

Comparative Studies Between *Mauritia flexuosa* and *Mauritiella armata*

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ABSTRACT

Mauritia flexuosa and *Mauritiella armata* belong to the family Arecaceae and are widely found in Brazil. **Aim:** In this work were evaluated: the phytochemical profile of the secretion popularly known as *M. flexuosa* wine, antioxidant activity of leaf, root and petiole hydroethanolic extracts of the two species, as well as the quantification of flavonoids and the chromatographic profile by means of High Performance Liquid Chromatography. **Materials and Methods:** The chromatographic profile was determined by high performance liquid chromatography, quantification of flavonoids and antioxidant activity, were performed by spectrophotometric method. **Results:** Antioxidant activity and presence of flavonoids were observed in the extracts of all the analyzed structures of the two species. The phytochemical profile of the wine evidenced the presence of secondary metabolites reported in other structures of *M. flexuosa*. In the chromatographic analysis, it was observed that the extracts evaluated have between three and nine compounds. **Conclusion:** Further studies should be performed to identify the active compounds in the two species.

Key words: Antioxidant activity, Arecaceae, Flavonoids, Phytochemical, Secondary metabolites.

Key Messages: In this work were evaluated: the phytochemical profile of the secretion popularly known as *M. flexuosa* wine, antioxidant activity of leaf, root and petiole hydroethanolic extracts of the two species *Mauritia flexuosa* and *Mauritiella armata* (Arecaceae), as well as the quantification of flavonoids and the chromatographic profile.

INTRODUCTION

The Arecaceae family is composed of 1500 species distributed in 200 genera, found mainly in tropical areas of the planet. They are part of this family the *Mauritiella armata* (buritirana ou xiriri)^{1,2} and also the *Mauritia flexuosa* (buriti, miriti, muriti or palmeira-do-brejo),³ this last secretes a substance called wine, which has easy fermentation and is used in folk medicine as a fortifier and in the control of intestinal problems.^{4,5}

These palm trees have wide distribution in Brazil.⁶ The fruits of both are important sources of food for local fauna,⁷ from the pulp of the fruits of *M. flexuosa* are produced ice cream, jellies, oil, sweets and wine and due to the antioxidant potential, can be added for example in cosmetic formulations or for the study of new drugs by the ability of phenolic compounds of inactivation of reactive species, preventing excessive oxidation which can lead to structural changes in molecules of the organism causing inflammation, cardiovascular diseases, neurodegenerative diseases, atherosclerosis, among others.^{3,8-11}

Faced with this, the objective of this work was to carry out phytochemical prospecting in the *M. flexuosa*

wine, to investigate antioxidant activity, to quantify flavonoids and to verify the chromatographic profile by means of high efficiency liquid chromatography (HPLC) of hydroethanolic extracts of *M. flexuosa* (wine, petiole, leaf and root) and *M. armata* (leaf, petiole and root).

MATERIALS AND METHODS

Collection of plant material

Samples of petiole, leaf and root of three different individuals of *M. flexuosa* and *M. armata* were collected in region of vereda in Bonito of Minas Gerais, the collection was carried out by the Doctor Maria Olivia Mercadante Simões. The plant material was oven dried at 40°C (±2) until it presents a constant mass, later it was pulverized and stored in paper bags in the refrigerator (5°C). Excisates were deposited in the Montes Claros Herbarium: MCMG and identified by Rubens Manoel dos Santos (*Mauritia flexuosa* Mart. (Buriti) n. 5777; *Mauritiella armata* (Mart.) Burret (Xiriri) n.5778a.

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Preparation of the extract

For each 10g of plant material 100 mL of 70% ethanol was added. The mixture was kept at controlled temperature at 35°C (± 2) for seven days, then filtered and placed in a greenhouse at 40°C (± 2) for three to five days for solvent drying, then they were stored in a refrigerator at -5°C until the time of analysis.

Phytochemical analysis

It was evaluated the presence of secondary metabolites: glycosides,¹² saponins, steroids and terpenes following the methodology of Royo et al.¹³ anthraquinones were identified according to Mello et al.¹⁴ and Falkenberg et al.¹⁵ Tests to verify the presence of alcohols were made as described by Andrade.¹⁶ The acidity, aldehyde and ketone tests were reproduced according to the procedures suggested by Cruz and Galhardo.¹⁷ The verification of reducing sugars was done using the methodology described by Beserra et al.¹⁸ and the thin layer chromatography (TLC) technique reported by Mercadante-Simões et al.¹⁹ with modifications.

High Performance Liquid Chromatography (HPLC)

Each extract was diluted in 0.1% trifluoroacetic acid solution: acetonitrile (90:10) at 1 mg/mL concentration, taken ultrasonic bath for 10 min, then filtered on a millex filter and injected into the liquid chromatograph (Waters), equipped with binary pump 1525, automatic injector 717, automatic fraction collector III, diode arrangement detector 2996 and Soft Ware Empower 1, previously stabilized. The column used was type C18 250×4.6 mm 5 micron Spherisorb. The mobile phase used was a 0.1% trifluoroacetic acid solution diluted in acetonitrile (99:1). The injection volume of the samples was 20 μ L, which ran in a flow of 0.6 mL/min. Readings of peak were taken at the wavelength of 220 nm. The analyzes occurred at a temperature of 30°C.

Quantification of flavonoids

The total flavonoid content was determined from the Alves and Kubota²⁰ spectrophotometric method with the use of aluminum chloride. The standard curve equations were obtained from reading the absorbances at the 425nm wavelength of the standard standard solution of rutin (20, 25.30, 35, 40, 45 μ g/mL). In 100 μ L of AlCl₃ methanolic solution (5% w/v), it was added 600 μ L of samples, at the concentrations of 100, 200, 300, 400, 500 μ g/mL and the volume was completed to 2.5 mL with methanol.

Antioxidant Activity

The evaluation of the antioxidant activity of the samples was measured by the DPPH free radical sequestering method (2,2-Diphenyl-1-picrylhydrazyl) described by Brand-Williams,²¹ with modifications. The stock solution of DPPH was prepared at the concentration of 40 μ g/mL and stock solutions of the samples prepared at 500 μ g/mL.^{21,22} Samples and DPPH were diluted in methanol.

In separate test tubes were pipetted 3 mL of DPPH and 0.5 mL of the solutions of each sample in the concentrations according to Table 1. Then the samples stayed for 30 min under cover the light and then was proceeded the spectrophotometer read at a wavelength of 517 nm. For white, methanol was used and for the negative control it was pipetted 3mL of DPPH and 0.5mL of methanol. Gallic acid was used as a positive control at concentrations from 0.33 to 1.66 μ g/mL. All the tests were performed in triplicate and the mean of the results was used for the calculation of percentage of antioxidant activity.

The percentage of antioxidant activity (% AA) was calculated with equation 1:

$$\%AA = \left(\frac{\text{AbsConst} - \text{AbsAmos}}{\text{AbsCont}} \right) \times 100 \quad (1)$$

Where *AbsCont* is the absorbance value of the control and *AbsAmos* represents the absorbance value of the sample.²²

RESULTS AND DISCUSSION

Phytochemical analysis of wine

They were identified: Glycosides, anthraquinones and saponins in the most concentrated dilutions of wine and still steroids and triterpenoids in extraction I, but not in extraction II. The *M. flexuosa* also has these metabolites in other structures, for the ethanol extract from its leaves has already been reported presence of steroids / triterpenoids, saponins, flavonoids and tannins.²³ In the pulp there are phenolic compounds, flavonoids and phytosteroids.²⁴ And in the roots there are flavonoids and triterpenes^{25,26} (Table 2).

Aldehydes were not detected as well as reducing sugars in both TLC and Fehling reactions. However, it was detected the presence of ketones and alcohols in a most intense way in the potassium dichromate test than in ferric chloride, which is directed to identification of phenols.²⁷ The verification of ketones, alcohols and the pH = 4 may be due to the fermentative process that consumed the sugars, where the primary alcohols produce acids and the secondary alcohols, the end product is the ketone.^{28,29}

Chromatographic analysis

In the extracts of the two species, the compound with retention time from 6.545 to 6.574 min was the majority for all analyzed parts. The compound with retention time between 5.257 and 5.351 min was the second most found in all structures, except for the root of *M. armata* where the second most prevalent compound is the one with retention time from 4.231 to 4.227 min, being this the third major in: leaves of both species and in the petiole of *M. flexuosa* (Table 3).

In the leaves of *M. flexuosa* were identified eight compounds, whereas this structure in *M. armata* has only three compounds, which coincide in prevalence in the two species. In the *M. flexuosa* root were detected six compounds and in *M. armata* seven. It is possible to verify that the compound with retention time 6.545 to 6.574 min is majority in the roots of both species and the compound with retention time 5.928 to 6.098 min is not present in the roots of *M. flexuosa*. The other compounds vary in quantity in the roots of the two plants. Among all the extracts the petiole of *M. armata* was the one with the highest number of compounds (9) (Table 3).

In *M. flexuosa* wine there are six compounds, the retention time of these substances was different from the retention times of the compounds found in the plant structures, as can be observed in Table 3.

Antioxidant activity and quantification of flavonoids

All the samples had antioxidant activity and it was possible to verify the presence of flavonoids, however when compared, the structures of *M. armata* showed higher antioxidant activity and amount of flavonoids than in the corresponding samples of *M. flexuosa* (Table 4).

In both palms, the leaf extracts were the ones with the highest EC₅₀ value as can be seen in Table 4. In *M. armata* and *M. flexuosa* the leaves exhibited greater antioxidant potential and flavonoid content than the leaves of *Calamus rotang* (EC₅₀ of 387,948 μ g/mL and flavonoid content of 2.68 EQ mg/g)³⁰ which is popularly used as antihelmintic and in the treatment of eye problems.³¹ However, they have less antioxidant activity than the leaves of *Phoenix dactylifera* (EC₅₀ corresponding to 7.44 to

Table 1: Sample concentrations in the antioxidant activity tests.

Sample	Part of the plant	Concentrations
<i>M. flexuosa</i>	Root	80,100, 120, 140, 160 µg/mL
	Petiole	100, 200, 300, 400, 500 µg/mL
	Leaf	80, 100, 120, 140, 160 µg/mL
	Wine	0.5% to 100% of its natural concentration
<i>M. armata</i>	Root	20, 40, 60, 80, 100 µg/mL
	Petiole	100, 200, 300, 400, 500 µg/mL
	Leaf	120, 140, 160, 180, 200 µg/mL

Table 2: Phytochemical profile of wine.

Test	Reaction	Result
Steroides	Extraction I (Bouchardart)	+++
	Extraction II	-
Glycosides	Ammonium hydroxide	+
Reducing sugar	Fehling A e B	-
	TLC	-
Anthraquinones	Direct reaction	+
	Reaction with previous acid hydrolysis	+
Alcohol	Potassium dichromate	++
	Ferric chloride	+
Aldehydes	Fehling	-
Ketones	Iodo from testing	+
Acidity	-	pH=4
Saponins	Persistent foam	1 2 3 4 5 6 7 8 9 10
		- - - - - - - + + +

(+) weak; (++) moderate; (+++) intense; (-) negative

12.61 µg/mL),³² parts of this plant are used in traditional medicine for the treatment of disorders of memory, fever, inflammation, paralysis and its leaves in particular possess antibacterial activity.³³ All these species mentioned belong to the family Arecaceae.

The best antioxidant performance of the leaves of *M. armata* (EC₅₀ of 50.6 µg/mL) may be due to the amount of flavonoids (7.92 mg/g.E) that was higher than that found in *M. flexuosa* (4.85 mg/g.E), in addition *M. armata* possesses only three compounds while *M. flexuosa* has eight (Table 4), this smaller amount of constituents in its extract reduces the possibility of interaction between the metabolites and potentiates the antioxidant activity of the extract.³⁴

The roots of *M. armata* and *M. flexuosa* had intermediate values of antioxidant activity when compared to the other analyzed structures, with EC₅₀ of 75.6 and 98.7 µg/mL respectively and although they presented antioxidant activity, the values are lower than that described for roots of *Borassus flabellifer* (15.75 µg/mL) that also belongs to the Arecaceae family.³⁵ The roots of the two species presented contents of flavonoids higher than those found in petioles, as can be observed in Table 4.

It was not possible to realize the tests of antioxidant activity of the wine at any of the concentrations. Initially, the samples were cloudy, not being possible the spectrophotometric analysis. Faced with this concentrations were reduced and it was centrifuged at 14,000 rpm and it was made white with the same centrifuged dilutions of the sample, however the absorbance was still higher than that of the control. The solvent methanol was changed to ethanol, but anyway there was no success.

The two species have phyto-constituents of interest, mainly flavonoids. It is important to perform analyzes to verify biological activities for both species, especially with *M. armata*, once the tests showed that the species has flavonoids and antioxidant activity higher than *M. flexuosa* and also because there are few studies of this plant related to the chemistry composition, besides, the prospection of these species and the proof of potential for use, can contribute to the valorization and conservation of the biome cerrado.

CONCLUSION

The presence of flavonoids allows the results of the antioxidant activity found for both species. The *M. flexuosa* wine evidenced secondary metabolites reported in other plant structures. Further studies should be performed to identify the active compounds.

Table 3: Chromatographic profile *M. armata* and *M. flexuosa*.

		Chromatographic profile results										
Time (mim)		4,231 – 4,227	4,762 – 4,780	5,257 – 5,351	5,928 – 6,098	6,545 – 6,574	6,967	8,500 – 8,891	11,172	12,278 – 12,373	13,218 – 13,331	16,191
Leafs	<i>Ma</i>	M3		M2		M1						
	<i>Mf</i>	M3	X	M2	X	M1			X	X	X	
Root	<i>Ma</i>	M2		M3	X	M1		X		X	X	
	<i>Mf</i>	X		M2		M1		M3		X	X	
Petiole	<i>Ma</i>	M3	X	M2	M3	M1		X		X	X	X
	<i>Mf</i>	X		M2	X	M1	X	X		X	X	
Time (mim)		4,495	5,149	5,592	6,330	7,493	14,052					
Wine (<i>Mf</i>)		M1	M2	X	X	M3	X					

Ma = *M.armata*; *Mf* = *M.flexuosa*; X - present; M1 = first major; M2 = second major; M3 = third major.

Table 4: EC₅₀ quantification of flavonoids (mg of rutin/g of extract) and line equations and correlation coefficients (R²) of standard curves of the samples.

Sample	Structure	EC ₅₀ µg/mL	Flavonoids (mg/g.E)	Equation of the line (R ²)
<i>M. flexuosa</i>	Root	98,7	3,83	y=0,495X + 1,12 (0,999)
	Petiole	309,8	0,5947	y=0,0693X + 28,53 (0,994)
	Leaf	94,4	4,85	y=0,506X + 2,24 (0,997)
<i>M. armata</i>	Root	75,6	5,93	y=0,5665X + 7,15 (0,999)
	Petiole	245,2	0,9286	y=0,0547X + 36,59 (0,9986)
	Leaf	50,6	7,92	y=0,3995X + 28,8 (0,9996)
Gallic acid	-	1,34	-	-
Rutin	-	-	-	y=0,0135X-0,0219 (0,9978)

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

ABBREVIATIONS



AbsAmos: Represents the absorbance value of the sample; **AbsCont:** Absorbance value of the control; **% AA:** Antioxidant activity; **AlCl₃:** Aluminum chloride; **DPPH:** 2,2-Diphenyl-1-1-Pricril-hydrazyl; **EC₅₀:** Half maximal effective concentration; **HPLC:** High efficiency liquid chromatography; **TLC:** Thin layer chromatography.

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GRAPHICAL ABSTRACT

Sample concentrations in the antioxidant activity tests, E.C₅₀, quantification of flavonoids (mg of rutin/g of extract).

Sample	Structure	Concentrations µg/mL	E.C ₅₀ µg/mL	Flavonoids (mg/g.E)	
 <i>Mauritia flexuosa</i>	Root	80,100,120,140,160	98,7	3,83	
	Petiole	100,200,300,400,500	309,8	0,5947	
	Leaf	80,100,120,140,160	94,4	4,85	
	Wine	0.5% to 100% of its natural concentration			
 <i>Mauritiella armata</i>	Root	20,40,60,80,100	75,6	5,93	
	Petiole	100,200,300,400,500	245,2	0,9256	
	Leaf	120,140,160,180,200	50,6	7,92	
Gallic acid			1,34	---	

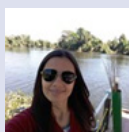
SUMMARY

- The current work were evaluated: the phytochemical profile of the *M. flexuosa* wine, antioxidant activity of leaf, root and petiole hydroethanolic extracts of the two species, as well as the quantification of flavonoids and the chromatographic profile by means of high performance liquid chromatography.
- Researchers have been stated the presence of flavonoids and antioxidant activity of were observed in the extracts of all the analyzed structures of the two species.
- Researchers have been stated the presence of phytoconstituents in wine.
- In the chromatographic analysis, it was observed that the extracts evaluated have between three and nine compounds.

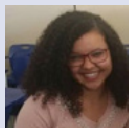
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Vanessa de Andrade Royo: Professor of the Postgraduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



Juliana Almeida Rocha: Master's degree from the Graduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



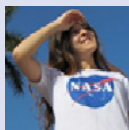
Kamylla Teixeira Santos: Student of the Graduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



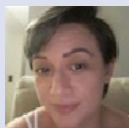
Jeane Ferreira Leal Freitas: Master's degree from the Graduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



Clarice Avelar Almeida: Student of the Biological Sciences Graduation Course of the Department of General Biology, State University of Montes Claros, BRAZIL



Bianca Ribeiro: Student of the Biological Sciences Graduation Course of the Department of General Biology, State University of Montes Claros, BRAZIL



Elytania Veiga Menezes: Professor of the Postgraduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



Dario Alves de Oliveira: Professor of the Postgraduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



Murilo Malveira Brandão: Professor of the Postgraduate Program in Biotechnology of the Department of General Biology, State University of Montes Claros, BRAZIL



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