The Open Food Science Journal

Content list available at: https://openfoodsciencejournal.com

RESEARCH ARTICLE

Evaluation of the Antimicrobial Activity of Buriti (*Mauritia Flexuosa*) Pulp Extracts

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Abstract:

Background:

Buriti (*Mauritia flexuosa*) is a palm tree typical of the Amazon region. This plant belongs to the *Arecaceae* family and is economically important because it contains substances important for the food, cosmetic and pharmaceutical industries. It has, in its fruits, compounds with antimicrobial potential.

Objective:

The objective of this study was to evaluate the minimal inhibitory concentration and minimum bactericidal concentration of the Buriti extracts against the four bacteria: *Salmonella* enterica serotype Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Methods:

15 extracts from buriti pulp were obtained varying in temperature, mass of buriti and ethanol content. The antimicrobial activity of these extracts was evaluated. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the methodology recommended by the Clinical and Laboratory Standards Institute was followed.

Results:

The results showed which buriti pulp extracts had strong inhibitory activity. Gram-positive results ranged from 21 to 78 $\mu g.mL^{-1}$. For Gram-negative, they ranged from 30 to 111 $\mu g.mL^{-1}$.

Conclusion:

The buriti extracts significantly slowed the growth of the tested bacteria.

Keywords: Buriti, Antimicrobial activity, Pulp extract, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, *Mauritia Flexuosa*.

Article History	Received: August 15, 2019	Revised: October 16, 2019	Accepted: November 07, 2019

1. INTRODUCTION

Food is required to meet the increasing demands in our daily life. Reproduction of microorganisms is the most important factor in the spoilage of perishable foods [1]. There is concern over groups of microorganisms responsible for food spoilage, leading to unpleasant smells, flavors and colors that decrease shelf life [2]. In addition, food borne outbreaks could cause serious economic losses [3]. So, preservatives that can maintain the quality of these foods for a longer time should be used. However, the industry still uses chemical preservatives that no

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longer meet the demand of consumers who want to consume safe, nutritious and tasty food [4]. Ingestion of synthetic additives has a cumulative effect on the organism causing toxicity and may even be carcinogenic. For this reason, interest in research of natural antimicrobial agents for food is growing, being an alternative to the use of artificial antimicrobial agents [5]. In this sense, the use of plant extracts may be an alternative because they have antimicrobial properties and are, generally, not toxic to humans [6].

Buriti (*Mauritia flexuosa)* is a palm tree typical of the Brazilian Amazon region, and belongs to the family *Arecaceae* that includes 34 species [7, 8]. Brazil produces 10,000 tons of buriti fruit annually according to the data from the Brazilian Institute of Geography and Statistics [9]. It is of economic importance because compounds such as β-carotene, tocopherols, and oleic acid can be applied in the cosmetic and food industries [10]. Generally, fruit pulps have a composition rich in phenolic compounds which act as antioxidants, which in turn are important in reducing cardiovascular disease and combating free radicals [11], as well as containing substances such as carotenoids, ascorbic acid and components such as phytosterols [12 - 14]. Its shell is a brown or reddish color [15] and its pulp is yellow [16]. In addition, buriti is considered an oleaginous species and its antimicrobial efficiency was been shown in previous studies [7]. In studies on rats, it showed to be efficient in the healing process of cutaneous wounds and its antibacterial activity against *Enterobacter aerogenes*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Staphylococcus aureus* was verified, which also proves its phytotherapeutic and antimicrobial activity [17]. The objective of this work was to obtain extracts under different conditions and evaluate their antimicrobial activity. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined for a number of bacteria, important for food safety.

2. MATERIALS AND METHODS

2.1. Source of Raw Material

The buriti samples (Fig. **1a**) were purchased at a local market in the town of Itapecuru Mirim-MA/Brazil, harvested in January 2016, transported by air in a cool box to Campo Mourão-PR/Brazil and sent to the Food Microbiology Laboratory of the Federal Technological University of Paraná (UTFPR), Campo Mourão Campus. Afterwards, they were cleaned and peeled as proposed by Sampaio & Carazza [16] and then the fruits were washed by brushing in running water and sanitized with sodium hypochlorite (20 ppm). After 15 minutes in this hypochlorite solution, the shells were removed manually using a knife. Following the removal of the shell, the pulp was removed. These pulps (Fig. **1b**) were put into polypropylene plastic bags (30 x 40 cm) with a capacity of 100 g and vacuum-packed using an SVB 400 packager (Sulpack, Caxias do Sul, Brazil).

2.2. Extractions

To assess the influence of temperature, ethanol percentage (ratio ethanol:water) and buriti mass on the antimicrobial activity of the extracts, 5 levels (−1.41(α), −1, 0, 1, +1.41(+α)), of these factors were tested as described in Table **1**. The assays were performed by varying the factors described in Table **2**. Thus, to perform the extractions, a certain mass of buriti was weighed in a falcon tube and diluted in 50 mL of ethanol:water. This solution was placed in a 713D mechanical propeller shaker (Fisatom Equipamentos Científicos, São Paulo, Brazil) for 30 minutes while kept at different temperatures. Using an SL-154 circulating thermostatic bath (Solab, Piracicaba, Brazil). Subsequently, an NT-815 centrifuge (Nova Técnica, Piracicaba, Brazil) with a rotation of 5242 G-force was used to remove the supernatant. Then, the ethanol fraction was evaporated at low pressure on a TE-211rotary evaporator (TECNAL, Maringá, Brazil) at 35 °C. This content was frozen in falcon tubes in a UFR30 vertical freezer (Liotop, São Carlos, Brazil) at -77 °C. Then the sample was lyophilized in an L101 freeze drier (Liotop, São Carlos, Brazil) for approximately 72 hours thus obtaining 15 extracts under different conditions.

2.3. Determination of Minimum Inhibitory Concentration (MIC)

To evaluate the antimicrobial activity of the extracts, the broth microdilution method was used following the protocol established by M100-S22/2012 document from the Clinical and Laboratory Standards Institute [18].

The assays were performed in 96 well microplates, which have markings indicating the position of each well, columns (1 to 12) and lines (A to H). One Gram-positive (*Staphylococcus aureus* ATCC 25923) and three Gram-negative bacteria (*Salmonella enterica* serotype Typhimurium 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were used as targets.

The bacterial isolates were grown at 35 °C for 24 hours on Hektoen agar for *S.* Typhimurium *and P. aeruginosa*; on Eosin Methylene Blue (EMB) agar for *E. coli*; and the Gram-positive *S. aureus* was cultivated on Mannitol Salt Agar (MSA) at 35 °C for 48 hours. Characteristic colonies were grown in Mueller Hinton broth (MHB) and incubated at 35 °C for 4 to 6 hours. The bacterial suspension was standardized in saline solution according to the McFarland standard in the range of 0.5, which corresponds to 5.10^8 $CFU.mL^{-1}$. To measure the MIC, lyophilized extracts were re-suspended in 0.1% Tween. The initial concentrations of each extract varied according to the weight of the lyophilized extract. The extracts at their initial dilution were added to the 96-well microplate and diluted 1:1 in serial dilutions. The microplates were incubated at 35 °C for 24 h. After the incubation, a visual reading was performed. A positive control containing MHB and the bacterial isolate was made and negative control was made with MHB and extracts in the absence of the bacterial isolates.

2.4. Determination of the Minimum Bactericidal Concentration (MBC)

For the determination of the Minimum Bactericidal Concentration (MBC), a 10 µL aliquot from each well in which no bacterial growth was visible in the MIC assay was transferred to the MHB and incubated at 35 °C for 18-24 h. Each test was performed in triplicate.

2.5. Experimental Design

A statistical experimental design was based on the Central Composite Design (CCD) with one dependent variable: antimicrobial activity. This response was measured according to the following variables: Temperature (°C), ethanol content (%) and buriti mass (g). These process variables were investigated at five levels (−1.41(−α), −1, 0, 1, +1.41(+α)), providing 15 combinations of assays. The range of the factors was 2.5 to 10 g, 15 to 65% and 30 to 70°C for pulp mass, ethanol content and temperature, respectively.

a) b)

Fig. (1). Photos taken of buriti fruit (**a**) and pulp (**b**).

Table 1. Levels of the independent variables evaluated to obtain the extracts that were used to determine the antimicrobial activity.

Independent Variables	Evaluated Levels				
	-α	**-1**	**0**	**1**	**+α**
Buriti mass (g)	2.50	4.02	6.25	8.48	10.00
Ethanol content (%)	15.00	25.13	40.00	54.87	65.00
Temperature (° C)	30.00	38.00	50.00	62.00	70.00

3. RESULTS

3.1. Assessment of Influencing Factors on the Inhibition of the Gram-positive Bacterium

As shown in Table **2**, all extracts, apart from that of assay 2, were able to inhibit the growth of *S. aureus* at the concentrations evaluated. The hydroalcoholic extract that was able to inhibit the growth of *S. aureus*, with a lower concentration of buriti pulp was Extract 9 with a MIC and an MBC of 21 μg.mL^{-1}. Extract 10 showed the highest concentration, with a MIC and an MBC of 96 μg.mL^{-1}.

3.2. Assessment of Influencing Factors on the Inhibition of the Gram-negative Bacteria

For all three Gram-negative bacteria tested, extract 4, whose extraction conditions were 4.04 g of buriti, 54.8% of ethanol and 62 °C, showed a MIC and an MBC value of 37 μg.mL^{-1}. While extracts 2 and 9 did not showed antibacterial effect for this kind of bacteria, even in the highest concentration tested.

For *S.* Typhimurium, extracts 2, 3, 7, 9 and 12 were unable to inhibit the growth of this microorganism at the highest concentrations evaluated. The hydroalcoholic extract that had the best MIC result was extract 5 with MIC and an MBC of 30 μg.mL^{-1}, and the extract with the highest concentration was extract 8 with a MIC and an MBC of 64 μg.mL^{-1}.

For *P. aeruginosa,* extract 8 had the best result with a MIC and an MBC of 32 μg.ml^{-1}, while the extract 7 showed the highest concentration to inhibit it, with a MIC and an MBC of 83 μg.mL^{-1}. However extracts 1, 2, 9, 11 and 14 did not inhibit the growth of this bacterium even at the highest concentrations evaluated.

For *E. coli,* extract 4 inhibits its growth with a MIC and an MBC of 37 μg.mL^{-1}, the best of all extracts tested. However, extract 10 only inhibited the growth of the bacterium with a MIC of 111 μg mL^{-1}. Extracts 2, 3, 8, 9, 11 and 12 at the highest concentrations tested were not able to inhibit the growth of this microorganism.

3.3. Discussion

It is known that the antimicrobial activity is associated with the phenolic compounds [19]. Table **3** shows the phenolic compounds present in the buriti pulp. Some phenolic compounds of this fruit [20] such as syringic acid, protocatechuic acid, catechin and ferulic acid present antimicrobial activity as reported by various authors [21 - 24]. In this way, the variables studied correlate with the phenolic content and with the antimicrobial activity. For instance, increasing the mass in the extraction process implies a lower solute-solvent interaction and so smaller masses lead to higher phenolic values [25, 26]. In the same way, an increase in the ethanol content [27, 28] and higher temperatures [28, 29] lead to greater amounts of phenolics. Moreover, the temperature increase affected the concentration of each phenolic compound as demonstrated by Rudke *et al.* [10], when studying extracts of buriti shells at different temperatures.

(Fig. **2**) shows the extracts which had the best results for MIC and MBC. Extracts 2 and 9 did not have any effect against Gram-negative bacteria at the concentrations evaluated. However, for the other extracts tested, there was a satisfactory result. Gram-negative bacteria is more resistant to natural compounds, which, according to Cruz *et al.* [30], is due to differences in the cell wall barrier properties of these bacteria.

Results reported by Soares [31] showed that the antibacterial activity of buriti oil inhibited growth of *S. aureus*, *E. coli* and *P. aeruginosa* with MICs of 74.8 μg.mL^{-1}, 623.2 μg.mL^{-1} and 312.5 μg.mL^{-1} respectively and MBCs of 309.4 μg.mL^{-1}, 1230 μg.mL^{-1} and 629.7 μg.mL^{-1} respectively, which demonstrates that the results obtained in this work with hydroalcoholic extract of buriti were more satisfactory. This difference between the results here in this work and the results obtained by Soares [31] could be cause she works only with the oil, while we used the hydroalcoholic extract, that could have more antibacterial compounds. In addition, the plant is cultivated in various places in Brazil, with different climates and varied geographical conditions which leads to pulps with different characteristics [32].

Table 2. Experimental design and determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration for the bacteria tested.

–	VARIABLES			Gram-positive	Gram-negative		
Extract	M (g)	E (%)	T (°C)	*S. aureus* (µg.mL^{-1})	*S.* Typhimurium (µg.mL^{-1})	*P. aeruginosa* (µg.mL^{-1})	*E. coli* (µg.mL^{-1})
1	4.02	25.13	38.00	39.00	42.00	-	42.00
2	4.02	25.13	62.00	-	-	-	-
3	4.02	54.87	38.00	32.00	-	35.00	-
4	4.02	54.87	62.00	29.00	37.00	37.00	37.00
5	8.48	25.13	38.00	70.00	30.00	60.00	60.00
6	8.48	25.13	62.00	60.00	59.00	59.00	59.00
7	8.48	54.87	38.00	78.00	-	83.00	83.00
8	8.48	54.87	62.00	63.00	64.00	32.00	-
9	2.50	40.00	50.00	21.00	-	-	-
10	10.00	40.00	50.00	96.00	55.50	55.50	111.00
11	6.25	15.00	50.00	40.00	36.00	-	-
12	6.25	65.00	50.00	29.00	-	35.00	-
13	6.25	40.00	30.00	43.00	49.00	49.00	49.00
14	6.25	40.00	70.00	40.00	44.00	-	44.00
15	6.25	40.00	50.00	51.00	50.00	50.00	50.00

M- mass; E-ethanol; T - Temperature.
-: there was no inhibition at the highest concentrations evaluated.

Table 3. Phenolic Compounds founded by Bataglion *et al.* [36], in buriti pulp.

PHENOLIC COMPOUNDS	Bataglion *et al.* [36]*
p-Coumaric acid	277.74 ± 12.44
Ferulic acid	184.66 ± 8.86
(+)-Catechin	961.21 ± 2.68
(−)-Epicatechin	1109.93 ± 4.24
Apigenin	102.48 ± 0.29
Luteolin	1060.90 ± 6.95
Myricetin	145.11 ± 5.15
Caffeic acid	895.53 ± 4.80
Kaempferol	41.54 ± 4.47
Quercetin	83.27 ± 1.01
Protocathecuic acid	2175.93 ± 18.01
Quinic acid[#]	230.74 ± 11.29
Chlorogenic acid	1154.15 ± 9,69

* Concentration in fruits µg.g^{-1} DWP. [#] Quinic acid is expressed in mg.g^{-1} DWP.

Corroborating these results, in studies with buriti extracts from trunk, leaves and fruits Koolen *et al.* [7], investigating the ability of the extract to inhibit the growth of *S. aureus, P. aeruginosa, E. coli* and *B. cereus,* found a minimum inhibitory concentration of 50 µg.mL^{-1} for *S. aureus* and *P. aeruginosa* and for the other two bacteria tested there was no inhibition.

The hydroalcoholic extract of buriti pulp showed activity with both Gram-positive and Gram-negative strains. The presence of saturated and unsaturated fatty acids may have been responsible for the inhibition of the strains [33, 34]. Buriti oil has a composition of unsaturated fatty acids and some compounds in smaller amounts, such as tocols, carotenoids and phenolics [35], where studies demonstrate the antimicrobial activity of this compound [17].The intensity of the extracts as antimicrobial agents is based on their MIC value, being classified as a strong inhibitor with MICs up to 500 µg.mL^{-1}, a moderate inhibitor with MICs between 600 and 1500 µg.mL^{-1} and a weak inhibitor with MICs above 1600 µg.mL^{-1} [35]. By this measure, the present study showed very intense inhibitory activity because the MIC values were all below 500 µg.mL^{-1}.

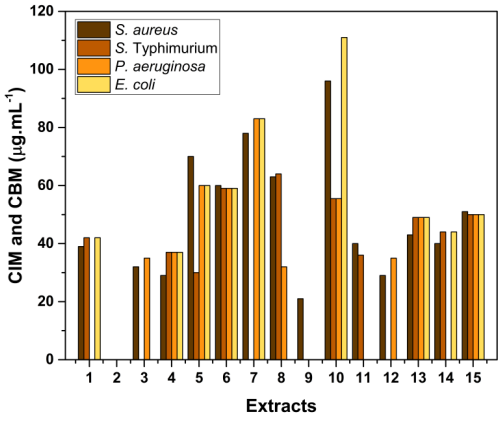

Fig. (2). CIM and CBM of the different extracts for the strains of bacteria tested.

CONCLUSION

This research shows that the hydroalcoholic extract of buriti (*Mauritia flexuosa*) tested showed antibacterial activity, significantly reducing the growth of *Salmonella enterica* serotype Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Future studies could be performed in different media and concentrations and directly with food, to verify the ability of the extract to inhibit bacteria. In this way, buriti could become very promising for applications in food prolonging the shelf life and maintaining the security of food.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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