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Evaluation of Antioxidant Activity *in Vitro* of Brazilian Cerrado Fruits

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Abstract

The objective of this study was to determine the evaluation of antioxidant activity in vitro of the pulps in the interest of better identifying the quality of this raw material from the Brazilian Cerrado. After the receipt of the fruits, the pulps were removed manually and, evaluate the antioxidant activity through DPPH• free radical, to quantify the 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 (pulps: ethyl alcohol), under continuous agitation, at room temperature. Then, the mixtures were filtered, and the supernatants were subjected to rotary evaporator 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: DPPH•; Total phenolic compounds; Ascorbic acid; Extracts

Introduction

The Brazilian Cerrado biome, typical of the tropical zone, is a savanna formation which occupies approximately 2.0 million km^2 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 Piaui, Rondônia and in one- fifth of Sao 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 [1]. This use may be due to the use of medicinal plants, wild bees breeding, wild animal management, ecotourism, rural tourism, condiments, handicrafts and fish farming.

Table 1: General characteristics of some species of Brazilian Cerrado fruits.

Fruits	General characteristics	References
Araticum *(Annona crassiflora)*	Tree from 4 to 8m 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 2.0g.	Clerici; Carvalho-Silva [2]; Lorenzi [3]
Buriti *(Mauritia Flexuosa)*	It is a palm tree more than 15m 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 50g.	Calbo; Moraes [4]; Ferreira [5]
Cagaíta *(Eugenia dysenterica)*	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.	Naves et al. [6]; Lorenzi [3]; Souza et al. [7]
Inga *(Inga edulis)*	It is a tree that can reach 40m 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 [8]
Jatoba *(Hymenaea courbaril)*	Tree from 4 to 6 m in height. Fruits between 6 and 18cm in length and 3 to 6cm in diameter with approximately 4 seeds weighing on average 3g each.	Almeida, [9]; Silva et al. [10]

Mangaba *(Hancornia speciosa)*	Medium-sized tree, 2 to 10m high, up to 15m; large canopy, 4 to 6m 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. [11]; Silva et al. [10]
Murici *(Byrsonima crassifólia)*	Hermaphrodite tree or shrub, measuring from 4 to 6m, often tortuous trunk with a diameter of up to 17cm, a pale gray color, with discontinuous and sinuous fissures forming irregular plaques. Cup with terminal branches of nodular growth. Leaves 14-20cm long by 6-12cm wide; flowers about 1.5cm in diameter, arranged in elongated ears; fruit of up to 2cm in diameter, globose drupe, glabrous, meaty mesocarp; pulp juicy and sweet; yellow at maturity	Castro Lorenzzi [12]; Silva Júnior [13]
Pequi *(Caryocar brasiliense)*	Tree about 10m high. Green leaves and yellow-white flowers. Green drupeid fruit with 4.2-6.4 x 6.5-7.8cm, has a fleshy coriaceous epicarp, surrounded by light yellow mesocarp and some seeds. Fruits weighing 50 to 250g.	Almeida [14]; Correia et al. [15]; Vera et al. [16]
Pitaya *(Hylocereus undatus)*	Cluster and fruit species, belonging to the family of cacti, are branched, with branches trialled, flattened on one side, being able to reach 20cm in length and 5 to 7cm in diameter. The flowers are lateral, 20 to 35cm 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.	Cordeiro et al. [17]; Fernandes et al. [18]
Seriguela *(Spondias purpúrea)*	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 5cm in length and about 2cm in diameter.	Leon [19]; Lira Júnior et al. [20]

Table 1 shows the general characteristics of fruits Brazilian Cerrado, high nutritional value, besides sensory attractiveness, such as color, flavor, peculiar and intense aroma, and they can be consumed freshly or used in the manufacture of 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 of raw materials and provide viable quantities for the industrial process [21].

Epidemiological studies have indicated that regular consumption of various fruits is associated with lower risk of chronic diseases, [22] due to the effects of the combination of vitamins, minerals, antioxidants and fibers [23-24].

The study of the evaluation of antioxidant activity of fruits that are native to Cerrado contributes to sustainable use, conservation, and selection of promising species, besides showing the economic importance of these foods. Thus, the aim of this study was to determine the evaluation of antioxidant activity in vitro of the pulps in the interest of better identifying the quality of this raw material from the Brazilian Cerrado.

Materials and Methods

Fruit collection

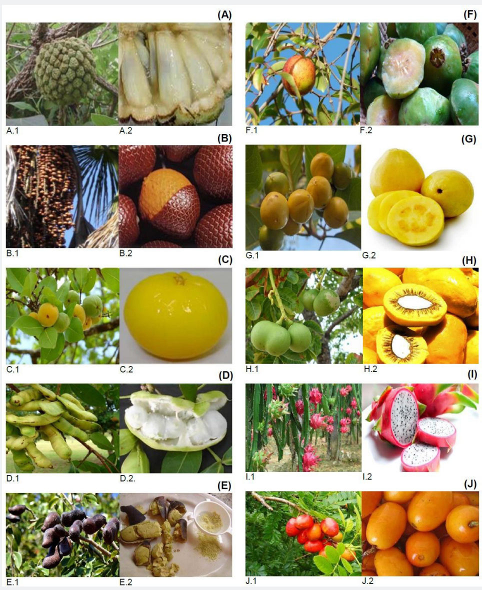

Figure 1: Brazilian Cerrado fruits. A: Araticum *(Annona crassiflora)*; A.1: Tree with mature fruit; A.2: Mesocarp (pulp); B: Buriti *(Mauritia flexuosa)*; B.1: Palm tree with mature fruit; B.2: Whole fruit; C: Cagaíta *(Eugenia dysenterica)*; C.1: Tree with mature fruit; C.2: Whole fruit; D: Ingá *(Inga 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 crassifólia)*; 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 purpúrea)*; J.1: Tree with mature fruit; J.2: Whole fruit.

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Fruits of the Brazilian Cerrado, as araticum *(Annona crassiflora)*, buriti *(Mauritia flexuosa)*, cagaíta *(Eugenia dysenterica)*, ingá *(Inga edulis)*, jatobá *(Hymenaea courbaril)*, mangaba *(Hancornia speciosa)*, murici *(Byrsonima crassifólia)*, pequi *(Caryocar brasilense)*, pitaya *(Hylocereus undatus)* e seriguela *(Spondias purpúrea)* 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).

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. 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 2h to reduce the moisture content, and then homogenized for further analysis, performed in triplicate.

Ethanolic extracts

The dried and triturated pulps were extracted with ethyl alcohol (95%) for 30 min, at a ratio of 1:4 pulps:ethyl alcohol, under continuous agitation at room temperature. Then, the mixture was filtered, and the supernatant subjected to rotary evaporator under pressure reduced to 40 °C, second method described by Roesler et al. [25].

Radical scavenging capacity (DPPH•)

This procedure was described by Brand-Williams et al. [26]. An ethanolic solution with 500mg mL^{-1} concentration of fruit seeds extract was prepared. Each sample of this solution (0.3mL) was added to 2.7mL of DPPH• standard solution (40mg mL^{-1}) in different concentrations (50, 100, 200, 300 and 400mg mL^{-1}). After 30 min of reaction, the absorbance was read at 515nm and converted into percentage of antioxidant activity (AA) by using the following formula: AA (%) = 100 - {[(Abssample - Absblank) x 100] / Abscontrol}, Abs = absorbance. Control was done with 2.7mL of DPPH• and the blank was performed with 0.3mL of ethanolic solution of the extract and 2.7mL of ethanol for each concentration. The extract concentration providing 50 % of radicals scavenging activity (EC50) was calculated from the graph of AA percentage against extract concentration.

Total phenolic compounds

The quantification of total phenolic compounds was determined by spectrophotometry, using the Folin-Ciocalteau reagent, according to the methodology described by Singleton and Rossi [27]. In this procedure, 100µL of natural extract solution was put in test tubes from a pipette and then 500µL of the Folin-Ciocalteau reagent was added. Next, 1.5mL of sodium carbonate 20 % saturated solution and 6mL of distilled water were also added. This mixture remained at rest for 2h at room temperature and the absorbance was determined at 765nm. 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 765nm and the coefficient of determination R_2 = 0.9985.

Determination of the ascorbic acid content

The ascorbic acid content was determined by the method from the Institute Adolfo Lutz [28], by titration with 0.01 N potassium iodide. One gram of each fruit is added in 20mL of 20% sulfuric acid, 1 mL of potassium iodide solution and 1mL of 1% starch solution. Then holder up the blue coloring. The results expressed as mg of ascorbic acid per 100grams of fruit pulp of the Brazilian Cerrado (mg/100g).

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.

Results and Discussion

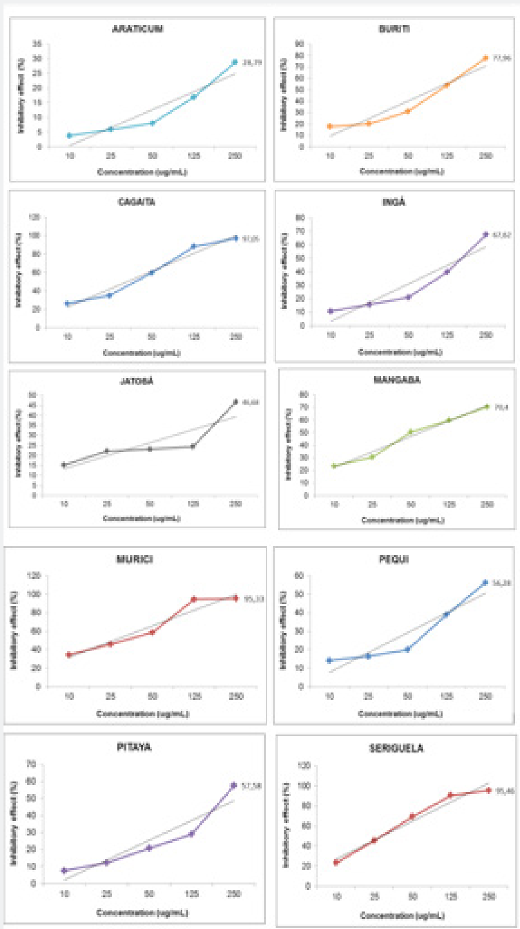

Figure 2: Percentage of DPPH radical inhibitory effect as a function of the concentration of Brazilian Cerrado pulps extracts Fruits. Values are means (n = 3).

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The antioxidant activity of the different fruit pulps 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.

The antioxidant capacity of the methanolic extracts of fruit pulps shows that the active compounds present act as donor of hydrogen to the radical, however this action can be differentiated among several fruits. For the purpose of classification, fruit pulps that exhibited the greatest capacity of sequestration, in the concentration of 250µg/mL, 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). (Figure 2)

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.

Table 2: Total phenolic compounds (TPC) of Brazilian Cerrado pulps extracts fruits.

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

*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).

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. 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).

The total phenolic contents of the extracts of fruit pulps of the Cerrado showed significant amounts of polyphenols, especially the 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 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 et al. [29] suggest that the extraction of the polyphenols is carried out by organic solvents of diversified polarity, using at least two extraction cycles, in order to consider also 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 the 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 of 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.

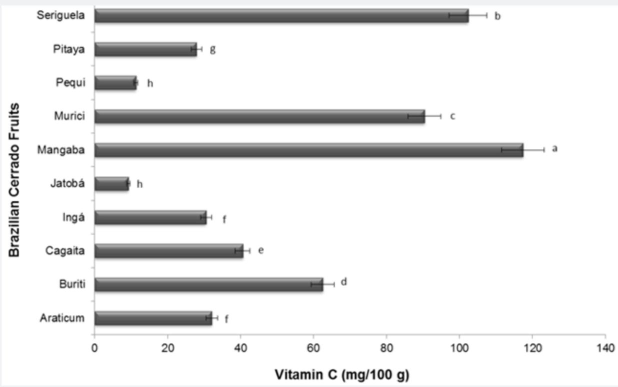

Figure 3: Average content of vitamin C (mg/100g) of Brazilian Cerrado pulps extracts fruits.

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The fruits that had lower prominence for vitamin C were inga, araticum, pitaya, pequi and jatoba with 30.50mg/100g; 32.10mg/100g; 27.90mg/100g; 11.30mg/100g and 9.20mg/100g, 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) (Figure 3).

The Ministry of Health recommends the daily intake of 60mg of vitamin C for adults [30]. Considering the results found, the consumption of 100g of fruit pulps of 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. [31], fruits considered as high sources of vitamin C contain 100 to 300mg/100g. Analyzing the results expressed 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 [32], several fruits of nutritional comparison, such as blackcurrant and red currant, grape, strawberry and apple, found a great difference in vitamin C content among the different fruits, with special attention black and red currants, being 181 and 41mg/100g, respectively. In the strawberry they found 56.7mg/100g, in the grape spina 27.7mg/100g and in the apple 5.7mg/100g.

Conclusion

In view of the results obtained, it is possible to conclude that fruits pulps of the Brazilian Cerrado is an important antioxidant and vitamin C potential, especially murici, seriguela and mangaba, and can be considered as good sources of natural antioxidants, which may be more effective and then the use of dietary supplements in protecting the body against cellular oxidative damage, so the consumption of these fruits should be encouraged and stimulated by society.

With this research project, it is concluded that it is possible to stimulate the consumption of fruits of Brazilian Cerrado, improving the nutritional quality of food, in order to present positive effects on human health, reducing the incidence of diseases and, with that, prolong the population life expectancy.

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