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Nutritional Potential and Bioactive Compounds in *Cereus jamacaru* DC: An Multifaceted Cactus From Brazilian Semiarid

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Abstract

Cereus jamacaru DC. is an endemic cactus of the Brazilian semiarid region empirically used by humans for forage, food and medicinal purposes. In this paper, we characterize the physicochemical and phytochemical profile of the *C. jamacaru* cladode and fruits, outlining their nutritional implications. We evaluate the basic physicochemical characteristic of the fruits and the main classes of chemical compounds present in aqueous and ethanolic extracts from cladode, peel, and pulp of ripe and semi-ripe fruits through qualitatively and quantitatively methods. We analyze the data through descriptive statistics and variance analysis. The fruits have appropriate pH, acidity, and total soluble solid levels for the fruit processing industry and fresh consumption. We identified the presence of saponins, tannins, flavones, flavonols, and xanthones in all samples, but alkaloids and steroids were both detected in cladodes exclusively. The phenolic compound and flavonoid contents vary according to the extraction method and sample class. The cladodes and pulp of ripe fruits have the highest flavonoid levels, while the content of phenolic compounds had a high level in peels of ripe and semi-ripe fruits. The presence of these bioactive compounds implies that

C. jamacaru products have relevant pharmacological interest and functionality for human (fruits) and domestic ruminant (cladodes) food. These applications can boost the agricultural-economic exploration of *C. jamacaru* and contribute to income generation, and improve human and animal nutrition.

Keywords: cactaceae, fruit, cladode, caatinga, secondary metabolites, semiarid region

1. Introduction

The Brazilian Seasonally Dry Tropical Forest, the so-called "Caatinga", covers an area of 912,529 km² in the semi-arid region of Northeastern Brazil (Silva, Leal and Tabarelli, 2017). The Caatinga supplies multiple environmental services and has potential for sustainable extractive activities of timber and non-timber forest products, including edible fruits and bioactive substances. However, a wide range of useful plants is still poorly explored, probably because their products were not characterized enough to drawn attention to the food and pharmaceutical sectors.

Cactaceae is a plant family abundant in the Caatinga (Sampaio and Costa, 2011) that contains several useful species for humans. *Cereus jamacaru* DC., popularly known as mandacaru, is one of this useful Cactaceae specie for humans (Sales et al., 2014) because its fruits are edible and the stem (cladode) is a source of domestic ruminant food.

The *C. jamacaru* cladodes are multi-articulate with candelabriform branches. Besides being an important food source for cattle during the dry season (Zara *et al*., 2012), the cladodes also have antimicrobial, vasodilatory (Messias, 2010), anti-inflammatory, and contraceptive actions (Andrade *et al.*, 2006), as experimentally demonstrated in rodents. The *C. jamacaru* fruit is an oblong ellipsoid-shaped berry, its pulp is white funicular and mucilaginous with black-colored seeds (Rocha and Agra, 2002) frequently consumed in natura or as juices and jellies by the locals.

Although the *C. jamacaru* phytochemical profile has been investigated before by several authors, including Bevilaqua *et al*. (2015), Santos *et al.* (2019), Dutra *et al.* (2019), and Medeiros *et al.* (2018), there are still other aspects to be studied, such as the phytochemical characterization of different parts of the plant using different extraction methods, as this would extend knowledge about its pharmacological and nutritional properties.

In order to emphasize the importance of this species as a powerful source of bioactive compounds and highlight its chemical composition versatility and the possibility of many uses, we evaluated the basic physicochemical characteristic of the fruits and the main classes of chemical compounds present in aqueous and ethanolic extracts of the *C. jamacaru* cladodes and fruits.

2. Method

2.1 Collection and Preparation of the Samples

We collected the fruit and cladode samples (Figure 1 D) in ten individuals (n = 10) from a *C. jamacaru* natural population located on private property (09 °01'49" S, 36 °24'07" W) in the municipality of Brejão, Pernambuco state, Brazil. The site climatic classification based on Köppen-Geiger is Cs'a-type (mesothermal including hot and dry summer). The predominant vegetation is Subcaducifolic and Deciduous Tropical Forest-types (Santos *et al.*, 2018).

After collection, we transported the samples under refrigeration to the processing site.

According to their color, we grouped the fruits in two maturation stages: ripe (predominantly red peel) and semi-ripe (reddish-green peel) (Fig. 1 A and B).

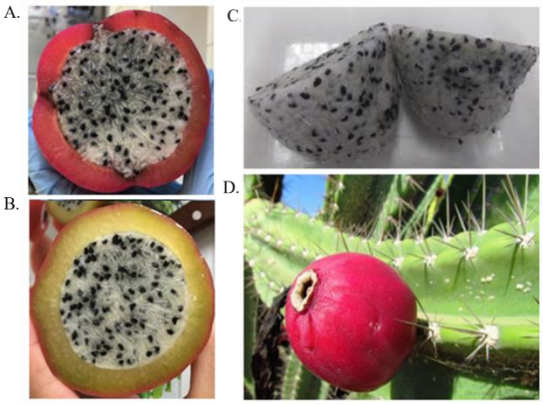

Figure 1. Ripe fruit (A), semi-ripe fruit (B), fruit pulp (C), fruit and cladode (D) of *C. jamacaru*

After fruits washed and sanitized with sodium hypochlorite (100 ppm), we manually separated the fractions of peel (epicarp + mesocarp) and pulp (endocarp + seeds) (Fig. 1A and C), resulting in four classes of samples afterward: ripe peel (RP), semi-ripe peel (SP), ripe pulp (RP), and semi-ripe pulp (SP). These samples were frozen (-10 ºC), lyophilized (Terroni Lyophilizer, model Series LS 3000), grounded, packed in hermetically-sealed glass pots, and, finally, stored at 25 ºC.

We properly cleaned the cladodes and removed their thorns manually. Then, they were horizontally sliced into 2.0 cm thick pieces and dried in a forced air circulation system (50 °C for 72 hours). The dry material was ground in a Cutting Mill (Willey®), packed in hermetically-sealed glass pots, and stored at 25 ºC.

2.2 Basic Physicochemical Characterization of the Fruits

For the physicochemical characterization, we determined: a) pH - using the potentiometric method; b) total soluble solids - using the refractometry on Brix scale (°Brix); c) titratable acidity - using the volumetric titration method (% citric acid/100g of the sample). Such methodologies are available in AOC (1992).

2.3 Phyto Chemical Prospection

2.3.1 Obtaining Extracts

The vegetal extracts were obtained through aqueous and ethanolic extractions. In aqueous extraction, we mixed 100g of the sample with 1.0 L of distilled water. Then, we constantly heated and shaken the mixture up to 90 °C, which remained at this temperature for 5 min. Right after, we placed the extract in hermetically-sealed glass pots and stored it at -10 ºC.

In ethanolic extraction, we mixed 100g of the sample with 1.0 L of ethanol (95%) - extracting solvent. The extraction was exhaustive, i.e., we put this sample and the extracting solvent together at 25 °C for eight days. We shook the mixture day after day, whereas we renewed the extracting solvent every two days. By using a rotary evaporator at 40 ºC, we filtered and concentrated the mixture afterward (Vizcaino, 2007). The extract was stored in hermetically-sealed glass pots at -10 ºC.

2.3.2 Identification of the Main Classes of Chemical Compounds

We identified the main classes of chemical compounds according to the methods proposed by Matos (2009) and Desoti *et al*. (2009). We used 1 mg/mL of extract (aqueous and ethanolic) for qualitative chemical tests. The steroids were detected by the Liebermann-Burchard reaction, while tannins by precipitation methods with iron salts, flavonoids through Shinoda and Taubouk reactions, as saponins by analysis of persistent foaming after extract agitation. In contrast, we performed chemical tests with specific reagents to check for the presence of phenols, flavones, flavonols, xanthones, catechins, anthocyanins, anthocyanidins, terpenoids, and flavanones, as described in table 1. The interpretation made was based on the characteristic visual patterns.

Table 1. Phytochemical prospecting of crude ethanolic and aqueous extracts from *Cereus jamacaru* DC

Secondary metabolites	**Characterization reactions**	**Changes observed in positive results**
Steroids	Liebermann-Burchard	Green coloring
Terpenoids	$Na_2SO_4/C_4H_6O_3$	Color change
Saponins	Formation in foam	Persistent foam
Alkaloids	CCD Reagent Dragendorff	-
Phenols	$FeCl^3$	Coloring ranging from blue to red
Tannins Condensable / hydrolyzable	Precipitation with iron salts	Blue or green precipitate
Flavones/flavonois /xanthones	Acidulation / alkalinization	Color change

Detection of the alkaloids chemical group was performed by thin-layer chromatography (CCD) using specific eluent and developer systems according to Cechinel Filho and Yunes (1998).

2.3.3 Content of Total Phenolic Compounds (CFT) and Total Flavonoids (FT)

We estimated CFT content through the modified Folin-Ciocalteau method by Roesler *et al.* (2007). We mixed 100 µL of extract (1:10 p / v) with 500 µL of the Folin-Ciocalteau reagent and 400 µL of Na_2CO_3 solution (7.5%). The samples were shaken and heated (50 ºC) for 5

min, then cooled at 25 ºC. The absorbance of the liquid fraction was measured at 760 nm using a UV-vis spectrophotometer (model 700 Femto® plus). A calibration curve of gallic acid from 50 to 300 μg mL^{-1} concentration (y = - 0.1267 + 0.0064x, R^2 = 0.9892) was used to estimate the CFT content in the samples. We performed determinations in triplicate, and the results were expressed in milligrams of gallic acid equivalents in 100g of dry matter (mg EAG/100g MS).

We estimated FT content according to the method described by Woisky and Salatino (1998). We mixed 0.5 ml of extract with 0.5 ml of $AlCl^3$ solution (5%). We left the mixture resting for 30 minutes at 25 ºC with no light. The liquid fraction absorbance was measured at 425 nm using a UV-vis spectrophotometer (model 700 Femto® plus). A quercetin calibration curve from 50 to 300 μg mL^{-1} concentration (y = 0.0011x + 0.0078, R^2 = 0.9885) was used to estimate the FT content in the samples. We carried out determinations in triplicate, and the results were expressed in milligrams of quercetin equivalents in 100g of dry matter (mg EQ/100g MS).

2.4 Data Analysis

We used the descriptive statistics (median, standard deviation, minimum, first quartile, second quartile, third quartile, and maximum) in boxplot for data analysis concerning to basic physicochemical characterization of the fruits. As these data also attended to the parametric analysis assumptions, we used the Variance Analysis (ANOVA) to compare the treatments through the statistical software BioEstat 5.0.

The data concerning both contents of total phenolic compounds and total flavonoids also attended to the parametric analysis assumptions and hence were analyzed through ANOVA in a double factorial scheme (5x2). Factor 1 concerns the sample class (cladode, ripe and semi-ripe peel, ripe and semi-ripe pulp), and the second factor concerns the extract type (aqueous and ethanolic). We compared the averages through the Tukey test at 5% probability level. We performed the analyses using the SISVAR 5.6 statistical program.

3. Results

3.1 Basic Physicochemical Characterization of the Fruits

We noticed a higher acidity level (P = 0.0032) in the peel of semi-ripe fruits (Figure 2A). However, the acidity (Fig. 2B) and pH (P = 0.01) levels in the pulp were similar between the two ripening stages (Fig. 2C and 2D). The content of soluble solids was higher (P<0.01) in the peel and pulp of ripe fruits (Fig. 2 - E and F).

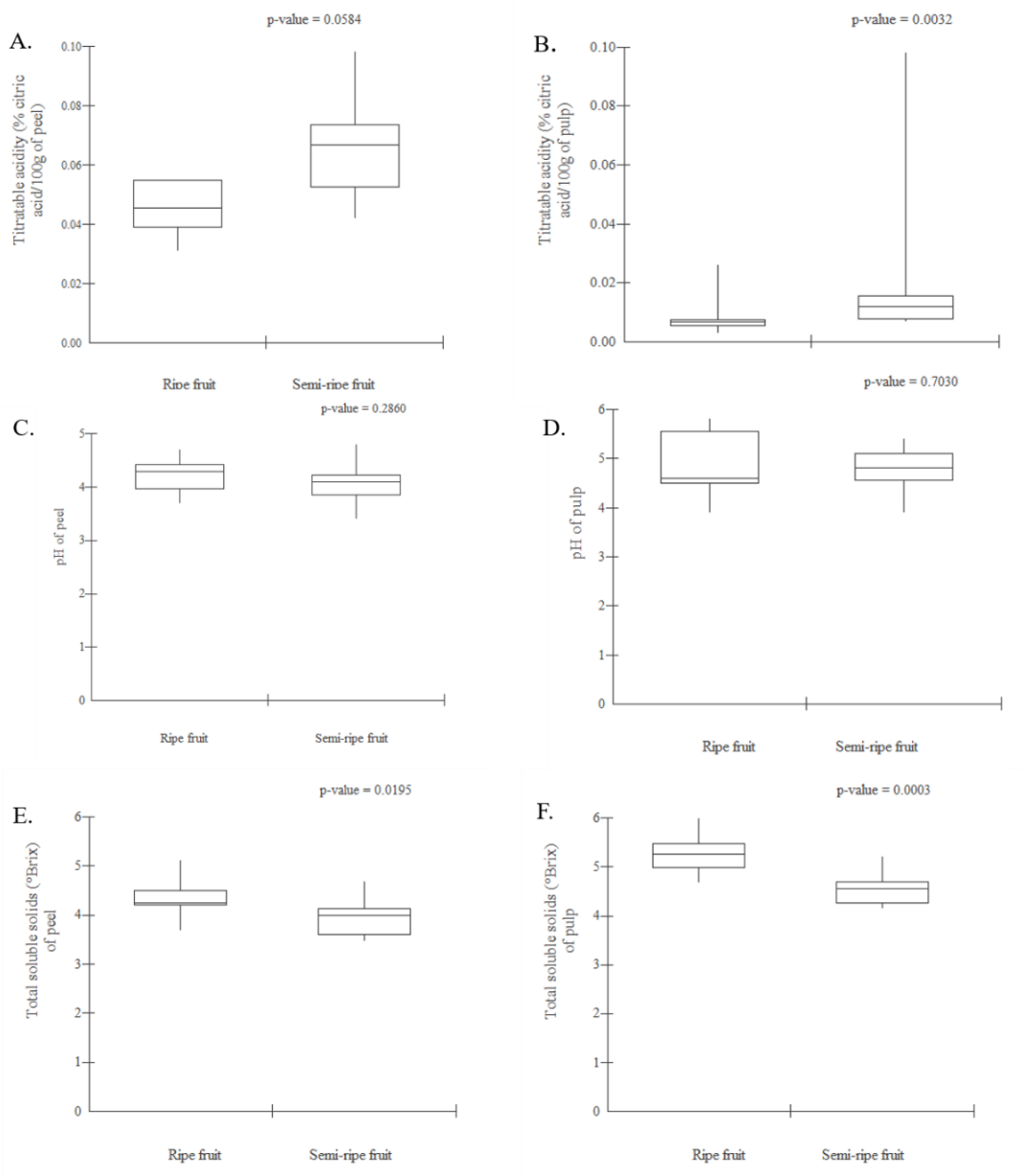

Figure 2. Physicochemical attributes of *C. jamacaru* ripe and semi-ripe fruits. A – the peel titratable acidity; B – the pulp titratable acidity; C – the peel pH; D – the pulp pH; E - the peel soluble solids content; F - the pulp soluble solids content

3.2 Phyto Chemical Prospection

We found in all samples the presence of phenols, flavones, flavonols, xanthones, and saponins (Table 2). The presence of steroids and alkaloids occurred in cladodes ethanolic extraction only. Moreover, we found Alkaloids in both ethanolic extractions of cladodes and ripe-fruit peels. Terpenoids, in turn, were found in peel and pulp of ripe and semi-ripe fruits only. We found no presence of anthraquinones, anthrones, coumarins and tannins (condensed and hydrolyzable).

Table 2. Qualitative phytochemical profile of the *C. jamacaru* cladode and fruit fractions in function of extraction methods

Sample class		Alkaloids	Steroids	Terpenoids	Saponins	Phenols	Flavones/Flavonoids/Xanthomas
Cladode	Aqueous extraction	-	-	-	+	+	+
Ripe peel		-	-	+	+	+	+
Semi-ripe peel		-	-	+	+	+	+
Ripe pulp		-	-	+	+	+	+
Semi-ripe pulp		-	-	+	+	+	+
Cladode	Ethanolic extraction	+	+	-	+	+	++
Ripe peel		-	-	+	++	+	++
Semi-ripe peel		-	-	+	+	+	+
Ripe pulp		-	-	+	+	+	++
Semi-ripe pulp		-	-	+	+	+	+

Reaction intensity: “+++” (high); "++" (mean); “+” (low), and “-” (negative reaction).

3.3 Content of Total Phenolic Compounds (CTP) and Total Flavonoids (TF)

There was an interaction (P<0.05) among the extraction methods and sample classes for the CTP and TF contents. The CTP contents in cladode and peel of ripe and semi-ripe fruit were higher through ethanolic extraction. However, the pulp of ripe and semi-ripe fruit samples showed the highest CTP contents through aqueous extraction (Fig. 3). The CTP contents in the peel of ripe and semi-ripe fruits were higher than in other samples.

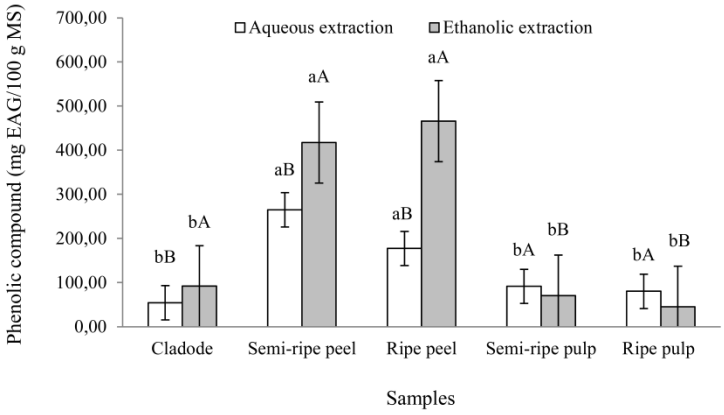

Figure 3. Phenolic compound contents of the *C. jamacaru* cladode and fruit fractions in function of extraction methods. Averages followed by the same letter do not differ from each other through the Tukey test at 5% probability. Vertical bars represent the average standard

error. Uppercase letters compare the extraction method, whereas lowercase ones compare the samples

Thus, the ethanolic extraction proved to be more efficient, as it provided a higher CTP yield (45.36 to 466.33 mg EAG/100 g MS) compared to aqueous extraction (54.48 to 265.10 mg EAG/100 g MS).

The cladode and pulp of ripe fruits had higher TF contents through aqueous extraction, but peel of ripe and semi-ripe fruits had higher TF contents through ethanolic extraction (Fig. 4). The cladode and pulp of ripe fruits had the highest levels of TF (Fig. 4).

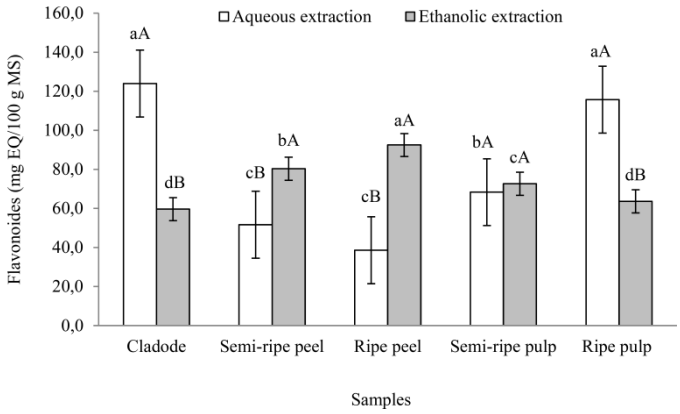

Figure 4. Total flavonoid contents through aqueous and ethanolic extractions in the *C. jamacaru* cladode and fruit fractions. Averages followed by the same letter do not differ from each other through the Tukey test at 5% probability. Vertical bars represent the average standard error. Uppercase letters compare the extraction method, whereas lowercase ones compare the samples

4. Discussion

In this study, we investigated the physicochemical characteristics and phytochemical composition in the *C. jamacaru* cladode and fruit fractions analyzing their pharmacological and nutritional implications accordingly.

4.1 Physicochemical Attributes of the Fruit Fractions

The pH (4.05 to 5.30) and titratable acidity (0.0087 to 0.1089% citric acid) ranges of *C. jamacaru* pulp indicate that this fraction has weak vulnerability to micro-organisms action, which could prolong its longevity after processing. While its total soluble solids content (SST) might be regarded as low (4.0 to 5.67 ºBRIX), it resembles other native fruits from Brazil (4.0 to 4.5 ºBRIX), such as *Psidium cattleyanum* S., *Mauritia flexuosa* Mart. and *Byrsonima crassifolia* L. Rich), being in concordance with the standard minimum criteria determined by the Brazilian legislation (BRASIL, 2016). Such characterization is relevant for the fruit processing industry because the greater the amount of SST, the greater the juice and pulp yield. In contrast, SST contents of *C. jamacaru* fruits (4.0 to 5.67 ºBRIX) were lower than those verified by Almeida *et al.* (2009) (10.50 and 11.5 ºBRIX), which might have happened due to the differences in the ripening stage, plant genotype, and local edaphoclimatic conditions.

There has also been a pulp SST content increasing according to the fruit maturation stage,

from 3.64 ºBRIX in the semi-ripe stage to 5.21 ºBRIX in the ripe one. Such increase was followed by an acidity decrease, from 0.0982% in the semi-ripe stage to 0.0570% in the ripe one. According to Damodaran *et al.* (2010), such change is due to the converting from citric and malic acids to sugars during the fruit ripening stage.

4.2 Qualitative Identification of the Main Compound Classes

We found several secondary metabolites in *C. jamacaru*, including alkaloids, terpenoids, phenols, and saponins. These compounds were also identified in the studies performed by Cartaxo *et al.* (2010), Schwarz *et al.* (2010), Bevilaqua *et al.* (2015), Santos *et al.* (2019), Dutra *et al.* (2019) and Medeiros *et al.* (2018).

The presence of these bioactive compounds suggests, therefore, *C. jamacaru* fruits have the functional potential for human food. In addition, the presence of terpenoids, saponins, phenols, flavones, flavonols, and xanthones in *C. jamacaru* cladodes also indicates that the product has the functional potential for domestic ruminants' food. A previous study performed by Davet (2005) has identified alkaloids and steroids as the main compounds contained in *C. jamacaru* cladodes, corroborating with the finding ones of this study and scientific literature for Cactaceae. More recently, Medeiros *et al.* (2018) have identified almost 24 phytochemicals in *C. jamacaru*, including tannins that have not been identified in our study.

4.3 CTP and FT Yield According to the Extraction Method and Class of Samples

We found that CTP and TF levels have varied according to the extraction method and class of samples. It probably happened because the extraction methods affect quantity, quality, and bioavailability of the metabolites (Miglio *et al.*, 2008). The extracting solvent influences the release/expression of flavonoid groups (Koubaa *et al.*, 2015) and phenolic compounds (Harbone, 1998), as they vary in polarity, acidity, hydrogen-bonding capacity of hydroxyl groups in the aromatic ring.

While most phenolic compounds can be soluble in water, some compounds present solubility only in organic solvents. In fact, ethanolic solvents may be more efficient in the extraction of total phenolic compounds than water (Vizzoto and Pereira, 2011; Tomsone *et al.*, 2012; Menezes Filho and Castro, 2019). However, the processing degree, the size of particles, the extraction duration, the temperature, and the extracting solvent concentration can all also influence the phenolic compound yields (Shahidi and Wanasundara, 1998).

4.4 Quantitative Analysis of the Total Phenolic Compounds and Total Flavonoids

CTP contents in *C. jamacaru* pulp (45.36 - 91.66 mg EAG/100g DM) were higher than those observed by Moreira et al. (2018) (28.35 mg EAG/100g MS), while CTP contents in peels (177.55 - 466.33 mg EAG/100g MS) were also higher than those observed in fruit peels of *Pilosocereus pachycladus* (173, 8 - 221.9 mg EAG/100 g MS) (Rodrigues *et al.* (2019), another endemic Cactaceae from Caatinga.

We also found that CTP contents in pulp of C. *jamacaru* fruits (45.36 - 91.66 EAG/100g DM) were higher compared to the fruits of cactus pear (*Opuncia ficus-indica*) (2.51 - 54.33 mg EAG/100g) (Mabrouki *et al.*, 2015) and lower compared to Pitaya (*Hylocereus* spp.) (165.24 mg EAG/100g) (Vizzotto *et al.*, 2014), both Cactaceae are cultivated for edible fruit production in Brazil.

We also believe the higher CTP content in peels (177.55 - 466.33 mg EAG/100g DM) regarding the *C. jamacaru* pulp (45.36 - 91.66 mg EAG/100g DM) is probably due to an accumulation of these metabolites in the fruit epidermis because, according to Daiuto *et al*. (2014), such metabolites act as protection against ultraviolet radiation.

Regarding the FT content in *C. jamacaru* cladode, the values obtained here (59.65 - 123.94 EQ /100g DM) are similar to those described by Dutra *et al.* (2019) who used the hydroalcoholic extraction method. In contrast, TF contents in fruit peels (38.56 - 92.5 mg EQ/100g DM) are higher than the ones observed by Araújo *et al.* (2008) (11.30 - 14.34 mg/500g), indicating existence of intraspecific variability in the content of this secondary compound. This variability in *Opuntia* spp. is attributed to local abiotic factors such as drought and soil fertility (Figueroa - Cares *et al.*, 2010), as they are capable of inducing changes in the composition of secondary compounds, including flavonoids (Pretti *et al.*, 2018). These polyphenols have antioxidant, anti-cancer (Dutra *et al.*, 2018), anti-inflammatory (Araújo *et al.*, 2008; Ferreira Júnior *et al.*, 2011), and antibacterial (Davet *et al.*, 2009) actions that are relevant to pharmacology.

5. Conclusions

The phenolic compound and flavonoid contents vary according to the extraction method and sample class. The cladodes and pulp of ripe fruits have the highest flavonoid contents, while the peels of ripe and semi-ripe fruits have higher phenolic compound contents. The presence of these bioactive compounds indicates that *C. jamacaru* products have great potential for human (fruits) and domestic ruminants (cladodes) foods and pharmacological use. These applications can boost the agricultural-economic exploration of *C. jamacaru* and contribute to income generation, and improve human and animal nutrition.

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