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## Antioxidant activity and physicochemical analysis of passion fruit (*Passiflora glandulosa* Cav.) pulp native to Cariri region

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**ABSTRACT.** The objective of this study was to determine the proximal composition, the physical and chemical characteristics and the *in vitro* antioxidant capacity (DPPH, ABTS and FRAP) of the pulp of the wild passion fruit (*Passiflora glandulosa* Cav.) from Cariri region, Ceara, Brazil. The results showed that the proximal composition and the caloric value of this passion fruit are similar to other species, but with a high ascorbic acid content. The fruit pulp showed low level of antioxidant activity and low level of polyphenolic compounds followed by three methodologies used. Due to high levels of titratable acidity (3.52) and total soluble solids (17.80° Brix), this fruit can be considered as a high value fruit for commerce. However, it is a species of passion fruit with few studies described in the literature, and more research is needed to assess its nutritional and functional potential.

**Keywords:** functional food, agricultural, dietary fiber, phenolics, natural antioxidants.

## Atividade antioxidante e análise físico-química da polpa do maracujá (*Passiflora glandulosa* Cav.) nativo da região do Cariri

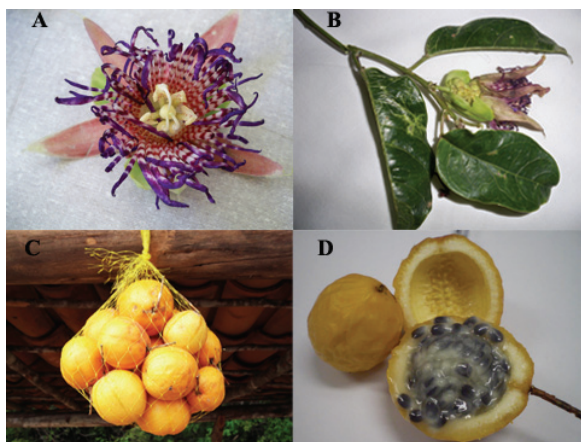
**RESUMO.** O objetivo do estudo foi determinar a composição centesimal, as características físicas e químicas e a capacidade antioxidante (DPPH, ABTS e FRAP) *in vitro* da polpa do maracujá-do-mato (*Passiflora glandulosa* Cav.) da região do Cariri, Ceará, Brasil. Os resultados demonstraram que a composição centesimal e o valor calórico desse tipo de maracujá são similares a outras espécies, mas com nível elevado de ácido ascórbico. A polpa apresentou baixo teor de polifenóis e baixa atividade antioxidante para as três metodologias aplicadas. Em razão dos altos níveis de acidez titulável (3,52) e sólidos solúveis totais (17,80° Brix), este fruto pode ser considerado como um fruto de alto valor para o comércio. Porém, esta é uma espécie de maracujá pouco descrita na literatura, sendo necessárias mais análises para avaliar seu potencial valor nutricional e funcional.

**Palavras-chave:** alimento funcional, agricultura, fibra dietética, fenólicos, antioxidantes naturais.

### Introduction

Tropical countries produce a large number of edible fruits, and Brazil has suitable climatic conditions for growing many fruit species, native and exotic, that are of potential interest to the agricultural industry and possible sources of income for the local population (Rosso, 2013). Worldwide fruit consumption has increased due to its nutritional potential and effects on health promotion (Vasco, Ruales, & Kamal-Eldin, 2008). In addition to their refreshing flavors and delicious aroma, fruits are sources of antioxidants, such as phenolic compounds, vitamins, carotenoids and minerals, which contribute chemo-preventive effects on human health (Vasco et al., 2008).

The species of passion fruit studied has ascendant plants and a perennial habit, with simple, full and ovate-oblong leaves. The flowers are hermaphrodite, usually isolated with white-purple petals and bloom from December to February. The fruits are globose, 4-5 cm in diameter, with an average weight of 22 g, yellow-orange when ripe and with white pulp. It is much appreciated by the locals, being consumed as a fresh fruit or as a juice. However, *P. glandulosa* Cav. is not cultivated, and the fruits are collected directly from forest during the fruiting period, thus hindering access to and knowledge of the fruit by the majority of the population (Figure 1).



**Figure 1.** Wild Passion fruit (*Passiflora glandulosa* Cav.) collected on the Araripe's Plateau, Ceara, Brazil: a) flower; b) sample fertile botany; c) fruit; d) pulp.

Passion fruit is a popular name given to several species of the genus *Passiflora* (the largest in Passifloraceae). In the Passifloraceae family, the genus *Passiflora* L. is the most diverse, with approximately 130 species native to Brazil (Cervi, 1997). Commercial cultivation is based almost exclusively on *Passiflora edulis* f. *flavicarpa* (yellow passion fruit), followed by *Passiflora alata* cultivation (sweet passion fruit) and *Passiflora edulis* Sims (purple passion fruit). Those three species have the highest commercial interest in Brazil (Instituto Brasileiro de Geografia e Estatística [IBGE], 2011). Evaluating the use of herbal medicines by the Brazilian population, some authors have found that *Passiflora* is among the most used (Marliére, Ribeiro, Brandão, Klein, & Acurcio, 2008).

Several studies have been conducted showing the potential of passion fruit (fruit, peel and seed) for various purposes. The most studied biological activity of the fruit of passion fruit is its antioxidant action that is attributed to polyphenols, particularly the flavonoids (Heim, Tagliaferro, & Bobilya, 2002). Moreover, a recent study showed that a pectin isolated from fresh peels of *Passiflora glandulosa* Cav has hypoglycemic effects in diabetic mice (Sousa et al., 2015).

This work reports for the first time the study of *Passiflora glandulosa* Cav. (maracujá-do-mato), a native of the Cariri region, Ceara, Brazil, and aims to determine the proximal composition, physical and chemical characteristics, energy value and evaluate the *in vitro* antioxidant activity of the pulp extract.

## Material and methods

Ripe passion fruits (6.0 Kg) were collected on the Araripe's Plateau, Ceara, Brazil, located at

7°10'22.21 " S and 39°35'38.54 " W, in February 2013. A voucher specimen was collected and deposited in the Herbarium Caririense Dárdano de Andrade-Lima, Universidade Regional do Cariri, under the number 9983. After collection, the fruits were pulped, and the pulp was then homogenized and stored at -20°C for later analysis. A portion of the sample was frozen, lyophilized and subjected to analysis of its proximal composition.

The proximal composition of the pulp of this wild passion fruit was determined according to the procedures of (Association of Official Analytical Chemists [AOAC], 2012), and total dietary fiber (TDF) was determined according to Prosky, Asp, Schweizer, DeVries, and Furda (1988). The content of total soluble solids, pH and titratable acidity of the pulp of wild passion fruit were determined according to the methodology adopted by the Adolfo Lutz Institute (2005). The ascorbic acid content of the pulp of the wild passion fruit was determined by titration using 2,6-dichlorophenolindophenol (DCPIP) according to Strohecker and Henning (1967). The results were expressed in mg ascorbic acid per 100 g fresh pulp.

The wild passion fruit pulp extract was prepared from 25 g of pulp and 40 mL of methanol: water (1:1). The mixture was homogenized and allowed to stand for 1 hour at room temperature. Then, the mixture was centrifuged at 15,000 rpm for 15 minutes, the supernatant was reserved, and 40 mL of 70% acetone was added to the residue. The solution was homogenized, allowed to stand for 1 hour at room temperature and then centrifuged at 15,000 rpm for 15 minutes. The supernatant was collected and added to the first supernatant, and the volume was made up to 100 mL with distilled water.

The content of phenolic compounds in the extract was determined by the method proposed by Waterhouse (2001), using the Folin-Ciocalteu reagent. Gallic acid was used as the standard solution at concentrations of 2, 5, 10, 15 and 20 ppm for the construction of the calibration curve. Total phenolic content was calculated from the equation of the line and expressed as mg gallic acid equivalents (GAEs) per 100 g pulp.

Total free radical scavenging capacity of the pulp extract was estimated by the DPPH radical capture method proposed by Brand-Williams, Cuvelier, and Berset (1995). Briefly, a solution of the radical is prepared by dissolving 2.4 mg DPPH in 100 mL methanol. A test solution (5 µL) was added to 3.995 mL of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min. in the dark. Absorbance of the reaction mixture was measured at 515 nm spectrophotometrically.

Absorbance of the DPPH radical without antioxidant, i.e. blank was also measured. All the determinations were performed in triplicate. The percentage scavenging radical was calculated from  $[(A_0 - A_1) / A_0] \times 100$ , where  $A_0$  is the absorbance of the blank at  $t = 0$  min, and  $A_1$  is the absorbance of the extract. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Trolox).

Free radical scavenging of the pulp extract was determined by ABTS radical cation decolorization assay (Re et al., 1999) as adapted by Rufino et al. (2010).  $ABTS^{\cdot+}$  cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 hours before use.  $ABTS^{\cdot+}$  solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5  $\mu$ L of pulp extract to 3.995 mL of diluted  $ABTS^{\cdot+}$  solution, the absorbance was measured at 30 min. after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times, and the results were expressed as micromoles of Trolox equivalents (TEAC) per gram of fresh weight ( $\mu$ mol TEAC  $g^{-1}$  f.w.).

The antioxidant activity of the pulp extract was measured using the iron reduction method (FRAP) according to Pulido, Bravo, and Saura-Calixto (2000) as adapted by Rufino et al. (2010). The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl and 20 mM  $FeCl_3 \cdot 6H_2O$  in the proportion of 10:1:1 at 37°C. Freshly prepared working FRAP reagent was pipetted using 1-5 mL variable micropipette (3.995 mL) and mixed with 5  $\mu$ L of the appropriately diluted pulp sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine ( $Fe^{3+}$  TPTZ) complex was reduced to ferrous ( $Fe^{2+}$ ) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 mL FRAP reagent + 5  $\mu$ L distilled water) after 30 min. incubation at 37°C. All the determinations were performed in triplicates and the results were expressed as mM of ferrous sulfate per gram of fresh weight ( $\mu$ M ferrous sulfate  $g^{-1}$  f.w.).

The results were expressed as the mean  $\pm$  standard deviation (SD). For the statistical analysis of the chemical composition of the fruit, analysis of variance (ANOVA) F (one-way) was applied, followed by the Tukey ( $p < 0.05$ ). The Pearson correlation coefficient between total phenolic compounds contribute and *in vitro* antioxidant capacity was calculated, and the software used for the statistical tests was GraphPad Prism 5.01.

## Results and discussion

The chemical composition of wild passion fruit pulp is described in Table 1 and it was compared with the average values found in the literature for the species *Passiflora alata* and *Passiflora edulis* f. *flavicarpa*. It has been noted that the chemical and physical characteristics of these fruits can be altered by many factors, such as harvest time, maturity, variety, climate, soil conditions and sun exposure (Amira et al., 2011).

**Table 1.** Proximate and nutritional composition of the freeze-dried pulp of *Passiflora glandulosa* Cav., *Passiflora alata* and *Passiflora edulis* f. *flavicarpa*.

Characteristics	<i>Passiflora glandulosa</i> Cav.	<i>Passiflora alata</i> <sup>1</sup>	<i>Passiflora edulis</i> f. <i>flavicarpa</i> <sup>2</sup>
Moisture	83.75 $\pm$ 0.14 <sup>a</sup>	84.12 $\pm$ 0.13 <sup>a</sup>	83.76 $\pm$ 1.09 <sup>a</sup>
Protein	1.00 $\pm$ 0.07 <sup>a</sup>	1.35 $\pm$ 0.00 <sup>a</sup>	1.46 $\pm$ 0.68 <sup>a</sup>
Lipids	0.12 $\pm$ 0.01 <sup>a</sup>	0.10 $\pm$ 0.04 <sup>a</sup>	1.03 $\pm$ 0.74 <sup>a</sup>
Carbohydrates	12.38 $\pm$ 0.26 <sup>a</sup>	13.05 $\pm$ 0.17 <sup>a</sup>	12.02 $\pm$ 0.90 <sup>a</sup>
Total dietary fiber	2.03 $\pm$ 0.01 <sup>a</sup>	0.70 $\pm$ 0.00 <sup>a</sup>	0.9 $\pm$ 0.26 <sup>a</sup>
Ash	0.72 $\pm$ 0.03 <sup>a</sup>	0.68 $\pm$ 0.09 <sup>a</sup>	0.83 $\pm$ 0.05 <sup>a</sup>
Energy (kcal)	54.60 $\pm$ 1.41 <sup>a</sup>	58.51 $\pm$ 0.34 <sup>a</sup>	65.98 $\pm$ 8.89 <sup>b</sup>

Mean  $\pm$  standard deviation (n=3). Means followed by the same letter do not differ by the Tukey test ( $p < 0.05$ ). <sup>1</sup> Determined by Souza, Pereira, Queiroz, Borges, and de Deus Souza Carneiro, et al. (2012). <sup>2</sup> Determined by: (UNICAMP, 2011) and (USDA, 2015a, 2015b).

In this study, it was determined that the pulp of *P. glandulosa* Cav. has a high moisture content: its average value is 83.75%, with mean values of 12.38, 1.00 and 0.12 g for carbohydrate, protein and lipid in each 100 g of pulp, respectively. The average content in the wild fruit observed for dietary fiber was 2.03%, and its ash content was 0.72%. Based on these results, the average caloric value was estimated to be 54.60 kcal per 100 grams of fresh pulp.

When comparing the values found in this study with the proximal composition of sweet passion fruit and yellow passion fruit, as described in the literature (Souza et al., 2012; UNICAMP, 2011), the wild passion fruit has similar levels of moisture, protein, carbohydrate and ash content. But it is distinguished by the absolute value of total dietary fiber (TDF) content and lower caloric value (Table 1). The results of the present investigation on TDF contents of wild passion indicate that the TDF values are much higher than the TDF values of corresponding fruits (Table 1), and all values were assessed by enzymatic and gravimetric methods (Prosky et al., 1988). The discrepancies observed between the results of the present study and those reported by Souza et al. (2012), Unicamp (2011) and USDA (2015a; 2015b) could be due to climatic or soil condition differences.

The pH value of the studied wild passion fruit pulp was 3.32, and its titratable acidity (TA) was 3.52. In the literature, it is observed that *P. alata*

has a pH value (3.31) similar to this passion fruit, whereas *P. edulis* genus has lower pH (2.93) than the passion fruit under study. *P. edulis* it is the closest to the wild passion fruit, with values between 3.91 to 4.68%; *P. alata* has a lower TA (2.00%) (Souza et al, 2012; De Marchi, Monteiro, Benato, & Silva, 2000). As for the content of soluble solids (SS), the value of 17.80° Brix was found, which is above the range of 13.08 to 15.00° Brix cited in the literature for other passion fruit species (De Marchi et al., 2000; Fortaleza, Peixoto, Junqueira, Oliveira, & Rangel, 2005). The SS/TA ratio of this wild passion fruit was determined to be 5.06, which is higher than the range of values from 2.26 to 4.40 cited in other studies of passion fruit (Raimundo, Magri, Simionato, & Sampaio, 2009). Therefore, it can be considered that *P. glandulosa* Cav. have a high probability of acceptance for consumption, as the fruits with the highest SS/TA are preferred by consumers (Silva, Sá, Mariguel, Barbosa, & Oliveira, 2002).

Moreover, the high content of titratable acidity is an important characteristic for the fruit pulp processing industry because it reduces the need for the addition of acidulants, avoiding significant changes in the sensory characteristics and degradation of nutritional compounds with bioactive action in the human body (Nascimento, Ramos, & Menezes, 1998).

The ascorbic acid content found in the species under study was 57.76 mg 100 g<sup>-1</sup> of fresh pulp. In the literature, lower amounts of ascorbic acid are found in *P. alata* 24.66 mg 100 g<sup>-1</sup> f.w. and *P. edulis* 27.02 mg 100 g<sup>-1</sup> f.w (De Marchi et al., 2000). According to the classification proposed by Ramful, Tarnus, Aruoma, Bourdon, and Baborun (2011), *P. glandulosa* Cav. of Cariri region is classified as having high ascorbic acid content (> 50 mg 100 g<sup>-1</sup> pulp).

The results regarding the total phenolic content and antioxidant capacity of *P. glandulosa* Cav. are summarized in Table 2. Based on the results, its pulp can be classified as having low concentration of total phenolics (20.55 mg GAEs 100 g<sup>-1</sup>). In the literature, a similar concentration of total phenolics (20.00 mg GAEs 100 g<sup>-1</sup>) in fresh passion fruit pulp (*Passiflora* sp.) (Kuskoski, Asuero, Troncoso, Mancini-Filho, & Fett, 2005), although values of 10.84 mg GAEs 100 g<sup>-1</sup> was found to *Passiflora alata* (Souza et al., 2012).

**Table 2.** Total phenolics, vitamin C and antioxidant capacity (ABTS, DPPH and FRAP) of the pulp of *Passiflora glandulosa* Cav.

<i>Passiflora glandulosa</i> Cav.	
Total phenolics (mg GAEs 100 g <sup>-1</sup> f.w.)	20.55 ± 0.96
Ascorbic acid (mg 100 g <sup>-1</sup> f.w.)	57.76 ± 0.40
ABTS (μmol TEAC g <sup>-1</sup> f.w.)	2.55 ± 0.31
DPPH (g mg DPPH <sup>-1</sup> )	39.70 ± 3.39
FRAP (μmol Fe <sub>2</sub> SO <sub>4</sub> g <sup>-1</sup> f.w.)	4.47 ± 0.36

Mean ± standard deviation (n=3); f.w fresh weight.

In the DPPH assay, the pulp of passion fruit exhibited stabilized absorbance 35 minutes after the start of the reaction. At this time, a decrease of 32.30% from the initial amount of DPPH radicals was observed. This result corroborates the Vasco et al. (2008) literature, which reports that passion fruit has low efficiency in capturing DPPH radicals, like watermelon, melon and mango (Melo, Maciel, Lima, & Nascimento, 2008).

Regarding the results obtained using the ABTS, the species studied showed an average antioxidant activity of 2.57 μmol TEAC g<sup>-1</sup> of f.w. A similar was result obtained in a similar study with frozen passion fruit pulp, where the average antioxidant activity was 2.7 μmol TEAC g<sup>-1</sup> of f.w. (Kuskoski et al., 2005). According to Souza et al. (2012), the antioxidant activity of sweet passion fruit (*Passiflora alata*) was determined in 10.84 TEAC g<sup>-1</sup> of f.w., and despite being greater than in *P. glandulosa* Cav., it is still considered a low-activity antioxidant. The antioxidant test by FRAP also revealed a low average antioxidant activity in the species studied (4.47 μM ferrous sulfate g<sup>-1</sup> of f.w.). Regarding the literature, it was described peel and seeds with 34.91 μM ferrous sulfate g<sup>-1</sup> of f.w activity (Infante et al., 2013). However, it is observed that fruit antioxidant analysis by this method are scarce, reinforcing the importance of the data obtained in this study (Table 2).

The antioxidant capacity of the fruit diversifies according to the contents of vitamin C, vitamin E, carotenoids, β-carotene (Rice-Evans & Miller, 1996), lycopene (Shami & Moreira, 2004), as well as flavonoids and other polyphenols (Saura-Calixto & Goñi, 2006). In this study, it was determined that the polyphenols content and DPPH are correlated negatively and significantly (r = -0.74; p < 0.01), supporting the results reported in the literature regarding the high correlation between phenolic content and antioxidant activity (Rufino et al., 2010).

## Conclusion

To our knowledge, this is the first study to describe the chemical composition and antioxidant activity of the pulp of *Passiflora glandulosa* Cav. native to Cariri region. And its pulp has high moisture content, low levels of proteins, lipids and low energy content. With

respect to its antioxidant potential, it has low levels of polyphenols and low antioxidant activity; however, it has a high probability of acceptance for fresh consumption due to its high level of titratable acidity.

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