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CHAPTER 11

Advanced extraction of bioactive compounds from mamacadela: An approach for efficient and sustainable process

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ABSTRACT

The Cerrado biome has numerous species of fruits little known and exploited, both by the population and the industry, and can be an alternative source of natural active compounds. This study aimed to optimize the process of extraction of antioxidants for mamacadela fruit (*Brosimum Gaudichaudii* Trécul) using the response surface methodology. The variables were: temperature (30 - 60 ºC), time (30 - 60 min), water or ethanol, and use or not of ultrasonic bath equipment. The quantification of antioxidant capacity was using the methods: ABTS, DPPH, FRAP, and total phenolic content. In the optimization of the extraction it was possible to identify that the extract with greater antioxidant capacity was with the use of water as solvent at 60ºC and with the shortest extraction time of 30 minutes, and without the utilization of an ultrasound bath. Thus, it is important to conduct more studies on the antioxidant potential of mamacadela fruit, seeking agroindustrial exploitation, besides adding value to food products.

Keywords: Antioxidants, Bioactive compounds, Experimental design.

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INTRODUCTION

The native fruits of the Brazilian Cerrado are widely used by the local population, especially in natural form, they occupy a prominent place in the Cerrado ecosystem, being marketed in regional fairs and with great popular acceptance. The characterization of bioactive compounds in fruits of the Cerrado is of great relevance for the search for alternative sources and that can group desirable attributes (antioxidant, antimicrobial, anticarcinogenic, antidegenerative and aging retardant properties). One of the market trends today is the consumer's search for products with sensory and nutritional qualities that provide healthiness and well-being, which made the food industries adapt to these market segments, seeking new formulations and innovative food products [1–5].

Mamacadela (*Brosimum Gaudichaudii Trécul*) is a fruit otherwise known as "mamacadela", "mamica-de-cadela" or "inharé", found in the cerrado, little known, and belongs to the family Moraceae. This family has 53 genera and about 1,500 species identified, with tropical prevalence, with more than 50% of the genera present in the Neotropical region, mainly in South America. Moraceae species are found in or near humid forests. Artocarpus, Brosimum, Ficus and Morus are among the best-known genera, which correspond to the widely known and consumed fruit plants of great nutritional and economic importance, such as Jaqueira, walnut, fig and blackberry [6,7].

Mamacadela leaves, bark and roots have ethnopharmacological uses in the treatment of various autoimmune skin diseases, especially vitiligo [8,9]. Root bark tea is used in baths, and ground root juice is used as an added component in ointments and lotions [10]. Even if the leaves and roots are already used, there is little information about the pulp extraction, about presence and antioxidant capacity of th e bioactive compounds of this fruit, thus the present work is innovative evaluating by the first time the use of an advanced extraction process for mamacadela pulp. It is worth highlighting that the extraction yield depends on the type of solvent, with variable polarity, extraction time, extraction temperature and sample-solvent ratio. So, the MSR (response surface methodology) is also useful for reducing the number of experimental trials and determining the effects of interaction between variables. Defining optimal conditions in the extraction process is essential to increase the effectiveness of the extraction of bioactive compounds [11–13]. Thus, this work aims to optimize the antioxidants extraction process from mamacadela pulp.

MATERIALS AND METHODS

EXTRACTS AND EXPERIMENTAL DESIGN

Fruits (approximately 5 kg) were collected in the rural area, in the farm Felicidade, in Jussara-Goiás - Brazil, with the following geographic coordinates Longitude: 50 52' 9" West. After washed, sanitized, and seed separation, the pulp was frozen (-18 °C) and transported to the State University of Maringá, where it remained frozen until the moment of analysis.

For the antioxidant extraction, the frozen pulp was subjected to drying at 50 °C in an oven with air circulation. For optimization, 10 g of dry sample was weighed and 25 mL of ethanol (+1) or distilled water (-1) was added during 30 (-1) or 60 (+1) minutes at a temperature of 30 (-1) or 60 (+1) (°C) under light, in a Dubnoff bath (-1) (Model TE-053 25 5 rpm) or with the use of ultrasonic bath (+1) (Unique model USC-1600ª, 100 W, 40 KHz). After the above procedures, each extract was filtered in 80G qualitative paper filter, 90 mm diameter and 90 mm Chiarotti porcelain funnel and then the supernatant was stored in an amber bottle and stored under freezing in a light shelter for further analysis. The extracts were submitted to antioxidant capacity analysis, and analyzed in triplicates. The independent variables tested (Table 1) were extraction temperature, extraction time, solvent (ethanol concentration in aqueous solution) and use of ultrasonic bath equipment. The dependent variables were: the antioxidant capacity quantified by the methods of DPPH radical sequestration, ABTS and FRAP and the content of phenolic compounds.

Table 1. Real and coded levels of variables.

Level	**-1**	**+1**
Extraction temperature (°C)	30	60
Extraction time (min)	30	60
Solvent	Water	Ethanol
Using the ultrasonic bath	Not	Yes

ANTIOXIDANT CAPACITY AND PHENOLIC COMPOUNDS

ABTS: An aliquot of 30 µl of extract was added to the tubes, along with 3 mL of already diluted ABTS•+ reagent [14]. The samples were incubated for 6 min, protected from light at room temperature and the reading was made at a wavelength of 734 nm in FEMTO spectrophotometer (Cirrus 80MB). The antioxidant capacity of the mamacadela extract was determined from the Trolox standard curve (2 mol/L) and expressed in mg Trolox/g of sample. The extract was diluted at a concentration of 200 mg/mL so that the ABTS values were within the standard curve ($y = -0.0003x + 0.6603$ / $R^2 = 0.9953$).

DPPH: An aliquot of 150 µl of the samples were added to 2.85 mL of a methanolic solution of DPPH, homogenized and maintained for 1 hour under shelter of light. Then, the absorbance values were measured at the wavelength of 515 nm in the FEMTO spectrophotometer (Cirrus 80 MB), the solvent methanol was used as white [15]. The analytical curve was prepared from ethanolic solutions of Trolox at concentrations ranging from 0 µmol/L to 900 µmol/L . The response was expressed in mg Trolox equivalents per gram of sample. The results were calculated using the standard curve of Trolox and expressed in mg Trolox/g of sample ($y = 0.1140x – 0,1125/R^2 = 0,9991$).

FRAP: The FRAP reagent was prepared using the 0.3 mol/L acetate buffer solution TPTZ 10 mol/L ferric chloride 20 mol/L in the ratio 10:1:1 (v:v). The 300 mol/L sodium acetate buffer solution was performed by adding 3.1 g of anhydrous sodium acetate in 16 mL glacial acetic acid

supplementing with distilled water to a volume of 1000 mL. The preparation of 10 mol/L TPTZ solution (2, 4, 6 tripiridyl-s-triazine solution) was performed by adding the mass of 0.312 g of TPTZ in 5 mL of HCl (hydrochloric acid) 40 mol/L. Following the preparation of the iron chloride solution at 20 mol/L [16]. The new working solution was prepared by mixing 25 mL of acetate buffer, 2.5ml of TPTZ solution and 2.5 mL of 20 mol/L iron chloride solution. An aliquot of 90 µl of sample extract, with 270 µl of distilled water and 2,7 mL of FRAP solution by incubation of 30 min sheltered light at a temperature of 37 oC. The FRAP reagent was used as white, the readings were at 595 nm in FEMTO Cirrus 80MB spectrophotometer. The Trolox standard was used for the development of the calibration curve (0-700 µmol/L; $y = 0.0013x + 0.0059$/ $R^2 = 0.9995$), the results were expressed in mg of Trolox/g of sample.

Phenolic compounds: The content of total phenolic compounds was determined by using the Folin-Ciocalteau method [17]. In light-proof test tubes, 125 µl of extract was placed and 125 µl of Folin 50% was pipetted. Then 2250 µl of sodium carbonate was added. For the white, the same process was used, only replacing the sample with distilled water. The solutions were incubated in the dark for 30 minutes for complete reaction of the reagent and readings at 725 nm, analysis was performed in triplicate. Gallic acid was used for the development of the calibration curve (0-300 µmol/L), the results were expressed in mg gallic acid equivalent per 100 g sample on dry basis (mg EAG.100 g-1 dry basis). For this analysis of phenolic compounds content, the extracts were diluted at a concentration of 200 mg/mL ($y = 0.0018x - 0.0182$/ $R^2 = 0.998$).

All results were submitted to the analysis of variance and Tukey's test for minimum significant difference ($p < 0.05$) between means using the statistical program Sisvar 5.6.

RESULTS AND DISCUSSION

Table 2 presents the results of DPPH, FRAP, ABTS, and phenolic compounds for mamacadela.

Table 2. DPPH, FRAP, ABTS analyses expressed in (µg Trolox/g sample) and phenolic compounds content (mg EAG/g sample) for mamacadela.

Treatment	Extraction parameters				Antioxidant capacity			
	T (°C)	Time (min)	Solv.	USD	DPPH	FRAP	ABTS	PC
1	30	30	Water	Not	1,77^{c} ±0,14	3,04^{de} ±0,17	38,51^{c} ±1,58	0,69^{ef} ±0,08
2	60	30	Water	Not	2,87^{ab} ±0,20	5,00^{c} ±0,18	43,93^{b} ±0,60	0,44^{f} ±0,23
3	60	60	Water	Not	2,47^{b} ±0,04	6,61^{b} ±0,25	11,56^{ef} ±0,00	0,71^{ef} ±0,02
4	60	60	Ethanol	Not	0,95^{de} ±0,03	3,63^{d} ±0,06	18,31^{d} ±0,65	0,43^{f} ±0,03
5	60	60	Ethanol	Yes	0,55^{efg} ±0,05	2,12^{ef} ±0,09	13,77^{e} ±0,46	2,40^{d} ±0,06
6	60	60	Water	Yes	0,98^{d} ±0,05	2,00^{gh} ±0,03	13,33^{h} ±1,34	0,53^{ef} ±0,22
7	60	30	Ethanol	Yes	0,52^{efg} ±0,02	0,81^{gh} ±0,04	8,90^{f} ±0,18	2,45^{d} ±0,07
8	30	60	Ethanol	Yes	0,26^{fg} ±0,03	0,41^{h} ±0,15	0,79^{h} ±0,20	3,44^{b} ±0,12
9	30	60	Ethanol	Not	0,22^{fg} ±0,01	0,38^{h} ±0,02	1,90^{h} ±0,60	3,46^{b} ±0,08
10	30	30	Water	Yes	0,56e^{fg} ±0,02	1,11^{gh} ±0,05	4,60^{h} ±090	0,53^{ef} ±0,26
11	30	30	Ethanol	Not	0,24^{fg} ±0,01	0,95^{gh} ±1,09	6,83^{fg} ±0,36	2,99^{c} ±0,10
12	30	60	Water	Not	0,82^{de} ±0,06	2,20^{fgh} ±0,03	21,73^{gh} ±0,19	0,78^{ef} ±0,10
13	30	60	Water	Yes	1,01^{d} ±0,03	2,61^{fg} ±0,06	18,83^{gh} ±0,67	0,92^{e} ±0,21
14	60	30	Ethanol	Not	0,64^{def} ±0,03	1,22^{gh} ±0,19	4,80^{h} ±0,38	4,27^{a} ±0,13
15	60	30	Water	Yes	3,09^{a} ±0,20	9,00^{a} ±0,83	52,63^{a} ±1,79	0,55^{ef} ±0,04
16	30	30	Ethanol	Yes	0,23^{g} ±0,03	0,58^{gh} ±0,02	8,78^{f} ±0,54	3,52^{b} ±0,20

* Letters in the same column indicate that there is a significant difference ($p<0.05$). The values are the mean standard deviation of 3 repetitions. T: temperature (°C); Solv: solvent; USD: ultrasound bath; PC: phenolic compounds.

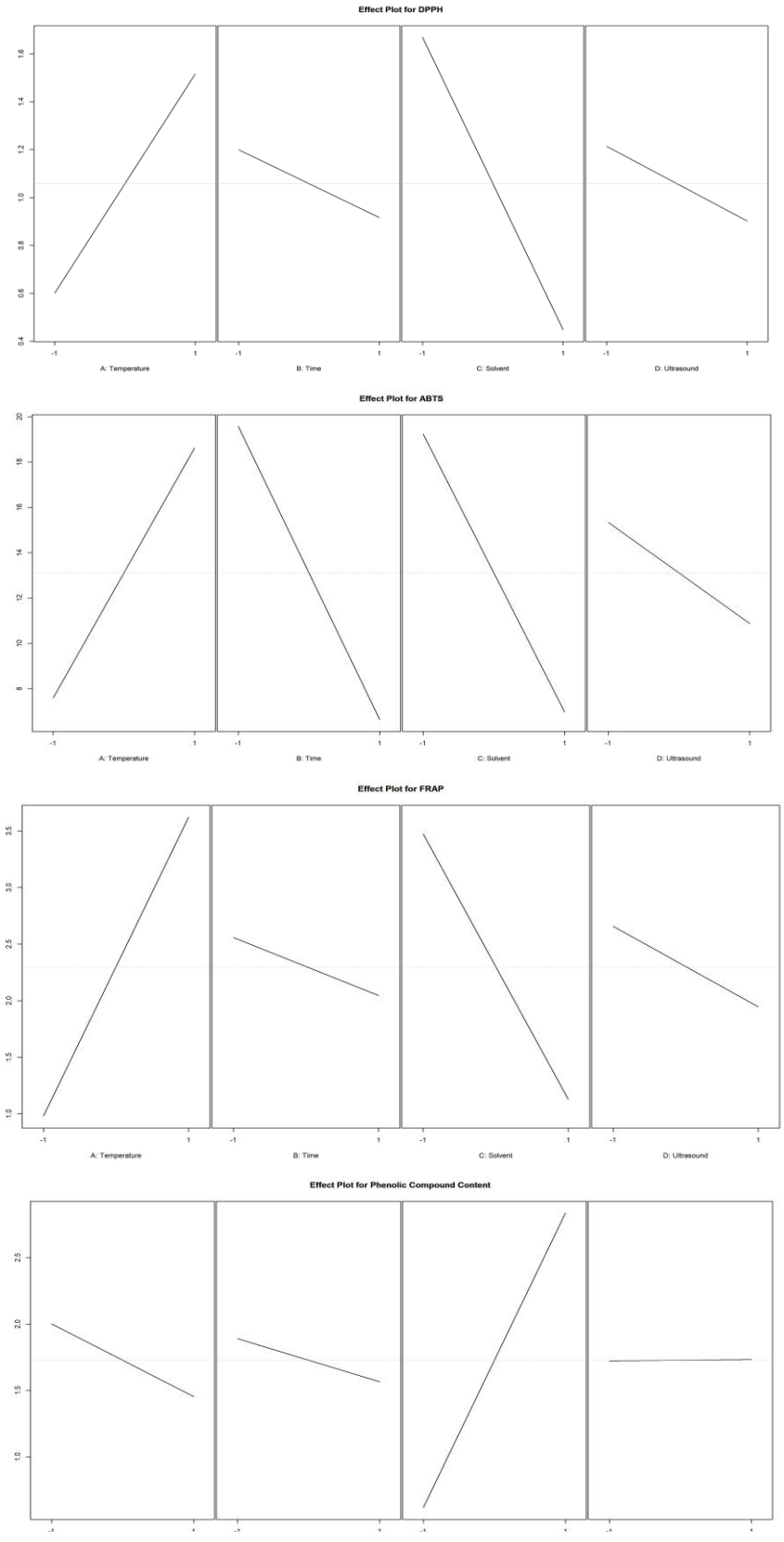
The highest values found for antioxidant capacity were for treatment 15, which uses a higher temperature (60° C), shorter time (30 minutes), water as a solvent, and ultrasound. However, for phenolic compound extraction, the highest values were for treatment 14, which uses a higher temperature (60° C), shorter time (30 minutes), ethanol as a solvent, and without ultrasound uses.

The antioxidant capacity found for mamacadela (Table 2) can be influenced by factors such as maturity, species, cultivation practices, geographical origin, growth stage, cultivation conditions, and storage process, as well as a preservation method and type of carrier agent [18–20]. Water as a solvent, higher temperatures, and the use of an ultrasonic bath were previously cited as great parameters for extracting antioxidant compounds from fruits [11,21–23].

Figure 1 shows the effects of extraction parameters for quantification of DPPH, ABTS, FRAP, and phenolic compound content. The data obtained show various interferences during the

extraction process. While the temperature had a positive effect, the use of ethanol had an inverse effect on the antioxidant extraction of mamacadela, for all methods used for quantification.

Figure 1. Effects of extraction parameters for quantification of DPPH, ABTS, FRAP and phenolic compound content. Temperature -1 = 30°C and +1 = 60°C; Time -1 = 30 min and +1 = 60 min; Solvent -1 = water and +1 = ethanol; Ultrasound -1 = not; +1 = yes.



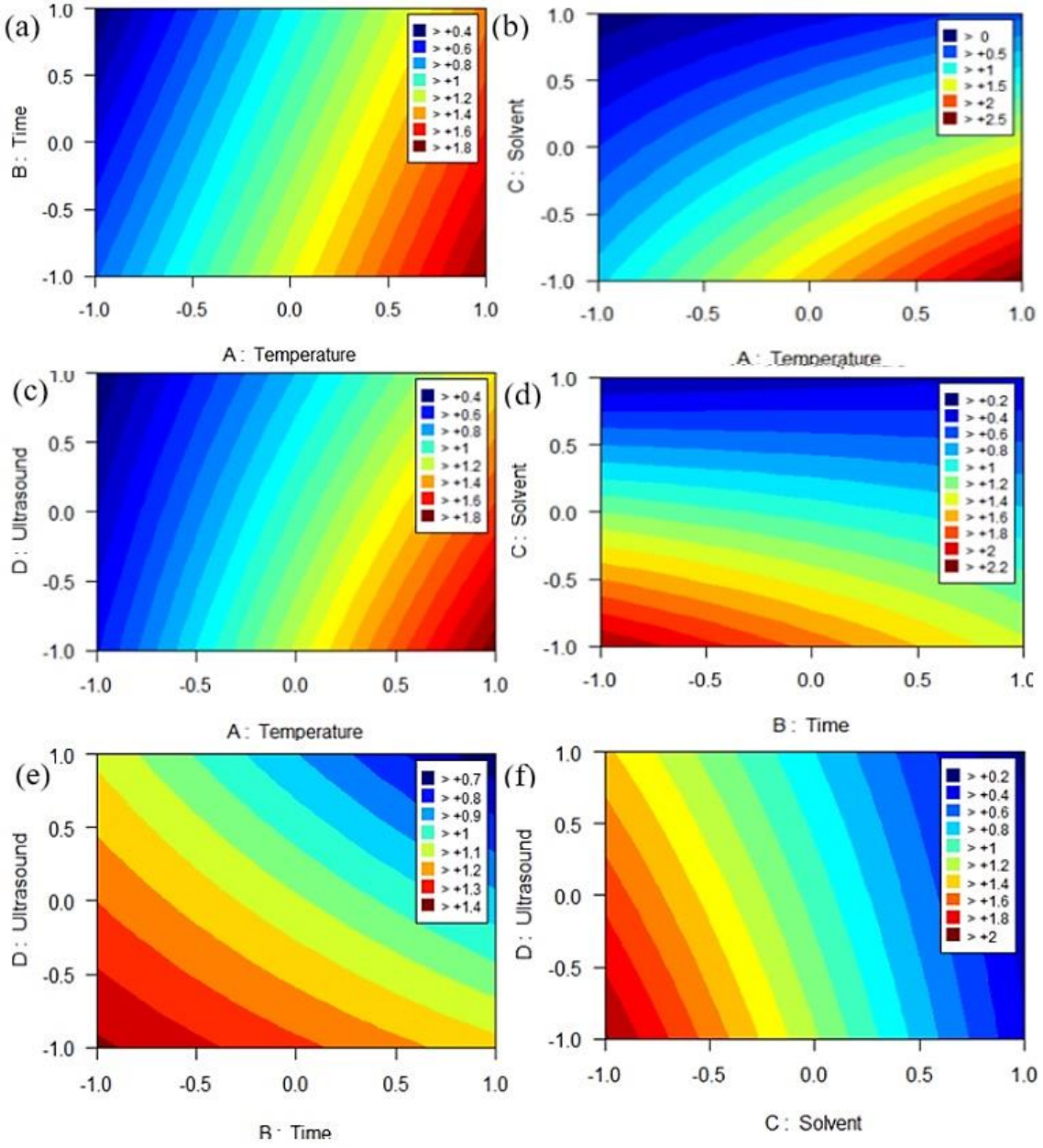
The time factor had a negative effect on the extraction of phenolic compounds and antioxidants. For the solvent type factor, water was the one that extracted the highest amount of antioxidant compounds. However, for the content of phenolic compounds ethanol presented better results.

In the DPPH analysis, the parameter that most interfered with quantifying the response variable was the use or not of ethanol, where the use of water showed the highest result. Therefore, mamacadela antioxidant compounds could be extracted using a sustainable green solvent, facilitating its extraction and reducing the cost of being a cheap solvent and easy availability.

For the ABTS methodology, the interaction between time and water as solvent showed greater significance, the increase in extraction time resulted in a decrease in antioxidant compounds present in the extracts. The FRAP analysis showed a positive relationship between the increase in extraction temperature and the increase in antioxidant compounds.

Figure 2 shows the response surface methodology of DPPH analysis, representing all other analyses that showed similar results. Double interactions were analyzed: temperature versus time, temperature versus solvent, temperature versus ultrasound, time versus solvent, time versus ultrasound, and solvent versus ultrasound.

Figure 2. Response surface for the analysis of DPPH in mamacadela fruits. Interaction of temperature versus time (a), temperature versus solvent (b), temperature versus ultrasound (c), time versus solvent (d), time versus ultrasound (e) and solvent versus ultrasound (f).



In all antioxidant analyses, the maximum temperature showed more significant results (Figure 1 and 2) This result may be related to the increase in diffusion rates and solubility of analytes, and a decrease in viscosity and surface tension of solvents. However, in some cases, a decrease in the extraction yield can be observed, since some compounds, such as phenolics, can be degraded when subjected to high temperatures [24–27].

The chemical composition and phytochemical compounds contained in plant materials have different solubility properties in different solvents, the ideal solvent for extraction will depend on the specific plant materials and the compounds that must be isolated [24,28]. In addition, the use of ultrasound is a promising technology for the extraction process [24,29–31].

CONCLUSION

It was possible to extract a representative amount of antioxidants from mamacadela fruits prioritizing a more sustainable process, using water as solvent. Overall, the conditions that maximized the extraction of antioxidants from the mamacadela fruit were using water as a solvent, at 60 °C, with the shortest extraction time (30 min). Finally, we emphasize the importance of conducting more studies on the antioxidant potential of mamacadela fruit, to seek to add value to these species and encourage their cultivation and genetic improvement, thus enabling the commercialization of these fruits to other Brazilian regions, increasing income for family farming and strengthening food and nutritional security of Brazilians.

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