Chemical characterization and evaluation of antioxidant and antimicrobial properties of the pulp oil of fruits of *Mauritia flexuosa* L. f.

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Abstract: This study aimed to characterize the chemical, antioxidant and antimicrobial properties of the pulp oil of *Mauritia flexuosa* L. f. (Arecaceae). Chemical identification was performed by gas chromatography coupled to mass spectrometry. The physicochemical properties were characterized. Antioxidant capacity has been verified eliminating free radicals, reducing and chelating iron. The antimicrobial activity was evaluated by the minimum inhibitory concentration and the modulatory action of antibiotics. The major fatty acids identified were stearic acid, palmitic acid and oleic acid. The acidity and the saponification index are within the limits established by the National Sanitary Surveillance Agency. The oil showed moderate antioxidant activity and antimicrobial activity against Candida strains. It also showed synergistic effects, especially on cefotaxime against Bacillus cereus. The results suggest the potential of the species as an antioxidant and in antimicrobial therapy.

Keywords: *Mauritia flexuosa* L; Antibiotic modulation; Antioxidant capacity; Buriti; MIC; Oil.
INTRODUCTION
Fruits and vegetables stand out due to their importance as components of a healthy diet, as well as the fact that, in adequate amounts, their consumption may reduce the risk of chronic-degenerative diseases such as cancer and cardiovascular problems. In addition, they are sources of micronutrients, fibers and other components with functional properties, which makes it able to establish a relationship between the ingestion of these foods and a better quality of life (Jaime et al., 2009; Vidal et al., 2012).

*Mauritia flexuosa* L. f. (Arecaceae), commonly known as buriti, is a palm tree that has a wide distribution in the North, Northeast, Central-West and Southeast regions of Brazil, inhabiting the banks of rivers, streams, lakes and springs (Ferreira, 2005). It has an elliptical to oval fruit, surrounded by a bark of reddish-brown triangular scales, with a thin, orange, fleshy and oily mesocarp, which is widely used in the manufacture of sweets, ice cream, juices, as well as jellies and fermented wine (Sampaio & Carazza, 2012).

The oil extracted from the pulp of the buriti fruit is known for its functional properties due to the high concentrations of monounsaturated fatty acids, in higher amounts than olive and Brazilian nut oils, recognized for being high quality nutritional oils and presenting high provitamin A carotenoids (911.4 ± 2.4 a 1003 ± 20 mg.kg⁻¹), having various applications for the food, pharmaceutical and cosmetic industries (Vieira et al., 2006; Silva et al., 2009; Aquino et al., 2012).

Fatty acids are compounds typically found conjugated to other molecules, such as glycerol, sugars or phosphate groups. These can be released from lipids, becoming free fatty acids, which have diverse and potent biological activities, such as protection against coronary heart diseases, diabetes and cancer, and also reduce the risk factors of these diseases, including hypertension, sensitivity to insulin, plasma lipoprotein concentrations and factors related to blood clotting (Lunn, 2007; Desbois & Smith, 2010).

The fixed oil of *M. flexuosa* is recognized for its biological activities. In the work of Leão *et al.* (2019) the antioxidant activity of nanoemulsions from buriti oil was evidenced, which is able to reduce ion iron and inhibit oxidative degradation. In addition, it was demonstrated the ability to inhibit the growth of multiresistant bacteria to antibiotics of various classes such as aminoglycosides, cephalosporins, sulfonamides, among others (Noble *et al.*, 2018).

The objective of this work was to obtain the chemical profile, to physico-chemically characterize and to evaluate the antioxidant and antimicrobial capacity of the pulp oil of the *Mauritia flexuosa* fruit.

**MATERIALS AND METHODS**

**Plant material and oil obtainment**
As described in the previous study (Nonato *et al.*, 2018), the *M. flexuosa* fruits were collected in the Environmental Protection Area (APA) of Chapada do Araripe (7°15′33.37″S 39°28′6.95″W) in the municipality of Crato, Ceará, Brazil in October 2016 and one exsicata was deposited in the Dárdano de Andrade-Lima Caririense Herbarium of the Regional University of Cariri under registration number 12620. The fresh pulp oil (OFB) was obtained by continuous extraction for 6 h in Soxhlet apparatus using hexane as solvent extractor and heating at 60°C. The solution was then concentrated under reduced pressure on a rotary evaporator at an average temperature of 50°C resulting in a 5.91% (w/w) yield.

**Analysis of fatty acids**
Fatty acids were indirectly determined using their corresponding methyl esters. The oil (0.2 g) was saponified for 2 h under reflux with methanolic potassium hydroxide solution (Hartman & Lago, 1973). After suitable treatment and pH adjustment, the free acids were methylated with methanol by acid catalysis to obtain the respective methyl esters. Analysis of the fixed constituents was performed by Gas Chromatography coupled to Hewlett-Packerd Mass Spectrometry (GC/MS), model 5971, using non-polar DB-1 capillary silica fused column (30 m x 0.25 mm id. 0.25 μm); carried by helium gas; flow rate 0.8 mL/min and split mode. The injector's temperature was 250°C and the detector's was 200°C. The column's temperature was programmed from 35°C to 180°C at 4°C/min then 180°C to 250°C at 10°C/min. Mass spectra were recorded from 30-450 m/z. The injected volume was 1 μL of 5 μg/mL solution in dichloromethane. The individual components were identified by matching their mass spectra, 70 eV, with those of the database using the library built through the spectrometer and two other computers using retention indices as a pre-selection.
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(Alençar et al., 1984; Alencar et al., 1990), as well as by visual comparison of standard fragmentation with those reported in the literature (Sienhagen et al., 1974; Adams, 2001).

Physical-chemical characterization
The oil was characterized according to the official methodologies of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008). The acidity value was determined with a solution of the oil in ethyl ether: ethanol (1:1) by titration with sodium hydroxide solution, using phenolphthalein as indicator, in which the result was given as the number of milligrams of KOH required to neutralize the free acids of 1 g of sample. To verify the saponification index, 2 g of the oil in 50 mL of alcoholic KOH solution was refluxed for 1 h. After cooling the solution, titration was performed with hydrochloric acid (0.5 M), also using phenolphthalein as indicator, and the result was expressed as the number of milligrams of KOH required to saponify 1 g of sample. The refractive index was determined in Abbé refractometer at 40°C. All analyzes were done in triplicate.

Antioxidant assays
DPPH free radical scavenging
The methodology proposed by Rufino et al. (2007) was followed. Concentrations ranging from 14 to 1400 μg/mL were evaluated. In a dark environment, a 0.1 mL aliquot of each concentration was transferred to test tubes with 3.9 mL of the DPPH radical solution (0.06 mM). BHT and ascorbic acid were used as positive control and methanol was used as the blank test. The solutions were incubated for 30 min protected from light and the spectrophotometer readings were performed at 515 nm. All test and controls were performed in triplicate. The results were given by linear regression and from this the IC₅₀ was determined and the equivalent of 1000 μM of the Trolox standard was substituted in the straight line equation of the absorbance graphic.

ABTS free radical capture
The concentrations tested ranged from 14 to 1400 μg/mL. Whilst protected from light, a 30 μL aliquot of each concentration was transferred to test tubes with 3.0 mL of the ABTS⁺⁺ radical. The reading was performed in a spectrophotometer at 734 nm after 6 minutes of reaction of the mixture. Trolox was used as positive control and methanol as blank control. All test and controls were performed in triplicate. In order to obtain total antioxidant activity, the equivalent of 1000 μM of the Trolox standard was substituted in the straight line equation of the absorbance graphic (Rufino et al., 2007).

FRAP (Ferric Reducing Antioxidant Power)
The FRAP reagent was obtained by mixing 25 mL of acetate buffer (0.3 M), 2.5 mL of a TPTZ solution (10 mM) and 2.5 mL of an aqueous ferric chloride solution (20 mM). An aliquot of 90 μL of each concentration (14-1400 μg/mL) was transferred into test tubes as well as 270 μL of distilled water and 2.7 mL of FRAP reagent, keeping in a heating bath at 37°C. The reading was performed after 30 minutes of reaction at 595 nm in spectrophotometer. The FRAP reagent was used as blank control and the ferrous sulfate as a positive control. All test and controls were performed in triplicate. The total antioxidant activity replaced in the absorbances' straight line equation was equivalent to 1000 μM of the ferrous sulfate standard (Rufino et al., 2006).

Chelation activity of Fe²⁺ ion
The chelation capacity was measured by the method proposed by Puntel et al. (2005), with adaptations. 100 μL of each concentration (14-1400 μg/mL) was added with 300 μL of the FeSO₄ solution (2 mM) and 336 μL 1.0M TRIS-HCl (pH 7.4). The test solutions were incubated protected from light for 5 minutes, whereafter 26 μL of phenanthroline (0.25%) was added. All test and controls were performed in triplicate. The reading was performed in spectrophotometer at 510 nm. The blank solution was prepared without the addition of the sample and by the absence of incubation.

Antimicrobial assays
Determination of minimum inhibitory concentration – MIC
The antimicrobial activity was tested by the microdilution method based on the document M7-A10 (CLSI, 2015). The assay was performed with four bacterial strains: Bacillus cereus INCQS 00303, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 and Salmonella cholaerasuis INCQS 00038, and three fungal strains: Candida albicans INCQS 40006, Candida krusei INCQS 40095 and Candida tropicalis INCQS 40042.

The oil was diluted with sterile distilled water
and dimethyl sulfoxide (DMSO) at a concentration of 1024 μg/mL. Serial dilutions were then performed by addition to the wells containing the suspension, reaching concentrations in the range of 512 to 8 μg/mL. The whole test was performed in triplicate and the plate incubated at 35 ± 2°C for 24 h. The reading was performed by colorimetry by the addition of 25 μL of resazurin indicator solution (0.01%) to each well after incubation, where a change from blue to pink indicates bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration capable of inhibiting the growth of microorganisms.

**Evaluation of direct contact modulation**
For the analysis of an oil action as a potentiator of aminoglycoside (amikacin and gentamicin) and beta-lactam (benzylpenicillin and cefotaxime) antibiotics for bacteria, and azole (fluconazole and ketoconazole) for fungi, the methodology proposed by Coutinho et al. (2008) was followed. The bacterial strains *Bacillus cereus* INCQS 00303 and *Salmonella choleraesuis* INCQS 00038, and the fungal strains *Candida albicans* INCQS 40006, *Candida krusei* INCQS 40095 and *Candida tropicalis* INCQS 40042 were used. The test was performed in the presence and absence of *M. flexuosa* fruit oil.

Inoculum cultures (MIC/8) in 10% specific culture medium were distributed in microdilution plates followed by addition of concentrations of the antibiotic solutions (1024 μg/ml) by serial dilution. The plates were incubated at 35 ± 2°C for 24 h and read by colorimetry by the addition of 25 μL of resazurin solution (0.01%).

**Statistical analysis**
The results of the antioxidant assays were evaluated through ANOVA and Tukey’s test, while the microbiological results were analyzed in bidirectional ANOVA and Bonferroni post-test using GraphPad Prism 6.0 software. The results with *p*<0.05 were considered as statistically significant.

**RESULTS**

**Fatty acids analysis**
The analysis by gas chromatography coupled to mass spectrometry (GC/MS) allowed to identify 97.39% of the constituents of the pulp oil of *M. flexuosa* fruits. The saturated fatty acids were predominant in relation to the unsaturated ones, with 80.44% and 16.95%, respectively. The major components of the oil were stearic acid (49.51%), followed by palmitic acid (22.14%) and oleic acid (16.58%) as shown in Table No. 1. Figure No. 1 shows the chromatographic profile of the oil.

**Physical-chemical characterization**
The values obtained for some of the main physical-chemical indices are shown in Table No. 2.
Table No. 1
Methyl ester profile of fatty acids identified in the fixed oil of *Mauritia flexuosa* fruit pulp

| Fatty acid                  | Rt (min) | %   |
|-----------------------------|----------|-----|
| Lauric Acid (C12:0)         | 9.89     | 0.49|
| Myristic Acid (C14:0)       | 12.36    | 1.26|
| Pentadecanoic Acid (C15:0)  | 13.52    | 0.53|
| Palmitoleic Acid (C16:1)    | 14.14    | 0.37|
| Palmitic Acid (C16:0)       | 14.61    | 22.14|
| Margaric Acid (C17:0)       | 15.67    | 1.39|
| Oleic Acid (C18:1)          | 16.43    | 16.58|
| Stearic Acid (C18:0)        | 16.68    | 49.51|
| Eicosanoic Acid (C20:0)     | 18.34    | 4.84|
| Docosanoic Acid (C22:0)     | 20.28    | 0.28|

Rt: Retention time.

Table No. 2
Physical-chemical characteristics of the pulp oil of *Mauritia flexuosa* fruits (OFB)

| Physical-chemical properties       | OFB             |
|-----------------------------------|-----------------|
| Acidity (mg KOH/g)                | 5.71 ± 0.01     |
| Saponification index (mg KOH/g)   | 210.97 ± 12.13  |
| Refractive index (nD^20)          | 1.467 ± 0.00    |

**Antioxidant assays**
In the DPPH free radical scavenging test, the oil presented low hydrogen donation capacity, with a maximum percentage of 9.38%, which was well below the positive controls ascorbic acid and BHT, with percentages of 96% and 88%, respectively, as can be seen in Figure No. 2.

![Figure No. 2](image)

**Figure No. 2**
Antioxidant activity by DPPH free radical scavenging of the pulp oil of *M. flexuosa* fruits (OFB) and of the positive controls ascorbic acid and BHT.
Considering the positive controls, the oil obtained a concentration capable of inhibiting 50% of moderate DPPH. Its IC$_{50}$ was only 4.76 times lower than BHT, while compared to the ascorbic acid its capacity was 38.67 times lower. The IC$_{50}$ values are elucidated in Table No. 3.

| Samples     | IC$_{50}$ (μg/mL) | Trolox equivalent |
|-------------|-------------------|-------------------|
| OFB         | 83.16 ± 5.09a     | 10.79 ± 1.07a     |
| Ascorbic Acid | 2.15 ± 0.05b   | 14.15 ± 0.04a     |
| BHT         | 17.46 ± 2.10c     | 11.78 ± 3.12a     |

Results are expressed as mean ± standard deviations (n=3). The means followed by different letters differ by the Tukey test at $p<0.05$.

The oil presented a moderate ability to capture the ABTS radical and to reduce the iron ion by the FRAP methodology, obtaining values of 69.26 ± 1.04 μM Trolox/g and 24.37 ± 0.75 μM Fe$_2$SO$_4$/g, respectively.

The oil showed good chelating activity, in which the response was concentration dependent (14-350 μg/mL) with significant difference between concentrations (Figure No. 3). In general, the response obtained a maximum percentage of 63.6%.

**Figure No. 3**
Effects of iron chelation on the oil of the *M. flexuosa* pulp. Values are expressed as mean ± standard deviation (n=6). *: $p<0.05$, ****: $p<0.0001$, (ANOVA and Tukey's test).

**Antimicrobial assays**
The values of the minimal inhibitory concentrations obtained from the tested strains are shown in Table No. 4.
Table No. 4
Minimum inhibitory concentration of the pulp oil of *M. flexuosa* fruits against bacterial and fungal strains.

| Tested microorganisms                  | MIC (µg/mL) |
|----------------------------------------|-------------|
| *Bacillus cereus* (INCQS 00303)        | ≥ 1024      |
| *Staphylococcus aureus* (ATCC 25923)   | ≥ 1024      |
| *Escherichia coli* (ATCC 25922)        | ≥ 1024      |
| *Salmonella choleraesuis* (INCQS 00038) | ≥ 1024      |
| *Candida albicans* (ATCC 40006)        | 853.33      |
| *Candida krusei* (ATCC 40095)          | 512         |
| *Candida tropicalis* (ATCC 40042)      | 512         |

ATCC: American Type Culture Collection; INCQS: National Institute for Quality Control in Health.

Regarding the modification of the antibiotic action against bacterial strains, the pulp oil of the *M. flexuosa* fruits showed both synergistic and antagonistic action, according to the data shown in Figures No. 4. The oil exerted synergistic effect on amikacin against *S. choleraesuis* decreasing MIC from 512 µg/mL to 213.33 µg/mL. On gentamicin, it also showed synergistic effect against *B. cereus*, with reduction of MIC from 512 µg/mL to 256 µg/mL, as compared to *S. choleraesuis*, with a decrease to 106.66 µg/mL.

On benzylpenicillin, the oil exerted an antagonistic effect against *B. cereus*, with an increase of MIC from 26.66 µg/mL to 256 µg/mL, and a synergistic effect against *S. choleraesuis*, reducing MIC to 1024 µg/mL for 512 µg/mL. The same result was obtained on cefotaxime for this strain. Still on cefotaxime, it exerted the most expressive synergistic effect of this study against *B. cereus*, with a reduction from 1024 µg/mL to 5.33 µg/mL.
DISCUSSION

Studies report oleic acid and palmitic acid as the main major components of the pulp oil of buriti fruits, with percentages ranging from 61% to 75.7% for oleic acid and 16.78% to 23% for the palmit acid, while the stearic acid presents low concentrations, ranging from 1.3% to 5.2% (Silva et al., 2009; Rodrigues et al., 2010; Damet et al., 2011; Pardauil et al., 2011; Aquino et al., 2012). The difference between the fatty acids synthesis of a species can be attributed to abiotic factors, such as water availability, habitat, among others (Kris-Etherton et al., 2000).

For oils and fats, the physico-chemical properties are evaluated and used to measure their quality (Lima et al., 2017). The acidity index aims to quantify the free fatty acids present in the oils, since high rates of these indicate changes that lead to hydrolytic rancidity (Ferreira et al., 2008; Bermejo et al., 2013). The acidity index obtained in this study (Table No. 2) was relatively higher than those reported in the literature for the oil of this species, with indexes ranging from 2.1 to 4.7 mg KOH/g (Vásquez-Ocmín et al., 2010; Aquino et al., 2012; Lima et al., 2017). However, it is within the limits allowed by the National Agency of Sanitary Surveillance (ANVISA), with a maximum of 10.0 mg KOH/g (Brasil, 2005).

The saponification index indicates the relative amount of high and low molecular weight fatty acids. Low molecular weight esters require more alkali for the saponification, so the saponification index is inversely proportional to the molecular weight of the fatty acids (Brasil, 2005). The saponification index obtained (Table No. 2) was higher than those found by Vásquez-Ocmín et al. (2010), which reported variable indices between 186.25 and 194.89 mg KOH/g for three morphotypes of the M. flexuosa fruit. The obtained index was also outside the range established by ANVISA (184-196 mg KOH/g), which shows a rate of low molecular weight fatty acids in the oil studied (Brasil, 2005).

The refractive index is characteristic for each type of oil and is related to the degree of saturation of the bonds, oxidation compounds and heat treatment (Mello & Pinheiro, 2012). The index found in this study is within the limit established by ANVISA, which ranges from 1.467 to 1.470 nD20 (Brasil, 2005). In the work of Aquino et al. (2012), comparing the physico-chemical properties of crude and refined buriti oil, a refractive index of 1.47 was obtained for both oils tested, these being values consistent with that obtained in this work.

The pulp oil of the buriti fruits tested in this study (Table No. 3) showed a relatively lower IC50 than the one reported in the literature for the crude and the refined buriti pulp oil with IC50 of 25.19 mg/mL and 50.98 mg/mL, in that order, as well as for the olive oil with IC50 of 11 mg/mL (Valavanidis et al., 2004; Aquino et al., 2012).

The antioxidant activity in foods depends on several factors, such as the oxidation conditions and stages, the formation and stability of the radicals, as well as the possible location of antioxidants and stability in different processing stages (Rockenbach et al., 2008).

The capture capacity of the ABTS radical showed greater effectiveness when compared to the edible oils of carrot, cranberry, cumin and hemp, with 8.90 ± 0.39 μM Trolox/g, 22.5 ± 1.22 μM Trolox/g, 30.8 ± 3.58 μM Trolox/g and 11.4 ± 2.08 μM Trolox/g, respectively (Yu et al., 2005).

The antioxidant activity of oils may be related to their content of tocopherols and carotenoids, which are considered lipophilic antioxidants (Chorilli et al., 2007; Borges et al., 2011). The literature reports a rich content of these compounds in buriti oil, with values of 1890 mg/kg, 1343 mg/kg and 918 mg/kg for total carotenoids, α-tocopherol and β-tocopherol, respectively (Silva et al., 2009). Castelo-Branco et al. (2016) indicate an essential role of tocopherols and a good performance in the evaluation by the ABTS method, as well as for the oxidative stability of vegetable oils, probably due to the associations between them.

Oil capacity to reduce the iron ion was lower than the patauá, Amazonian palm, which obtained 584.9 ± 5.3 μM Fe3SO4/g (Hidalgo et al., 2016). The antioxidant efficiency analyzed by the FRAP method is dependent on the redox potentials of the compounds under study, which are characterized by the complexity of their molecules, as well as the ability to reduce phenolic compounds, such as tocopherols, depending on the level of hydroxylation and extension of their conjugations (Pulido et al., 2000).

Mello & Pinheiro (2012), analyzing the chelating capacity of the olive oil of the Arbequina variety from two different cultures obtained 25.00 ± 1.11% and 24.22 ± 2.40%, showing a lower activity
than the buriti oil studied (Figure No. 3). Chelating agents reduce the availability of transition metals and inhibit radical-mediated oxidative chain reactions in biological or food systems, which improves human health, quality, stability and food safety (Yu et al., 2005).

Regarding the antibacterial assays, the oil had MIC ≥ 1024 μg/mL for all strains tested (Table No. 4), which does not demonstrate clinically relevant effect, since it requires very high concentrations of the natural product to reach serum levels (Houghton et al., 2007). For the fungal strains, the oil had higher MIC of 853.33 μg/mL, showing moderate activity (Holetz et al., 2002).

Bacterial resistance involves biochemical and genetic mechanisms, such as inactivation of the antimicrobial agent by chemical changes, target modification, changes in the efflux pump and external permeability of the membrane, as well as the enzymatic inhibition of the antibiotic target (Guimarães et al., 2010). These mechanisms may be related to the non-effectiveness of the oil on the bacteria tested.

The literature shows the activity of fatty acids against strains of Candida albicans, such as oleic acid, caprylic acid and lauric acid, in which this activity occurs through the disorganization of the cytoplasm by the rupture or disintegration of its plasma membranes (Bergsson et al., 2001; Pinto et al., 2017).

Antibiotic activity modifiers are drugs that modulate or revert microbial resistance to certain antibiotics, through alteration of susceptibility to inhibition of the efflux pump (Costa et al., 2008). Other mechanisms of resistance may also be altered by the combination of antibiotics with natural products, such as increased membrane permeability, modification of the antibiotic receptor, and enzymatic inhibition (Wagner, 2011).

Aminoglycoside antibiotics are widely used for the treatment of infections, however, nephrotoxicity due to its continued use remains a common clinical problem, toxicity being one of the factors that limit its dosage (Watanabe et al., 2004). Thus, the combination of these antibiotics with natural products leads to a decrease in their therapeutic dose and consequently their toxicity (Figueroedo et al., 2013).

Beta-lactams represent a class of antibiotics widely used due to their tolerance by the body, but their use generates one of the main mechanisms of resistance to drugs used by bacteria, which is the production of inactivating enzymes called betalactamases (Zeba, 2005). The combination of these with natural products may be an alternative to overcome this resistance barrier.

The antibiotic action modification test against fungal strains did not present significant results. This may be related to resistance mechanisms of Candida genus to azoles, such as efflux pumps, point mutations in the synthesis and overexpression of ergosterol, as well as mutations that deny membrane rupture, such as by-pass pathways (Pfaller, 2012).

CONCLUSION
The results obtained demonstrate that the pulp oil of the M. flexuosa fruit has moderate primary antioxidant activity and good iron ion chelation capacity, as well as being a significant source in antibacterial therapy combined with antibiotics. It exhibits physico-chemical characteristics within the established parameters for edible oils, can be used as functional food. Thus, given the importance of this species, the data obtained can be a starting point for new in vivo assays, aiming to understand its biological activities and the possible development of new complementary therapies.

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