0.027$
60	$4.209^{a} \pm 3.068$	$0.291^{c} \pm 0.014$
80	$0.831^{c} \pm 0.849$	$0.329^{b} \pm 0.015$

Means ± standard deviations followed by different letters differ from each other by Tukey's test ($p<0.05$).

The extract obtained by maceration with ultrasound at 60°C for 1 hour showed better results in terms of concentration of total phenolic compounds and consequently antioxidant activity, an expected fact, since most phenolic derivatives are good antioxidant agents (BOROSKI et al., 2015; MORAES et al., 2020). An extract with high potential to scavenge free radicals has low IC_{50} value, requiring a small amount of extract to decrease the initial concentration of the DPPH radical by 50%.

The use of water as solvent was reported in the work by Rodrigues (2016) where IC_{50} of 1.78 mg/mL was found for the sample subjected to a temperature of 95 °C for 1 hour, under agitation. This result was almost 7 times worse when compared to the IC_{50} value found in our study (0.291 mg/mL). This points to temperature as an important process variable, indicating that high extraction temperatures should be avoided to minimize the decomposition of thermosensitive substances with antioxidant properties. In the study by Torres et al. (2022), it was possible to obtain extract with good antioxidant activity (IC_{50} of 0.31 mg/mL) using pressurized liquid extraction at 10 mPa and 4 mL/min and lower temperature (80 °C).

The ultrasound-assisted extraction technique was also efficient in obtaining extracts with antioxidant activity, as it can increase the diffusibility between solvent and biological material without drastic increase in temperature.

Conclusion

Ora-pro-nobis leaves (*Pereskia aculeata* Mill.) are an important source of minerals and proteins, with low molecular weight peptides. The aqueous extracts obtained by ultrasound--assisted maceration at a temperature of 60°C for one hour of extraction present high content of total phenolic compounds and good antioxidant activity, suggesting their use as natural preservative and nutraceuticals in the food industry, aiming at a more balanced and healthy diet.

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REFERENCES

ALVES, A. N. **Extração de proteases de ora-pro-nóbis (*Pereskia aculeata* Miller) e purificação parcial em sistemas aquosos bifásicos formados por peg+ fosfato de sódio+água**. 2020. 65 f. Dissertação (Mestrado em Engenharia e Alimentos)- Universidade Estadual do Sudoeste da Bahia- UESB, Itapetinga, Bahia, 2020.

ALMEIDA, M. E. F. DE; CORRÊA, A. D. Utilização de cactáceas do gênero Pereskia na alimentação humana em um município de Minas Gerais. **Ciência Rural**, v. 42, p. 7451-756, 2012.

ALMEIDA, M. E. F.; JUNQUEIRA, A. M. B.; SIMÃO, A. A.; CORRÊA, A. D. Caracterização química das hortaliças não-convencionais conhecidas como ora-pro-nobis. **Bioscience Journal**, v. 30, n.1, p. 431-439, 2014.

AOAC - Association of Official Analytical Chemists. Official Methods of Analysis. 14. ed. v. 1. 1995.

AOAC. Association of Official Analytical Chemists. Official methods of analysis of the association analytical chemists. 18. ed. Maryland: AOAC, 2005.

AOAC - Association of Official Analytical Chemists. Official methods of analysis of the Association of the Analytical Chemists. 17th ed. Virginia, 2000.

BARREIRA, T. F.; PAULA FILHO, G. X.; PRIORE, S. E.; SANTOS, R. H. S.; PINHEIRO-SANT'ANA, H. M. Nutrient content in ora-pro-nóbis (*Pereskia aculeata* **Mill.): unconventional vegetable of the Brazilian Atlantic Forest. Food Science and Technology**, v. 41, n. 1, 2021.

BOROSKI, M.; VISENTAINER, J. V.; COTTICA, S; M.; MORAIS, D. R. **Antioxidantes: Princípios e Métodos Analíticos.** Editora 1. ed. Curitiba- PR, 2015.

CECCHI, H. M. **Fundamentos teóricos e práticos em análise de alimentos**. 2. ed. Campinas, SP: UNICAMP, 2007.

CUNHA, M. A. D.; PINTO, L. C.; SANTOS, I. R. P. D.; NEVES, B. M.; CARDOSO, R. D. C. V. Plantas Alimentícias Não Convencionais na perspectiva da promoção da Segurança Alimentar e Nutricional no Brasil. **Research, Society and Development**, v. 10, n. 3, p. e20610313306, 2021.

DALA PAULA, B. M. **Química & Bioquímica de Alimentos**. Editora Universidade Federal e Alfenas, 2021.

DRIs - The Dietary Reference Intakes. 2001. Disponível em: https://web.archive.org/web/20121031115637/http://www.crnusa.org/about_recs4.html.

DUARTE, M. R.; HAYASHI, S. S. Estudo anatômico de folha e caule de *Pereskia aculeata* Mill. (Cactaceae). **Revista Brasileira de Farmacognosia**, v. 15, n. 2, p. 103-109, 2005.

FEYZI, S.; VARIDI, M.; ZARE, F.; VARIDI, M. J. Fenugreek (*Trigonella foenum-graecum*) seed protein isolate: Extraction optimization, amino acid composition, thermo and functional properties**. Journal of the Science of Food and Agricultu**re, v. 98, n. 511, p. 3165-3176, 2015.

FOOD INGREDIENTS BRASIL. Dossiê Proteínas. **Revista FIB**, n. 22, p. 58-74, 2012.

GHASEMZADEH, A.; GHASEMZADEH, N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. **Journal of Medicinal Plant Research**, v. 5, n. 31, p. 6697-6703, 2011.

GIRÃO, L. V. C.; SILVA FILHO, J. C.; PINTO, J. E. B. P.; BERTOLUCCI, S. K. V. Avaliação da composição bromatológica de ora-pro-nóbis. **Horticultura Brasileira**, v. 21, n. 2, p. 411, 2003.

LAEMMLI, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Review in Nature**, v. 227, p. 680-685, 1970.

LAGO, A. M. T.; NEVES, I. C. O.; OLIVEIRA, N. L.; BOTREL, D. A.; LUIS ANTONIO MINIM, L. A.; DE RESENDE, J. V. Ultrasound-assisted oil-in-water nanoemulsion produced from Pereskia aculeata Miller mucilage. **Ultrasonics Sonochemistry**, v. 50, p. 339-353, 2019.

MAZIA, R. S.; SARTOR, C. F. P. Influência do tipo de solo usado para o cultivo de *Pereskia aculeata* sobre propriedade protéica. **Revista Saúde e Pesquisa**, v. 5, n.1, p. 59-65, 2012.

MERCÊ, A. L. R.; LANDALUZE, J. S.; MANGRICH, A. S.; SZPOGANICZ, B.; SIERAKOWSKI, M. R. Complexes of arabinogalactan of *Pereskia aculeata* and Co2+, Cu2+, Mn2+, and Ni2+. **Bioresource Techonology**, v.76, n.1, p.29-37, 2001.

MIRANDA, A. L. P.; FRAGA, C. A. M. Atividade Sequestradora de Radical Livre Determinação do Potencial Antioxidante de Substâncias Bioativas. *In*: MONGE A., GANELLIN, C. R. (Ed). **Practical Studies for Medicinal Chemistry**. Genebra: IUPAC, 2006.

MORAES, T. V.; DE SOUZA, M. R. A.; SIMÃO, J. L. S.; ROCHA, C. B.; MOREIRA, R. F. A. Potencial antioxidante da espécie *Pereskia aculeata* Miller: uma análise bibliométrica. **Brazilian Journal of Surgery and Clinical Research**, v. 29, n.1, p.79-85, 2020.

NRC - NATIONAL RESEARCH COUNCIL. 1989. Recommended dietary allowances. 10. ed. Washington: National Academic. 284p.

OLIVEIRA, D. C. S.; WOBETO, C.; ZANUZO, M. R.; SEVERGNINI, C. Composição mineral e teor de ácido ascórbico nas folhas de quatro espécies olerícolas não-convencionais. **Horticultura Brasileira**, v. 31, p.472-475, 2013.

PERERA, C. O.; ALZAHRANI, M. A. J. Ultrasound as a pre-treatment for extraction of bioactive compounds and food safety: A review. **LWT – Food Science and Technology**, v.142, p.111114, 2021.

RODRIGUES, A. R. **Atividade antioxidante e antimicrobiana de extratos de ora-pro-nóbis (pereskia aculeata mill.) e sua aplicação em mortadela**. 2016. 91 f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) – Universidade Federal de Santa Maria (UFEM), Faculdade de Engenharia de Alimentos, Santa Maria- RS, 2016.

SANTOS, I. C.; PEDROSA, M. W.; CARVALHO, O. C.; GUIMARÃES, C. D. C; SILVA, L.S. **Ora-pro-nobis**: da cerca à mesa. EPAMIG. Circular técnica. v. 177, 2012.

SILVESTRI, J. D.; PAROUL, N.; CZYEWSKI, E.; LERIN, L.; ROTAVA, I.; CANSIAN, R. L.; MOSSI, A.; TONIAZZO, G.; OLIVEIRA, D. DE; TREICHEL, H. Perfil da composição química e atividades antibacteriana e antioxidante do óleo essencial do cravo-da-índia (*Eugenia caryophyllata* Thunb.). **Revista Ceres**, v. 57, n. 5, p. 589-594, 2010.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. **Methods in Enzymology**, v. 229, p. 152-178, 1999.

SOUZA, T. C. L., **Perfil de compostos fenólicos extraídos de folhas de ora-pro-nóbis (*Pereskia aculeata* Miller)**. 2014. 84 f. Dissertação (Mestrado em Engenharia de Alimentos) – Universidade

Estadual de Campinas, Faculdade de Engenharia de Alimentos, Campinas- SP, 2014.

SOUSA, R. M. F.; LIRA, C. S.; RODRIGUES, A. O.; MORAIS, S. A. L.; QUEIROZ, C. R. A. A.; CHANG, R.; AQUINO, F. J. T.; MUÑOZ, R. A. A.; OLIVEIRA. A. Antioxidant activity of ora-pro-nobis (*Pereskia aculeata* Mill.) leaves extracts using spectrophotometric and voltammetric assays in vitro. **Bioscience Journal**, v. 30, n.1, p. 448-457, 2014.

SUBRAMANIAN, R., GAYATHRI, S., RATHNAVEL, C., RAJ, V. Analysis of mineral and heavy metals in some medicinal plants collected from local market. **Asian Pacific Journal of Tropical Biomedicine**, v.2, n. 1, p. 74-78, 2012.

TAKEITI, C. Y.; ANTONIO, G. C.; MOTTA, E. M. P.; COLARES-QUEIROZ, F. P.; PARK, K. J. Nutritive evaluation of a non-convencional leafy vegetable (*Pereskia aculeata* Miller). **International Journal of Food Science and Technology**, v. 60, n.1, p. 148-160, 2009.

TORRES, T. M. S.; ÁLVAREZ-RIVERA, G.; MAZZUTTI, S.; SÁNCHEZ-MARTÍNEZ, J. D.; CIFUENTES, A.; ELENA IBÀNEZ, E.; FERREIRA, S. R.S. Neuroprotective potential of extracts from leaves of ora-pro-nobis (*Pereskia aculeata*) recovered by clean compressed fluids. **The Journal of Supercritical Fluid**, v. 179, p. 105390, 2022.