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Bioactive profile of mandacaru fruits and cytotoxicity against the L929 cell line

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Mandacaru (*Cereus jamacaru* DC. Subsp. Jamacaru) is native to the Brazilian Caatinga and is part of the Cactaceae family. Mandacaru fruits are attractive in taste and flavour, although they are not widely commercialised. However, there is limited scientific literature on the composition of this fruit. Therefore, the present work was aimed at analysing the physicochemical composition, as well as *in vitro* antioxidant activity, phytochemical profile, and cytotoxicity of mandacaru fruit from three different locations in the Brazilian State of Sergipe. The pulp and peel of mandacaru fruit used in this study presented low Vitamin C concentration, mean values between batches 18.2 mg. 100 g^{-1} (pulp) and 27.5 mg. 100 g^{-1} (peel). The pulp of mandacaru fruit from the region of Monte Alegre presented the highest concentration of apparent phenolic compounds (117.2 mg. EAG g^{-1}) and antioxidant activity evaluated by the inhibition of the ABTS radical (22.4 µmol. trolox g^{-1}). The chemical profile of mandacaru fruit consisted of *o*-coumaric and *p*-coumaric acids, but they were not considered to be cytotoxic; thus, this native fruit is suitable for consumption, exhibiting relevant amounts of apparent phenolic compounds and antioxidant activity. Further studies are needed to prolong the shelf life of the fruit for commercialisation.

Key words: *Cereus jamacaru*, antioxidant activity, phytochemical profile, Caatinga.

INTRODUCTION

Caatinga is one of the Brazilian biomes responsible to produce native and exotic Brazilian fruits. It is an exclusively and unique Brazilian biome, with the presence of dry vegetation, covering a wide area of the Brazilian Northeast region; in addition, it is associated with a diverse cultural heritage. This region is home to a diverse variety of fruitful cacti. However, some of these species are still underexplored (Albuquerque et al., 2007; Lucena et al., 2013).

Owing to the decrease in water resources in the world,

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cacti are considered as the food of the future because of their ability to adapt to dry climates, with great potential for generating income because of their wide range of applications (Santos et al., 2019; Shetty et al., 2012).

Cereus jamacaru DC subsp. Jamacaru is among the fruit species present in Caatinga, part of the cactaceae family and popularly known as mandacaru. This cactus species produces attractive fruits in colour and flavour, with a thick and red pericarp, a white pulp, and small black seeds, which are used in the production of jam by the local community (Silva and Alves, 2009; Torres et al., 2009).

Mandacaru fruits grow in large quantities between the months of February and September. Nevertheless, they are not widely commercialised, differently to Koubo *(Cereus peruvianus*), fruit of the cactacea family, grown in Israel and sold to Europe, which bears a great resemblance to the mandacaru fruit (Mizrahi, 2014; Santos et al., 2019). The sustainable use and production of mandacaru fruit can generate an alternative source of income to the local community, promoting this important caatinga biome.

Accordingly, the present work was aimed at analysing the physicochemical composition, phytochemical profile and *in vitro* antioxidant activity of the pulp and peel of the mandacaru fruit, as well as the cytotoxicity of the fruit pulp.

MATERIALS AND METHODS

Collection of plant

Three batches of ripe mandacaru fruits (*C. jamacaru* DC. subsp. *jamacaru*) were collected from three different municipalities in the Brazilian State of Sergipe: Aquidabã (10°16'18.0"S and 37°02'39.5"W), in January 2019 (batch 1); Monte Alegre (10°05'21.7" S and 37°39'5,17" W), in February 2019 (batch 2); and Porto da Folha (9°55'35.9"S and 37°15'47.5"W), in February 2019 (batch 3), according to data from the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGen) (National System for the Management of Genetic Heritage and Associated Traditional Knowledge). The samples of mandacaru fruit were identified and stored in the Herbarium of Federal University of Sergipe (ASE) under record number 42279.

Batches 1, 2 and 3 contained 17, 28 and 27 fruits, respectively. The samples were then sent to the Chemical and Biochemical Food Laboratory in the Department of Food Technology of the Federal University of Sergipe, where the fruits were previously sanitised with running water and immersed in water with sanitiser at 200 ppm for 10 min. The fruits were subsequently re-immersed in sanitising solution at 3 ppm for the removal of excess chlorine. The pulp and the peel were manually pulped and weighed, corresponding to a final mass of 1.55 kg of pulp and 2.02 kg of peel (batch 1), 2.21 kg of pulp and 2.6 kg of peel (batch 2), 2.37 kg of pulp and 2.76 kg of peel (batch 3). They were then stored in a freezer at -20^{o}C for further analysis.

Physicochemical and centesimal characterisation

Six fruits were randomly selected from each batch, and their dimensions were determined using a digital pachymeter, in the perpendicular and parallel directions to the central axis and expressed as millimetres (mm).

The colorimetric analysis of mandacaru fruit was carried out at four different points of the fruits. The readings of CIELAB parameters L*a*b* were carried out with a CR-10 colorimeter (Konica Minolta), where L* represents the lightness of the colour measured (L* = 100 = white; L* = 0 = black). In turn, a* expresses the variation between red and green (more negative a*=greener; more positive a* = redder). Coordinate b* represents the variation between blue and yellow (more negative b* = bluer; more positive b* = yellower) (Melo et al., 2017).

The centesimal compositions of mandacaru fruit pulp and peel were determined in the Technological Institute of Research from the State of Sergipe (ITPS), according to the analytical standards of the Instituto Adolfo Lutz (IAL, 2008). Moisture (method no. 012/IV), ashes (standard no. 018/IV), proteins (standard no. 036/IV), lipids (standard no. 032/IV), carbohydrates (determined by difference) and total energy value (TEV) (calculation) were then determined.

The chemical analyses were carried out in the Chemical and Biochemical Food Laboratory in the Food Technology Department from the Federal University of Sergipe, according to the analytical standards of the IAL (2008), having determined pH (standard no. 017/IV), total soluble solids (TTS) (standard no. 315/IV) and mandacaru fruit pulp-peel ratio (316/IV), titratable acidity in organic acid (standard no. 312/IV) for mandacaru fruit pulp and volumetric titratable acidity (310/IV) for the fruit peel. Water activity (a_w) was determined for the fruit pulp followed by the fruit peel, according to the standards established by Association of Official Analytical Chemists (AOAC, 1995).

Vitamin C

For the mandacaru fruit pulp and peel, Vitamin C was determined in triplicate by titrimetric analysis, according to AOAC (1997), as described by Oliveira et al. (2010), with modifications, using 2% oxalic acid titrated with 2,6-dichlorophenolindophenol (DCPIP) solution, and then standardized with ascorbic acid solution.

Extract production

Initially, the mandacaru fruit pulp was lyophilised for 72 h for storage owing to its high perishability. To obtain the extract, 6.5 g of the lyophilised fruit (equivalent of 50 g of pulp *in natura*) was macerated in 1,000 mL 50% hydroethanolic solution (v/v), remaining in an ice-cold solution for 72 h in the dark, as described by Sonaglio et al. (2004). The extract was then filtered in a Büchner funnel and concentrated in a rotary evaporator until reaching 50% of the initial volume. Finally, the extract produced was lyophilised and stored in a freezer at -20°C for subsequent analyses.

Apparent phenolic compounds

The phenolic compounds present in mandacaru fruit pulps were estimated in triplicate using the Folin-Ciocalteu spectrophotometric method, according to the procedures described by Boroski et al. (2015).

The sample extracts were prepared in a 50% hydroethanolic solution in the dark, reaching a final concentration of 5 mg mL^{-1}.

Firstly, 250 µL of extract solution was added together with 250 µL of Folin-Ciocalteu reagent (1:1), 500 µL of saturated sodium carbonate solution (35 g in 100 mL) and 4 mL of distilled water in test tubes.

The test tubes were stirred and remained at room temperature in the dark for 25 min, and then they were subsequently centrifuged for 10 min at 3,000 rpm. Maximum absorbance at 725 nm was then determined in a UV-Vis SP-220 (Bioespectre) spectrophotometer.

Gallic acid was used to determine the external standard curve, plotting the different concentrations (0 to 150 $mg.L^{-1}$) in a scatter plot and determining the respective linear regression equation. The results were expressed as gallic acid equivalent per g of the sample (mg $GAE.g^{-1}$).

Antioxidant activity

ABTS• radical scavenging assay

The antioxidant activity of mandacaru fruit pulps was analysed by ABTS scavenging assay [2,2'–azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], as described by Boroski et al. (2015).

At first, an analysis of the Trolox standard (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was carried out to build the calibration curve, with dilutions between 100 and 2,000 $\mu mol.L^{-1}$. One aliquot of 30 µL was added in the test tubes. Then, 3.0 mL of $ABTS^{+}$ cation radical was added in the dark (which corresponds to 5 mL of 7.0 $\mu mol.L^{-1}$ ABTS stock solution with 88 µL of 140 μmol^{-1} potassium persulfate solution). The solution was incubated at room temperature in the dark for 16 h. Subsequently, 1 mL of solution was diluted in ethanol, to obtain an absorbance of 0.7 ± 0.05 in 734 nm, and then homogenised. Readings were taken after 6 min at 734 nm using a UV-Vis SP-220 (Bioespectre) spectrophotometer. The external Trolox standard curve was then obtained, generating the respective linear regression equation.

The analysis of mandacaru fruit pulps was carried out after three dilutions of the extracts with 50% (v/v) hydroethanolic solution (5, 10 and 15 $g.L^{-1}$), in triplicate, according to the same procedure used to obtain the calibration curve.

A graph was plotted from the absorbances obtained for the dilutions, generating a linear regression equation, where the absorbance for 1000 $\mu mol.L^{-1}$ of Trolox was replaced in the straight-line equation obtained from the dilutions. The results were expressed as µmol $Trolox.g^{-1}$ of sample (Boroski et al., 2015).

Ferric reducing antioxidant power (FRAP)

Antioxidant activity was estimated using the FRAP method, as described by Boroski et al. (2015). The FRAP reagent was prepared as follows: 100 mL of 300 $mmol.L^{-1}$ sodium acetate buffer solution, pH 3; 10 mL of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution; 10 mL of 20 $mmol.L^{-1}$ iron (III) chloride hexahydrate.

The extracts of mandacaru fruit pulp were prepared using distilled water in triplicate and in the dark. Subsequently, 3 mL of FRAP reagent was added in a test tube, together with 100 µL of the sample and 300 µL of distilled water, then homogenised and incubated in a water bath at 37°C for 10 min. Absorbance was determined at 593 nm.

Iron (II) sulphate heptahydrate $FeSO_4.7H_2O$ ranging between 0 and 2000 $\mu mol.L^{-1}$ was used for the external calibration curve. The results were obtained from the calibration equation and expressed as µmol of Fe (II) g^{-1} of sample, then converted into µmol of Fe (II) 100 g^{-1} of sample.

Chromatographic analysis

Reagents

LC-grade methanol (Honeywell, Muskegon, MI, USA), isopropyl alcohol (JT Baker, Philipsburg, PA, USA) and formic acid (JT Baker, Philipsburg, PA, USA) were used for HPLC analysis. Deionised water was purified by a Milli-Q system (Millipore, São Paulo, SP, Brazil). All the solvents were filtered through nylon 0.45 µm membranes (MFS) and degassed by ultrasonic bath before use.

High-performance liquid chromatography–photodiode array detection analysis

A Shimadzu liquid chromatograph (Kyoto, Japan) Prominence Model equipped with a DGU-20A3 vacuum degassing, SIL-20AHT autosampler, two high pressure pumps LC-20AT, a CTO-20A column oven and an SPD-M20A photodiode array detector (DAD) system coupled with a CBM-20A system interface was used for chromatographic analysis. Data collection was performed using Shimadzu LC Solution software.

The extracts were prepared by dissolving 100 mg in 20 mL of isopropyl alcohol: deionised water (80:20) (5 $mg.mL^{-1}$) and concentrated in a rotary evaporator at 45°C until reaching approximately 2 mL. The extract was then filtered, diluted with isopropyl alcohol: deionised water (80:20) until 4 mL, immersed in ultrasonic bath for 5 min and analysed by HPLC-DAD.

The chromatographic profile of the extracts of mandacaru were obtained using a Phenomenex Luna C_{18} column (250 × 4.6 mm i.d., 5 µm of particle diameter) under the following conditions: at 35°C, flow rate 1 mL/min, injection volume of 25 µL and a mobile phase consisting of 0.5% (v/v) aqueous formic acid (A) and methanol (B). The gradient elution for samples was as follows: 5-100% B for 60 min, 100% isocratic B for 10 min, and the system was returned to the initial conditions in 5 min. The column was conditioned before the next injection for 35 min Photodiode array detector was set at 270 nm for acquiring chromatograms, and ultraviolet spectra were recorded between 200 and 400 nm.

Samples of standards (benzoic acid, caffeic acid, catechin, cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, ellagic acid, gallic acid, gentisic acid, protocatechuic acid, quercetin, and vanillic acid) were dissolved in isopropyl alcohol: deionised water (80:20) (0.5 $mg.\ mL^{-1}$). Identification was based on comparisons of absorption spectra and co-injection with standard compounds. For co-injection, the concentration of the standards used was 0.002 $mg.mL^{-1}$ for the *o*-coumaric acid standard and 0.004 $mg.mL^{-1}$ for the *p*-coumaric acid.

Cytotoxicity

L929 cells (murine fibroblasts) were grown and kept in DMEM culture medium (Sigma) supplemented with 10% foetal bovine serum (Gibco) and 1% antibiotic (penicillin/streptomycin) and maintained in a humified oven with 5% CO_2 at 37°C for 24 h.

The cells were detached from the harvesting flasks using 0.25% Trypsin (dissolved in DMEM medium), counted, and deposited in 96 well plates at a density of 2×10^4 cells/well. After 24 h of incubation in the oven, for better adherence of the cells to the plate, the cells were treated with 50% (v/v) hydroethanolic extracts at concentrations ranging from 12.5 to 200 $\mu g.\ mL^{-1}$ for 24 h. Cell viability was analysed by the methyl-thiazolyl-tetrazolium (MTT) colorimetric assay, based on the reduction of MTT by viable cell mitochondria into blue Formazan crystals.

Therefore, after treatment, the plates were washed twice with PBS, with 200 µL of MTT and subsequently added in each well. The plate was then incubated for 3 h and the formazan crystals dissolved with DMSO for 10 min, with readings carried out in a spectrophotometer at a wavelength of 570 nm (Alley et al., 1988; Denizot and Lang, 1986; Mosmann, 1983). The result was expressed as % of cell viability, through the following equation: % of cell viability = $[(ABS_{sample}) / (ABS_{control}) \times 100]$.

Statistics

The experiments were carried out in triplicate and the results underwent analysis of variance (ANOVA) and Tukey's test ($p<0.05$), using the software Sisvar 5.6. The results were expressed as mean

Table 1. Physicochemical characteristics and Vitamin C concentration of mandacaru fruit peel.

Parameter	Batch 1	Batch 2	Batch 3
pH	4.8±0.2[a]	5.2±0.0[a]	5.6±0.0[a]
a_w	0.9±0.0[a]	0.9±0.0[a]	0.9±0.0[a]
TA (% citric acid)	0.2±0.0[a]	0.3±0.0[a]	0.2±0.0[a]
SS (°Brix)	2.3±0.5[a]	2.3±0.0[a]	3±0.0[a]
Ratio (SS/TA)	8.5±2.5[a]	7.2±2.6[a]	13.9±0.8[a]
Moisture (g. 100g^{-1})	92.2±0.4[a]	92.0±0.1[a]	93.1±0.6[a]
Ashes (g. 100g^{-1})	0.7±0.0[a]	1.0±0.0[a]	0.6±0.0[a]
Vit C (mg. 100g^{-1})	8.3±0.1[a]	8.6±0.5[a]	5.7 ±1.1[a]
PTN(g. 100g^{-1})	3.5±0.1[a]	1.4±0.0[a]	1.6±0.0[a]
LIP (g. 100g^{-1})	0.6±0.0[a]	0.3±0.0[a]	0.5±0.0[a]
CHO (g. 100g^{-1})	2.88[a]	5.19[a]	4.02[a]
TCV (Kcal. 100g^{-1})	31.1[a]	29.6[a]	27.3[a]
Longitudinal Diameter (cm)	86.6±17.6[a]	97.5±13.1[a]	92.5±7.3[a]
Transverse Diameter 1 (cm)	65.9 ± 14.3[a]	58.4±6.5[a]	63.6±7.0[a]
Transverse Diameter 2 (cm	61.8±14.1[a]	55.5±9.1[a]	60.6±6.5[a]
L*	24.0±0.9[a]	25.6±0.4[a]	21.8±0.4[a]
a*	24.5±2.4[a]	28.5±4[a]	21.5±4.2[a]
b*	18.0±1.3[a]	20.2±1.3[a]	19.9±0.6[a]

Batch 1= mandacaru fruit collected in the municipality of Aquidabã, Batch 2= mandacaru fruit collected in the municipality of Monte Alegre, Batch 3= mandacaru fruit collected in the municipality of Porto da Folha, a_w = water activity, TA = Titratable acidity, SS= Soluble solids, Vit C= Vitamin C LIP= Lipids, CHO= Carbohydrates, TCV= Total caloric values. Results expressed as mean values (n=3) ± standard deviation. Mean values followed by different letters in the same row differ statistically according to Tukey's test ($p<0.05$).

± standard deviation.

RESULTS

Physicochemical and centesimal characterization

Tables 1 and 2 show the physicochemical characteristics of the fruits, as well as Vitamin C concentration of mandacaru fruit pulp and peel.

In the physical characterisation of mandacaru fruits (Table 1), no significant difference ($p> 0.05$) was found between the batches. The fruits had an average longitudinal diameter of 92.2 cm. Regarding the colour of all batches, there was a predominance of red (a *) over yellow (b *) and light luminosity.

The mandacaru fruits have a little acid character, showing mean values of 5.3 and 5.2 for pulp and peel, respectively. The values of centesimal composition were similar for all batches, with mean caloric value of 58.7 and 29.3 Kcal 100 g^{-1} for pulp and peel, respectively. The bark moisture was higher than the pulp, according to Tables 1 and 2.

Total phenolic compounds and antioxidant activity

The content of apparent phenolic compounds in the pulp of mandacaru fruits ranged from 98.6 to 117.2 mg. EAG g^{-1}, and batches 2 and 3 differed significantly from batch 1 ($p <0.05$), similarly to the antioxidant activity by the ABTS radical capture method, with batch 2 being superior to the others (22.4 µmol. trolox g^{-1}). For iron reduction capacity, the results were similar for all batches ($p> 0.05$) with mean values of 386.85 µmol Fe (II) g^{-1} (Table 3).

Phytochemical identification

Complementing the determination of phenolic compounds, an analysis was made of the extracts of the pulp of the mandacaru fruit from the batches under study in HPLC-DAD. The results indicate that there are no profile differences between the evaluated batches, with differences in peak intensity only.

Of the twelve compounds investigated (catechin, quercetin, protocatechuic acid, vanillic acid, o-coumaric acid, p-coumaric acid, gallic acid, gentisic acid, cinnamic acid, ellagic acid, benzoic acid and caffeic acid) with the use of commercial standards, p-coumaric (peak 4) and o-coumaric (peak 5) acids were positively identified by comparing their respective retention times with the retention time of the standards and by co-injection of the standards into the sample.

Figure 1 shows the chromatograms of the sample from batch 1 (p-coumaric acid) and p-coumaric acid standard.

Table 2. Physicochemical characteristics and Vitamin C concentration of mandacaru fruit pulp.

Parameter	Batch 1	Batch 2	Batch 3
pH	5.3±0.4[a]	5.1±0.3[a]	5.5±0.1[a]
a_w	0.9±0.0[a]	0.9±0.0[a]	0.9±0.0[a]
TA (% citric acid)	0.1±0.0[a]	0.2±0.0[a]	0.1±0.0[a]
SS (°Brix)	8.4±0.1[a]	10.4±0.6 [a]	9.1±0.3[a]
Ratio (SS/TA)	53.6±15.5[a]	47±23.3[a]	50±9.9[a]
Moisture (g. 100g^{-1})	84.3±13 [a]	85.2±0.9[a]	86.3±2.0[a]
Ashes (g. 100g^{-1})	0.5±0.0[a]	0.4±0.0[a]	0.3±0.0[a]
Vit C (mg. 100g^{-1})	21.5±1.5[a]	18.1±0.4[a]	15.1± 0.5 [a]
PTN (g. 100g^{-1})	1.7±0.1[a]	1.9±0.0[a]	1.3±0.1[a]
LIP (g. 100g^{-1})	0.2±0.0[a]	0.2±0.0[a]	0.4±0.0[a]
CHO(g. 100g^{-1})	13.1[a]	12.1[a]	11.4[a]
TCV (Kcal. 100g^{-1})	62.2[a]	58.6[a]	55.5[a]

Batch 1= mandacaru fruit collected in the municipality of Aquidabã, Batch 2= mandacaru fruit collected in the municipality of Monte Alegre, Batch 3= mandacaru fruit collected in the municipality of Porto da Folha, a_w = water activity, TA = Titratable acidity, SS= Soluble solids, Vit C= Vitamin C LIP= Lipids, CHO= Carbohydrates, TCV= Total caloric values. Results expressed as mean values (n=3) ± standard deviation. Mean values followed by different letters in the same row differ statistically according to Tukey's test ($p<0.05$).

Table 3. Concentration of apparent phenolic compounds and antioxidant activity of mandacaru fruit pulp.

Method	Batch 1	Batch 2	Batch 3
Total phenolic C. (mg. EAG g^{-1})	98.6±5.4[b]	117.2± 2.4[a]	109.7± 4.6[a]
ABTS (µmol. trolox g^{-1})	10.2±0.1[b]	22.4 ±0.1[a]	21.9 ±0.1[a]
FRAP (µmol. Fe(II) g^{-1})	374.6±25.5[a]	400.9±7.5[a]	384.9±21.2[a]

Batch 1= mandacaru fruit collected in the municipality of Aquidabã, Batch 2= mandacaru fruit collected in the municipality of Monte Alegre, Batch 3= mandacaru fruit collected in the municipality of Porto da Folha. Results expressed as mean values (n=3) ± standard deviation. Mean values followed by different letters in the same row differ statistically according to Tukey's test ($p<0.05$).

Based on these chromatograms, similar retention times were found between the standard and the sample (analyte), eluting in 24 min. This was confirmed with the co-injection of the standard into the sample (Figure 1), as there was an increase in the intensity of absorption of the related peak of the analyte (t = 24 min) when fortifying with the standard p-coumaric acid.

A similar procedure was used to confirm the identity of the analyte eluted at 30 min. Co-injection of the sample (Figure 2) with the o-coumaric acid standard showed the coincidence of retention time, as well as the increase in absorption for the chromatographic peak of the target analyte (Figure 2). It can be inferred that there is possibly o-coumaric acid in the sample.

In an attempt to identify other compounds, which do not coincide with the standards employed, the UV-vis spectra were analysed using the comparison with the previously identified spectra (Campos and Markham, 2007), with peaks 1 (t = 3 min; UV λ_{max}: 278), 2 (t = 5 min; UV λ_{max}: 257), and 3 (t = 5.8 min; UV λ_{max}: 254) (Figure 3) being similar to the phenolic acid spectra, suggesting that they may be part of this class of compounds.

Cell viability

Figure 4 shows the results of the cytotoxicity test of the hydroethanolic extracts of the mandacaru fruit pulp regarding the L929 cell culture (fibroblast) viability through absorbance readings of the MTT test.

DISCUSSION

The physical parameters of the mandacaru fruits studied in the present work agree with the data reported in the literature (Almeida et al., 2009; Melo et al., 2017). Diameter and colour are important attributes, as the appearance of fruits is one of the most relevant parameters for attracting consumers, and it is of

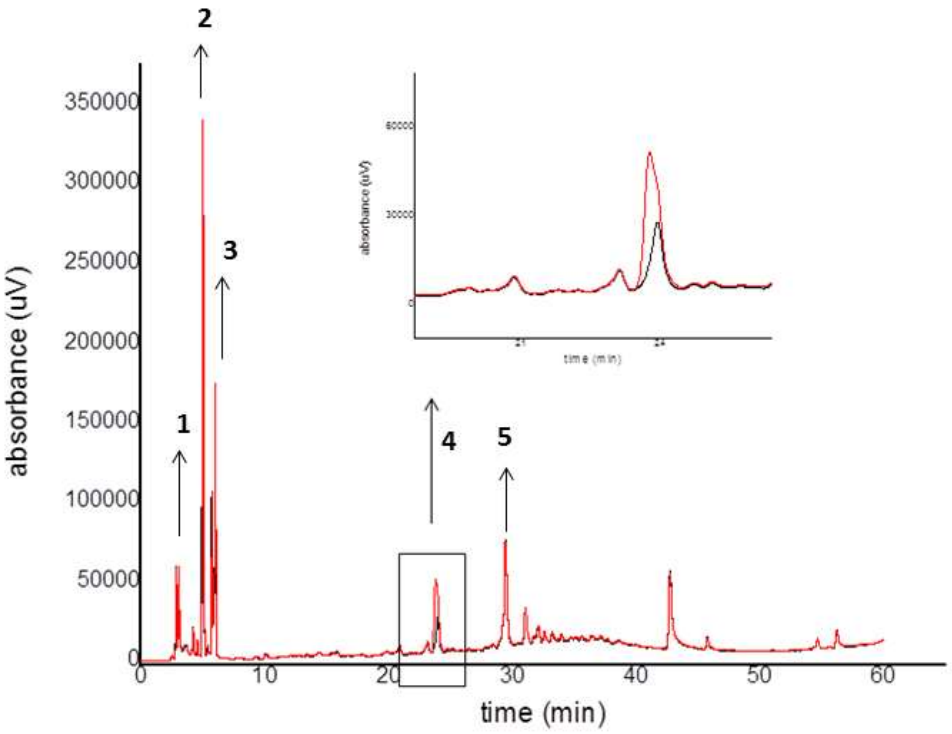

Figure 1. Chromatograms corresponding to the overlap between the chromatogram of the mandacaru fruit extract (black) and co-injection with the standard *p*-coumaric acid (red) (peak 4)

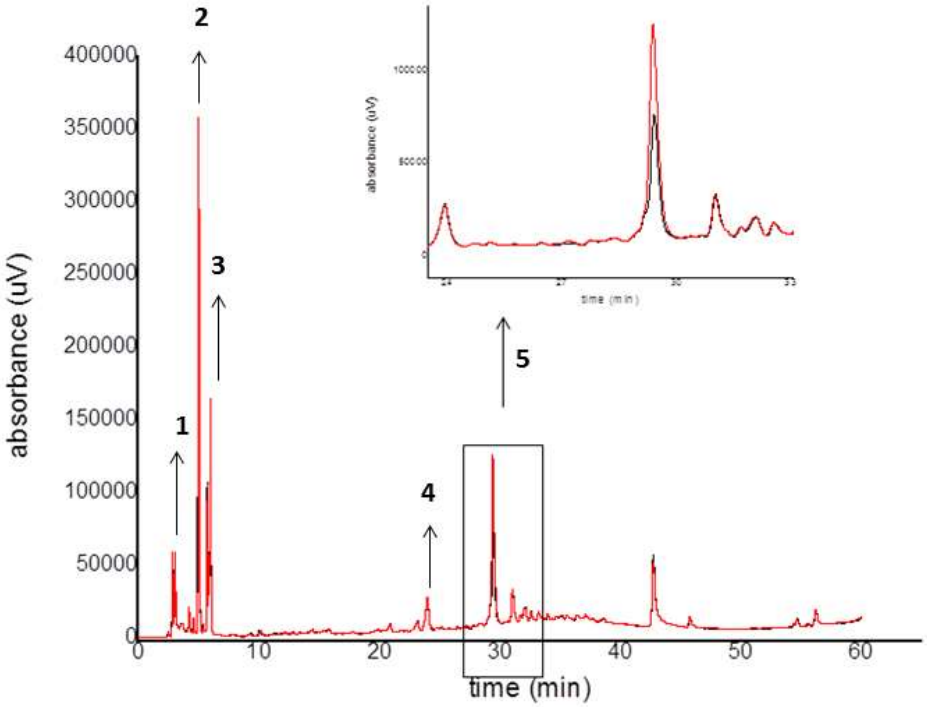

Figure 2. Chromatograms corresponding to the overlap between the chromatogram of mandacaru fruit extract (black) and co-injection with the standard *o*-coumaric acid (red) (peak 5).

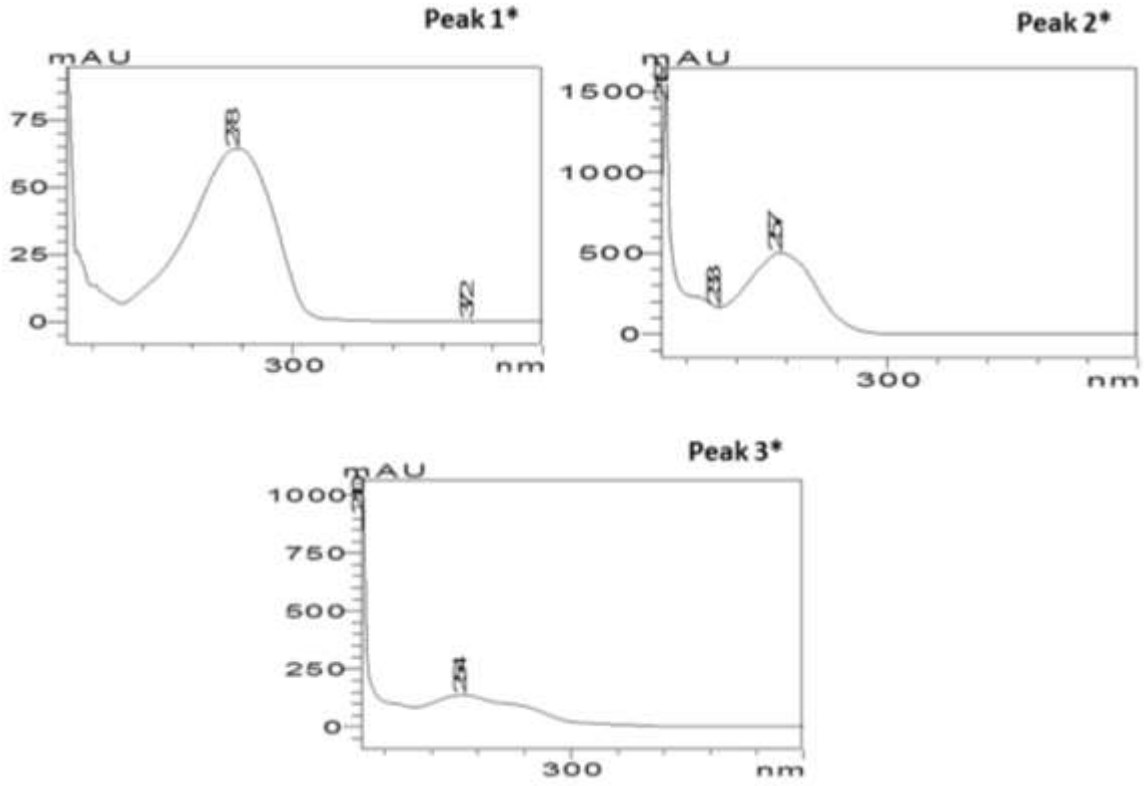

Figure 3. UV-vis spectra of peaks 1, 2 and 3. *Peak numbers are in accordance with Figures 1 and 2.

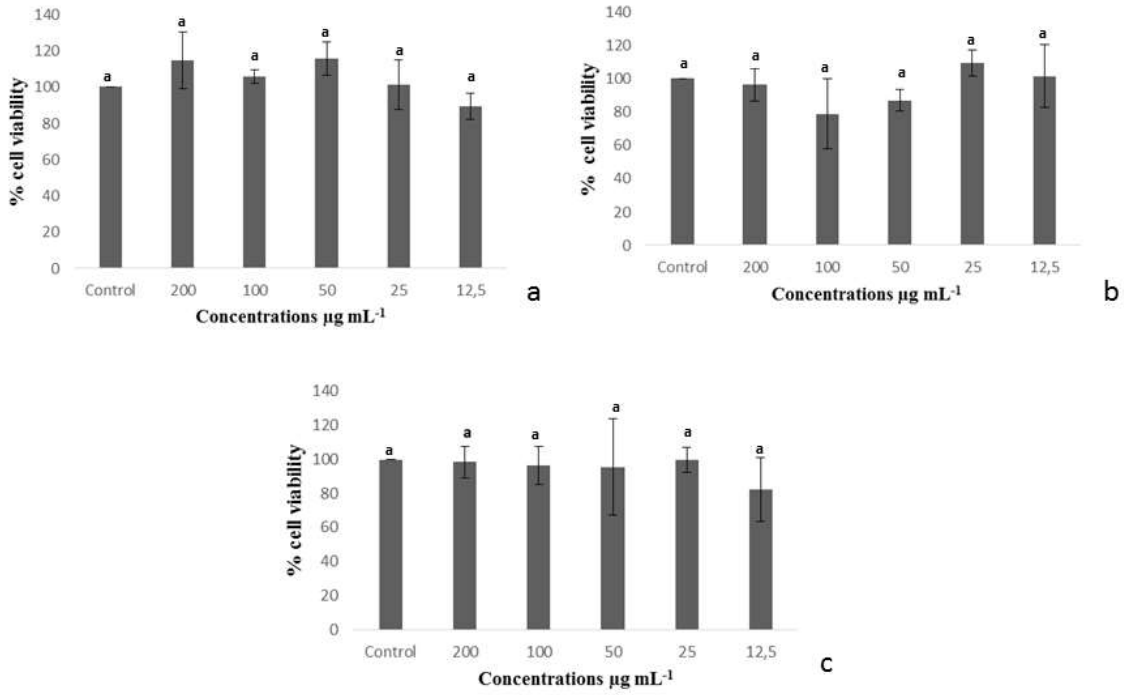

Figure 4. Viability percentage of L929 cell cultures treated with mandacaru fruit pulp extract from the different batches (Batch 1 (a), Batch 2 (b) and Batch 3 (c)). The percentage of viable cells is presented as mean ± standard deviation. The lines at the top of the bars represent the standard deviation. Different letters differ statistically by Tukey's test ($p<0.05$). The cells were treated with mandacaru pulp extract for 24 h.

commercial added value to the fresh fruit (Aguiar et al., 2015).

The results of the (SS/TA) ratio for mandacaru fruit pulp demonstrate that the fruits obtained were in a more advanced ripening stage, which creates a greater sense of sweetness to consumers.

Other Cactaceae fruits produced in Brazil also presented a high (SS/TA) ratio, such as pitaya fruit (*Hylocereus polyrhizus,* 45.31), cultivated in the North of Minas Gerais and Indian fig (*Opuntia fícus-indica,* 101.66), grown in the State of Bahia (Cordeiro et al*.,* 2015; Silva et al., 2017). For pitaya fruit, *Hylocereus undatus* species, produced in Mexico, a lower ratio was found (33.5) (Yah et al*.,* 2008), suggesting that a high value of this parameter can be characteristic of Brazilian Cactaceae fruits, as such parameters are influenced by edaphoclimatic conditions where the fruit is produced.

According to the results for pH, mandacaru pulp and peel are not considered to be acid (pH<4.5), which makes the fruit more prone to microbial development (Baruffaldi and Oliveira, 1998; Nascimento et al*.,* 2011). In addition, the pulp and peel exhibit high moisture concentrations and water activity. These parameters characterise foods as highly perishable (Fellows, 2006).

The physicochemical analysis of the peel was carried out to verify the potential for reuse in future applications, since there are no reports of the complete characterisation of the fruit in the literature. For the industrialisation of the fruit, these characteristics are important for processing purposes.

Mandacaru fruit also presents a low calorific value, similarly to other popularly commercialised fruits, such as apples (63 kcal. 100 g^{-1}), pears (61 kcal. 100 g^{-1}) and guava (54 kcal. 100 g^{-1}) (Núcleo de Estudos e Pesquisas em Alimentação, 2011), which can be an option for consumption in calorie-restricted diets.

Vitamin C concentration determined for mandacaru fruit was low mean values 18.2 mg. 100 g^{-1} (pulp) and 27.5 mg.100 g^{-1} (peel), when compared to other fruits also found in Caatinga, such as mangaba (193.07 mg. 100 g^{-1}) (Paula et al*.,* 2019), with the concentration found for mandacaru pulp being twice the result found in the fruit peel.

Despite the low Vitamin C concentration found in mandacaru fruits, the consumption of this vitamin through this fruit, even if in small concentrations, can complement one's diet in achieving the daily 75 mg a day of Vitamin C required for adults, according to Dietary Reference Intake (DRIs) (Institute of Medicine, 2002).

In relation to the total apparent phenolic content, the fruit pulp from batches 2 and 3 differed significantly from batch 1 ($p < 0.05$), with batch 2 presenting the highest concentration. This result is an indication that the region of Monte Alegre, in the State of Sergipe, can contribute to an increase in the presence of phenolic compounds when compared with batch 1, which was produced in another region, and are superior to those reported in other cactus species.

Cayupán et al. (2011) reported values of 0.6 mg GAE. g^{-1}, on average, of *O. ficus-indica* fruits (Indian figs) at different ripening stages. Kim et al. (2011) found values between 3.51 and 4.91 mg GAE.g^{-1} for *H. undatus* and *Hylocereus costaricensis* pulps (white-fleshed and red-fleshed pitayas), respectively.

This variation can be a result of phenological and environmental factors, such as prolonged periods of drought and high temperatures, which are characteristic of Caatinga biomes. These factors can have an impact on the increased production of phenolic compounds, as a defence and resistance mechanism of plants (Barba et al., 2017; Ignat et al., 2011).

The antioxidant activity, property of phenolic compounds, is one of the main elements by which vegetables provide health protection, delaying or preventing the oxidation process in substrates by means of one or more mechanisms, such as neutralisation of free radicals and metal complexation (Boroski et al., 2015; Burillo et al., 2018; Carocho et al., 2018).

The antioxidant activity evaluated by the FRAP method showed the highest values, while the results for ABTS point to a similar behaviour between batches 2 and 3, collected in the same region and at the same time, confirming the findings for phenolic compounds.

The difference in results between FRAP and ABTS is due to the extractive power of each of the solvents in use, hydroalcoholic for ABTS and aqueous for FRAP, which vary according to polarity. The methods used in this research to assess antioxidant activity, are only models that employ different chemical mechanisms. Thus, even if the extracts exhibited similar composition and concentration levels, they could have different responses. In this regard, the solvents that best fit the models were selected for the study.

The results found in the present study are within the range of values referred by Almeida et al. (2011) for the different fruits of caatinga, according to the ABTS assay, such as umbu (1.07 µmol trolox g^{-1}), murici (15.73 µmol trolox g^{-1}) and mangaba (10.84 µmol trolox g^{-1}).

For the correlation between total apparent phenolic and antioxidant activity, Pearson's correlation coefficient was used. The results showed that in both the aqueous extract evaluated by the FRAP method ($r = 0.9720$) and the hydroalcoholic extract evaluated by the ABTS method ($r = 0.9312$), there is a strong correlation between the concentration of phenolic compounds and antioxidant activity. The extract was evaluated by the FRAP method, which showed the strongest correlation between phenolic compounds and antioxidant activity.

The mandacaru fruit represents a good source of phenolic compounds among the exotic fruits found in the Brazilian Northeast region, with considerable antioxidant activity, not only for the presence of phenolic compounds, but also because of other bioactive compounds that were not investigated in this study.

Phytochemical analysis allows to identify phenolic compounds (*o*- and *p*-coumaric acids), reported for the first time for *Cereus jamacaru.* To date, there are no reports on the phytochemical composition of mandacaru fruits determined by HPLC-DAD. However, other cactus fruits have been evaluated in the literature in terms of their phytochemical profile, such as pitayas (*Stenocereus thurberi, Stenocereus stellatus* and *Stenocereus pruinosus*), which contain a series of phenolic compounds such as quercetin, ferulic acid, caffeic acid and *p*-coumaric acid, the latter being identified in that work (Castro-Enríquez et al., 2020; García-Cruz et al., 2017).

El-Hawary et al. (2020), when studying prickly pear (*O. ficus-indica*), also showed phenolic acids such as quinic, malic, piscidic, diferuloyl-syringsic, eucomic and dicaffeoylferulic acids.

Researchers' interest in phenolic compounds in native and exotic fruits has increased significantly in recent decades. Over the years, phenolic compounds have become popular for their potential application in reducing chronic diseases, owing to their role as mediators in oxidative reactions, which can be used to reduce premature aging, in addition to other diseases associated with the action of free radicals (Ksouri et al., 2009; Lin et al., 2016; Martins et al., 2011).

El-Hawary et al. (2020), when applying *in vivo* tests, showed that the bioactive compounds of the Indian fig (*O. ficus-indica*) are related to the reduction of oxidative stress, which is one of the main factors that contribute to the onset of neurological diseases. Thus, it is an interesting candidate for treatment of neurological diseases such as Alzheimer's disease.

Kang et al. (2013) investigated the effects of *Sasa quelpaertensis* Nakai extract and its main constituent, *p*-coumaric acid, in adipogenesis in 3T3-L1 cells. The authors found that *p*-coumaric suppressed the proliferation of adipocytes, which suggests that the extract analysed might have anti-obesity effects, which should be investigated.

The first parameter used to investigate the clinical applications of a compound is the *in vitro* cytotoxicity test. Based on this analysis, one can establish the potential of a sample in presenting adverse reactions at a cell level (Garcia, 2012; Wennberg et al., 1979).

The hydroethanolic extracts of mandacaru fruits of all batches were not toxic to the L929 cell culture in the concentrations analysed, as none of the concentrations analysed showed cell viability of less than 70%, with no significant difference to the control ($p>0.05$).

The samples are considered cytotoxic when the percentage of viable cells is lower than 70% (Teixeira et al., 2005). However, cell proliferation was stimulated. This factor can be associated with a certain substance present in the extract, since a crude extract was used, rather than isolated compounds.

Therefore, the authors suggest that the samples analysed should be assessed in other cell cultures. Moreover, other compounds present in the extract should be identified, as it was found in the identification of the bioactive profile.

Conclusion

The pulp and peel of the mandacaru fruit studied in the present research have low calorific value, as well as low vitamin C content, in both batches. The hydroalcoholic extracts of the fruit pulp exhibited high antioxidant capacity, by *in vitro* tests, ABTS and FRAP. Among the compounds responsible for antioxidant activity, o-coumaric and p-coumaric acids were identified in all the study batches. The pulp extracts of the mandacaru fruit did not dissipate the potential for cytotoxicity for the cultivation of L929 cells. Investigation of the composition of the hydroalcoholic extract chemistry using mass spectrometry should be carried out to fully elucidate this point, aiming to deepen the understanding of those compounds probably involved in the antioxidant action.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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