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Bromatological analysis, phytochemical and antioxidant potential of carnauba (*Copernicia prunifera* (Mill.) H.E. Moore) fruit

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Chemical composition as well as phytochemicals and antioxidant activity of underutilized plants, such as carnauba palm (*Copernicia prunifera*), provide an informed decision on how to maximize their benefits. This study aimed to evaluate the chemical composition and antioxidant activity of the fruit of carnauba. The total phenolic content (gallic acid equivalents, GAE) of the ethanol extract of the whole fruit, pulp and kernel were 44.6000, 0.0447 and 0.6930 mg GAE/g extract, respectively. The respective total tannin content was 1590.932, 2977.724 and 147.650 ppm in the whole fruit, pulp and kernel. The fruit of carnauba showed 9.84 mg anthocyanins/50 g of fresh pulp. Ethanol extract of whole fruit had a potent antioxidant activity. Thus, the fruit of carnauba has add-value in food industry.

Key words: Secondary compounds, carnauba ethanol extract.

INTRODUCTION

Carnauba palm or Brazilian tree of life [*Copernicia prunifera* (Miller) H. E. Moore, Arecaceae family] is a palm tree found in the northeastern Brazil and Cerrado biomes, located in some Brazilian regions (D'alva, 2004). The extractive exploitation of the resources offered by This palm was first described around 1648 by Maregravius and Piso, and subsequently ratified by R. Müller, in 1768, upon reporting the first experiments on carnauba wax for candles. Carnauba wax is extracted from the leaves, and used extensively in various

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Figure 1. The unripe fruits of carnauba (*C. prunifera*) used, before full maturity when the external coloring of the bunches were predominantly green.

industrial applications, such as a protective and polishing agent, glazing for paper, coating and encapsulation of pharmaceuticals, and food additives. It is exported to more than forty countries, although the United States, Japan and Germany are the major destinations (Oliveira and Gomes, 2006).

The fruit of carnauba contains protein (5.46% on pulp; 6.89% on kernel), fat (6.25% on pulp; 13.65% on kernel), carbohydrates (64.32 % on pulp; 63.29% on kernel), cellulose (5.81% on pulp; 4.07% on kernel), vitamins (96,20 mg.100g^{-1} on fruit) and other constituents, such as phenols (33,93 mg.100 g^{-1} for flavonoids; 2,42 mg.100 g^{-1} for anthocyanin) (Braga, 1976; Nogueira, 2009). Moreover, this fruit has been evaluated for its antioxidant (DPPH radical scavenging activities with inhibitory concentration of 50% from 15.3±0.4 and 41.9±0.8 µg.ml^{-1} on mesocarp and epicarp, respectively; 3549±184 g.g DPPH^{-1} on fresh matter, and 4877±24.3 g.g DPPH^{-1} on dry matter of whole fruit) (Silva et al., 2005; Rufino et al., 2010, 2011), and antibacterial activities against Gram-positive bacteria (*Staphylococcus aureus*) (Ayres et al., 2008).

The fruit of carnauba can be used for different things, including obtaining milk from the pulp, as well as producing flour from the extracted and dried pulp or the green fruit, for use in biscuit manufacturing (Pereira and Carneiro, 2006; Ferreira, 2009). The mature fruit can be used, for example, to manufacture jelly for human consumption (Nogueira et al., 2009). Furthermore, the ground and roasted kernel may be used as coffee substitute (Pio Correa, 1931) or yield oil, which can also be used in food (Lorenzi, 1996). Recently, use as animal feed (Silva et al., 2015a). Besides that, carnauba leaves are noted for their elevated content of wax which has many applications in the pharmaceutical industry, food industry, cosmetics, and lubricants (Barman et al., 2011).

In this context, in addition to standard chemical analysis to determine the protein, fiber, lipids and mineral salts, the phytochemical approach, which involves the study of the properties of secondary metabolites (phenols, flavonoids, anthocyanins, tannins, among others) present in the food has gained prominence. Namely, the antioxidant (Schenkel et al., 2007), antimicrobial (Kooti et al., 2016), antifungal, antiparasitic, (Camacho et al., 2004) anti-inflammatory (Raso et al., 2001), hypocholesterolemic, immunostimulant (Cozzolino, 2009) and insecticidal properties (Menezes, 2005) demonstrate the various biological effects posed by these bioactive secondary metabolites. .

The aim of this study was to determine the chemical composition, phytochemical profile and antioxidant activity of the extracts obtained from the fruit of the carnauba palm and its separate parts, namely: the pulp and kernel.

MATERIALS AND METHODS

Plant materials

The unripe fruits of *C. prunifera* (Mill.) H. E. moore were collected in the Aracati, Ceará state (northeast of Brazil) (Figure 1). The fruit was then transported to the experimental farm where a sample was immediately frozen at -20°C until use. A separate sample was

Table 1. Evaluation of color tests for anthocyanins, anthocyanins, and flavonoids.

Constituents	Color		
	Acid (pH 3)	Alkaline (pH 8.5)	Alkaline (pH 11)
Anthocyanins and anthocyanidins	Red	Lilac	Blue-crimson
Flavones, flavonols and xanthones	-	-	Yellow
Flavonoids	-	-	Orange-red

Source: Matos (2009).

dehydrated using an outdoor dryer that was revolved several times a day to allow homogeneous dehydration to occur. The dried sample was ground and stored at room temperature until analysis. Analyses were performed on the whole fruit of the dried sample, and the pulp and kernel of the frozen fruit.

Chemical analysis of the fruit

The dry matter (DM), crude protein, ether extract and mineral matter were analyzed according to the procedures described by Silva and Queiroz (2002). The neutral detergent fiber, acid detergent fiber, lignin and cellulose contents were analyzed according to Van Soest et al. (1991).

Phytochemical detection

The whole fruit, kernel and pulp extracts were obtained by respective pulverization. An initial hexane extraction was performed followed by an ethanol extraction, and then the sample left in contact with the solvent for 7 days. This process was repeated three times. After, the extracts were filtered (filter paper, Qualy® - diameter 9 cm) and concentrated by rotary evaporation (model:801, FISATOM, Sao Paulo, Brazil) under reduced pressure, thus obtaining the hexane and ethanol extracts. Detection of phytochemicals were performed according to the method by Matos (2009). The hexane and ethanol extracts were qualitatively analyzed for phenols and tannins, anthocyanins, proanthocyanidins, flavonoids, saponins, catechins, triterpenes and steroids (Libermann–Burchard test), and leucoanthocyanidins.

Detection for phenols and tannins

Three drops of an alcoholic solution of 1% ferric chloride III ($FeCl_3$) was added to 10 mg of extract. Then, the mixture was stirred well for visual assessment of variations in color and precipitate formation, indicating the presence or absence of phenols and tannins (Matos, 2009).

Detection of anthocyanins, anthocyanidins and flavonoids

Approximately, 0.2 mg of extract was added to a test tube in triplicate. The first tube was acidified using 10% HCl until pH 3 was attained, the remaining extracts were basified using 10% (w/v) aqueous sodium hydroxide (NaOH) solution until pH 8.5 and 11, were reached, respectively. The color development in the tubes was evaluated according to Table 1.

Detection of saponins

In a test tube, 50 mg extract was added to 5 to 10 mL of water. Then, the tube was shaken vigorously for 2 to 3 min, and the presence or absence of foam was observed.

Steroids and triterpenoids detection

Based on the Liebermann-Burchard test, 0.2 mg of extract was placed in a vial and then 1 to 2 mL of chloroform added for dissolution. After, the solution was filtered using a small funnel closed with cotton into a second test tube. Then, 1 ml of acetic anhydride was added, and the solution stirred gently before adding three drops of concentrated sulfuric acid (H_2SO_4; 18 M) and stirred again. The development of color was then observed (Matos, 2009).

Leucoanthocyanidins, catechins and flavones detection

Approximately, 0.2 mg of extract was added to two test tubes, respectively. HCl was added to one tube, until pH 1 to 3 was attained. The remaining tube was basified using NaOH until pH 11 was attained. Then, the tubes were heated at 60 to 70°C in a water bath for 3 min, observing changes in color, according to Table 2 (Matos, 2009).

Flavonols, flavanones, flavonols and xanthones detection

Approximately, 10 mg of magnesium ribbon and 0.5 mL of concentrated HCl was added to a test tube with 10 mg of extract. After completion of the reaction, indicated by the end of the effervescence, a color change indicative of the presence of the compounds was observed (Matos, 2009). .

Preparation of extracts

The unripe fruits (whole fruit, pulp and kernel) were initially immersed in hexane for 7 days. After, the mixture was filtered (filter paper - diameter 9 cm) and the organic liquid phase was concentrated on a rotary evaporator under reduced pressure, resulting in the hexane extract. Then, the plant material was placed at room temperature to evaporate any residual hexane and the plant material then immersed in ethanol (70% ethyl alcohol) for 7 days (this procedure was repeated twice with the same plant material to ensure maximum removal of the constituents). After, the liquid phase was concentrated on a rotary evaporator under reduced pressure, resulting (non-volatile portion) in the ethanolic extracts (Freire et al., 2015).

Table 2. Evaluation of colors for leucoanthocyanidins, catechins and flavones.

Constituents	Natural color	
	Acid	Alkaline
Leucoanthocyanidins	Red	-
Catechins (catechin tannins)	Yellow-gray	-
Flavanones	-	Red-orange

Source: Matos (2009).

Determination of total phenols

According to the Folin-Ciocalteu method (Obanda and Owuor, 1997), each extract was dissolved in methanol to a final concentration of 5 mg/ml. Then, 100 µl of the final solution was vortexed with 500 µl of Folin-Ciocalteu reagent and 6 ml of distilled water for 1 min. Then, 2 mL of 15% sodium carbonate (Na_2CO_3) was added and the mixture vortexed for 30 s. Then, 10 mL of distilled water was added, and after 2 h at room temperature, the absorbance was measured in a spectrophotometer (T-2000, TEKNA, Brazil) at 750 nm. Quantitation was done in triplicate and the results expressed as gallic acid equivalents (GAE) per gram of crude extract.

Determination of anthocyanins

According to Teixeira et al. (2008), organic solvent (ethanol/water) in the ratio 70:30 v/v was added to approximately 50 g sample, followed by sufficient HCl to adjust the pH to 2. The material was allowed to stand for 24 h at low temperature (27°C) in the dark light. After, the material was filtered (filter paper, Qualy® - diameter 9 cm) and transferred to a 100 ml volumetric flask, and then centrifuged at 2000 rpm for 10 min. The extract was purified using a mixture of ethyl ether and hexane in the ratio 1:1 to remove chlorophyll (three successive extractions). Finally, an aliquot was transferred to a 10 ml flask and brought to volume using a mixture of ethanol/HCl in the ratio 85:15 v/v. The absorbance was measured at 535 nm (T-2000, TEKNA, Brazil) using ethanol/HCl (85:15 v/v) as the blank. The anthocyanin content was calculated according to equation 1.

Equation 1: Equation for obtaining anthocyanin content of the vegetable extracts of carnauba.

$$AntT\ (mg/50\ g\ sample) = \frac{OD\ x\ VE1\ x\ VE2\ x\ 1000}{Valq\ x\ W\ x\ 982}$$

where,
OD is optical density of the diluted extract, *VE*1 is the total volume of the concentrated extract, *VE*2 is the total volume of the diluted extract, *Valq* is the aliquot volume used to dilute the concentrated extract, *W* is the sample weight, and 982 is the average extinction coefficient of anthocyanins by the single pH method.

Determination of tannins

100 mg aliquot of the plant material was extracted with 1 ml of acetone: water solution (70:30 v/v) in water bath at 30°C, and vortexed every 5 min. Then, the contents were centrifuged at 4000 rpm for 5 min, and the supernatant was collected. The extractions were repeatedly performed until achieving a maximum extraction. The extracts were then evaporated in a water bath at 60°C until near completely dried, at which time 2.5 ml of methanol was added. Then, 0.1 ml of this mixture was added to a test tube containing 0.9 ml of methanol. After, 5 ml of vanillin reagent was added (2.5 mL of a solution containing 1 g of vanillin in 100 mL of methanol, followed by 2.5 mL of a solution containing 8 ml of concentrated HCl in 100 mL of methanol). The tubes were heated in a water bath at 60°C for 20 min. The sample control contained 4% HCl solution in methanol. The absorbance was then measured at 500 nm (T-2000, TEKNA, Brazil) and the tannin content obtained from the calibration curve, previously performed using catechin (Costa et al., 2003).

Determination of condensed tannins (CT)

Powdered sample (200 mg) was placed in a beaker, and 10 mL of 70% acetone was added. The mixture was then transferred to a test tube, which was capped and then vortexed for 20 min, before centrifugation at 3000 rpm for 10 min. The supernatant was collected and placed in an ice bath. A capped test tube containing 0.5 ml extract, 3 ml of butanol-HCl solution (95:5 v/v) and 0.1 ml of 4% ferric reagent (2.1 g of NH4Fe (SO4)2.12 H_2O + 17 mL concentrated HCl + 33 ml methanol), was then placed in a water bath at 100°C for 60 min. A control sample without extract (no heat treatment was required) was prepared simultaneously. After the tubes had been cooled, the absorbance at 550 nm was measured (T-2000, TEKNA, Brazil). The level of CT was expressed as leucocyanidin equivalents using the following formula:

Reading x 78.26 x dilution factor/DM% (Porter et al., 1986).

Antioxidant potential assessment

The antioxidant potential was evaluated using the DPPH free radical assay, as described by Almeida et al. (2006) with some modifications. Briefly, the extracts were dissolved in methanol and then further diluted with methanol to yield the following concentrations: 10, 20, 40, 70, 100, 200, 600 and 700 ppm. A 1 mL aliquot of each concentration was combined with 1 ml methanolic solution of DPPH 60 µmol L-1. The samples were left to rest for 30 min in the dark at room temperature. Then, the absorbance was measured at 520 nm (T-2000, TEKNA, Brazil) against a blank sample containing methanol. The process was performed in triplicate. The inhibition of the DPPH radical was calculated from the ratio of absorbance of each extract concentration against a reference solution containing 1 mL methanol and 1 mL of DPPH. The percentage inhibition of each extract was then obtained from Equation 2.

Table 3. Chemical analysis of the whole carnaúba fruit, its kernel and pulp.

Chemical composition (%)	Whole fruit	Pulp	Kernel
Dry matter	87.60	96.65	34.81
Crude protein	6.30	5.21	4.50
Ethereal extract	6.36	0.65	4.95
Mineral matter	5.32	6.44	2.07
Neutral detergent fiber	61.03	67.29	50.11
Acid detergent fiber	42.33	37.35	47.69
Lignin	16.08	14.81	11.64
Cellulose	25.21	22.91	35.32

Equation 2: Equation for obtaining the percent inhibition of the DPPH radical of the vegetable extracts of carnauba.

$$\%In = \frac{(Abs_{DPPH} - Abs_{sample})x\ 100\%}{Abs_{DPPH}}$$

Where,

% In is the free radical inhibition percentage of the extract, Abssample is the absorbance of the extract and AbsDPPH is the absorbance obtained from the sample DPPH/methanol.

The respective concentration of the extract (mg/ml) required to inhibit 50% of the free radical scavenging ability was calculated by linear regression, using Origin 7.0 software, of plots, where the x-axis represented the various concentrations of the extracts, while the y-axis represented the average percentage of free radical scavenging ability obtained from the triplicates.

RESULTS AND DISCUSSION

Chemical analyses of foods provide insight into the nutritional composition of such foods, facilitating their maximal efficacy. In the present study, the chemical composition of whole carnauba fruit, and its pulp and kernel were independently assessed, as shown in Table 3.

Silva et al. (2015b) evaluated several fruit species belonging to the Arecaceae family, and observed that for most species, the total protein contents of the pulp (1.50 to 20.60 g 100 g^{-1}) and kernel (2.60 to 11.70 g 100 g^{-1}) resented a wide variation, but was typically higher in the kernel relative to the pulp. One exception was Syagrus *cearensis*, which had the highest content in the pulp as seen in kernel (20.60 g 100 g^{-1} versus 4.30 g 100 g^{-1}) (Silva et al., 2015b). Braga (1960) reported that values of protein relatively close to those obtained in the present study for the pulp content (5.46%) and higher values for the kernel (6.89%), compared to those found in present study (6.30, 5.21 and 4.50% in the dried whole fruit, the pulp and fresh kernel, respectively).

Braga (1960) also assessed the fat content of the fruit pieces, and found 13.65 and 6.25% in the almond and pulp, respectively. The present work presented comparatively lower values of 0.65 and 4.95% in the kernel and pulp, respectively. The ash content of the pulp was 2.07%, similar to that reported in the literature (2.95%) for the pulp, however, the ash content of the kernel was significantly different, at 6.44% in the present study compared to 1.55% reported Braga (1960).

In this instance, the drying process used in this study may have influenced the outcome, as the fruit drying process reduces the fat content and sodium content as described by Alasalvar and Shahidi (2013). Furthermore, it is known that fruit maturation stage influences various physico-chemical characteristics and therefore, nutritional changes in the content of sugars, protein, vitamin C, minerals, as well as pH, among other features (Brito and Narain, 2002; Nogueira et al., 2002; Tlili et al., 2014).

The phytochemical analysis of *C. prunifera* is reported in Table 4. Positive results were obtained for phenols and tannins, and anthocyanins, anthocyanidins and flavonoids at pH 11 (except for the hexane extract of the whole fruit), saponins (exclusive to the ethanol extract of the kernel), free steroids (except for the ethanol extracts of the pulp and almond), leucoanthocyanidins, catechins and flavones (solely in the ethanol extract of the whole fruit), and flavonols, flavanones,flavonoids and xanthones (only in the ethanol extract of the kernel).

Phenols are responsible for the flavor, odor and color of various plants (Vizzoto et al., 2010). They constitute a major chemical group of plants and can be divided into three subclasses, namely phenolic acids, stilbenoids and flavonoids, these being the most studied. Several studies have demonstrated the beneficial effects of diets rich in flavonoids, including anti-inflammatory, anti-cancer, cardiovascular disease prevention, oxygen free radical reduction, anti-allergic and antiviral (Middleton, 1998; Egert and Rimbach, 2011; Mccullough et al., 2012). Rufino et al. (2010) observed that the presence of

Table 4. Qualitative phytochemical analysis of the whole fruit, kernel and pulp hexane and ethanol extracts.

Compounds	Whole fruit		Pulp		Kernel	
	HE	EE	HE	EE	HE	EE
Phenols and tannins	(+)	(+)	(+) for flaboblenics tannins	(+) for flaboblenics tannins	(+) for flaboblenics tannins, condensed or catechin	(+) for flaboblenics tannins, condensed or catechin
Anthocyanins, anthocyanidins and flavonoids	(-)	(-) for pH 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11
Saponins	(-)	(-)	(-)	(-)	(-)	(+)
Steroids and triterpenoids	(+) for free steroids	(+) for free steroids	(+) for free steroids	(-)	(+) for free steroids	(-)
Leucoanthocyanidins, catechins and flavones	(-)	(+)	(-)	(-)	(-)	(-)
Flavonols, flavanones, flavanonols and xanthones	(-)	(-)	(-)	(-)	(-)	(+)

EE = Ethanol extract; HE = hexane extract; (+) = Positive; (-) = Negative.

flavonoids in the pulp of the fruit of carnauba. As this class of compounds (flavonoids) have many beneficial health properties (Sucupira et al., 2012; Moo-Huchin et al., 2015; Cao et al., 2016), their presence in the fruits of the carnauba is a positive factor that contributes to increase their potential use in animal nutrition.

Flavonoids represent an attractive example of plant bioactives with promising uses, including in animal feed (Vasta and Luciano, 2011). As shown in Table 5, the total phenolic content in the ethanol extracts obtained in the present study, was 44.60 mg GAE/g in the whole fruit extract, 0.04 mg GAE/g extract in the pulp and 0.69 mg GAE/g extract in the kernel. In the hexane extract, there was 33.2000 mg GAE/g in the whole fruit extract, 0.8326 mg GAE/g in the kernel extract and none detected in the pulp. These data shows that the phenolic compounds have more solubility in ethanol, justified by the interactions of the hydrogen bond type of the hydroxyl of the alcohol with the hydroxyl groups characteristic of the phenol, presenting, therefore, a higher concentration of phenols in the extract in ethanol Table 5.

The total polyphenol content of carnauba evaluated by Rufino et al. (2010), observed that the aqueous extract of the DM was 830±28.3 mg GAE/100 g. Coimbra and Jorge (2012) evaluated, among other parameters, the total phenolic compounds in three different Brazilian fruits, including the macaúba (*Acrocomia aculeata*), which belongs to the same family as *C. prunifera* (Mill.) H.E. Moore. In the analysis of macaúba, Coimbra and Jorge (2012) found 4.38±0.08 mg GAE/g in the kernel, and 2.21±0.02 mg GAE/g in the pulp. Tannins are polymers with valuable functions in plants, contributing to the taste, odor and color, and responsible for the astringency of

Table 5. Total phenols and tannins in the whole fruit, pulp and kernel extracts, and the anthocyanin content of the pulp and kernel extracts of carnaúba.

Evaluation	Whole fruit	Pulp	Kernel
Phenols (mg GAE/g extract)			
Ethanol extract	44.6000	0.0447	0.6930
Hexane extract	33.2000	Not detected	0.8260
Tannins			
Total	1590.9262 ppm	2977.7234 ppm	147.6531 ppm
Condensed	15.4950 mg eq leucocyanidin/100 g DM	-	-
Anthocyanins (mg/50 g sample)	-	9.84	5.63

Table 6. Antioxidant activity of whole fruit of carnaúba.

Extracts	Antioxidant activity
Ethanol extract	15.41 ppm
Hexane extract	1845.80 ppm

many products. For these reasons, tannins are economically important flavors and colors in foods and beverages (Simões et al., 2007). Plants rich in tannins are used in traditional medicine to treat various diseases because both the hydrolysable and condensed tannins have the ability to complex iron, vanadium, manganese, copper and aluminum, and they display antioxidant and free radical scavenging abilities (Simões et al., 2007). The total tannin content was 1590.93, 2977.72 and 147.65 ppm in the whole fruit, pulp and kernel, respectively. The tannin content in plants can vary according to the climatic and geographic conditions, and they may present a varied chemical composition that is often poorly understood (Battestin et al., 2004). The presence of tannins in the plant kingdom is well known, and this class of compounds has been related to several therapeutic properties, knowing that foods that have the same composition have functional properties in organisms (Sirdaarta et al., 2015).

The fruit of carnauba showed 9.84 mg anthocyanins/50 g of fresh pulp. Evaluating several tropical fruits, including carnauba, Rufino et al. (2010, 2011) found a relatively higher value in the fruit pulp of carnauba, reporting 4.10±0.10 mg anthocyanins/100 g DM. However, the immature green fruit may be one reason for the difference in values between this study and that by Rufino et al. (2010, 2011). The fruit of carnauba was also characterized for their antioxidant capacity. Antioxidants are substances which retard or inhibit oxidative degradation reactions by various mechanisms, such as inhibiting free radicals and complexing metal ions.

In this study, the antioxidant activities of the ethanol and hexane extracts of the whole fruits were 15.4 and 1845.8 ppm, respectively, as shown in Table 6. These data show that the extract in ethanol has antioxidant ability expressively higher than that of the extract in hexane. This is possibly related to the fact that phenols compounds known for their good antioxidant ability (Boulekbache-Makhlouf et al., 2013; Bicudo et al., 2014), are present in a higher proportion in the ethanol extract, justifying the result. The antioxidant capacity of various tropical fruits was studied by Rufino et al. (2010), who reported the pulp of the fruit of carnauba presented a high antioxidant activity of 3549±184 g/g DPPH, for the fresh matter and 4877±24.30 g/g DPPH, based on DM.

The antioxidant activity seems to be directly related to the phenolic content, as noted by Vasco et al. (2008), who evaluated some main fruits of Ecuador and observed that the fruits with relatively higher antioxidant activity were those with high or medium phenol contents.

Conclusion

The chemical and phytochemical analysis of the carnauba fruit showed a significant potential in terms of energy content and a protein content of more than 5% linked to the presence of secondary metabolites such as phenols that have recognized beneficial properties as antioxidants. The fruit of the carnauba is carrying an alternative with potential use in animal feeding, especially in the region where its production is consolidated (northeastern Brazil), which often suffers from prolonged droughts that put the animal feed at risk. Therefore, the strategic use of the fruit of the Carnauba as a food source paved way for expanding the possibilities of using this tree in a sustainable and optimized way.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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