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In vitro antimicrobial activity and fatty acid composition throughgaschromatography-massspectrometry (GC-MS) of ethanol extracts of *Mauritia flexuosa* (Buriti) fruits

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In this study, the chemical composition of the peel and pulp of *Mauritia flexuosa* fruits were analyzed and the antimicrobial activity of ethanolic extracts from these fruits was evaluated using *in vitro* tests. Chemical composition analysis with gas chromatography-mass spectrometry (GC-MS) indicated the presence of saturated and unsaturated fatty acids. The peel extracts (ECBU) presented 54.41% and the pulp (EPBU) presented 94.05% of the saturated fatty acids lauric, myristic, palmitic, stearic, oleic and linoleic acids. The antimicrobial activities were performed using the diffusion and micro-dilution (MIC) methods. ECBU was active against the bacteria *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* at a concentration of 200 mg mL^{-1}, but it was not active against the yeasts *Candida albicans* and *Candida parapsilosis* using the diffusion method. The MIC results showed that ECBU was active against the tested bacteria at concentrations > 12.5 mg mL^{-1} and EPBU was active at concentrations > 25.0 mg mL^{-1}. This was probably due to higher sensibility of the method. The results indicated that the peel and pulp extracts of *M. flexuosa* present antibacterial activity and that ECBU is an especially promising potential candidate for the prospection of new pharmaceutical compounds.

Key words: *Mauritia flexuosa*, Buriti, anti-bacterial agents, fatty acids.

INTRODUCTION

The vast availability and indiscriminate use of antimicrobial compounds has led to a selection of micro-organisms that are resistant to these drugs. These drugs exert influence both in the patient under treatment and

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the ecosystem, with significant repercussions in the result of the disease and also in the increase in resistant environmental bacterial strains and species (Avorn and Solomon, 2000). In order to supply an increasing demand for new antimicrobial drugs, research on new sources of substances, including plants, has grown (Caetano et al., 2002). Bioactive compounds from plants have presented high specificity against a broad spectrum of bacteria (Dixon, 2001). The Cerrado and Amazonian biomes present 20% of all the biodiversity in the world (Calixto, 2005), which includes great diversity of plants with well-known therapeutic properties and chemicals that can be used in biological studies. *Mauritia flexuosa* L.f. (buriti) belongs to the Arecaceae family and is considered one of the most abundant oleaginous palms in Brazil, where it is native. The fruits of buriti are spherical or oval with seasonal fruiting (Storti, 1993), are rich in vitamin A and carotenoids which gives them their characteristic yellowish/reddish color (Albuquerque et al., 2003) and are traditionally consumed *in natura* (Barbosa et al., 2010). The commercialization of products from this palm tree in regions where it is native provides income for the local population and helps maintain the integrity of the "veredas" ecosystem, its main habitat. The indigenous Brazilian people call this species "the tree of life", due to the use of most of its parts, from the leaves to the root. Ribeiro et al. (2014) found 40 different uses for buriti among traditional native communities in Northwest Brazil. The studies of bioactive compounds with antimicrobial activities from buriti fruits are very rare. Buriti oil is reported as presenting antimicrobial properties as a soap formula (Soares et al., 2017). Koolen et al. (2013) and Batista et al. (2012) showed antimicrobial activity of extracts of leaves, trunk and fruits of *M. flexuosa*. Melhorança Filho and Pereira (2012) report antimicrobial activity against *Staphylococcus aureus* by seeds of two other Amazonian palms, *Eutherpe oleracea* and *Bactris gassipaes.* Barros et al. (2014) showed that buriti cream was effective in healing of skin lesions in mice. Due to the economic importance of *M. flexuosa* for indigenous Brazilian people, the objective of this study was to carry out *in vitro* antimicrobial activity tests of the ethanol extracts from the pulp and the fruit peel against human pathogens and to analyze the chemical composition of the fatty acids presented in gas chromatography coupled to a mass spectrometer. There are few studies on the antimicrobial activities of the chemical components (GC-MS) of the peel and pulp of this palm tree's fruits.

MATERIALS AND METHODS

Chemicals

Ethanol, Aluminum chloride ($AlCl_3$), Sodium chloride (NaCl), and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mueller Hinton Broth and Sabour aud culture media were obtained from Kasvi (Curitiba, Paraná, Brazil). The water used in all analyses was ultrapure produced by a Milli-Q, Millipore system (Bedford, USA). Other reagents used in this study were of analytical grade.

Plant materials

Ripe fruits were collected from *M. flexuosa* (Figure 1a and b) in October 2015, in "vereda" ("veredas" are well-defined ecosystems that occur within the Brazilian Cerrado biome, and are characterized by the presence of buriti palm trees in semi-waterlogged conditions) site in the State of Tocantins, Brazil (9°58'2.078934"S 48°17'28.64502"W), at an altitude of 488 m. A voucher specimen of *M. flexuosa* (10.952) was deposited at the HTO herbarium of *Universidade Federal do Tocantins* (Federal University of Tocantins - UFT).

Sample preparation

The *M. flexuosa* fruit peels were removed manually after immersing the fruit in warm distilled water (40ºC), and were separated from the pulp using a stainless steel knife (Figure 1c to e). Thereafter, the materials were dried in an oven with air circulation (Fanem, São Paulo, Brazil) at 40°C for 48 h and crushed in a home processor (Arno, São Paulo, Brazil). Samples of approximately 10 to 30 g were weighed on a precision analytical scale (Shimadzu do Brazil, São Paulo, Brazil) and placed in cellulose cartridges in a Soxhlet apparatus with 200 mL of ethanol solvent (Vetec, 99.8% P.A.) for extraction over five h. In the end of the process, the solvent was removed using a rotary evaporator (Cienlab, São Paulo, Brazil) with a reduced pressure of 45°C. The crude extracts from buriti's pulp (EPBU) and peel (ECBU) were stored in a sterile bottle and refrigerated (10 to 15°C).

Gas chromatography–mass spectrometry (GC-MS)

In order to analyze the chemical compounds presented in the plant extracts, they were derivatized (esterification reaction) by acid catalysis of boron trifluoride in methanol with heating (Meher et al., 2006). Analyses were carried out using a Shimadzu GC/MS QP Model 2010 Ultra chromatograph equipped with an HP-5MS (30 m × 0.25 mm × 0.25 µm) fused silica capillary column. Standards for the GC–MS were saturated alkanes (C11 - C40) The program temperature for the standards used was 50°C (0 min); 5°C min^{-1} reaching 310°C (20 min), in which the retention time of $C_{11}H_{24}$ is 10.020 min and that of $C_{13}H_{28}$ is 15.535 min in Split mode: 1:25. The heating ramp had been programmed for a temperature range of 50°C (0 min); 5ºC min^{-1} up to 300°C (10 min) at a speed of 3°C min^{-1}. Injection temperature: 300°C; Interface temperature: 250ºC in Split mode: 1:25. Helium gas was used as a carrier gas at a speed of 1.2 mL min^{-1}. The energy of the electron was 70 eV and the temperature of the ion source was 250°C. The compounds were identified by comparing the mass spectrometer and their GC retention data with standards. Further identifications were made by comparing the mass spectrometer with those of the NIST-08 (National Institute of Standards and Technology) libraries and those cited in the literature (Adams, 2017).

Antimicrobial assays

ATCC-type strains (American Type Collection Culture) were kindly provided by collection from the National Institute for Quality Control in Health at the Oswaldo Cruz Foundation (INCQS/FIOCRUZ – Rio de Janeiro, Brazil). The used bacteria used were: *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 25922), *S. aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853) and the yeasts used were: *Candida albicans* (ATCC 10231) and

Figure 1. *Mauritia flexuosa* is a palm tree that grows in and near swamps and other wet areas; (a) ripe fruit (b) fruit immersed in water (c) peeled fruit (d) and (e) shells separated for drying.
Source: Photos by the author.

Candida parapsilosis (ATCC 22019), microorganisms that are usually recommended for use in antimicrobial assays (Alves et al., 2008; Silva et al., 2012).

Antimicrobial sensitivity testing

The antimicrobial assays were performed in triplicate using the well diffusion method (CLSI, 2012) in Petri (140 × 15 mm) dishes with 50 mL of Muller Hinton Agar medium for bacteria and the same amount of Saboraud Agar medium for the yeast tests. Inoculum solutions were prepared using 3 to 4 colonies of the isolated strain in plates and diluted in 0.85% saline solution before reaching the corresponding turbidity of 0.5 on the McFarland scale (CLSI, 2003); that is, around 1.5×10^8 Colony Forming Units ($CFU.mL^{-1}$) of bacteria and 2.0×10^6 CFU mL^{-1} (Pelissari et al., 2010) of yeasts. A 10% solution of Dimethyl sulfoxide (DMSO) was used as the negative control, and 30 µg mL^{-1} of Fluconazole for the yeasts or 30 µg mL^{-1} of Chloramphenicol for the bacteria was used as the positive control. The solutions containing the inocula were swabbed on the surface of the media and the wells were made with a sterile cork borer. The wells were then filled with 50 µL of the tested extract diluted in 10% DMSO at concentrations of 200, 100 and 50 mg mL^{-1}, and with the positive and negative controls. After 24 h of incubation at 37°C (bacteria) and 25°C (yeasts), the microbial growth inhibition halos were measured in millimeters with a digital caliper.

Determination of the minimum inhibitory concentration (MIC): Determination of the minimum inhibitory concentration (MIC) was done using the broth microdilution technique as recommended by the Clinical and Laboratory Standards Institute (CLSI) (Lima et al., 2006). The tests were performed in a “sensitive microtiter” plate with 96 sterile wells only for microorganisms that presented inhibition in the well test (*E. faecalis*, *E. coli*, *S. aureus* and *P. aeruginosa)*. Initially, 100 µL of Muller Hinton growth medium was added to each well, followed by the extracts that were added by performing serial dilution as recommended by Benfatti et al. (2010), thus obtaining a range of concentrations of the pulp or peel extracts (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 $mg.mL^{-1}$). A solution of 2000 µg mL^{-1} of Chloramphenicol was used as the positive control, leading to serially diluted concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8 µg mL^{-1}. The negative control was 10% DMSO. Bacteria viability was tested using serial dilutions from a starting solution of 10^7 CFU mL^{-1}. In addition, control of media sterility was also executed. The 5 µL inoculum of the 10^7 CFU mL^{-1} bacterial solution was added to all except the sterility control wells. The plates were covered with plastic film and incubated at 37°C for 24 h. After the incubation period, 30 µL of a 1% aqueous reazurine (7-hydroxy-10-oxidophenoxazin-10-ium-3-one) solution was added to each well for 1 h. A resulting blue color in the well was read as growth inhibition and a reddish pink as non-inhibition.

RESULTS

Extract yields

The yield of the pulp extract (EPBU) was 14.13% and the

Table 1. Fatty acid composition (%) of the ethanol extract from *Mauritia flexuosa* peel (EPBU) and pulp (ECBU).

Fatty acid composition	ECBU % area	EPBU % area
12:0 lauric acid	38.52	84.08
14:0 myristic acid	-	3.97
16:0 palmitic acid	15.20	2.02
18:0 stearic acid	1.69	3.98
18:1 oleic acid	41.17	5.56
18:1 trans-11 vaccenic acid	0.77	-
18:2 linoleic acid	2.65	0.39

Table 2. Mean diameter of growth inhibition (in millimeters (mm)) of bacterial strains in susceptibility tests using the ethanolic extracts ECBU and EPBU (concentration: 50, 100 and 200 mg mL^{-1}) from *M. flexuosa* fruits.

Microorganism	Diameter of the inhibition halo (mm)					
	ECBU (mg mL^{-1})			EPBU (mg mL^{-1})		
	50	100	200	50	100	200
E. faecalis	9.38 mm± 0.267	11.23 mm ±0.416	12.88 mm ±0.181	-	-	-
E. coli	-	11.63±0.559	14.22 ±0.498	-	-	-
S. aureus	10.55 mm ±0.280	12.61 mm ±0.200	15.50 mm ±0.434	-	-	-
P. aeruginosa	-	-	9.56 mm ± 0.223	-	-	-
C. albicans	-	-	-	-	-	-
C. parapsilosis	-	-	-	-	-	-

ECBU = Ethanolic extract from *M. flexuosa* fruit peel, EPBU = Ethanolic extract from *M. fleuxuosa* fruit pulp.

yield of the peel (ECBU) was 22.30%.

Fatty acid determination by gas chromatography

The values obtained by gas chromatography for the chemical composition of fatty acids in the crude extracts are presented in Table 1. The ethanolic extracts of *M. flexuosa* fruit peels contained both saturated (55.41%) and unsaturated fatty acids (44.59%). The saturated fatty acid was primarily lauric (38.52%) acid, while unsaturated fatty acids included oleic (41.17%) and linoleic (2.65%) acids. The ethanolic extract of the pulp had a high content of saturated fatty acids (94.05%) and unsaturated fatty acids (5.95%). Saturated fatty acids in pulps included lauric (84.08%), myristic (3.97%) and stearic (3.98%) acids, and unsaturated fatty acids including oleic (5.56%) and linoleic (0.39%) acids.

Antimicrobial activity of crude extracts

The antimicrobial activity test was performed with the crude ethanolic extracts ECBU and EPBU from *M. flexuosa* (Table 2) in which EPBU showed no inhibition halo against the bacteria tested. The extract ECBU presented an inhibition halo ranging from 0 to 15.5 mm for all bacteria at a concentration of 200 mg/mL. The largest inhibition halo occurred against *S. aureus* and the smallest against *P. aeruginosa*. At a concentration of 100 mg/mL, all bacteria were inhibited except *P. aeruginosa*. The extract was able to inhibit *E. faecalis* and *S. aureus* at concentrations as low as 50 mg/mL, but was not able to inhibit the other tested strains.

Minimum inhibitory concentration (MIC)

The MIC results from the extracts ECBU and EPBU are shown in Table 3. The used extract concentrations used in the test ranged from 50 to 0.39 mg/mL. The ECBU extract presented an MIC of 12.5 mg/mL against *E. faecalis*, 25 mg/mL against *S. aureus,* and 50 mg/mL against other tested bacteria, with an inhibitory response in lower concentrations than EPBU, which had an MIC between 25 mg/mL against *E. coli,* and 50 mg/mL against the other tested bacteria.

DISCUSSION

The ethanolic extracts obtained from the peels and pulp

Table 3. Minimum inhibitory concentration (MIC) in mg/mL of crude ethanolic extracts from the peel (ECBU) and the pulp (EPBU) of *M. flexuosa* with antimicrobial activities.

Crude extract	*E. faecalis*	*E. coli*	*S. aureus*	*P. aeruginosa*
ECBU	12.5	50	25	50
EPBU	50	25	50	50

of *M. flexuosa* fruits were shown to be available and easily obtainable source of antimicrobials active against a range of bacterial strains. The Soxhlet system was chosen to obtain the extracts because it is a standard method in which the temperature and nature of the solvent determine and favor the extraction efficiency of the active compounds. Ethanol was the solvent chosen because it is affordable, comes from a renewable source, has low toxicity and is capable of extracting a wide range of polar compounds and some non-polar compounds (Bastos et al., 2010). EPBU yield was 14.13%, which is lower than values of 23.55% found in the literature (Carvalho et al., 2011) probably because the extraction method used hexane as the solvent instead of ethanol for 12 h in a Soxhlet extractor. On the other hand, the ECBU yield of 22.30% was greater than that found by Fuentes et al. (2013) of 13% using hexane as the solvent over 8 h.

The differences in yields obtained may be related not only to the nature of the solvents, but also to other factors such as temperature, soil type, humidity, and general sanity of the tree, etc. which can cause the plant to produce different substances. For example, Vasquez-Leon et al. (2017) showed that bioactive compounds in *Moringa oleifera* Lam. leaves are influenced by climatic factors, soil, and tree age. Milanez et al. (2018) discussed that buriti fruits harvested at different stages of ripening produced different quantities of total phenolic compounds, especially among fruits harvested at the ripened stage, where the levels of these compounds were higher.

The comparison between extracts obtained using ethanol and hexane shows that the percent of saturated fatty acids (55.41%) in ethanolic extracts of ECBU was lower than that extracted from the same fruit biomass when using hexane as the solvent (59%) (Forero-Doria et al., 2016). However, the percent of unsaturated fatty acids of ECBU (44.59%) was higher than what is reported by Darnet et al. (37.9%) (Forero-Doria et al., 2016), using hexane as the solvent. The percent of lauric acid in the ethanolic extract was higher (38.52%) than that obtained using hexane as a solvent (0.7%) (Fuentes et al., 2013). The obtained values for oleic acid (41.17%) and linoleic acid (2.65%) from ECBU were similar to the ones shown by Fuentes (2013), which has 33.4% for oleic acid and 3.7% for linoleic acid. Extraction using ethanol is a viable means of obtaining compounds from *M. flexuosa* fruits, especially the unsaturated fatty acids.

EPBU presented a higher percent of saturated acids (94.05%) than the values found in the literature [21.9% (Darnet et al., 2011) and 21.76% (Manhães and Sabaa-Srur, 2011)] and a lower percent of unsaturated acids (5.95%) compared to the values obtained for the hexane-extracted substrate (78.01 and 78.18%) (Manhães and Sabaa-Srur, 2011). The percent of oleic acid (5.56%) in ethanol-extracted EPBU was below what is commonly found in buriti pulp and lower than in hexane-extracted oil [75.7 and 73.32% (Manhães and Sabaa-Srur, 2011)]. The higher concentration of saturated fatty acids in the two ethanolic extracts (ECBU and EPBU) compared to extracts obtained using hexane is probably explained by the temperature increase during ethanol extraction (P.E. 78.37°C) as compared to hexane (68°C), which favored the extraction of the saturated compounds that are more resistant to oxidation and more stable at higher temperatures.

Antimicrobial activity tests were carried out with the agar dilution method that is widely used, since it presents simple execution and low cost, and could easily demonstrate the spectra of activity for both of the tested extracts. ECBU demonstrated activity against both G+ (*E. faecalis* and *S. aureus*) and G- strains (*E. coli* and *P. aeruginosa*), which indicates broad spectrum inhibitory activity against bacteria. However, it did not show activity against the yeasts tested (*C. albicans* and *C. parapsilosis*). The literature (Batista et al., 2012) reported an inhibition activity for the *M. flexuosa* pulp extract obtained with hexane extraction against *S. aureus* ATCC 6538. Silveira et al. (2005) showed that both ethanolic and hexanic extracts of *M. flexuosa* fruits were active against *S. aureus* and *P. aeruginosa*, but did not significantly inhibit *E. coli*.

Huang et al. (2011) demonstrated that fatty acids exhibit patterns of inhibition against oral bacteria with specificity that relates more to the bacterial species than the general structural characteristics of the microorganisms. This study also showed that fatty acids were much less effective against *C. albicans* than the oral bacteria, with effectiveness limited to hexanoic, octanoic, and lauric acids (Huang et al., 2011). We were not able to correlate the fatty acid composition to the halo of antimicrobial activity of the fruit since crude extracts were used for the testing of antimicrobial activity. Further studies of the antimicrobial activity of the combined or isolated fatty acids detected are needed to allow correlation of inhibition zone and fatty acid composition. It is also possible that the inhibition may be correlated not to a specific compound but to conjugated groups. Sugar

based surfactants conjugated with fatty acid chains are an emerging broad group of highly biocompatible and biodegradable compounds with established and potential future applications in the pharmaceutical, cosmetic and food industries. Lucarini et al. (2016) showed that synthetic lactose palmitoleate and lactose nervonate were shown to exhibit antimicrobial activity versus eight pathogenic species belonging to G+ and G- microorganisms and fungi.

EPBU showed no activity against the bacteria when tested with the well diffusion method. This result is different from (Mekonnen et al., 2016) probably because conditions in this experiment such as the extraction solvent and the microbial species and strains differed from other studies. The same EPBU extract presented a positive result in the MIC test and this may be related to the fact that this method allows for greater solubility of polar compounds (Miranda-Arámbula et al., 2017) that are present in the extract and better dispersion favoring interaction with the tested microorganisms (Valgas et al., 2007). It is also approximately 30 times more sensitive than the other methods described in the literature (Ostrosky et al., 2008). The MIC is widely used for simplicity, low cost, reproducibility, sensitivity and for using a minimum amount of reagents, which allows for a greater number of replicates, increasing the reliability of the results and leaving a permanent record.

The presence of fatty acids in *M. flexuosa* extracts could have been contributed to their antimicrobial activity. The antimicrobial effect of these acids occurs because they affect the cell wall, interfering with mechanisms of bacterial virulence such as the prevention of biofilm formation and inhibition of toxin and enzyme production (Ogidi et al., 2015). The entire process of investigation that included information retrieval, botanical identification of the species, research and experimentation provides subsidies for the production of efficient and inexpensive products. In addition, it could also be a social and economic reinforcement for families in the regions where the fruit is found and widely consumed.

Conclusion

Buriti (*M. flexuosa*) fruits and their products present great economic and social importance in the geographic areas where this plant is autochthonous. The obtained ethanolic extracts from the pulp and peel of these fruits showed antibacterial activity against the human pathogens studied. The gas chromatographic analysis (GC-MS) identified the fatty acids: lauric, myristic, palmitic, stearic, oleic and linoleic. Therefore, this study concludes that ECBU and EPBU present potential for pharmaceutical and technological applications due to the presence of bioactive compounds with antibacterial activity and has brought forward new information on the biotechnological potential of this Brazilian palm tree.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

ECBU, Ethanolic extract of Buriti bark; **EPBU,** ethanolic extract of Buriti pulp; **MIC,** minimum inhibitory concentration; **G+,** gram positive; **G-,** gram negative; **GC-MS,** gas chromatography coupled to mass spectrometer; **DMSO,** Dimethylsulfoxide; **ATCC,** American type collection culture; **CFU,** colony forming unit; **CLSI,** Clinical and Laboratory Standards Institute.

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