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ORIGINAL ARTICLE

Ora-pro-nobis (*Pereskia aculeata* Miller): a potential alternative for iron supplementation and phytochemical compounds

Ora-pro-nobis (Pereskia aculeata Miller): uma fonte potencial de ferro e compostos fitoquímicos

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Abstract

Ora-pro-nobis (OPN) (*Pereskia aculeata* Miller) is a non-conventional edible plant rich in protein, fibres and minerals. The innovation of this work is based on the sustainability production of the aqueous extract (green solvent) containing iron and bioactive compounds as well as employing a native plant from Brazil. A screening of phytochemicals components, phenolic compounds, flavonoids and tannins contents were performed using OPN extract. The antioxidant activity of the OPN aqueous extract was determined by three different assays as following: 2,2-diphenyl-1-picrylhydrazyl (DPPH•); Ferric Reducing Antioxidant Power (FRAP); and Oxygen Radical Absorbance Capacity (ORAC). The OPN dried leaves presented high protein and minerals contents. Indeed, the Fourier-Transform Infrared Spectroscopy (FTIR Spectroscopy) analysis performed in OPN aqueous extract confirmed the appearance of representative functional groups of the bioactive compounds. Overall the results suggested that it is possible to use simple aqueous solvent to produce OPN extract rich in iron, bioactive compounds and within antioxidant activity that could be potentially used as functional food ingredient.

Keywords: Non-conventional edible plant; Aqueous extract; Bioactive compounds; Experimental design; Antioxidant activity; Food ingredient.

Resumo

Ora-pro-nobis (OPN) (*Pereskia aculeata* Miller) é uma planta alimentícia não convencional rica em proteínas, fibras e minerais. A inovação deste trabalho é baseada na produção sustentável de um extrato aquoso contendo ferro e compostos bioativos utilizando uma planta nativa brasileira. Uma triagem dos componentes fitoquímicos e a determinação dos compostos

fenólicos, flavonoides e taninos foram realizadas usando extrato de OPN. A atividade antioxidante do extrato aquoso de OPN foi investigada empregando-se três diferentes métodos: DPPH•, FRAP e ORAC. Folhas secas de OPN apresentaram elevados conteúdos de proteínas e minerais. A análise de FTIR realizada no extrato aquoso de OPN confirmou a presença de grupos funcionais representativos de compostos bioativos. Os resultados sugerem que é possível usar um solvente aquoso simples para produzir um extrato de OPN rico em ferro, compostos bioativos e com atividade antioxidante, o qual poderia ser potencialmente utilizado como ingrediente alimentar funcional.

Palavras-chave: Planta alimentícia não convencional; Extrato aquoso; Compostos bioativos; Planejamento experimental; Atividade antioxidante; Ingrediente alimentício.

1 Introduction

Research involving non-conventional vegetables have been developed as an alternative food source for consumers. Non-conventional edible plants are not part of the daily diet of the population, being underutilized and unknown despite their high nutritional value (Kinupp & Lorenzi, 2014). Among the non-conventional edible plant, it can be highlighted ora-pro-nobis (OPN) that present considerable content of protein and minerals (Takeiti et al., 2009).

The OPN, *Pereskia aculeata* Miller, also known popularly as American gooseberry or Barbados goosebery (Martin et al., 2017), belongs to the Cactacea family. It is native from the American tropics (southern region of the United States of America (USA) and Brazil) (Conceição et al., 2014). Studies have been developed associating the consumption of OPN to the prevention of health problems such as constipation, osteoporosis and iron deficiency anaemia (Almeida & Corrêa, 2012), anti-inflammatory (Pinto et al., 2015) and antioxidant activities (Pinto et al., 2012). Furthermore, there are studies involving the use of OPN to produce mucilage (Lima Junior et al., 2013; Conceição et al., 2014), nanoemulsion (Lago et al., 2019), films (Oliveira et al., 2019) and food products such as pasta (Sato et al., 2019), breads (Silva et al., 2015), sausage (Silva Sobrinho et al., 2015) and fermented milk (Amaral et al., 2018).

In fact, the OPN leaves are considered great potential alternative source of protein, minerals and vitamins (Takeiti et al., 2009). The protein content (20-30%) observed in OPN leaves (Lima Junior et al., 2013) is higher than the various type of food such as meat, fish and eggs (Latham, 1997). The content of minerals, particularly iron and calcium, is higher than other vegetables such as broccolis, spinach and chard. The fibre content (Almeida Filho & Cambraia, 1974; Takeiti et al., 2009) and not possessing toxic properties observed in OPN leaves (Mercê et al., 2001) make it a helpful and considerable food source. In addition, the presence of phytochemical compounds could improve and expand its application.

The significant iron content found in OPN leaves could make it a plant with an alternative potential ingestion for iron deficiency anaemia. This is the most common type of anaemia (around 75% of cases) in developing countries, and one of the most frequent health worldwide problems (Soleimani & Abbaszadeh, 2011).

Studies involving the extraction of different compounds mainly related to the production of mucilage/hydrocolloids from OPN leaves have been reported (Lima Junior et al., 2013; Conceição et al., 2014; Lago et al., 2019). However, there was not found information in the literature with respect to the iron and bioactive compounds aqueous extraction from OPN leaves. Flavonoids and phenolic compounds were found in mucilage (Carvalho et al., 2014) and in methanolic extract produced from OPN leaves (Pinto et al., 2012).

This work aimed to produce an OPN aqueous extract rich in iron and bioactive compounds. The simple and rapid aqueous solvent was used instead of methanol/ethanol due to its sustainability and green chemistry rules. OPN extract was elaborated considering the binomial time-temperature using a central composite design and characterised in function of the phytochemical compounds, total phenolic content, tannins, flavonoids, potential antioxidant activities (2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing

Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays) and Fourier-Transform Infrared Spectroscopy (FTIR Spectroscopy) analysis.

2 Materials and methods

2.1 Ora-pro-nobis (OPN) leaves

The OPN leaves were harvested during summer season (February/2015) in a Brazilian state (São Luís Farm, Conceição do Araguaia, Pará, Brazil), located in the North region of the country. The plant specimen was identified generating a voucher specimen (No. ESA136618) deposited in the Herbarium E.S.A., Piracicaba, Brazil.

2.2 Preparation of flour from ora-pro-nobis leaves

The OPN leaves were collected from the same plant, manually pre-selected (leaves without spots or yellowish colour) and washed in running water to remove organic materials, dust, and other soils that could be adhered. A second wash with sodium hypochlorite solution (NaOCl, 5.0%) was used as sanitising solution. OPN leaves were dried under the sun during two periods of 10 h using an apparatus to avoid contamination and accumulation of soil on the leaves. OPN dried leaves were ground using a knife mill (Marconi, model MA 340, Brazil), stored in plastic containers suitably identified and conditioned at room temperature (25 ± 1 °C).

2.3 Characterisation of OPN leaves

2.3.1 Dry Matter (DM)

The DM was determined according to Silva & Queiroz (2002). OPN dried and triturated leaves were weighed (2 g) and placed in an oven at 105 °C during 4 h. The sample was weighed for determination of DM, according to the Equation 1:

$$DM\,(\%) = \left(m'/m\right)100 \quad (1)$$

where m' is the dry mass of sample (g of OPN dried leaves) and m is the fresh mass of sample (g of OPN fresh leaves). The analysis was performed in triplicate.

2.3.2 Chemical composition

Chemical composition (moisture content, proteins, lipids and ash) of OPN leaves was determined by Association of Official Analytical Chemists (1990). The analyses were carried out in three independent experiments. Total carbohydrate content (%) was determined by difference (Instituto Adolfo Lutz, 2008).

2.3.3 Total dietary fibre

Total dietary fibre (soluble and insoluble) was determined according to the methodology 985.29 (Association of Official Analytical Chemists, 2012).

2.4 Preparation of OPN aqueous extract

The OPN dried and triturated leaves (1 g) were homogenised in 100 mL of sodium chloride (50 mM, Synth, Brazil) solution and kept under constant magnetic stirring (IKA, model C-MAG HS 7, Germany), considering the conditions of central composite design. Afterwards, two steps of filtration were carried out as following: first employing Buchner filters (four different sizes 1, 2, 3 and 4), followed by the filtration

using two types of membranes (Química Moderna, Brazil) and porous size (0.8 µm, ester mixing membrane filters; 5.0 µm, cellulose nitrate filters. The filtrate was named as OPN aqueous extract.

A central composite design was performed to evaluate the effects of the variables time (1, 3, 5 h) and temperature (45 ºC, 60 ºC, 75 ºC) on the extraction process. A ratio 0.01 g mL^{-1} (OPN dried leaves/50 mM NaCl solution) was employed. The factorial planning matrix consisted of 7 trials (2^2 experiments and 3 central points). The response was the iron content in the OPN aqueous extract. The iron quantification was performed using a UV/Vis spectrophotometer (PerkinElmer, model Lambda 35, USA) reading at 510 nm, using the phenanthroline method proposed by Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1999).

2.4.1 Preliminary phytochemical screening in OPN aqueous extract

Phytochemical analysis (qualitative) of OPN aqueous extract was based on the identification of alkaloids, anthraquinones, catechin, flavonoids, phenols compounds, steroids, and tannins according to Barbosa et al. (2004) and coumarin by Costa (1994). The phytoconstituents were classified following the parameters: presence (+) and absence (–).

2.4.2 Total phenolic compounds

It was determined using the classical methodology (Folin-Ciocalteau colourimetric assay) proposed by Singleton et al. (1999). Aliquot (0.5 mL) of the extract was placed into tube containing Folin-Ciocalteau reagent (2.5 mL, Sigma-Aldrich, BCBV9444 lot, Switzerland). After 5 min, 2.0 mL of the sodium carbonate (Dinâmica, Brazil) solution (Na_2CO_3, 7.5%) was placed into the tube, homogenized in vortex (IKA, Vortex 1 V1 model, Germany) and kept for 2 h in the absence of light. The absorbance was measured in spectrophotometer (Perkin-Elmer, Lambda 35 model, USA) at 740 nm. The blank was prepared using the solvent 50 mM NaCl, following the same proceedings described for the sample. A standard curve was elaborated using gallic acid (Sigma-Aldrich, CAS number 149-91-7, Germany, 0.02 to 0.06 mg mL^{-1}). Total phenolic compounds were express in mg Gallic Acid Equivalents (GAE) g^{-1} OPN extract. The tests were carried out in three separate trials.

2.4.3 Quantification of flavonoids

It was determined according to Miliauskas et al. (2004), with modifications. Aliquot (3 mL) of OPN extract was homogenized in 2.0 mL of methanolic aluminium chloride ($AlCl_3$ 5%, Êxodo, Brazil) solution. The resultant solution was incubated during 30 min at 25 °C and absence of light. The absorbance was performed at 441 nm using a spectrophotometer (Perkin-Elmer, Lambda 35 model, USA). Blank sample was made homogenising 3.0 mL of sample with 2.0 mL of methanol. Rutin (Sigma-Aldrich, CAS number 207671-50-9, Germany, 2.0 to 20 µg mL^{-1}) was employed as the standard. Flavonoids content was express in mg Rutin Equivalents (RE) g^{-1} OPN extract. The experiments were performed in three independent replicates.

2.4.4 Determination of tannins

It was performed according to Burns (1963). Aliquots (1.0 mL) of OPN extract were placed in tubes. HCl (8%) in methanol was homogenized with vanillin (4%) in methanol using the same volume for both solutions. The resultant solution was denominated vanillin-HCl reagent and it was added (5.0 mL) into the tubes containing the samples. They were kept in the absence of light during 20 min. The measurement of the transmittance was performed in spectrophotometer (Perkin-Elmer, Lambda 35 model, USA) at 500 nm. Catechin (Sigma-Aldrich, CAS number 7295-85-4, Germany, 0.03 to 0.40 mg mL^{-1}) was used as the standard tannin quantification. The content of tannins was express in mg catechin equivalents (CE) g^{-1} OPN extract. Results were calculated from the mean of three independent replicates.

2.4.5 Antioxidant activities

2.4.5.1 DPPH• radical scavenging assay

It was performed according to Brand-Williams et al. (1995) methodology. Samples of OPN extract at different dilutions (0.1 mL) were placed in 3.9 mL of the DPPH solution (Sigma-Aldrich, CAS number 1896- 66-4, Germany, 6.0×10^{-5} M), and homogenized using vortex (IKA, Vortex 1 V1 model, Germany). Samples were kept at absence of light at 25 ± 2 °C during 9 min (stablished from preliminary tests). The determination of antioxidant potential was quantify by Effective Concentration (EC_{50}). The absorbance was measured using a spectrophotometer (Perkin Elmer, Lambda 35 model, USA) at 515 nm. Results were express in µMol Trolox Equivalents (TE) g^{-1} OPN extract. Results are the average of three independent experiments.

2.4.5.2 Ferric Reducing Antioxidant Power (FRAP)

It was carried out based on the methodology proposed by Benzie & Strain (1996). Ferric chloride was used as oxidant agent. Fe^{2+} ion generated from the redox reaction produces a coloured product with 2,4,6-tris(2-pyridyl)-s-triazine (Sigma-Aldrich, CAS number 3682-35-7, Germany). FRAP reagent was obtained from mixing the following solutions (considering the ratio 10:1:1): acetate buffer (300 mM), 2,4,6-tris(2-pyridyl)-s-triazine solution (10 mM of 2,4,6-tris(2-pyridyl)-s-triazine in 40 mM HCl) and 20 mM $FeCl_3$. Aliquot of OPN diluted extract (0.1 mL) was added in 2.9 mL of FRAP reagent, homogenized in vortex (IKA, Vortex 1 V1 model, Germany) and kept in water bath (Marconi, MA-127 model, Brazil) at 37 °C during 30 min. The absorbance was measured in spectrophotometer (Perkin Elmer, Lambda 35 model, USA) at 593 nm. Trolox (Sigma-Aldrich, CAS number 53188-07-1, Germany, 2.5 to 22.5 µM) was used as the standard. Results were express in µMol TE g^{-1} OPN extract. Experiments were performed in three distinct replicates.

2.4.5.3 Oxygen Radical Absorbance Capacity (ORAC) assay

The activity of free radical capture by the ORAC was carried out according to Ou et al. (2001), with minor modifications. In a 96-well polystyrene black microplates, 150 µL of 81 nM fluorescein ($C_{20}H_{12}O_5$, Sigma-Aldrich, CAS number 518-47-8, USA) prepared with sodium phosphate (NaH_2PO_4, Synth, Brazil) buffer (75 mM, pH 7.4) and 25 µL of different dilutions of the OPN extract (1:1, 1:10; 1:50, 1:100, 1:500 and 1:1000) in sodium phosphate buffer were placed into each well. The mixture was preincubated at 37 °C for 10 minutes in a fluorimeter (BMG Labtech, FLUOstar Optima model, Germany). After incubation, 25 µL of 2,2 Azobis(2-methylpropionamide)-dihydrochloride (AAPH, Sigma-Aldrich, CAS number 2997-92-4, USA) 152 mM was added into each well. The microplate was immediately placed in the equipment and agitated prior to each reading. The fluorescence was recorded from the top in the fluorimeter (BMG LABTECH, model FLUOstar OPTMA, Germany) previously programmed to read the fluorescein at an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm every 1 minute over 120 min interval. The plate reader was controlled by Gen 5 software. A blank with fluorescein and AAPH using sodium phosphate buffer (75 mM, pH 7.4) instead of the antioxidant solution and Trolox to perform the calibration curve (8.0 to 96.0 µM) as antioxidant were employed in each assay. Results were expressed as µMol TE g^{-1} OPN extract. The experiments were performed in triplicate.

2.4.6 FTIR analysis

The FTIR is an important and potent tool to identify functional groups. Spectra of the samples (OPN leaves and OPN lyophilized extract) were documented from dried samples homogenized within potassium bromide (KBr, Synth, Brazil) in order to obtain a translucent sample disc. A spectrophotometer (Thermo Scientific, Nicolet 6700 model, USA) was used to record a scan in the range of 4100 to 600 cm^{-1} with resolution of 4 cm^{-1}.

2.4.7 Determination of iron

Total iron content, ferrous iron and ferric iron (subtracting ferrous iron from total iron) were performed using a UV/Vis spectrophotometer (PerkinElmer, model Lambda 35, USA) reading at 510 nm, using the phenanthroline method described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1999). Ferrous ammonium sulphate was used to generated a standard curve for the determination of the total iron content.

2.5 Statistical analysis

The Analysis of Variance (ANOVA) and the effects of the two variables (time and temperature) used in the central composite design were performed in the program Statistic (Statistic Inc., version 7.0, USA) with a level of significance $p \leq 0.05$. Differences between the means were detected by the Tukey's test. Means ± Standard Deviation was used to express the results.

3 Results and discussion

3.1 Chemical composition and quantification of iron in OPN leaves

Chemical composition of OPN leaves was determined (Table 1). The content of protein was similar to that mentioned in the literature by Almeida Filho & Cambraia (1974), Rocha et al. (2008) and Silva et al. (2013), 17.40-25.40%, 22.93% and 24.73%, respectively.

The protein content observed in our study was higher than those found in meat, which is around 20.00% (Feiner, 2006). The consumption of vegetable sources rich in proteins could contribute to prevent or treat protein deficiencies, especially for the groups of population that have a limited access to animal proteins (Almeida et al., 2014). It is relevant to consider that there is a main difference respected to the quality of meat and vegetable proteins: the amino acids composition. Generally, the protein from vegetables source do not present all essential amino acids. In the case of OPN leaves, according to Conceição et al. (2014) and Takeiti et al. (2009), the protein presented high digestibility (75% to 85%) and all essential amino acid recommended by the Food and Agriculture Organization (FAO) of the United Nations as necessary for human consumption.

Regarding the total dietary fibre content, OPN leaves showed higher values (Table 1) than those found by Rocha et al. (2008) (12.64 ± 1.38%) and similar to those verified by Takeiti et al. (2009) (39.10%). The lipid content (~ 8.00%) was in accordance to the range of values obtained by Morton (1987) (6.80% to 11.70%). Meanwhile, Rocha et al. (2008) found values (3.64 ± 0.41%) lower than those observed in our study. The carbohydrate content (51.61 ± 0.44%) was higher than values found by Silva et al. (2013) (40.64 ± 1.21%) and Rocha et al. (2008) (36.18 ± 4.27%). Ash content around 12.00% was found for OPN leaves which was lower than those presented by Takeiti et al. (2009), of 16.10% (dry basis).

Table 1. Determination of chemical composition (dry basis) and iron content in OPN leaves.

Analysis	OPN dried leaves
Dry matter (%)	93.13 ± 0.05
Moisture (%)	6.87 ± 0.03
Ash (%)	11.90 ± 0.11
Protein (%)	21.81 ± 0.10
Lipids (%)	7.81 ± 0.30
Total dietary fibre (%)	37.23 ± 0.25
Carbohydrate (%)	51.61 ± 0.44
Iron content (mg 100 g^{-1})	47.81 ± 0.22

The iron content (47.81 mg 100 g^{-1}) found in our study was higher than those mentioned in the literature (Almeida et al., 2014 (20.56 mg 100 g^{-1}); Oliveira et al., 2013 (8.10 mg 100 g^{-1}); Takeiti et al., 2009 (14.20 mg 100 g^{-1})). It could be considered high mainly when compared with traditional sources (Universidade Estadual de Campinas, 2011) such as spinach (6.67 mg 100 g^{-1}), broccoli (6.76 mg 100 g^{-1}) and bean (5.50-21.88 mg 100 g^{-1}), and similar to cress (50.82 mg 100 g^{-1}) and purple lettuce (58.14 mg 100 g^{-1}). OPN leaves could provide high level of iron, considering the dietary recommendation by FAO/WHO for adults (14.00 mg day^{-1}).

3.2 OPN aqueous extract

3.2.1 Experimental design

In Table 2 is presented the iron content found at different conditions of extraction.

Table 2. Central composite design matrix and the iron content responses.

Trial	Temperature (°C)	Time (h)	Iron content (µg iron g^{-1} OPN extract)
1	-1 (45)	-1 (1)	0.97
2	+1 (75)	-1 (1)	1.64
3	-1 (45)	+1 (5)	1.58
4	+1 (75)	+1 (5)	2.04
5	0 (60)	0 (3)	1.31
6	0 (60)	0 (3)	1.35
7	0 (60)	0 (3)	1.39

The regression coefficients were calculated and only non-interaction parameters presented statistical significance ($p \leq 0.05$). A first-order model describing the total iron content as a function of the temperature and time of extraction was generated (Equation 2).

$$Iron\,content = 73.55 + 14.15t + 12,56T \quad (2)$$

where: t = time and T = temperature.

The significance of the model was verified by ANOVA test. Results shown a low pure error, suggesting a reproducibility of the experimental data. F value is a ratio of the mean square from the regression to the mean square from the real error. Results indicated a predictive model with the good enough regression coefficient (R = 0.86) and the F test calculated value (12.77) was higher than the table F value ($F_{0.95;\,2;\,4} = 6.94$) (Haaland, 1989).

Coetzee et al. (2014) evaluated the extraction of phytochemical compounds (including polyphenols) from the rooibo extract – *Aspalathus linearis* (Burm. f.) R. Dahlgren (known as herbal tea), and concluded that the time, temperature, and extraction method variables significantly influenced the concentration of aqueous extracts. Marume et al. (2017) noticed extraction of minerals (iron, magnesium, calcium, potassium and phosphorus) from medicinal plants (leaves and stem) using aqueous methanol (50%) in an ultrasonic bath during 1 h.

Based on these results, the optimum condition to obtain high iron content in the extract from OPN leaves was 75 °C for 1 h. It was selected for all further experiments.

3.2.2 Screening of phytochemicals compounds

Screening tests of OPN aqueous extract indicated the presence of coumarin, phenols compounds, flavonoids and tannins. Qadir et al. (2015) reported a screening of phytochemical compounds found in seeds

extracts of the *Anamirta cocculus* Linn. using seven different solvents (acetone, benzene, chloroform, ethanol, methanol, a mixture of methanol and ethyl acetate (1:3) and petroleum ether). They could verify important differences comparing the solvents and their polarities: chloroform and methanol presented higher amounts of phenolic compounds and saponins, whereas methanol indicated the presence of flavonoids. Parveen et al. (2018) analysed the phytochemical compounds present in aqueous and methanolic extract of triphala. They observed that methanol used as a solvent was more effective than water to extract phytochemicals. Junsathian et al. (2018) noticed relevant results for phenolic compounds, flavonoids and tannins from Thai plant aqueous extract.

Considering our results and the mentioned works above, it was noted and evidenced that the solvent and method of extraction and the food matrix used to prepare an extract directly influences in its final composition.

3.2.3 Total phenolic compounds, flavonoids, tannins and antioxidant properties

Phenolic compounds, flavonoids and tannins were previously identified in OPN extract (screening of phytochemicals compounds) and then, quantified (Table 3). The total phenolic compounds were the major component found in OPN extract.

There is no information in the literature regarded to the levels of phenolic compounds, flavonoids and tannins in OPN aqueous extract. Gavamukulya et al. (2014) determined the phytochemical composition in ethanolic and water leaves extracts of *Annona muricate* L., reporting values of phenolic compounds ranging from 373 to 684 µg GAE mL^{-1} extract. They also observed higher phenolic contents in aqueous extract than ethanolic extract.

Table 3. Quantification of phytochemical compounds of ora-pro-nobis (OPN) aqueous extract and determination of different antioxidant assays.

Determination	Results
Total phenolic compounds (mg GAE g^{-1} OPN extract)[1]	151.503 ± 3.345
Tannins (mg CE g^{-1} OPN extract)[2]	4.636 ± 0.119
Flavonoids (mg RE g^{-1} OPN extract)[3]	0.145 ± 0.006
DPPH (mg mL^{-1})	68.695 ± 2.850
FRAP (µMol TE g^{-1} OPN extract)[4]	0.909 ± 0.026
ORAC (µMol TE g^{-1} OPN extract)[4]	36.691 ± 2.722

[1]GAE: Gallic acid equivalents. [2]CE: Catechin equivalents. [3]RE: Rutin equivalents. [4]TE: Trolox equivalents.

Garcia et al. (2019) evaluated the total phenolic compounds in OPN hydromethanolic extract and found relevant values (23.75 mg g^{-1} extract) with similar amount of phenolic acids and flavonoids. Souza et al. (2016) determined in OPN leaves extract the total phenolic contents based on mg GAE g^{-1} extract using different solvents. They found for the methanol extract high phenolic content (15.04 mg GAE g^{-1} extract), proceeded by the ether (11.78 mg GAE g^{-1} extract) and chloroform extracts (5.17 mg GAE g^{-1} extract). The results presented in our study were 5-10 fold higher than those noticed by Garcia et al. (2019) and Souza et al. (2016) for OPN extract and also 1.5-fold higher than those obtained for aqueous extract of *Feijoa sellowiana* (O. Berg) O. Berg leaves (Ebrahimzadeh et al., 2008), indicating the OPN aqueous extract as a potential source of phenolic compounds. According to Sánchez-Rangel et al. (2013) and Huang et al. (2005), other types of compounds potentially widely found in plant extracts such as vitamin C and reducing sugars, could reduce the Folin-Ciocalteu reagent, which would result in overestimated values of total phenolic compounds employing the mentioned method. However, the total phenolic content determined by Folin-Ciocalteu assays is simple, convenient, and reproducible (Huang et al., 2005).

In fact, the OPN extract presented flavonoids (traces) and tannins contents (Table 3). Our results were lower than some those reported in the literature regarding to flavonoids contents that evaluated different solvents,

including water. It could be related to the different raw material as well to the solvent employed. Poojary et al. (2015) developed an extraction of phytochemicals from the root bark (*Mammea suriga* Kosterm.) using different solvents, including water. They observed high content of flavonoids in chloroform, ethyl acetate, ethanol and aqueous extracts. Majouli et al. (2017) performed phytochemical analysis in *Hertia cheirifolia* L. roots extracts using various solvents (butanol, ethyl acetate, hydro-methanol and petroleum ether) and obtained minimum and maximum extraction of flavonoids in petroleum ether and ethyl acetate, respectively.

We employed three different antioxidant methodologies aiming to investigate the potential antioxidant properties of the OPN aqueous extract. The importance of using different assays to assess the antioxidant capacity of plant extracts is related to the antioxidant compounds that act by distinct and specific mechanisms (Gonçalves et al., 2019). The antioxidant properties of OPN aqueous extract were determined (Table 3) and presented similar values to the other works (Guo et al., 2018; Junsathian et al., 2018). According to Nollet & Gutierrez-Uribe (2018) phenolic compounds were correlated with antioxidant activity, showing the important ability to scavenge free radicals prevenient from different sources such as cells function or exogenous root.

Studies involving the potential antioxidant properties of OPN leaves and its aqueous extract using different assays are scarce in the literature. Garcia et al. (2019) elaborated hydroethanolic extract of OPN leaves and noticed relevant values of antioxidant capacity using different assays (DPPH and ABTS). Souza et al. (2016) noticed lower DPPH values assessing a methanolic extract of OPN leaves. Silva et al. (2018) observed an antioxidant activity of 44.99 g TE kg^{-1} of fresh plant employing a methanolic extract of OPN leaves. Pinto et al. (2012) recorded the potential antioxidant activity in OPN leaves using a DPPH method of indication. They investigated different solvent to extract the antioxidant compounds and found phenols as the major compounds (49.11 ± 3.30 mg tannin acid equivalents g^{-1} sample, using dichloromethane as the solvent). Gavamukulya et al. (2014) determined the *in vitro* antioxidant activity (DPPH assay) in ethanolic and water extracts of *A. muricata* leaves and found 2.04 mg mL^{-1} and 0.91 mg mL^{-1}, respectively. Parveen et al. (2018) evaluated the antioxidant properties (DPPH and FRAP) in triphala extracts. They noticed that methanolic extract was more powerful free radical scavenger than aqueous extract and it was attributed to the polarity of the solvent and phenolic compounds.

OPN aqueous extract presented lower results for FRAP (Table 3) antioxidant activity assay (0.91 ± 0.03 µMol TE g^{-1} OPN extract) in comparison with others plant extract (Parveen et al., 2018; Junsathian et al., 2018). Elfalleh et al. (2019) evaluated the antioxidant potential of *Stachys tmolea* Boiss. aqueous extract and verified FRAP values higher than those shown in the present work.

The ORAC results presented in Table 3 was lower than overall reported by Lin et al. (2018) for several plant extracts and Tian et al. (2018) in extracts of berries, leaves, and branches.

3.2.4 FTIR analysis

The FTIR spectral for OPN aqueous extract and flour of OPN leaves are presented in Figure 1. The data indicated the appearance of different functional groups in both of samples. The data of the aqueous extract and OPN leaves revealed the presence of potential bioactive compounds represented by functional groups such as –CHO, –COOH, –COOR, >NH and –OH. Both samples shown characteristic broad peak for hydrogen bonded –OH stretching (zone about 3400 cm^{-1}) in functional group region. According to Poojary et al. (2015) the hydroxyl group functionality is an integral part of the most phytochemicals compounds such as flavonoids and tannins. Furthermore, note that some peaks disappeared (region 2800 to 2900 cm^{-1} and 1200 to 1500 cm^{-1}) when compared the spectra obtained for leaves and extract of OPN. It could be related to the slight extraction of phytochemicals compounds from the OPN leaves.

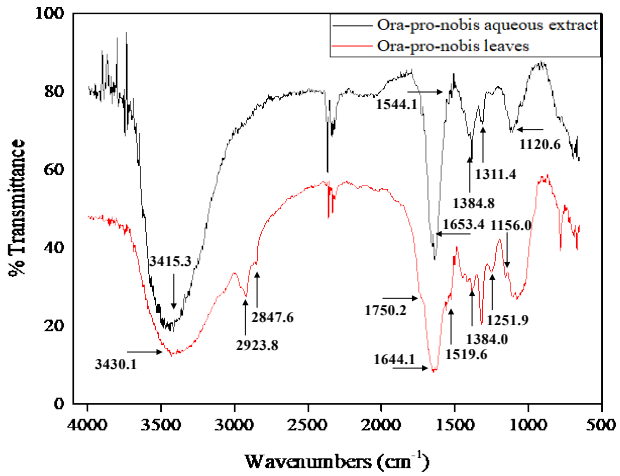

Figure 1. FTIR spectrum of OPN.

Indeed, the OPN leaves presented a spectrum with peaks at 3430.1 (H-bonded O–H stretching; or –N–H stretching), 2923.8 (aldehyde –C–H stretching), 2847.6 (–C–H stretching), 1750.2 (aldehyde/ketone –C = O stretching), 1644.1 (–C = C– stretching), 1519.6 (–NH bending), 1384.0 (–CH3 bending), 1251.9 (–C–H in-plane bending), 1156.0 (amine C–N stretching). It suggests that the probable phytochemicals compounds found in OPN leaves would be alkaloids, flavonoids, poly phenols, carboxylic acid containing phytochemicals, tannins (Poojary et al., 2015).

OPN aqueous extract noticed a spectrum with peaks at 3415.3 (H-bonded O–H stretching), 1653.4 [alkene C = C stretch (conjugated)], 1544.1 (–NH bending), 1384.8 (–CH3 bending), 1311.4(–C–H in-plane bending), 1120.6 (amine C–N stretching). It indicated that the potential phytochemicals compounds noticed in OPN aqueous extract would be alkaloids, flavonoids, poly phenols (Poojary et al., 2015).

FTIR results assessed in this work for OPN leaves were similar to those obtained by Conceição et al. (2014), that prepared powdered gum derived from OPN aqueous extract and Mercê et al. (2001), that developed studies involving OPN leaves to obtain biopolymers.

4 Conclusion

The results obtained indicated that the ora-pro-nobis (OPN) leaves presented a high concentration of proteins, fibres and iron, which can be an important source of nutrients using non-conventional edible plant. The simple, rapid and inexpensive process using a solvent 50 mM NaCl solution was able to extract iron and bioactive compounds from the OPN leaves. The OPN aqueous extract presented potential antioxidant activities demonstrated by FRAP, ORAC and DPPH• methods. The OPN aqueous extract containing iron and bioactive compounds obtained in this work could be applied in carrier systems as a complementary alternative to combat the iron deficiency and radical scavenger. It could be inferred from this work that the OPN may be widely explored to obtain compounds related to antioxidant activity. In addition, the high concentration of iron in the extract can enable important applications in the development of new products rich in iron from a natural source."

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