Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00360

Antioxidant characterization of fruits of the Family Cactaceae: *Hylocereus undatus*, *Selenicereus megalanthus*, and *Hylocereus polyrhizus*

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Abstract

In this study, the antioxidant capacity of the peel and pulp of three Pitaya species was investigated: *Hylocereus undatus*, *Selenicereus megalanthus*, and *Hylocereus polyrhizus*. The methods used include the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, the thiobarbituric acid reactivity assay (TBARS) for inhibition of lipid peroxidation, and the Folin-Ciocalteau method for determination of total phenolic content. Regarding the TBARS results, the peel sample of 20% *H. polyrhizus* showed the highest antioxidant capacity in both solvents (water and alcohol) analyzed. Regarding the pulp, the *H. undatus* sample in the aqueous extract was the sample with the highest antioxidant potential (12.5%). In the ethanolic extract, the sample of *H. polyrhizus* pulp (25%) showed the highest antioxidant potential, as did the peel at a concentration of 20%. Using the DPPH method, the peel sample of the 5% aqueous extract of *H. undatus* can be highlighted with a greater antioxidant capacity. Regarding the pulp, the sample with the highest potential was the 20% *H. undatus* in the aqueous extract. In the ethanolic extract, the *H. undatus* samples from the peel and pulp showed the highest antioxidant potential with 25 and 50%, respectively. In terms of phenolic content, no significant difference was found between the peel samples, but among the pulp samples, *H. undatus* had the lowest concentration. It can be concluded that the different pitaya species exhibited significant antioxidant capacity, suggesting that pitaya consumption could potentially provide benefits to human health due to its bioactive compounds.

Keywords: pitaya; bioactive compounds; free radicals; oxidative stress; antioxidant.

Practical application: Pitaya species can provide benefits to human health through its bioactive compounds.

1 INTRODUCTION

Pitaya, a rustic plant from the Cactaceae family, originates from the tropical regions of Mexico, Central, and South America (Freitas & Mitcham, 2013). Its primary scientific name is *Hylocereus undatus* (white pitaya). Other species include *Selenicereus megalanthus* (yellow pitaya) and *Hylocereus polyrhizus* (red or purple pitaya) (Abreu et al., 2012; Correia et al., 2016). The fruit is rich in bioactive and functional compounds such as vitamins B1, B2, and B3, beta-carotene, lycopene, vitamin E, phenolic compounds, ascorbic acid, potassium, magnesium, and carbohydrates (Abreu et al., 2012).

Free radicals are molecules produced by the body's metabolic processes and contain highly unstable and reactive electrons (Vasconcelos et al., 2014). While they are produced from essential oxidative metabolic processes, such as those that activate our immune system (Schneider & Oliveira, 2004), an excess of free radicals is linked to neurodegenerative diseases like Alzheimer's and Parkinson's, premature aging, and oxidative stress (Mahan et al., 2012).

Harman (1956) proposed the theory of oxygen radicals, suggesting that aging might be a consequence of oxidative stress. Such stress can catalyze reactions with lipids, proteins, and DNA, leading to progressive changes in tissues and the genetic code (Ferreira & Matsubara, 1997; Mahan et al., 2012). Cells have developed two primary defense mechanisms against damage caused by excess free radicals. The first pathway aims to detoxify harmful agents before they cause injury, leveraging both endogenous enzymes and exogenous antioxidants like reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase (GSH-Px), and vitamin E. The second defense pathway has the function of repairing the injury that already has occurred, consisting of ascorbic acid, glutathione reductase, and GSH-Px (Ferreira & Matsubara, 1997). This second protection process is related to the removal of damage to the deoxyribonucleic acid (DNA) molecule and the reconstitution of damaged cell membranes. The antioxidants obtained from the diet, such as vitamins C, E, and A, and the phenolic compounds and carotenoids present in fruits and vegetables are extremely important in the interception of free radicals (Bianchi & Antunes, 1999),

Received: June 25, 2024.
Accepted: July 12, 2024.
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Conflict of interest: nothing to declare.
Funding: Coordination for the Improvement of Higher Education Personnel—Financial Code 001; National Council for Scientific and Technological Development; and Research Support Foundation of the State of Rio Grande do Sul.

as they reduce the risk of developing a wide range of diseases by reduction of the body's production of free radicals (Costa & Rosa, 2016).

Because pitaya is a fruit recently introduced into the regular diet of the Brazilian population, the scarcity of studies regarding its antioxidant content, and the divergence of information about this fruit, the need for further studies on the subject is justified. Thus, this study aimed to analyze the antioxidant capacity through the inhibition of lipid peroxidation and capture of free radicals, as well as to determine the levels of total phenolic compounds in the peel and pulp of different species of the fruit: *H. undatus* (white pulp pitaya), *S. megalanthus* (yellow pulp pitaya), and *H. polyrhizus* (pitaya with red or purple flesh).

2 MATERIALS AND METHODS

2.1 Sample characterization

White pulp pitaya (*H. undatu*) and red or purple pulp pitaya (*H. polyrhizus*) were commercially obtained in the city of Pelotas, in the state of Rio Grande do Sul; the pitaya with yellow pulp (*S. megalanthus*) was obtained in the city of Rio de Janeiro, in the state of Rio de Janeiro. Three samples of each fruit species were collected. The selection process for the acquisition of the fruits involved aspects of appearance, diameter, and maturation, which were selected visually. The samples were collected in plastic bags, allocated in a thermal bag, preserved at a temperature of 10°C for transport, and taken to the Food Testing Laboratory of the Faculty of Nutrition of the Universidade Federal de Pelotas, where they were sanitized, and the peel was separated from the pulp and subsequently stored in an ultra-freezer at -80°C until the analyses were carried out.

2.2 Extract preparation for antioxidant analysis

The extract of *H. undatus*, *S. megalanthus*, and *H. polyrhizus* fruits was prepared for the *in vitro* analysis using distilled water and ethyl alcohol (99.8%) at different concentrations (10, 20, and 30%).

2.3 Evaluation of in vitro antioxidant activity

2.3.1 Thiobarbituric acid reactive substance

The reaction to thiobarbituric acid (TBA) was determined according to the methodology of Ohkawa et al. (1979). First, test tubes containing Milli-Q water and Extra Virgin Olive Oil were incubated in a water bath at 80°C and submitted to oxidation by 100 µM of ferrous sulfate for 10 min. Subsequently, the sample with different concentrations and extraction temperatures was added to each tube, along with sodium lauryl sulfate 8.1%, acetic acid buffer pH 3.44, and TBA 0.6%. Then, it was incubated again in a water bath at 100°C for 1 h. The reaction products were determined by absorbance measurement at 532 nm, in a spectrophotometer. The TBARS concentration was calculated using a standard curve with known concentrations of 1,1,3,3-tetramethoxypropane, and the results were expressed in nanometers of malondialdehyde (MDA) per gram of the sample. The experiment was carried out in triplicate.

2.3.2 Antioxidant capacity by the DPPH method (2,2-difenil-1-picrilhidrazil)

The DPPH method used was described by Brand-Williams et al. (1995), based on the capture of the DPPH radical (2,2-diphenyl-1-picryl-hydrazyl) by antioxidants, producing a decrease in absorbance at 515 nm. DPPH was used at a concentration of 60 µM, dissolved in methyl alcohol. In a dark environment, an aliquot of 0.1 mL of sample was transferred to test tubes containing 3.9 mL of the DPPH radical (60 µM) and then homogenized. A control solution, consisting of 0.1 mL of 50% methyl alcohol (40 mL), 70% acetone (40 mL), and water (20 mL), was used with 3.9 mL of the DPPH radical (60 µM). After the preparation, the samples were stored in a dark environment for 45 min. As a blank, methyl alcohol was used, and the standard curve was performed from the initial DPPH solution (60 µM), varying the concentration from 10 to 50 µM. Results were expressed in EC50 (µg mL^{-1}). The experiment was carried out in triplicate.

2.4 Analysis of total phenolic compounds by the Folin-Ciocalteau method

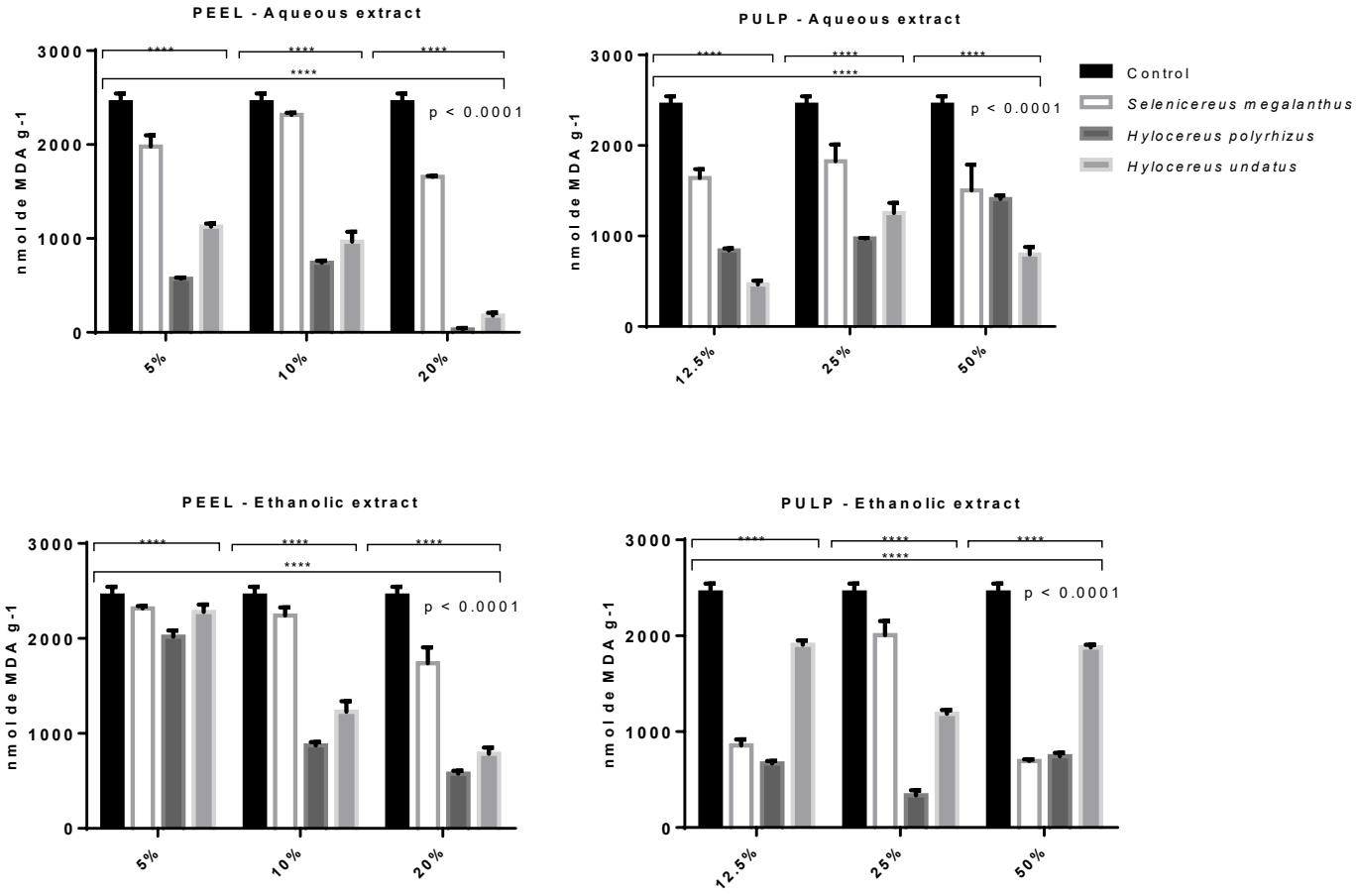
The quantification of phenolic compounds was determined by the method of Swain and Hillis (1959) with slight modifications. A total of 0.5 g of each sample was homogenized with 20 mL of solvent (methanol). The samples were centrifuged at 10,000 rpm for 15 min. A volume of 250 µL of the supernatant was collected, and 4 mL of distilled water and 250 µL of Folin-Ciocalteau (1:1) were added to the sample. The tubes were shaken, and after 5 min, 0.5 mL of Na_2CO_3 (7%) was added. After 2 h, absorbance was measured at 725 nm. The amount of phenols in the extract of *H. undatus*, *S. megalanthus*, and *H. polyrhizus* was quantified using a standard curve prepared with gallic acid between concentrations of 10–100 µg mL^{-1}. The quantification of phenolic compounds was performed in triplicate, and the results were expressed in micrograms of gallic acid per gram of the sample (µg g^{-1}).

2.5 Statistical analysis

The data were analyzed using analysis of variance and Tukey's multiple comparison test, based on significance levels greater than 95% ($p < 0.05$), with the aid of the GraphPad Prism 6 Software for Windows to compare the different parameters evaluated in the experiment.

3 RESULTS

The results of the lipid peroxidation inhibition assessment by the TBARS of the peel and pulp with aqueous and ethanolic extracts of the three fruit species in different concentrations are presented in Figure 1. Thus, a significant difference was observed between all treatments and species analyzed when compared to the control. Furthermore, when analyzing the interaction between the different species of pitaya, there was a significant difference between all samples in both extracts. It is also noteworthy that, among the samples of the peel in both analyzed extracts (aqueous and ethanolic), it was highlighted



*Significant differences between samples.

Figure 1. Analysis of the inhibition of lipid peroxidation of the peel and pulp of different pitaya species. *Selenicereus megalanthus* (yellow pulp pitaya), *Hylocereus polyrhizus* (red or purple pulp pitaya), and *Hylocereus undatus* (white pulp pitaya) were analyzed with two solvents (water and ethanol) at different concentrations by the thiobarbituric acid reaction method (TBARS). Data were expressed as mean ± standard deviation in nanomoles of malondialdehyde (MDA) per gram of the sample.

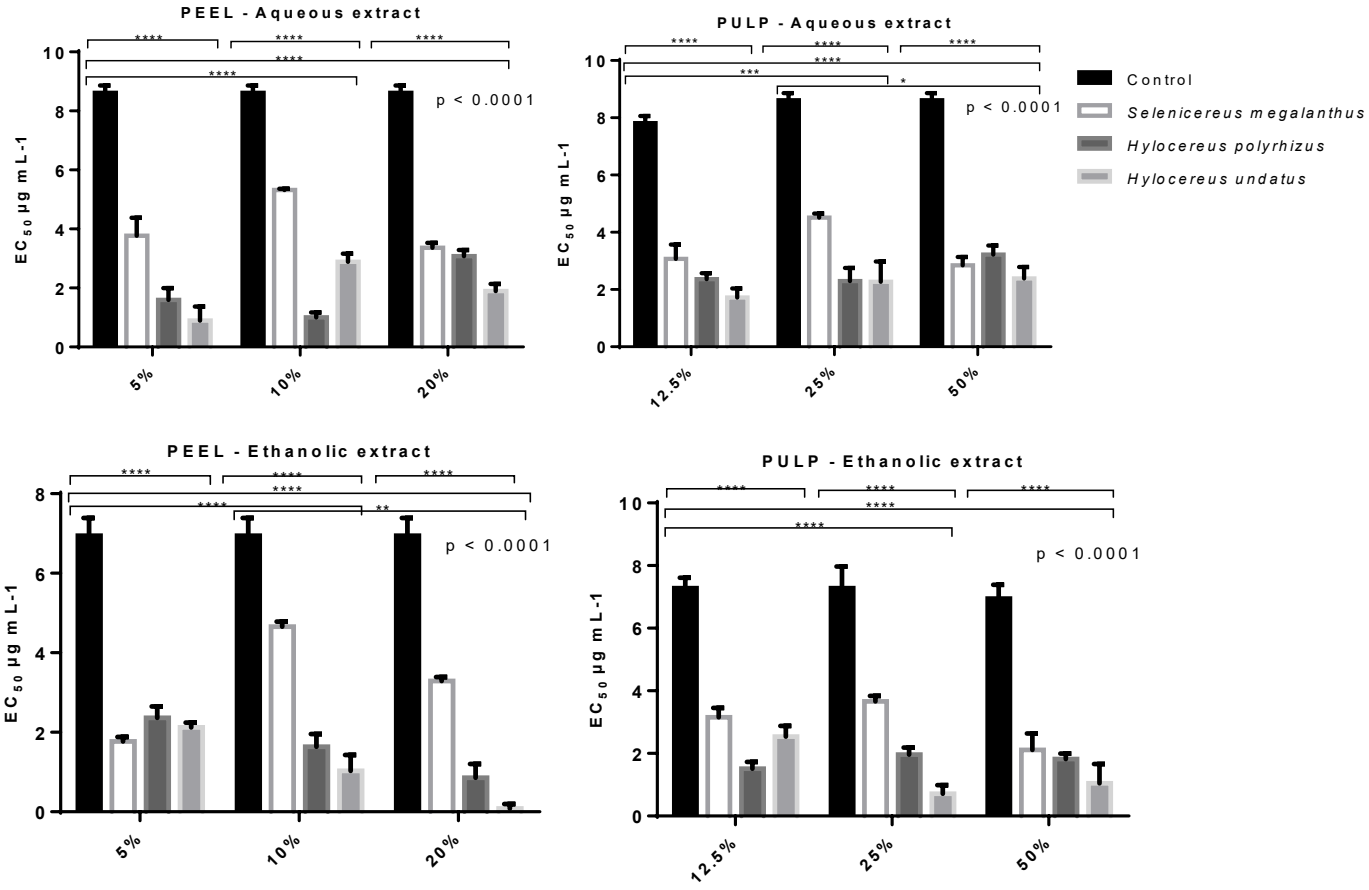
that the sample with the greater antioxidant potential was the 20% *H. polyrhizus*, followed by the *H. undatus* sample in the same concentration.

Regarding the pulp, a significant difference was observed between the different concentrations and species analyzed when compared to the control. Similarly, when analyzing the interaction between the different species of pitaya, there was a significant difference between all samples in both extracts. It was also observed that the pulp samples with higher antioxidant potential were the 12.5% *H. undatus*, followed by the 50% *H. undatus* and 12.5% *H. polyrhizus* samples in aqueous extracts. Regarding the ethanolic extracts, the samples of 25% *H. polyrhizus* were highlighted, followed by samples of 12.5% *H. polyrhizus* and 50% *H. undatus*, with greater antioxidant potential.

Figure 2 shows the results of the DPPH radical capture analysis of the different species, extracts, and concentrations of the fruit pulp and peel. Through the analysis of the aqueous and ethanolic extracts from the peel, a significant difference was observed between all treatments and species when compared to the control. However, when analyzing the interaction between both species, it was observed that in the peel samples in aqueous extract, there was a significant difference only between the species *S. megalanthus* and *H. polyrhizus* and *S. megalanthus* and *H. undatus*. When analyzing the interaction of the peel in ethanolic extract, a significant difference was observed between all species. Among the peel samples in both extracts analyzed, the 5% *H. undatus* sample stood out with the highest antioxidant capacity, followed by the 10% *H. polyrhizus* sample.

The pulp presented significant differences between the different concentrations and species analyzed when compared to the control. When analyzing the interaction between species in the aqueous extract, a significant difference was observed in all samples; however, when analyzing the interaction between species in the ethanolic extract, there was interaction only between *S. megalanthus* and *H. polyrhizus* and *S. megalanthus* and *H. undatus*. The 20% *H. undatus* pulp samples showed the highest antioxidant potential, followed by the *H. polyrhizus* sample at the same concentration in the aqueous extract. Regarding the ethanol extracts, samples of *H. undatus* presented greater antioxidant potential at concentrations of 25 and 50%, respectively.

Furthermore, the quantification of total phenolic compounds was carried out, and the results are shown in Figure 3. It was observed that there was no significant difference in the concentration of total phenolic compounds between the samples



*Significant differences between samples.

Figure 2. Analysis of the antioxidant capacity of the peel and pulp of different pitaya fruit species, *Selenicereus megalanthus* (yellow pulp pitaya), *Hylocereus polyrhizus* (red or purple pulp pitaya), and *Hylocereus undatus* (white pulp pitaya), analyzed with two solvents (water and ethanol) at different concentrations by the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical capture method. Data were expressed as mean±standard deviation in EC_{50} µg mL^{-1}.

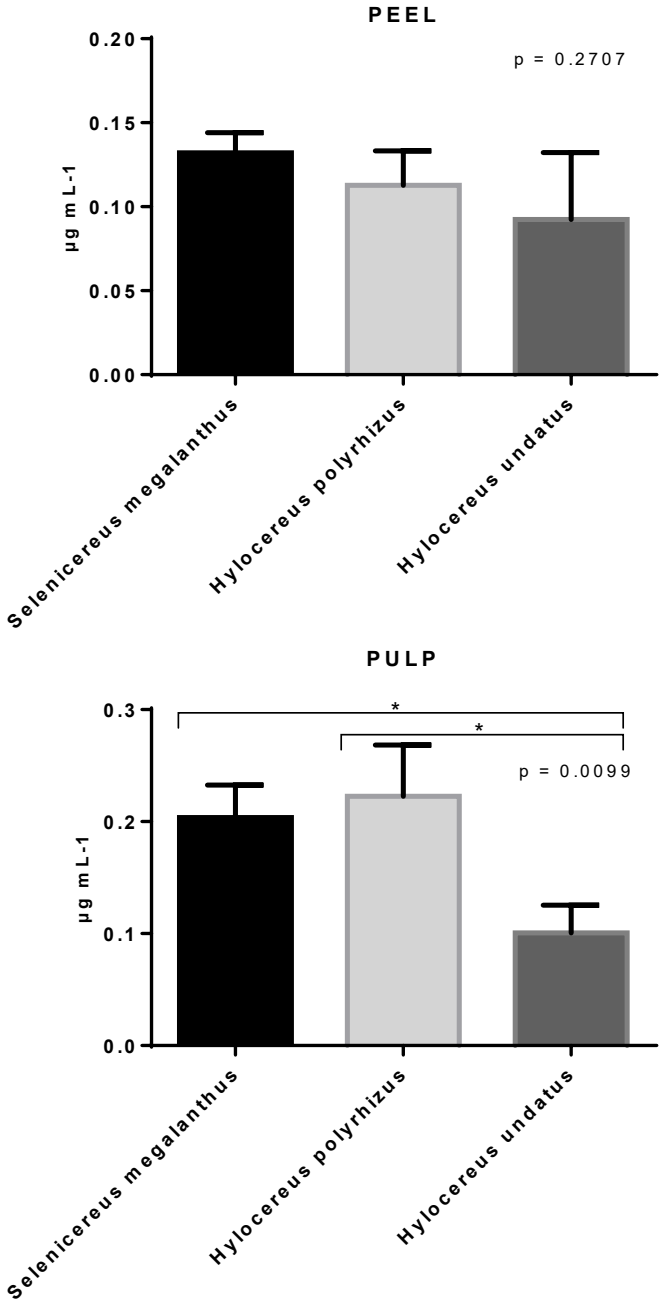
of the peel of the three analyzed fruit species (*S. megalanthus, H. polyrhizus*, and *H. undatus*). However, when analyzing the pulp samples of the three fruit species, a significant difference was observed between *S. megalanthus* and *H. undatus* and between *H. polyrhizus* and *H. undatus*.

4 DISCUSSION

In living organisms, lipid peroxidation is defined as the oxidation of the lipid layer of the cell membrane, caused by the imbalance between pro-oxidant and antioxidant agents, causing changes in the normal physiological activity of the cell (Filaire et al., 2011). Phenolic compounds can scavenge free radicals and pro-oxidant metals, preventing damage to DNA, proteins, and lipids. Additionally, they modulate gene expression in cell signaling mechanisms associated with non-communicable diseases (Costa & Rosa, 2016).

From the lipid peroxidation inhibition analysis (TBARS), it was observed that the aqueous extracts of varying concentrations exhibited a significant difference ($p < 0.0001$) compared to the control. This underscores the antioxidant potential of the species *H. undatus, H. polyrhizus*, and *S. megalanthus*. In both extracts at the different concentrations analyzed, it can be observed that the sample with the highest antioxidant potential was the peel of the 20% *H. polyrhizus* in aqueous extract, followed by the sample of the same concentration and extraction method of the species *H. undatus*. Choo and Yong (2011) reported similar results, with the pulp of the *H. polyrhizus* species showing greater antioxidant capacity than *H. undatus*. Manihuruka et al. (2017) observed a robust antioxidant capacity in the peel of *H. polyrhizus* in the TBARS assay with significant oxidative inhibition in meat sausages and attributed this effect to the phenolic compounds present in the fruit.

In this study, antioxidant capacity was observed in both extracts (aqueous and ethanolic) in samples of *S. megalanthus, H. polyrhizus*, and *H. undatus* at the different concentrations analyzed when compared to the control. Abreu et al. (2012) demonstrated that *H. polyrhizus* had the highest antioxidant capacity when analyzing the pulp, associating this result with the high content of betacyanins present in the fruit pulp since the levels of phenolic compounds found in the pulp of this species were similar to the levels found in the pulp of *H. undatus*. In contrast, Obenland et al. (2016) detected little or no betacyanin content in *H. undatus*, thus demonstrating a low antioxidant potential of the fruit. According to Santos et al. (2016), the antioxidant activity decreases as the fruits ripen, likely due to



*Significant differences between samples.

Figure 3. Analysis of the concentration of total phenolic compounds in extracts of *Selenicereus megalanthus* (yellow pulp pitaya), *Hylocereus polyrhizus* (red or purple pulp pitaya), and *Hylocereus undatus* (white pulp pitaya) by the Folin-Ciocalteu method. Data were expressed as mean ± standard deviation in micrograms of gallic acid per milliliter.

decreasing levels of phenolic compounds and vitamin C, both of which serve as potent antioxidants.

The antioxidant capacity of the fruits was also evaluated by the DPPH method. This widely recognized method measures the sample's ability to donate hydrogen to the DPPH radical. Therefore, the higher the antioxidant content present in a fruit and its respective extract, the greater the inhibition/reduction of the DPPH solution (Choo & Yong, 2011). Notably, the *H. undatus* peel, at a 20% concentration in aqueous extract, exhibited the most substantial antioxidant potential by the DPPH method. However, Abreu et al. (2012) reported lower antioxidant potential for pitaya species analyzed with the DPPH method, with values less than those identified in our study.

Among the plant-derived compounds with the greatest antioxidant potential are the phenolic compounds, which are structurally divided into two groups: phenolic acids and flavonoids. These molecules are involved not only in plant antioxidant defense but also in growth control, antimicrobial activity, pH control, metabolism, and hormonal activity, among others. Its antioxidant activity is mainly mediated through the transfer of its electron and/or hydrogen atom to the free radical, inactivating it (Cheynier et al., 2013; Olszowy, 2019). Thus, to evaluate the content of total phenolic compounds in this study, the Folin-Ciocalteau method was used. No significant differences were observed in the phenolic content between the peel samples of various pitaya species. However, a notable difference was evident between the pulp samples of *H. undatus* and *H. polyrhizus*. This contrasts with the findings of Abreu et al. (2012), who reported no significant difference in phenolic compound levels between these two species.

In this study, the pulp of *H. polyrhizus* had a notably higher content of phenolic compounds than *H. undatus*. This contrasts with the findings of Choo and Yong (2011), who reported a significantly higher phenolic compound content in *H. undatus* compared to *H. polyrhizus*. A significant difference in phenolic compound content was noted between *S. megalanthus* and *H. undatus*. Of the two, *S. megalanthus* exhibited a higher concentration. Moreover, Lima et al. (2013) observed different results from those found in this study, where the concentration of polyphenols and flavonoids was higher in the *H. undatus*.

Various factors can impact the content of secondary metabolites in plants, such as phenolic acids. These include seasonality, temperature, water availability, ultraviolet radiation, nutrient supplementation, atmospheric pollution, mechanical damage, and pathogen attacks (Gobbo-Neto & Lopes, 2007), as well as different degrees of maturation, origin, and climate (Abreu et al., 2012). According to Kaur and Kapoor (2001), the composition of phenolic compounds in fruits can also be modified by post-harvest factors, including storage and processing. Prolonged processing and storage can lead to enzymatic and chemical oxidation of these compounds, reducing their levels and thereby altering the antioxidant potential of the final product.

5 CONCLUSION

Therefore, based on the results of this study, it can be concluded that the pitaya fruit species *H. undatus, H. polyrhizus*, and *S. megalanthus* presented significant antioxidant capacity in both their peel and pulp. This capacity was observed in both aqueous and ethanolic extracts, evaluated by lipid peroxidation inhibition and DPPH radical capture. The notable content of total phenolic compounds in the fruit likely underlies its robust antioxidant potential. Consequently, these results hint at the potential health benefits of pitaya consumption due to its bioactive properties. However, further research is required to confirm these potential benefits.

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