

PHYTOCHEMICAL AND ANTIOXIDANT COMPOSITION IN ACCESSIONS OF THE MANGABA ACTIVE GERMPLASM BANK¹

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ABSTRACT - Mangaba (*Hancornia speciosa* Gomes) is a fruit species native to Brazil, belonging to the Apocynaceae family. Its cultivation is predominantly extractivist and its antioxidant properties have been recently reported. The objective of this study was to evaluate the bioactive compounds and antioxidant activity in fruits of accession from the Active Germplasm Bank of Embrapa Coastal Tablelands (BI, CA, LG, PR, PT and TC). The extracts were analyzed by high-performance liquid chromatography (HPLC), which identified chlorogenic acid (93.71 - 131.66 mg.100g⁻¹), ferulic acid (0.85 - 2.27 mg.100g⁻¹) and rutin (238.59 - 442.94 mg.100g⁻¹). The accessions CA and PR showed the highest values of total phenols (1179.39 and 1167.05 mg GAE.100g⁻¹, respectively). The accessions CA and TC had the highest concentration of the major compound rutin (436.78 and 442.94 mg.100g⁻¹, respectively). Antioxidant activity values ranged from 125.95 to 158.67 g.g⁻¹ DPPH. Principal component analysis grouped the accessions into four groups, due to the genetic variability verified in previous studies. The results will be useful to guide actions of selection and future breeding program of the species.

Keywords: Brazilian fruits. Phenolic compounds. Rutin. Antioxidant activity.

COMPOSIÇÃO FITOQUÍMICA E ANTIOXIDANTE EM ACESSOS DO BANCO ATIVO DE GERMOPLASMA DE MANGABA

RESUMO - A mangabeira (*Hancornia speciosa* Gomes) é uma espécie frutífera nativa do Brasil, pertencente à família Apocynaceae. Seu cultivo é predominantemente extrativista e suas propriedades antioxidantes têm sido recentemente relatadas. O objetivo do presente trabalho foi avaliar os compostos bioativos e atividade antioxidante em frutos de acessos oriundos do Banco Ativo de Germoplasma da Embrapa Tabuleiros Costeiros (BI, CA, LG, PR, PT e TC). Os extratos foram analisados em cromatografia líquida de alta eficiência (HPLC) e identificou-se o ácido clorogênico (93,71 - 131,66 mg.100g⁻¹); ácido ferúlico (0,85 - 2,27 mg.100g⁻¹) e rutina (238,59 - 442,94 mg.100g⁻¹). Os acessos CA e PR apresentaram os maiores valores de fenóis totais (1179,39 e 1167,05 mg EAG.100g⁻¹, respectivamente). Os acessos CA e TC apresentaram a maior concentração do composto majoritário rutina (436,78 e 442,94 mg.100g⁻¹, respectivamente). Os valores da atividade antioxidante variaram entre 125,95 e 158,67 g.g⁻¹ DPPH. A análise de componentes principais agrupou os acessos em quatro grupos, em consequência da variabilidade genética previamente verificada em estudos anteriores. Os resultados serão úteis para direcionar ações de seleção e futuro programa de melhoramento genético da espécie.

Palavras-chaves: Frutas brasileiras. Compostos fenólicos. Rutina. Atividade antioxidante.

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INTRODUCTION

The great biodiversity of Brazilian native fruit species, with nutritional and pharmacological potential, is still little explored. These species include mangaba (*Hancornia speciosa* Gomes), of the Apocynaceae family, which occurs naturally in the Cerrado and Atlantic Forest. The term ‘mangaba’ means ‘good thing to eat’ in Tupi-Guarani (SILVA JÚNIOR, 2004). In addition to fresh consumption, its fruits are widely used in the production of frozen pulp, sweets, ice cream and liqueurs.

The consumption of mangaba and its products are associated with the quality of antioxidant compounds, aromatic compounds and gastroprotective effect (MORAES et al., 2008; LIMA et al., 2015). Chemical characterization of secondary metabolites of plants enables the tracking of bioactive substances in germplasm, besides ensuring better quality of raw material used in phytotherapy programs and pharmaceutical industry (ALVES et al., 2010).

The Mangaba Active Germplasm Bank of Embrapa Coastal Tablelands, a registered depository of the species (MMA/CGEN/ 02000.002581/2014-53), was implemented in 2006 and is composed of 27 accessions represented by 299 genotypes. The first fruiting occurred in 2013, and Silva et al. (2015) identified accessions whose fruits had high vitamin C content (226.63 and 244.43 mg.100 g⁻¹FM) and evaluated the total polyphenols (from 88.81 to 148 mg.100g⁻¹) and antioxidant activity (between 3.91 and 12.20 µM Trolox/g). In 2017, with a higher number of adult plants and in the fruiting stage, Santos et al. (2017) observed accessions with vitamin C content greater than 400 mg/100 g⁻¹FM and suggested their use in future breeding programs of the species.

Results of the study conducted by Bastos et al. (2017) suggest that mangaba fruit is a source of valuable phenolic compounds. The chemical composition can be influenced by climate and

genetic factors. The search for knowledge about the characterization of chemical composition together with genetic variability is extremely important to know the best genotypes with desired traits (COSTA et al., 2011), such as high vitamin C content and that best adapt to the growing environment, for example.

Studies on antioxidant compounds and their use in the prevention or control of some diseases, such as arthritis, diabetes and cancer, have aroused the interest of the scientific community and general population. Among foods that contain natural antioxidants, fruits and vegetables are the ones that most contribute to the dietary supply of these compounds, associated with beneficial effects on human health (SUCUPIRA et al., 2012).

Growing epidemiological evidence of the role of antioxidant foods in disease prevention has led to the development of a large number of methods to determine antioxidant capacity (PÉREZ-JIMÉNEZ; SAURA-CALIXTO, 2006). The DPPH method (BRAND-WILLIAMS; CUVELIER; BERSET, 1995) is based on the scavenging of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) by antioxidants, producing a decrease in the absorbance of the DPPH free radical reaction with the antioxidant agent at 515 nm (RUFINO et al., 2007).

The objective of this study was to evaluate the bioactive compounds and antioxidant activity in fruits of mangaba accessions of the Active Germplasm Bank of Embrapa Coastal Tablelands.

MATERIAL AND METHODS

The fruits used came from six accessions in the production stage, from the Active Germplasm Bank of mangaba of Embrapa Coastal Tablelands (Table 1). The Active Germplasm Bank was implemented in 2006, in a non-irrigated area of restinga, in soil classified as *Espodossolo Humilúvico* (Spodosol), in the municipality of Itaporanga D’Ajuda, SE, Brazil (11°06’40” S; 37° 11’15” W; altitude 9 m).

Table 1. Origin (state and municipality), coding and number of plants of the accessions of the Active Germplasm Bank of mangaba of Embrapa Coastal Tablelands in fruiting stage.

State	Municipality	Accessions/Coding	Number of plants
Bahia	Jandaíra	Costa Azul (CA)	6
	Conde	Barra de Itariri (BI)	6
	Mata de São João	Lagoa Grande (LG)	6
Sergipe	Indiaroba	Terra Caída (TC)	6
		Preguiça (PR)	6
		Pontal (PT)	6

Lyophilized mangaba pulps were extracted by ultrasound, using 5 g of pulp with 100 mL of methanol:water solvent (9:1, v/v) in an ultrasonic washer (Unique Ultrasonic Cleaner USC-2800), with

dimensions of 300 x 240 x 150 mm, maximum power of 145 W and frequency of 40 KHz, for 30 minutes at 35 °C. The extractions were performed in triplicate and the temperature was monitored with a

digital thermometer. The crude hydroalcoholic extract (9:1 v/v, methanol/water) obtained was subjected to simple filtration, concentrated in rotary evaporator and stored at -20 °C.

Total phenols were determined in a UV-VIS spectrophotometer (1800 model, Shimadzu, Japan). The phenolic compounds of mangaba methanolic extracts were measured using the colorimetric method with Folin-Ciocalteu (SINGLETON; ROSSI, 1965). In a test tube, 50 µL of extract were added with 3 mL of deionized water and 250 µL of Folin-Ciocalteu (1 N). The solution was homogenized in vortex mixer, with subsequent rest for 8 minutes. Then, solution of Na₂CO₃ (sodium carbonate) (20% m/v) and 950 µL of deionized water were added. After 30 minutes in the dark, reading was performed at 765 nm on a spectrophotometer. The procedure for analysis of total phenols was performed in triplicate, in a light-free environment. Quantification was carried out by the external standardization method. The gallic acid standard was used as a reference for the calibration curve, and the concentration of phenolic compounds was expressed in milligrams of gallic acid equivalents (GAE).100g⁻¹ of dry mass.

The determination of antioxidant activity through the DPPH radical followed the methodology of Rufino et al. (2007), based on the scavenging of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) by antioxidants. The DPPH radical solution (60 µM) was prepared with 2.4 mg of diluted DPPH, completed with methanol in 100-mL volumetric

flask, homogenized and transferred to amber vial. In test tubes, three different dilutions of the extracts obtained by ultrasound extraction (1:1, 1:5 and 1:10) were prepared. 3.9 mL of DPPH radical solution were added to the 0.1 mL volume of dilution and vortex mixed. After 30 min, reading was performed at 515 nm in a UV-VIS spectrophotometer (1800 model, Shimadzu), using methyl alcohol as blank. Antioxidant activity was calculated from the standard DPPH curve in µM and the results were expressed in g.g⁻¹ DPPH.

Analysis of extracts of mangaba accessions was carried out using a high-performance liquid chromatograph (Agilent Technologies® 1290 Infinity). The analytes were separated in a C18 reverse phase column (Phenomenex, USA), with 150 nm in length, 4.6 mm internal diameter, 5 µm particle size and C-18 guard column (Phenomenex, USA) with 20 mm length and 4.6 mm internal diameter, kept inside an oven with controlled temperature of 30 °C. Wavelengths were 254, 310 and 325 nm for rutin, ferulic acid and chlorogenic acid, respectively. The elution system in gradient mode with mobile phase was composed of acetic acid solution (5%) in water (A) and methanol (B) (Table 2), mobile phase flow rate of 1,000 mL.min⁻¹, injection volume of 20 µL of the solution, and running time of 80 min. The identified phenolic compounds were quantified by the external standardization method. The results were expressed in mg.100g⁻¹ of mangaba extract.

Table 2. Gradient elution program in HPLC-DAD developed for the analysis of phenolic compounds in mangaba.

Time (min)	Acetic Acid (5%)	Methanol (%)
1	80	20
10	80	20
65	40	60
70	20	80
75	20	80
78	80	20
80	80	20

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied to the study of the similarity of individuals based on the distribution of chemical composition and antioxidant activity using R software, version 3.2.5.2016, with Princomp and factoextra packages.

The experimental design was completely randomized, with six treatments and six replicates. The data were subjected to analysis of variance by F test, and the means were compared by the Bonferroni test, at 5% probability level. Statistical analyses were

carried out using the program SAS®.

RESULTS AND DISCUSSION

Extraction yields - There was a significant difference in the yield of the different accessions of mangaba evaluated. The values ranged from 39.36 to 47.09 g.100g⁻¹. Fruits of the accessions BI and LG, from the state of Bahia, had the highest averages (46.21 and 47.09 g.100g⁻¹), respectively (Table 3).

Table 3. Ultrasound extraction yield of the evaluated mangaba accessions.

Accession	Extract yield (g.100g ⁻¹)
PR	43.47 ± 1.98 ab
PT	44.72 ± 2.23 ab
TC	42.83 ± 1.85 ab
BI	46.21 ± 2.61 a
CA	39.36 ± 2.03 b
LG	47.09 ± 2.06 a
CV (%)	4.87
P	0.01

CV (%) = Coefficient of variation.

*Means followed by the same letter in the same column, do not differ by Bonferroni test at 5% probability.

Total phenolic compounds content and antioxidant activity - Fruits of the accessions PR and CA showed higher content of total phenolics (1167.05 and 1179.39 mg GAE.100g⁻¹, respectively) than those of the other accessions (Table 4). The values found in the present study were higher than those reported by Lima et al. (2015) (490 mg GAE.100g⁻¹), Rufino et al. (2010) (169 ± 21.5 mg GAE.100g⁻¹) and Almeida et al. (2011) (98.8 ± 5.6 mg GAE.100g⁻¹). For antioxidant activity, there was no significant difference between accessions. The values ranged from 125.95 to 158.67 g.g⁻¹ DPPH

(Table 4), which are lower than those reported by Rufino et al. (2010) (890 g.g⁻¹ DPPH) and Vidal (2010) (5,792.66 g.g⁻¹ DPPH). Despite the significant difference between the values of phenolic compounds, the DPPH free radical scavenging activity was not significant, possibly because the determination of antioxidant activity is related to the combined effect of these compounds in the sample. There was a synergistic effect of the antioxidant compounds in the sample that were not identified and quantified by HPLC.

Table 4. Total phenolic content and antioxidant activity in pulp extracts of mangaba accessions of the Active Germplasm Bank of Embrapa Coastal Tablelands.

Accession	Total phenolic content (mg GAE.100g ⁻¹)	Antioxidant activity (g.g ⁻¹ DPPH)
PR	1167.05 ± 80.17 a	158.67 ± 20.71 a
PT	993.45 ± 43.16 ab	156.02 ± 7.80 a
TC	955.96 ± 79.48 b	150.54 ± 0.95 a
BI	899.68 ± 90.86 b	125.95 ± 11.70 a
CA	1179.39 ± 46.34 a	153.00 ± 20.04 a
LG	903.15 ± 38.93 b	143.51 ± 1.96 a
CV (%)	6.54	9.60
P	0.0004	0.34

CV (%) = Coefficient of variation.

*Means followed by the same letter in the same column, do not differ by Bonferroni test at 5% probability.

Identification and quantification of bioactive compounds by HPLC - Three phenolic compounds were identified and quantified by high-performance liquid chromatography (HPLC), one flavonoid (rutin) and two phenolic acids (ferulic acid

and chlorogenic acid). Rutin was the compound with highest concentration and ferulic acid was the one with lowest concentration (Table 5). The presence of rutin and chlorogenic acid has also been reported by Lima et al. (2015) and Rufino et al. (2010).

Table 5. Concentrations of chlorogenic acid, ferulic acid and rutin obtained in pulp extracts of mangaba accessions of the Active Germplasm Bank of Embrapa Coastal Tablelands.

Accession	Chlorogenic acid (mg.100g ⁻¹)	Ferulic acid (mg.100g ⁻¹)	Rutin (mg.100g ⁻¹)
PR	93.71 ± 14.51 b	0.93 ± 0.29 b	236.90 ± 20.39 c
PT	96.83 ± 4.03 b	0.90 ± 0.29 b	234.56 ± 16.36 c
TC	112.98 ± 14.88 ab	2.27 ± 0.13 a	436.78 ± 3.09a
BI	116.14 ± 8.58 ab	1.23 ± 0.07 b	238.59 ± 12.01 c
CA	131.66 ± 14.18 a	0.85 ± 0.03 b	442.94 ± 19.60 a
LG	115.03 ± 8.46 ab	1.22 ± 0.03 b	319.39 ± 14.23 b
CV (%)	10.05	14.75	4.48
P	0.02	0.0001	0.0001

CV (%) = Coefficient of variation

*Means followed by the same letter in the same column, do not differ by Bonferroni test at 5% probability.

There was a significant difference between the mangaba accessions for the concentrations of chlorogenic acid, ferulic acid and rutin (Table 5) and these values were correlated with the origin of the individuals.

Chlorogenic acid is a secondary metabolite synthesized by phenylpropanoid pathway of phenylalanine via cinnamic acid. It is the ester of caffeic and L-quinic acids (3-caffeoylquinic acid). Its biological activities include antioxidant properties (SATO et al., 2011), antimicrobial effects (PUUPPONEN-PIMIA et al., 2001) and antiviral activity (WANG et al., 2009). For this bioactive compound, the accession CA had the highest concentration (131.66 mg.100g⁻¹). The accessions PR (93.71 mg.100g⁻¹) and PT (96.83 mg.100g⁻¹) obtained the lowest averages and did not differ statistically from TC (112.98 mg.100g⁻¹), BI (116.14 mg.100g⁻¹) and LG (115.03 mg.100g⁻¹).

The study of chlorogenic acid concentration in different genotypes of guelder-rose (*Viburnum opulus*) was reported by Kraujalytė et al. (2013), who found different values of chlorogenic acid content (0.54 to 6.93 mg/mL) among genotypes of the same cultivar. In mangaba, Gomes et al. (2013) observed chlorogenic acid concentrations of 11.34 mg.100g⁻¹ and Lima et al. (2015) found values of 18.09 ± 1.68 mg.100g⁻¹.

For ferulic acid, which can be used in the treatment of Alzheimer's disease, cancer, cardiovascular diseases, diabetes mellitus and skin diseases (MANCUSO; SANTANGELO, 2014), the highest concentration was observed in the accession TC (2.27 mg.100g⁻¹).

For rutin, the accessions TC and CA obtained the highest averages (436.78 and 442.94 mg.100g⁻¹,

respectively). The mean value found in the present study for rutin was 318.19 mg.100g⁻¹, much higher than that reported by Lima et al. (2015), 10.260 ± 1.13 mg.100g⁻¹. The accessions TC and CA had the highest concentrations of rutin and can be selected for future breeding programs, showing that genetic factors can modulate the composition and concentration of phytochemicals. Rutin is a flavonoid with antioxidant, anti-inflammatory and anticancer activities and great therapeutic importance for improving the resistance and permeability of capillary vessels (BECHO; MACHADO; OLIVEIRA, 2009).

Although all fruits were harvested at the same time and at the same maturity stage, there was a variation in the contents of phenolic compounds, which can be explained by the genetic variability existing among the accessions studied, already reported by Costa et al. (2011).

In addition to the compounds observed in this study, Lima et al. (2015) worked with fresh mangaba pulp and identified by HPLC-DAD gallic acid, catechin, vanillic acid, p-coumaric acid and rosmarinic acid, with predominance of chlorogenic acid (18.09 mg.100⁻¹) and rutin (11.34 mg.100⁻¹).

Principal component analysis (PCA) – This analysis enabled a multivariate study of the experimental data, facilitating the visualization of the correlation between the six mangaba accessions and the variables - total phenols, antioxidant activity, and concentrations of ferulic acid, chlorogenic acid and rutin. The accessions were grouped into four groups: Group 1 (G1), formed by CA; Group 2 (G2), formed by TC; Group 3 (G3), formed by PR and PT, and group 4 (G4), formed by BI and LG (Figure 1).

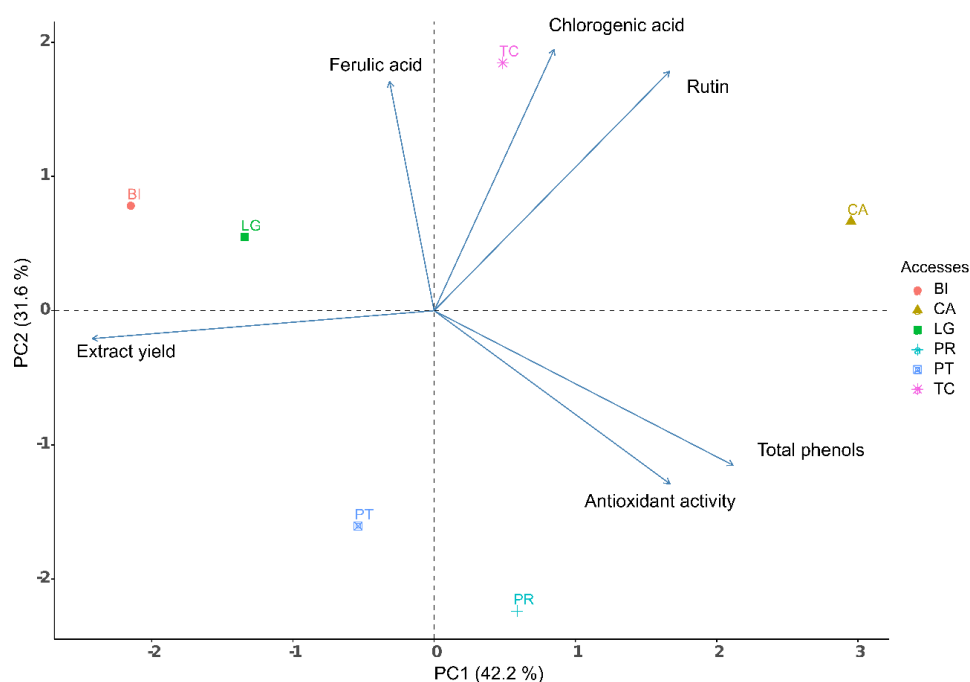


Figure 1. Principal Component Analysis (PCA) of the six mangaba accessions (BI, CA, LG, PR, PT and TC) of the Active Germplasm Bank evaluated in relation to total phenols, antioxidant activity and concentrations of ferulic acid, chlorogenic acid and rutin.

The first two principal components represented approximately 74% of the total variation of the information contained in the mean values of the five variables analyzed (total phenols, antioxidant activity and concentrations of ferulic acid, chlorogenic acid and rutin). The first principal component (PC1) represented 42.2% of all information for the six accessions (PR, PT, TC, BI, CA and LG). In this component, Group 3 (PR and PT accessions) was characterized by having the highest value of total phenol content. The second principal component (PC2) corresponded to 31.6% of the variation, in which Group 2 (TC accession) stood out with the highest positive weights for the ferulic acid concentration variable. The accessions CA and TC were clearly separated by the highest and

lowest ferulic acid contents, respectively (Figure 1).

For the chemical composition based on the content of total phenols, ferulic acid, chlorogenic acid, rutin and antioxidant activity, the accessions were classified into four distinct groups by hierarchical cluster analysis (HCA): Group 1 (G1), composed of the accession CA and characterized by having the highest concentrations of chlorogenic acid and rutin; Group 2 (G2), formed by the accession TC, with the highest concentration of ferulic acid; Group 3 (G3), formed by PR and PT, which had the highest antioxidant activity by the DPPH method, and Group 4 (G4), with the accessions BI and LG, characterized by having similar values of chlorogenic acid and ferulic acid (Figure 2).

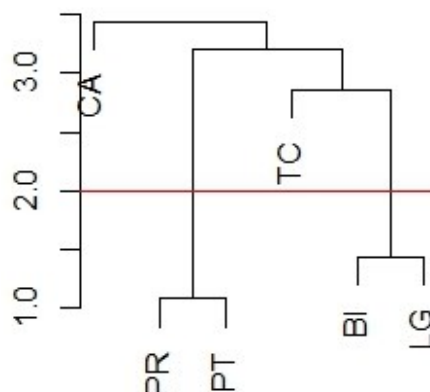


Figure 2. Hierarchical Cluster Analysis (HCA) of the six mangaba accessions (BI, CA, LG, PR, PT and TC) of the Active Germplasm Bank studied in relation to chemical composition and antioxidant activity.

The CA accession was the furthest from the others, indicating that it has well-defined chemical characteristics and genetic traits. In this analysis, Group 2 with the accession TC, separated from the others by the PCA, was close to Group 4, containing the accessions BI and LG, and the two multivariate analyses show the same grouping.

CONCLUSIONS

This was the first report on the quantification and identification of bioactive compounds in fruits of the accessions of the Active Germplasm Bank of Embrapa Coastal Tablelands, showing correlation between the total phenol content, antioxidant activity and quantity of phenolic compounds.

The antioxidant activity through the DPPH radical was lower than those reported in the literature and did not vary among accessions.

The accessions CA (from Bahia) and TC (from Sergipe) have higher concentration of the major compound rutin and can be indicated for selection in future mangaba breeding programs.

The extracts of the accessions CA and TC are the best ones for future pharmacological applications because they have the highest contents of the major compound rutin, and also of chlorogenic acid and ferulic acid.

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