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## EVALUATION OF ANTIOXIDANT ACTIVITY OF BRAZILIAN CERRADO FRUITS IN VITRO

AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE IN VITRO DE  
FRUTOS DO CERRADO BRASILEIRO

EVALUACIÓN DE LA ACTIVIDAD ANTIOXIDANTE IN VITRO DE  
FRUTOS DEL CERRADO BRASILEÑO

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## ABSTRACT

The objective of this study was to determine the evaluation of antioxidant activity of the pulps in vitro from the Brazilian Cerrado in the interest of identifying the quality of these raw materials. After receiving the fruits, the pulps were removed manually and, the antioxidant activity was evaluated through 1,1-difenil-2-picrilhidrazil - DPPH• free radical total phenolic compounds and ascorbic acid content. To obtain the extracts, dried and crushed pulps were extracted with ethanol (95%) for 30 minutes, in a ratio of 1:4 (pulp: ethyl alcohol), under continuous agitation at room temperature. Then, the mixtures were filtered, and the supernatants were subjected to rotary evaporation under pressure reduced to 40°C. Pulps from Brazilian Cerrado fruits showed relevant antioxidant activity and may have positive effects on human health, reducing the incidence of diseases and, therefore, prolonging the life expectancy of the population.

## KEYWORDS

ascorbic acid, DPPH•, fruits extracts, total phenolic compounds.

## RESUMO

O objetivo deste estudo foi determinar a avaliação da atividade antioxidante in vitro das polpas do cerrado brasileiro, com o objetivo de identificar a qualidade dessa matéria-prima. Após o recebimento dos frutos, as polpas foram removidas manualmente e avaliadas a atividade antioxidante por meio do radical livre 1,1-difenil-2-picril-hidrazil - DPPH•, compostos fenólicos e o teor de ácido ascórbico. Para obtenção dos extratos, as polpas secas e trituradas foram extraídas com etanol (95%) por 30 minutos, na proporção de 1:4 (polpas: álcool etílico), sob agitação contínua, à temperatura ambiente. Em seguida, as misturas foram filtradas e os sobrenadantes foram submetidos a evaporador rotativo sob pressão reduzida a 40°C. As polpas dos frutos do cerrado brasileiro apresentaram atividade antioxidante relevante e podem ter efeitos positivos na saúde humana, reduzindo a incidência de doenças e, portanto, prolongando a expectativa de vida da população.

## PALAVRAS-CHAVE

ácido ascórbico, DPPH•, extratos de frutas, compostos fenólicos totais.

## RESUMEN

El objetivo de este estudio fue determinar la evaluación de la actividad antioxidante in vitro de pulpas del cerrado brasileño, con el objetivo de identificar la calidad de esta materia prima. Después de recibir los frutos, se despulparon manualmente y se evaluó la actividad antioxidante a través del contenido de radicales libres 1,1-difenil-2-picrilhidrazilo - DPPH•, compuestos fenólicos y ácido ascórbico. Para la obtención de los extractos, las pulpas secas y trituradas se extrajeron con etanol (95%) durante 30 minutos, en proporción 1:4 (pulpa: alcohol etílico), en agitación continua, a temperatura ambiente. Luego, las mezclas se filtraron y los sobrenadantes se sometieron a un evaporador rotatorio a presión reducida a 40°C. Las pulpas de frutas del cerrado brasileño mostraron una actividad antioxidante relevante y pueden tener efectos positivos en la salud humana, reduciendo la incidencia de enfermedades y, por lo tanto, prolongando la esperanza de vida de la población.

## PALABRAS CLAVE

ácido ascórbico, DPPH•, extractos de frutas, compuestos fenólicos totales.

## 1 INTRODUCTION

The Brazilian Cerrado biome, typical of the tropical zone, is a savanna formation which occupies approximately 2.0 million km<sup>2</sup> and corresponds to 23.1% of the Brazilian territory. It comprises the states of Goiás, Tocantins, Mato Grosso do Sul and Minas Gerais, as well as southern Mato Grosso, western Bahia and the Federal District. It extends even beyond Central Brazil, in the form of islands, in southern Maranhão, northern Piauí, Rondônia and in one-fifth of São Paulo. In Minas Gerais, it occupies more than 50% of the territory.

According to the “Cerrado Sustentável” Program, the Cerrado presents significant biodiversity that can be exploited through sustainable use as a viable alternative for conservation of significant areas and as a means of generating income, food security and quality of life for traditional communities and farmers (BRASIL, 2006). This is done through the use of medicinal plants, wild bee breeding, wild animal management, ecotourism, rural tourism, condiments, handicrafts and fish farming (BORDIGNON et al., 2019).

Table 1 shows the general characteristics of Brazilian Cerrado fruits, such as the high nutritional values these fruits possess, sensory attractiveness, such as color, flavor, peculiar and intense aroma, and if they can be consumed freshly or used to make sweets, jams, liqueurs, juices, ice cream, cakes, breads, and biscuits. In recent years, a considerable interest in the chemical composition of wild fruits and pulps has been generated. Results have shown that some plants are rich in oils, ascorbic acid and antioxidants, with the possibility of being alternative sources for raw materials and provide viable quantities for the industrial process (VIEIRA; COSTA, 2007).

Epidemiological studies have indicated that regular consumption of various fruits is associated with lower risk of chronic diseases, Gogus and Smith (2010) because of the combination of vitamins, minerals, antioxidants and fibers (GUO et al., 2022; BORDIGNON et al., 2019).

Evaluating the antioxidant activity of fruits native to the Cerrado contributes to sustainable use, conservation, and the selection of promising species, besides showing the economic importance of these foods. Thus, the aim of this study was to evaluate the antioxidant activity in vitro of the pulps in the interest of better identifying the qualities of these raw materials from the Brazilian Cerrado.

**Table 1** - General characteristics of some species of Brazilian Cerrado fruits.

Fruits	General characteristics	References
Araticum ( <i>Annona crassiflora</i> )	Tree from 4 to 8 m in height. Crass-membranous leaves and yellowish-green flowers. The fruit is sub-globulous, of green color when developing and brown, when ripe. The pulp has a coloration ranging from white to yellow, with seeds of average weight between 1.5 and 2.0 g.	LORENZI (2000); CLERICI; CARVALHO-SILVA (2011)
Buriti ( <i>Mauritia flexuosa</i> )	It is a palm tree more than 15 m high. Fruit with hard, red and scaly peel, covering the soft and oily pulp, whose coloration varies from dark yellow to reddish. The fruits are ovoid and weigh on average 50 g.	CALBO; MORAES (1997); FERREIRA (2018)

Fruits	General characteristics	References
Cagaíta ( <i>Eugenia dysenterica</i> )	Tree from 4 to 10 meters high, they have deciduous leaves and their trunk is grooved, with a strong presence of cork. Its flowers are axillary, solitary or arranged in three arrangements. They are hermaphrodite and complete. The fruits are globose, bagaceous, pale yellow, slightly acidic, membranous epicarp.	LORENZI (2000); NAVES; BORGES; CHAVES (2002); SOUZA et al. (2002)
Inga ( <i>Inga edulis</i> )	It is a tree that can reach 40 m high, with relatively low trunk and wide canopy. Composite leaves (leaves divided into leaflets) with three to four pairs of leaflet. fruits have seeds covered by sarcotesta (white pulp).	PENNINGTON (1997)
Jatoba ( <i>Hymenaea courbaril</i> )	Tree from 4 to 6 m in height. Fruits between 6 and 18 cm in length and 3 to 6 cm in diameter with approximately 4 seeds weighing on average 3 g each.	ALMEIDA; SILVA; RIBEIRO (1997); SILVA et al. (2001)
Mangaba ( <i>Hancornia speciosa</i> )	Medium-sized tree, 2 to 10 m high, up to 15 m; large canopy, 4 to 6 m in height and diameter; The leaves are simple, of varying shape and size, are hairy or glabrous and short-petiolate. The flowers are hermaphrodite The fruits are of the berry type, of size, shape and varied colors, usually, ellipsoidal or rounded, yellowish or greenish, with or without red pigmentation.	LEDERMAN et al. (2000); SILVA et al. (2001)
Murici ( <i>Byrsonima crassifolia</i> )	Hermaphrodite tree or shrub, measuring from 4 to 6 m, often tortuous trunk with a diameter of up to 17 cm, a pale gray color, with discontinuous and sinuous fissures forming irregular plaques. Cup with terminal branches of nodular growth. Leaves 14-20 cm long by 6-12 cm wide; flowers about 1.5 cm in diameter, arranged in elongated ears; fruit of up to 2 cm in diameter, globose drupe, glabrous, meaty mesocarp; pulp juicy and sweet; yellow at maturity	CASTRO; LORENZZI (2005); SILVA JÚNIOR (2005)
Pequi ( <i>Caryocar brasiliense</i> )	Tree about 10 m high. Green leaves and yellow-white flowers. Green drupeid fruit with 4.2-6.4 x 6.5-7.8 cm, has a fleshy coriaceous epicarp, surrounded by light yellow mesocarp and some seeds. Fruits weighing 50 to 250 g.	ALMEIDA (1998); VERA et al. (2005); CORREIA et al. (2008)

Fruits	General characteristics	References
Pitaya ( <i>Hylocereus undatus</i> )	Cluster and fruit species, belonging to the family of cacti, are branched, with branches trialled, flattened on one side, being able to reach 20 cm in length and 5 to 7 cm in diameter. The flowers are lateral, 20 to 35 cm long, complete. The fruit presents pulp (mesocarp), the edible part of the fruit, formed by a mass of mucilaginous texture, with small and soft seeds, homogeneously distributed.	FERNANDES et al. (2010); CORDEIRO et al. (2015)
Seriguela ( <i>Spondias purpurea</i> )	It is a medium-sized tree, reaching up to 7 meters in height and its fruits, isolated or in bunches, can, when mature, have a yellow or reddish-yellow color, measuring from 2.5 to 5 cm in length and about 2 cm in diameter.	LIRA JÚNIOR et al. (2010); GOMES et al. (2014)

Source: Elaborated by the authors

## 2 MATERIALS AND METHODS

### 2.1 FRUIT COLLECTION

Fruits from the Brazilian Cerrado, such as araticum (*Annona crassiflora*), buriti (*Mauritia flexuosa*), cagaíta (*Eugenia dysenterica*), ingá (*Inga edulis*), jatobá (*Hymenaea courbaril*), mangaba (*Hancornia speciosa*), murici (*Byrsonima crassifolia*), pequi (*Caryocar brasiliense*), pitaya (*Hylocereus undatus*) and seriguela (*Spondias purpurea*) were collected from regions which are representative of their productivity in the Brazilian Cerrado (in the Southeast region of Brazil, city of Frutal, Minas Gerais), and they were acquired at different periods during the growing seasons of 2015/2016 (Figure 1).

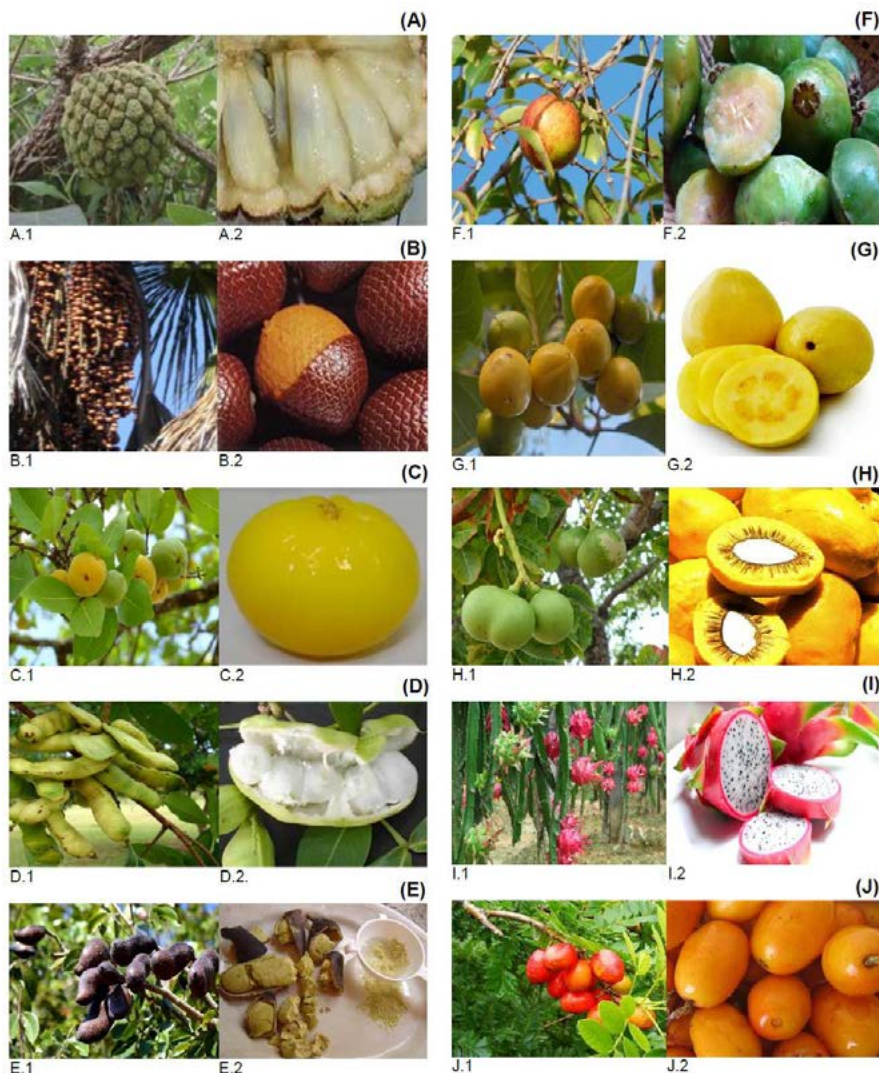
### 2.2 SAMPLE PREPARATION

After a period of maturation, the fruits were harvested and those that had cracks, or had been damaged by insects and/or attacked by animals or birds were disposed of. After, their pulps were removed manually at the Laboratory of Physical-Chemical Analysis, Department of Exact and Earth Sciences, Minas Gerais State University (UEMG). Once selected, the pulps were placed in an oven at 35°C, for a period of 2 h to reduce the moisture content, and then homogenized for further analysis, performed in triplicate.

### 2.3 ETHANOLIC EXTRACTS

The dried and triturated pulps were extracted with ethyl alcohol (95%) for 30 min, at a ratio of 1:4 pulp: ethyl alcohol, under continuous agitation at room temperature. Then, the mixture was filtered and the supernatant subjected to rotary evaporation under pressure reduced to 40°C, according to the method described by Roesler and collaborators (2007).

**Figure 1** - Brazilian Cerrado fruits. A: Araticum (*Annona crassiflora*); A.1: Tree with mature fruit; A.2: Mesocarp (pulp); B: Buriti (*Mauritia flexuosa*); B.1: Palm tree with mature fruit; B.2: Whole fruit; C: Cagaíta (*Eugenia dysenterica*); C.1: Tree with mature fruit; C.2: Whole fruit; D: Ingá (*Inga edulis*); D.1: Tree with mature fruit; D.2: Whole fruit; E: Jatobá (*Hymenaea stigonocarpa*); E.1: Tree with mature fruit; E.2: Whole fruit; F: Mangaba (*Hancornia speciosa*); F.1: Tree with mature fruit; F.2: Whole fruit; G: Murici (*Byrsonima crassifolia*); G.1: Tree with mature fruit; G.2: Whole fruit; H: Pequi (*Caryocar brasiliense*); H.1: Tree with mature fruit; H.2: Mesocarp (yellow pulp); I: Pitaya (*Hylocereus undatus*); I.1: Tree with mature fruit; I.2: Whole fruit; J: Seriguela (*Spondias purpurea*); J.1: Tree with mature fruit; J.2: Whole fruit.



## 2.4 DPPH•

This procedure was described by Brand-Williams, Cuvelier and Berset (1995). An ethanolic solution with  $500 \text{ mg mL}^{-1}$  concentration of fruit seed extract was prepared. Each sample of this solution (0.3 mL) was added to 2.7 mL of DPPH• standard solution ( $40 \text{ mg mL}^{-1}$ ) in different concentrations (50, 100, 200, 300 and  $400 \text{ mg mL}^{-1}$ ). After 30 min of reaction, the absorbance was read at 515 nm and converted into percentage of antioxidant activity (AA) by using the following formula:  $AA (\%) = 100 - \{[(Abs_{\text{sample}} - Abs_{\text{blank}}) \times 100] / Abs_{\text{control}}\}$ , Abs = absorbance. Control was done with 2.7 mL of DPPH• and the blank was performed with 0.3 mL of ethanolic solution of the extract and 2.7 mL of ethanol for each concentration. The extract concentration providing 50% of radical scavenging activity ( $EC_{50}$ ) was calculated from the graph of AA percentage against extract concentration.

### 2.4.1 TOTAL PHENOLIC COMPOUNDS

The quantification of total phenolic compounds was determined by spectrophotometry, using the Folin-Ciocalteu reagent, according to the methodology described by Singleton and Rossi (1965). In this procedure, 100  $\mu\text{L}$  of natural extract solution was put in test tubes from a pipette and then 500  $\mu\text{L}$  of the Folin-Ciocalteu reagent was added. Next, 1.5 mL of sodium carbonate 20% saturated solution and 6 mL of distilled water were also added. This mixture remained at rest for 2 hours at room temperature and the absorbance was determined at 765 nm. The gallic acid standard was used to make the calibration curve and the result was expressed in milligrams of equivalents of gallic acid per gram of extract (mg/g). The equation of the gallic acid calibration curve was  $C = 0.001 A + 0.012$ , in which  $C$  is the concentration of gallic acid,  $A$  is the absorbance at 765 nm and the coefficient of determination  $R^2 = 0.9985$ .

## 2.5 DETERMINATION OF THE ASCORBIC ACID CONTENT

The ascorbic acid content was determined by the method from the Adolfo Lutz Institute (2005), by titration with 0.01 N potassium iodide. One gram of each fruit is added in 20 mL of 20% sulfuric acid, 1 mL of potassium iodide solution and 1 mL of 1% starch solution. Then holder up the blue coloring. The results are expressed as mg of ascorbic acid per 100 grams of fruit pulp of the Brazilian Cerrado (mg/100 g).

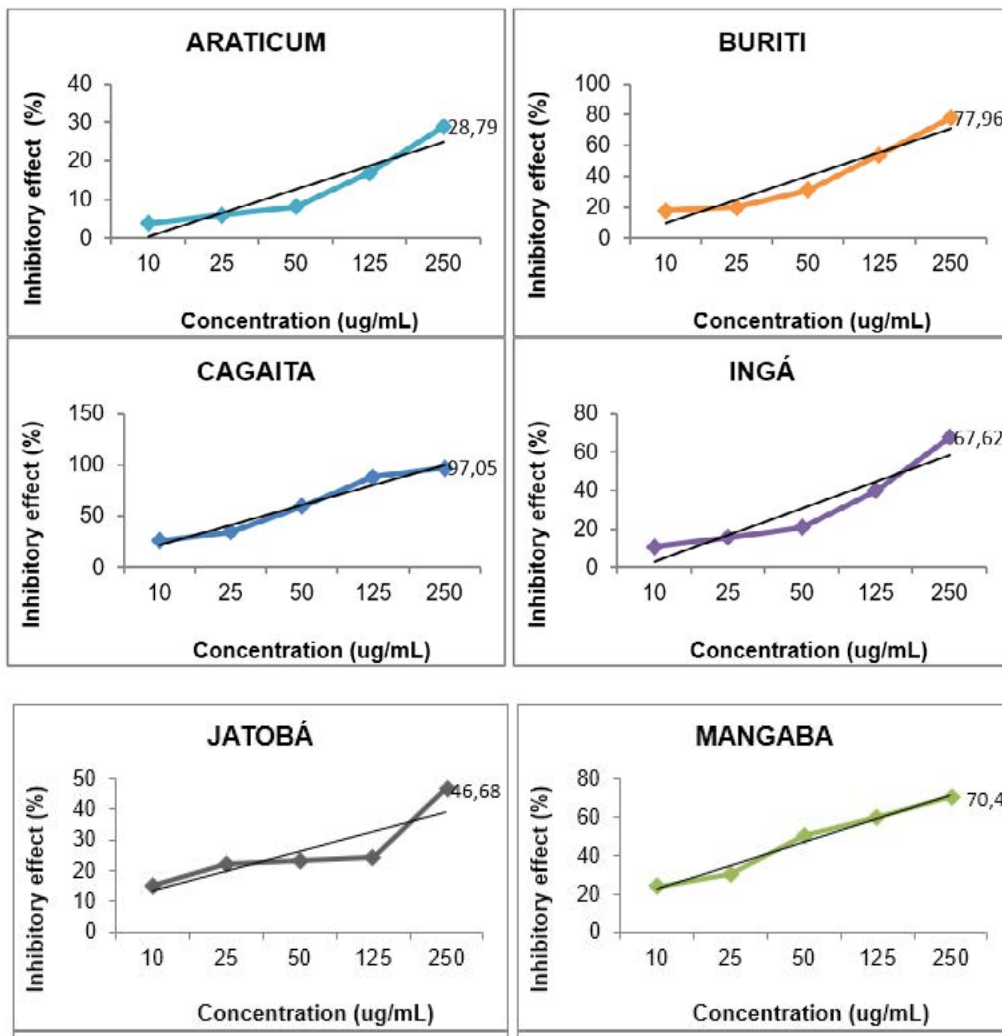
## 2.6 STATISTICAL ANALYSIS

The results of the analytical determinations, in triplicate, were submitted to analysis of variance and the differences between the averages will be tested at 5% probability by the Tukey test, through the ESTAT program, version 7.7 beta.

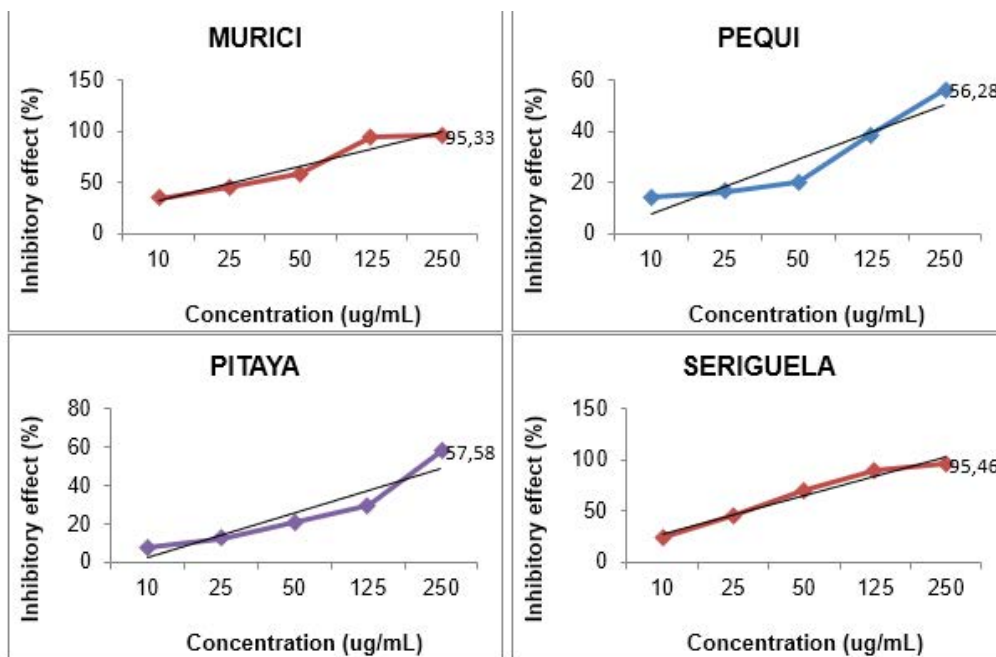
### 3 RESULTS AND DISCUSSION

The antioxidant activity of the different fruit pulp extracts from the Brazilian Cerrado is presented in Figure 2, through the free radical sequestration, where it presents the antioxidant power of the pulps in terms of the DPPH radical inhibitory effect, which increased as the extract concentration also increased.

**Figura 2** - Percentage of DPPH radical inhibitory effect as a function of the concentration of antioxidant activity of Brazilian Cerrado fruit pulp extracts. Values are means (n = 3).







Source: Research Data.

The antioxidant capacity of the ethanolic extracts of fruit pulps shows that the active compounds present act as a donor of hydrogen to the radical; however, this action can be differentiated among several fruits. For the purpose of classification of Scherer, Godoy (2009), fruit pulps that exhibited the greatest capacity of sequestration, in the concentration of  $250 \mu\text{g mL}^{-1}$ , can be classified, as:

- above 70% with strong sequestration capacity (buriti, murici, seriguela and cagaita);
- between 50 and 70% with moderate sequestration capacity (pequi, pitaya, inga and mangaba);
- below 50% with poor sequestration capacity (araticum and jatoba).

The pulp of the fruit of cagaita was the one that obtained a better blocking effect, that is, a greater antioxidant activity, followed by the murici and seriguela pulps.

In general, the fruit pulp of the Brazilian Cerrado studied showed a good antioxidant activity, being in agreement with several studies that report the high contribution of the fruits as a bioactive food, rich in antioxidants (ANJOS CRUZ, J. M. et al., 2022; BORGES et al., 2022). This aspect is important in the promotion of health and inclusion of the same in the diets with a view to the prevention of several chronic non-communicable diseases and also to mitigate the damages caused by oxidative stress.

Table 2 shows the results of the averages for the total phenolic compounds of Brazilian Cerrado fruit pulp extracts.

**Table 2** - Total phenolic compounds (TPC) of Brazilian Cerrado fruit pulp extracts.

Brazilian Cerrado Fruits	TPC (mgEGA <sup>-1</sup> g)*
Araticum ( <i>Annona crassiflora</i> )	64,29 ± 0,10 <sup>f</sup>
Buriti ( <i>Mauritia flexuosa</i> )	138,41 ± 0,14 <sup>d</sup>
Cagaita ( <i>Eugenia dysenterica</i> )	185,02 ± 0,21 <sup>c</sup>
Inga ( <i>Inga edulis</i> )	56,65 ± 0,12 <sup>g</sup>
Jatoba ( <i>Hymenaea courbaril</i> )	3,35 ± 0,10 <sup>i</sup>
Mangaba ( <i>Hancornia speciosa</i> )	38,60 ± 0,08 <sup>h</sup>
Murici ( <i>Byrsonima crassifolia</i> )	234,29 ± 0,15 <sup>a</sup>
Pequi ( <i>Caryocar brasiliense</i> )	0,24 ± 0,16 <sup>j</sup>
Pitaya ( <i>Hylocereus undatus</i> )	73,12 ± 0,10 <sup>e</sup>
Seriguela ( <i>Spondias purpurea</i> )	227,82 ± 0,20 <sup>b</sup>

\*The results represent the mean ± standard deviation of three determinations followed by the same letters do not differ by the Tukey test ( $p > 0.05$ ).

Source: Research Data.

The total phenolic contents of fruit pulp extracts showed significant amounts of polyphenols, especially murici, seriguela, cagaita and buriti, which exhibited the highest contents of these constituents, followed by the fruits of pitaya, araticum, inga, mangaba, jatoba and pequi, which differed by the Tukey test at 5% probability ( $p < 0.05$ ).

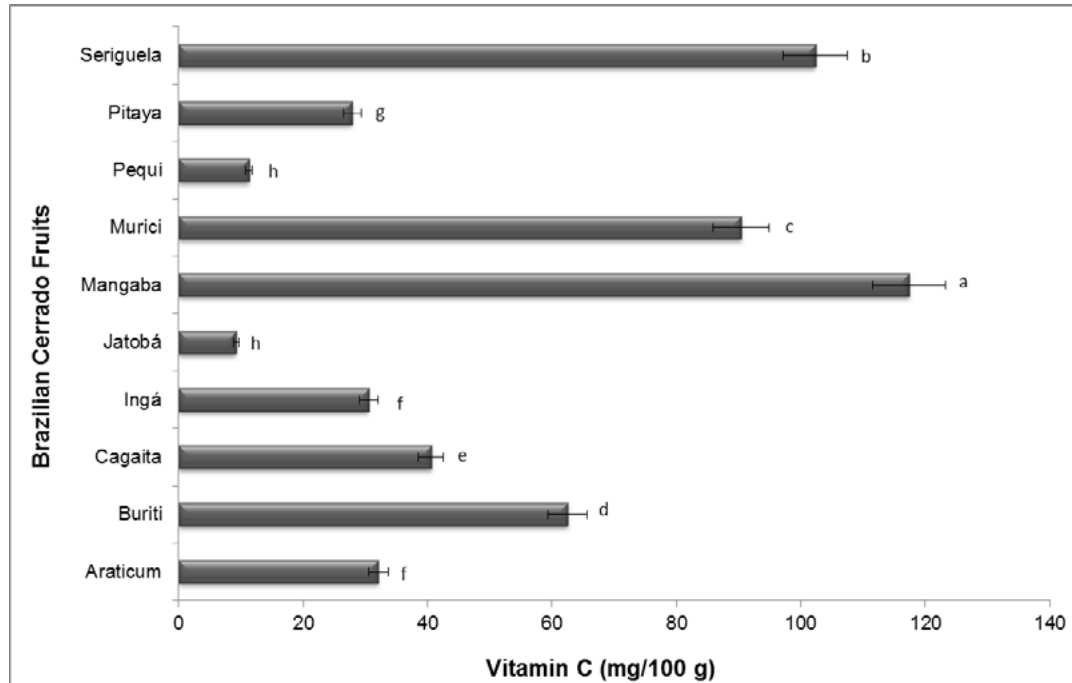
It can be seen that a mixture of different phenolic compounds with diversified polarity is found in the fruits, the highest proportion of these constituents being soluble in methanol.

Phenolic compounds by the action of digestive enzymes and / or intestinal flora can be released from the food matrix and act as an antioxidant. Thus, Pérez-Jiménez and collaborators (2008) suggest that the extraction of the polyphenols be carried out by organic solvents of diversified polarity, using at least two extraction cycles, in order to also consider the antioxidant action of the compounds present in the residue.

The results obtained may serve as a basis for future work. The characterization and knowledge of the genes that control the formation of phenolic compounds, as well as the knowledge of their antioxidant activity or blocking effect, allows researchers to develop and select fruit varieties with greater antioxidant activity and with that, to improve the nutritional quality of the Brazilian population.

Figure 3 shows the average content of vitamin C concentration in fruit pulps from the Brazilian Cerrado. The results showed that the samples of mangaba, seriguela and murici presented the highest vitamin C content, with a statistically significant difference by the Tukey test at 5% probability ( $p < 0.05$ ) between the analyzed vitamin contents.

**Figure 3** - Average content of vitamin C (mg 100 g<sup>-1</sup>) of Brazilian Cerrado pulps extracts fruits.



Source: Research Data.

The fruits that had lower prominence for vitamin C were inga, araticum, pitaya, pequi and jatoba with 30.50 mg100 g<sup>-1</sup>; 32.10 mg100 g<sup>-1</sup>; 27.90 mg100 g<sup>-1</sup>; 11.30 mg100 g<sup>-1</sup> and 9.20 mg100 g<sup>-1</sup>, respectively. These low levels of vitamin C may be related to the characteristics of the raw material (cultivar, degree of maturation, climate, and cultivation practices).

The Ministry of Health recommends the daily intake of 45 mg of vitamin C for adults (BRASIL, 2003). Considering the results found, the consumption of 100 g of fruit pulp from the Brazilian Cerrado provides 15% for fruits with low content to 195% of the daily recommendations of vitamin C for adults.

According to Gama et al. (2002), fruits considered as high sources of vitamin C contain 100 to 300 mg100 g<sup>-1</sup>. Analyzing the results shown in Figure 3, it is possible to state that the fruits of mangaba and seriguela can be classified within this category.

In a study conducted by Hummer and Barney (2002), several fruits of nutritional comparison, such as black currant and red currant, grape, strawberry and apple, found a great difference in vitamin C content among the different fruits, with special attention given to black and red currants, being 181 and 41 mg100 g<sup>-1</sup>, respectively. In the strawberry, they found 56.7 mg100 g<sup>-1</sup>, in the grape, spina 27.7 mg100 g<sup>-1</sup>, and in the apple, 5.7 mg100 g<sup>-1</sup>.

## 4 CONCLUSIONS

In view of the results obtained, it is possible to conclude that fruit pulps from the Brazilian Cerrado have important antioxidant and vitamin C potential, especially murici, seriguela and mangaba. They can thus be considered as good sources of natural antioxidants, which may be more effective than the use of dietary supplements in protecting the body against cellular oxidative damage. Therefore, the consumption of these fruits should be encouraged and stimulated by society. Doing so would, based on the results, improve the nutritional quality of food in order to positively affect human health, reduce the incidence of diseases and, with that, prolong the population's life expectancy.

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