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In vitro antibacterial action on methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* and antitumor potential of *Mauritia flexuosa* L. f.

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Mauritia flexuosa **L.f. (Arecaceae), commonly known as the buriti palm, is used in traditional medicine in Brazil. Oil from fruits is used for wound healing of skin burns and as an anti rheumatic agent. The stem pith is used to treat dysentery and diarrhea. The aim of this study was to evaluate the *in vitro* antibacterial and cytotoxic activities of *M. flexuosa* L.f. extracts and fractions. Crude ethanol extracts (EtOH) from fruits, leaves, and stems were submitted to liquid-liquid partition to yield four different soluble fractions: hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and aqueous (Aq). The antibacterial activity was evaluated using the broth micro dilution method against methicillin-susceptible *S. aureus* (MSSA - ATCC 29213) and methicillin-resistant *S. aureus* (MRSA – clinical sample 155). Cytotoxicity activity was determined against five human tumor cell lines (HL-60, Jurkat, THP-1, MCF-7, and HCT-116) and Vero cell as a model for toxicity control, using MTT colorimetric assay. Across all extracts and fractions assayed, only DCM fractions from leaves and stems were active against *S. aureus* (MSSA and MRSA) at concentrations ranging from 31.3 to 62.5 µg/ml, and against tumor cell lines with IC_{50} values ranging from 20.3 to 79.0 µg/ml. The DCM fraction from stems was purified by gel permeation chromatography (GPC) to yield G1 to G12 groups. Groups G7 to G12 showed antimicrobial activity against MSSA and MRSA with MIC values of 31.3 to 125.0 µg/ml and four groups (G3, G9, G10, G12) exhibited cytotoxic activities in the range of 20 µg/ml against at least one tumor cell line. Using the hyphenated methodology, liquid chromatography-mass spectrometry (LC-MS), it was possible to identify several phenolic compounds in subgroups G7 to G10 that can be associated with the medicinal properties of stems from buriti.**

Key words: *Mauritia flexuosa*, antibacterial activity, antitumoral activity, phenolic compounds.

INTRODUCTION

Mauritia flexuosa L.f. is a palm that can reach up to 30 and 40 m in height and is known as the buriti palm in Brazil. It grows in flooded areas where it forms extensive clusters, and also occurs in the humid savannas of the central-west, as well as in the north and northeast regions of Brazil (Nascimento, 2010).

The buriti palm is of great cultural and economic importance to the riverside communities in Northern Brazil, particularly in the Pará and Amazon states where it provides many domestic uses such as wine, cooking oil, the production of artisanal crafts and toys, and for constructing the walls of dwellings (Plotkin and Balick, 1984; Santos and Coelho-Ferreira, 2011). Additionally, the plant is traditionally used as vermifuge and anti-rheumatic in the healing of skin burns (Souza and Felfili, 2006), and also to treat dysentery (Plotkin and Balick, 1984).

Despite the popular use of *M. flexuosa*, few studies have investigated its biological properties. Previous studies have shown that a cream containing 10% buriti oil administrated in Wistar lineage rats increased the production of fibroblasts and collagen fibers, promoting re-epithelialization (Batista et al., 2012). It was also shown that buriti oil inhibited the growth of four bacteria (*Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 1304, *Klebsiella pneumoniae* ATCC 10031 and *Staphylococcus aureus* ATCC 6538), using an agar diffusion method (Batista et al., 2012).

S. aureus is the most commonly isolated human bacterial pathogen, and is a prominent causer of skin infections, pneumonia, endocarditis, toxic shock syndrome, scalded skin syndrome, osteomyelitis, and sepsis. This pathogen is associated with high morbidity and mortality, mainly in hospitalized patients suffering from immune deficiencies or viral infections (Otto, 2014). Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other β-lactam antimicrobial drugs, thus the costs associated with treatment, and consequences associated with the infections caused by this pathogen are considerable (Rubin et al., 1999).

Cancer chemotherapies have shown clinical benefits such as recovery and prolonging patient survival. However, several studies have reported that tumor heterogeneity and additional mutations of cancer cells create challenges in the development of effective targeted therapies (Joo et al., 2013). The influence of natural products or compounds directly derived from them as approved drugs for the treatment of the cancer between 1981 and 2010 is quite outstanding (Newman and Cragg, 2012). The successes of these small molecules demonstrate that the identification of new drugs is still an adequate approach, since in the future the therapies will be based on tumor phenotype or specific markers present in the patients (Joo et al., 2013).

Considering the biological activity of buriti oil and the search for new drugs from natural products, the main objective of the present study was to perform a comparative evaluation of the antimicrobial activity of the *M. flexuosa* extracts and partitioned fractions from fruits, leaves, and stems against MSSA and MRSA. It also aimed at evaluating differential antiproliferative activity against five human tumor cell lines that included T lymphocyte (Jurkat), promyelocytic leukemia (HL60), acute monocytic (THP-1), colorectal carcinoma (HCT-116) and breast cancer (MCF-7) cell lines.

MATERIALS AND METHODS

Plant

The stems, leaves and fruits of *M. flexuosa* L. f. (Arecaceae) were collected at “Chapada Gaúcha”, Minas Gerais, Brazil (15° 33’ 690 S; 45° 23” 849 W). The voucher specimen (BHCB 173387) was deposited in the herbarium of the Institute of Biologic Sciences of the Federal University of Minas Gerais, Minas Gerais, Brazil.

Preparation of extracts

Collected stems, leaves, and fruits were washed in running water before being dried at room temperature. The fresh plant material was chopped into small pieces of approximately 5 × 5 cm^2, and extracted three times with absolute ethanol at room temperature, with a three day period between extractions. The mixtures were filtered through cellulose filter paper and evaporated to dryness under reduced pressure using a rotary evaporator at 40°C. The residual solvent was removed in a vacuum centrifuge at 40°C to yield crude ethanol extracts of stems, leaves, and fruits.

Preparation of *M. flexuosa* partitioned fractions

An aliquot of the crude ethanol extract of each part of the plant was suspended in MeOH:H_2O (8:2) using an ultrasonic bath and extracted successively by solvent-solvent partitioning to give the soluble fractions: hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and aqueous (Aq), respectively.

Procedure for gel permeation chromatography (GPC)

GPC was carried out using two coupled glass columns (Büchi column n° 17980) filled with Sephadex LH-20™ gel (GE Healthcare, U.S.A.) and absolute ethanol as mobile phase pumped at flow rates of 120 ml/h. Fractions were collected at 20 ml/tube and pooled after thin layer chromatography (TLC) analysis.

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Procedure for TLC analyses

TLC analyses were carried out on pre-coated commercial silica gel plates G-60/F_{254} (0.25 mm, Merck, Darmstadt, Germany). TLC plates were eluted in a pre-saturated chamber, using solvents mixtures in different proportions of: (a) ethyl acetate: methanol: water (EtOAc:MeOH:H_2O/70:10:2 v/v); or (b) dichloromethane: methanol (DCM:MeOH/95:5 v/v). The spots were visualized under UV light at 254 or 360 nm, alternatively by means of spraying the plates with a mixture (1:1 v/v) of ethanol solution of vanillin 1% w/v and sulfuric acid at 10% v/v or 2-aminoethyl diphenylborinate/polyethylene glycol 4000 (NP/PEG) solution in ethanol at 2% (w/v).

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analyses

LC-HRMS (MSMS) analyses were performed on a Nexera UHPLC-system (Shimadzu) hyphenated to a maXis ETD high-resolution ESI-QTOF (ElectroSpray Ionization - Quadrupole Time of Flight) mass spectrometer (Bruker, Germany) and controlled by the Compass 1.5 software package (Bruker, Germany). Solutions of the samples at 200 µg/ml in ACN:H_2O (1:1 v/v) were injected on a Shimadzu Shim-Pack XR-ODS-III column (C18, 2.2 µm, 80 Å, 2.0 × 200 mm) with a flow rate of 200 µl/min. The samples were eluted with mixtures of water with 0.1% formic acid (A), and acetonitrile plus 0.1% formic acid (B) using 10% B for 10 min and a linear gradient from 10% B to 100% B in 40 min and 100% B for 5 min. Ion-source parameters were set to 500 V end plate offset, 4500 V capillary voltage, 2.0 bar nebulizer pressure, 8.0 L/min dry gas flow at 200°C. Data dependent precursor fragmentation was performed at collision energies of 30 eV. Ion cooler settings were optimized for average sensitivity in a 40 to 1000 m/z range using a solution of 10 mM sodium formate in 2-propanol/0.2% formic acid (1:1, v/v) as calibration solution. Mass calibration was achieved by initial ion-source infusion of 20 µl calibration solution and post-acquisition recalibration of the raw data. Compound identification was performed by chromatographic peak dissection with subsequent formula determination according to exact mass and isotope pattern (MS^1) and database comparison of compound fragment spectra (MS^2) as well as by comparison of compound fragment spectra and co-elution with standard compounds from Sigma-Aldrich. Sources of reference ESI fragmentation pattern spectra consisted of an in-house database of commercial or isolated and identified compounds as well as the public spectra database MassBank (Horai et al., 2010). Spectral similarity indices (fit scores) above 60% were considered and manually proved.

Preparation of extracts and fractions for biological analyses

Stock solutions of the samples were prepared in dimethyl sulphoxide (DMSO; Sigma-Aldrich) at 20 mg/ml, resulting in homogeneous solutions.

Antimicrobial assays

Antibacterial activity was evaluated using the following microorganisms, methicillin-susceptible *S. aureus* (MSSA - ATCC 29213) and methicillin-resistant *S. aureus* (MRSA – clinical sample 155).

Minimum inhibitory concentration (MIC) was determined based on the broth micro dilution method, according to the methodology proposed in document M07-A9 (Clinical and Laboratory Standards Institute - CLSI 2012), with modifications. Briefly, samples were serially diluted two-fold in 100 µl Brain Heart Infusion (BHI) Broth (Himedia) in flat-bottomed 96-well polystyrene microtiter plates, in order to achieve concentration ranges from 0.98 to 1000 µg/ml.

The bacterial inoculum (100 µl) was then added into each well to reach a final concentration of approximately 5 × 10^5 CFU/ml. Growth and sterility controls were included for each assay and tests were performed in triplicate. The solvent (DMSO) served as negative control and was used at a final concentration of ≤5% v/v. Gentamicin and kanamycin (Sigma-Aldrich) were used as positive controls.

The micro dilution plates were incubated under aerobic conditions at 37°C for 24 h. The MIC was defined as the lowest concentration of sample that completely inhibited visible growth of microorganisms in the micro dilution wells.

Assays with human tumor cell lines

Five human tumor cell lines were used: Jurkat (human immortalized line of T lymphocyte), HL-60 (human promyelocytic leukemia), THP-1 (acute monocytic), MCF-7 (breast cancer) and HCT-116 (colorectal carcinoma). The Vero cell (African green monkey kidney cells) was used as a model for toxicity control. All lines were maintained in the logarithmic phase of growth in RPMI 1640 or D-MEM supplemented with 100 µg/ml penicillin and 100 µg/ml streptomycin enriched with 2 mM of L-glutamine. HL-60 cells were cultured in RPMI and 10% (v/v) fetal bovine serum. The adherent cells were maintained in D-MEM enriched with 5% (v/v) of fetal bovine serum. All cultures were maintained at 37°C in a humidified incubator with 5% CO_2 and 95% air. The media were changed twice weekly and the cells were regularly examined. All cell lines were used for a maximum of 20 replications.

Cell lines were inoculated at the densities: 1 × 10^4 cells (MCF-7, HCT-116 and Vero), 5 × 10^4 cells (HL-60) and 1 × 10^5 cells (Jurkat and THP-1) per well. The plates were pre-incubated for 24 h at 37°C to allow the adaptation of cells prior to the addition of the samples. The half maximal inhibitory concentration (IC_{50}) was determined over a range of concentrations (eight nonserial dilutions: from 100 to 1.5 µg/ml). All cell cultures were incubated in a 5% CO_2/95% air-humidified atmosphere at 37°C for 48 h. The negative control consisted in a treatment of 0.5% DMSO. Cell viability was estimated by measuring the rate of mitochondrial reduction of tetrazolium–dye (MTT). All samples were tested in triplicate, in three independent experiments (Monks, 1991). The cytotoxicity of the controls (etoposide, doxorubicin and cisplatin) were evaluated under the same experimental conditions as positive controls.

RESULTS AND DISCUSSION

Bioguided fractionation

Bioguided fractionation is a typical method for identifying active components of crude extracts. Figure 1 depicts the flowchart used in the purification of the *M. flexuosa* crude ethanol extract from stems. The yield of the crude ethanol extracts relative to the weight of dry plant material ranged from 8.6% (w/w) for fruits and leaves, to 18.2% (w/w) for the ethanol extract from stems (Figure 2).

The components of *M. flexuosa* extracts were initially fractionated by liquid-liquid partition with solvents of increasing polarity. Partitioning of crude ethanol extract from fruits revealed a large dispersion of compounds with different polarities as shown by the yield of the Hex (39.9%) and Aq (37.6%) fractions (Figure 2). Compared

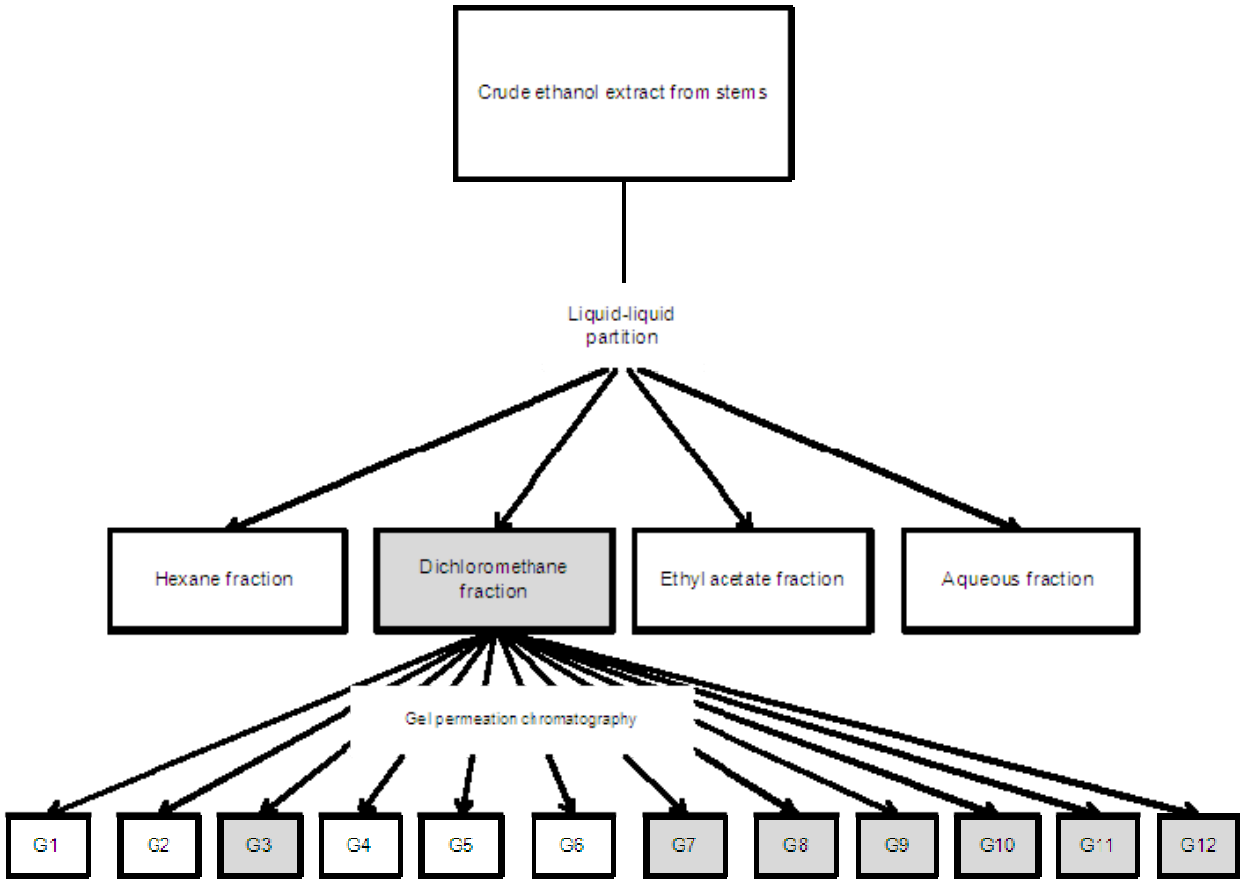

Figure 1. The flowchart presents the methodology adopted for bioguided fractionation of the *M. flexuosa* crude ethanol extract from stems. Fractions and groups in gray showed biological activity.

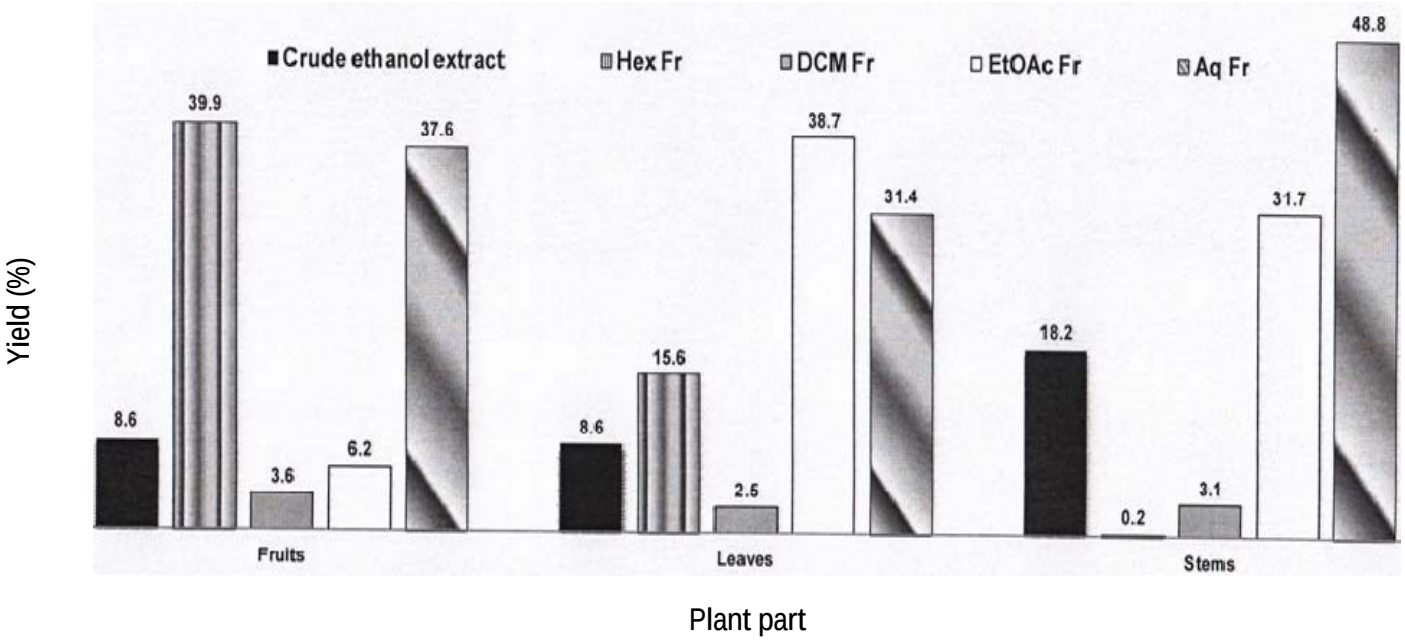

Figure 2. Yield of extraction/partition. Percentage yield of crude ethanol extract (w/w) was estimated as dry crude ethanol extract weight/dry initial weight×100. Yield percentage of fractions (w/w) was estimated as dry fraction weight/dry starting crude ethanol extract weight×100.

Table 1. Antimicrobial and cytotoxic activities from *Mauritia flexuosa*.

Sample	MIC (µg/ml)		IC_{50} (µg/ml)***					
	MRSA*	MSSA*	*HL-60*	*Jurkat*	*MCF-7*	*HCT-116*	*THP-1*	*Vero*
Leaves								
DCM fraction	62.5	>1000	20.3±0.7	62.3±8.7	>100	65.1±12.7	>100	>100
Stems								
DCM fraction	31.3	31.3	27.7±1.4	55.5±1.6	79.0±17.2	74.6±17.0	>100	>100
Controls								
Kanamycin	256	<0.5	-	-	-	-	-	-
Gentamicin	256	2.0	-	-	-	-	-	-
Etoposide**	-	-	1.8 ± 0.7	-	-	-	2.1 ± 1.2	>100
Doxorubicin**	-	-	-	-	12.9 ± 2.3	3.6±0.2	-	57.2 ± 6.6
Cisplatin**	-	-	0.06 ± 0.02	17.1 ±4.3	-	38.1 ± 5.3	-	72.7 ± 16.5

*MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin-susceptible *Staphylococcus aureus.* **Concentration in µM.
***Values referred as media±SD.

across all Hex fractions, the fruits yielded 39.9% of nonpolar compounds, while the stems produced only 0.2% of these components. In addition, it was observed that the crude ethanol extracts from the stems and leaves contained a great quantity of polar compounds represented by their EtOAc and Aq soluble fractions.

Antibacterial and cytotoxicity screening of the crude ethanol extracts and partitioned fractions

Across all extracts and fractions tested, only DCM fractions from leaves and stems were able to inhibit the growth of *S. aureus*. DCM fraction from leaves exhibited MIC values of 62.5 µg/ml against MRSA and DCM fraction from stems showed MIC values of 31.3 µg/ml against both strains. Preliminary cytotoxic studies with the extracts and fractions were carried out in a constant concentration of 20 µg/ml. Only the DCM fractions from leaves and stems were able to inhibit the growth of the lineages by 50 to 80% (data not shown), thus they were tested for the determination of the IC_{50} values (dose-response effect) in a concentration range from 0.1 to 100 µg/ml. They showed IC_{50} ranging from 20.3 ± 0.7 to 79.0 ± 17.2 µg/ml (Table 1) and were nontoxic against THP-1 and Vero cell lines (IC_{50} > 100 µg/ml) in the range of concentrations tested. The HL-60, a human pro-myelocytic leukemia cell line, was the most susceptible to both DCM soluble fractions which exhibited IC_{50} values in the range of 20 µg/ml against this tumor cell line.

According to the literature, investigation of the pharmacological properties and chemical composition of *M. flexuosa* L.f. extracts is still limited. Although previous work has shown that buriti oil is able to inhibit the growth of *S. aureus* (ATCC 6538) using the agar diffusion method (Batista et al., 2012), any nonpolar fraction, including the hexane fraction from fruits, that was found to be an oil, did not show activity against the strains of *S. aureus* (ATCC 29213 and clinical sample 155) by micro dilution assays. In addition, polar extracts previously defatted from the fruits, leaves, and trunks of the buriti palm had weak and moderate activity against *S. aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Micrococcus luteus* (ATCC 4698), with minimal inhibitory concentrations ranging from 200 to 50 µg/ml. The extracts were found to inhibit *S. aureus* in the range of 50 to 100 µg/ml (Koolen et al., 2013b).

Zanatta et al. (2008, 2010) showed that buriti oil from fruits has low irritant properties to the skin through *in vitro* assays using human keratinocyte cell line (HaCaT) and mouse embryonic fibroblast cell line (3T3). Recently, two triterpenes isolated from the roots of *M. flexuosa* L.f. were tested against several bacteria and fungi, including *S. aureus* and *Staphylococcus epidermidis*. They showed high MIC values; in general above 50 µM. In this same investigation, the triterpene mauritic acid exhibited cytotoxic activity against OVCAR-8, PCM3 and NCIH358M cell lines with IC_{50} values of 3.02, 2.39, and 6.19 µM, respectively (Koolen et al., 2013a).

Preliminary fractionation of DCM fraction from stems and biological activities

DCM fraction from stems was active against four tumor cell lines and yielded the best results for both strains of *S. aureus*. In addition, the greatest yield from crude ethanol extract was returned (Figure 2). This fraction was submitted to the GPC followed by TLC analysis, from which twelve groups (G1 to G12) were obtained. They were

Table 2. Antimicrobial and cytotoxic activities of the groups from dichloromethane fraction from *Mauritia flexuosa* stems.

Group	MIC (µg/ml)		IC_{50} (µg/ml)**					
	MSSA	MRSA	*HL-60*	*Jurkat*	*MCF-7*	*HCT-116*	*THP-1*	*Vero*
G3	>500.0	>500.0	2.0±0.5	67.8±11.5	28.9±0.7	6.0±2.0	7.4±2.0	>100
G7	31.3	125.0	56.8±10.3	75.7±21.8	69.5±10.4	78.4±14.3	57.3±4.0	70.7±21.3
G8	31.3	125.0	38.0±15.7	80.6±35.1	104.5±59.2	83.3±24.6	74.3±12.5	79.8±35.6
G9	31.3	62.5	21.6±12.6	42.2±21.8	43.2±21.0	58.3±36.8	38.0±6.4	39.4±22.6
G10	31.3	62.5	23.5±15.6	35.7±20.6	100.5±23.3	16.0±7.15	21.6±5.2	49.4±19.0
G11*	31.3	31.3	-	-	-	-	-	-
G12	31.3	31.3	33.8±12.6	89.2±53.8	33.3±15.5	10.1±4.2	39.1±10.8	34.1±15.2

*Not tested against any cell line. **Values referred as media ± SD.

were tested against both strains of S. aureus and their IC50 values were determined against five human tumor cell lines. Groups 7 to 12 showed antibacterial activity with MIC values of 31.3 µg/ml for MSSA and MIC values ranging from 31.3 to 125.0 µg/ml for MRSA. Groups 3, 7 to 10 and 12 showed IC_{50} values ranging from 2.0 to 104.5 µg/ml against human tumor cell lines. Four groups (G3, G9, G10 and G12) exhibited cytotoxic activities in the range of 20 µg/ml against at least one tumor cell line. Group 3 was the most active against HL-60, HCT-116 and THP-1 cell lines, and showed IC_{50} values within the cutoff below 20 µg/ml (Table 2). Groups G1, G2, G4 to G6 showed no activity against any cell line. Group G3 was the most selective group, active only against tumor lines. G11 was active only against strains of *S. aureus* and was not tested against any cell line.

As the determination of the IC_{50} was performed after an assessment of samples with activity greater than 50% at 20 µg/ml, it was expected that the IC_{50} values had 20 µg/ml as concentration limit, however values greater than 20 µg/ml were obtained in these assays. Variation in the results were observed due to several factors, for example loss of power of the active principles associated with the cycles of freezing and thawing and the stock of the solutions in DMSO for long periods. Experiments carried out at different times could be subject to greater variation in the results for these factors (Kozikowski et al., 2003).

The crude ethanol extracts contain components whose concentration increase when the partition is performed, thus, purified fractions show higher activity than crude extracts. On the other hand, active extracts can lose their activity after purifications when the mechanism of the activity is due to synergic effects and the purification disrupt this association between compounds.

A study conducted at Centers for Disease Control and Prevention (CDC) estimated that in 2005 more than 1,300,000 MRSA infections occurred in that year in the United States (David and Daum, 2010). There are relatively few antibiotic agents available to treat MRSA infections, thus it is essential that the development of new drugs be prioritized where the current therapies are outdated, present low efficiency, high toxicity, operational difficulties in therapeutic procedures (such as long periods of hospitalization), or high associated costs. The infections caused by strains of MRSA and MSSA fit this profile, and additionally, MRSA infections are more expensive and difficult to treat than infections caused by methicillin-susceptible *S. aureus* (MSSA) (Purcell et al., 2006). Our results showed that DCM fraction from stems had activity at 31.3 µg/ml for both strains (Table 1), which is an important result because of the complexity of therapy for these strains.

According to American Cancer Society (2013), the estimated number of new cancer cases and deaths in the United States in 2013 was around 1,660,290 and 580,350, respectively. Although many types of cancer can be prevented, such as those caused by the use of cigarettes and alcoholic beverages; these numbers are quite significant. The five types of tumor cells tested represent a significant fraction of the main types of cancer recorded worldwide. Even though there are specific therapies for these types of cancer, our study showed great potential for development of new drugs in the tests carried out. Even if the concentrations of active components are not in the same order of magnitude as the drugs commonly used, it is important to note the low toxicity of the extracts and fractions compared to controls, doxorubicin and cisplatin (Table 1).

This study justifies and reinforces the use of this plant in traditional medicine in Brazil. Additional studies are necessary to explore in full its potential benefits to other biological systems.

Characterization of secondary phenolic metabolites by LC-MS of bioactive groups from DCM fraction

By means of TLC analysis and visualization of the spots by NP/PEG as developer reagent, orange, light green and yellow spots, with an intense fluorescence under UV, characteristic of phenolic compounds and/or flavonoids were observed in some groups from the DCM fraction of stems. Several compounds from these classes of natural products, mainly represented by glycoside flavonoids, were

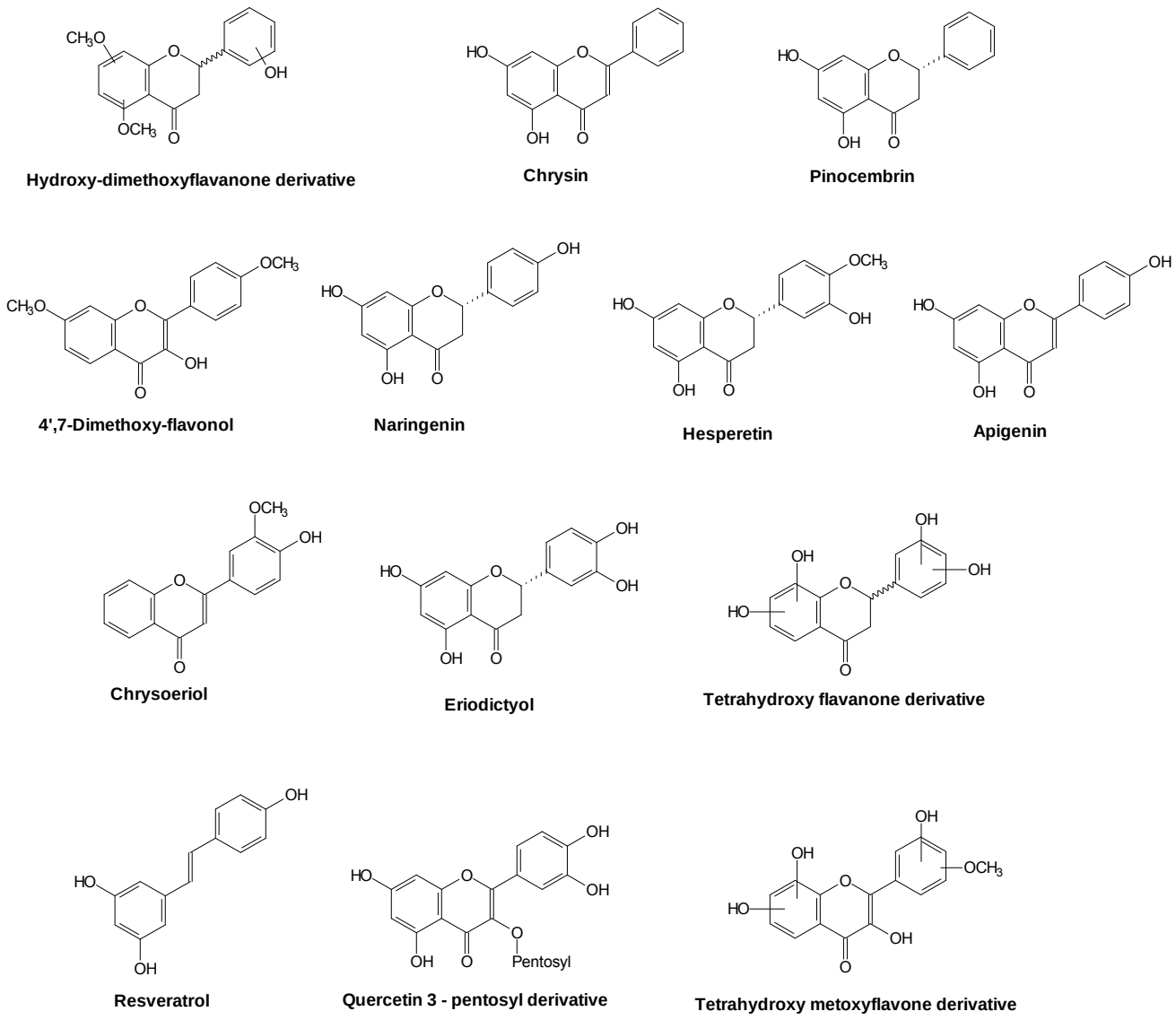

Figure 3. Phenolic compounds found in DCM fractions from *Mauritia flexuosa* stems.

were isolated from polar extracts of this species, in the previous works (Koolen et al., 2012; 2013b). The active groups G7-G11 that showed the presence of phenolic compounds by TLC analysis, were analyzed by ESI-LC-MS in the positive mode. Groups G3 and G12 did not show any metabolite with similarity index greater than 60% comparable with the compounds in the database nor presented similar fragmentation profiles with known standards.

As shown in Table 3, it was possible to identify phenolic compounds, mainly flavonoids (Figure 3). Groups G7 to G8 presented isobars eluted at 25.7 and 33.8 min, respectively. These compounds are referred to as hydroxy'-dimethoxyflavanone derivatives because it was not possible to identify the position of the hydroxyl and methoxyl groups linked to the aromatic moiety.

Chrysin, naringenin, hesperetin and apigenin were identified by means of co-injection with standards. The structures of phenolic compounds from G10 were compared with the spectra of the in-house mass bank database and showed similarities with resveratrol and eriodictyol.

Group G11 presented a glycoside (pentosyl) flavonol, eluting at 19.0 min. Mass fragmentation analysis revealed a aglycone moiety which was assigned to quercetin, but it was not possible to identify the pentose moiety linked to the aglycone. The compounds eluted at 20.8 and 26.9 min presented hydroxyl and/or methoxyl groups linked to the aromatic rings. The proposals of the structures of these substances, as well as of other derivatives, were based on the pattern of fragmentation of compounds whose structures are known (in-house databank and

Table 3. Results of LC-HRMS of the dichloromethane fraction from Mauritia flexuosa stems.

Group	Rt/ min	*m/z* $[M+H]^+$	Formula	Fragment ions (abundance)	Compound	Identified by	Fit score (Purity)
G7	25.7	301.1081	$C_{17}H_{16}O_5$	181.05 (100) 147.04 (8)	Hydroxy-dimethoxy flavanone derivative	MassBank	991
	31.2	255.0659	$C_{15}H_{10}O_4$	153.02 (10) 129.03 (2) 147.04 (1)	Chrysin	Standard/ in-house DB	997
G8	29.9	257.0825	$C_{15}H_{12}O_4$	153.02 (100) 131.05 (30) 103.05 (4) 173.06 (1)	Pinocembrin	MassBank	956
	33.8	301.1089	$C_{17}H_{16}O_5$	181.05 (100) 147.04 (35) 119.05 (3) 123.04 (2)	Hydroxy-dimethoxy flavanone derivative	MassBank	945
	35.1	299.0929	$C_{17}H_{14}O_5$	284.07 (25) 256.07 (2) 181.05 (2)	4',7-Dimethoxy flavonol	MassBank	961
G9	23.5	273.0757	$C_{15}H_{12}O_5$	153.02 (100) 147.04 (39) 119.05 (6) 123.04 (3)	Naringenin	Standard/ in-house DB	999
	24.1	303.0871	$C_{16}H_{14}O_6$	153.02 (100) 177.06 (46) 145.03 (28) 179.03 (6)	Hesperetin	Standard/ in-house DB	823
	26.3	271.0606	$C_{15}H_{10}O_5$	153.02 (7) 119.05 (2) 106.09 (2)	Apigenin	Standard/ in-house DB	984
	27.0	301.0711	$C_{16}H_{12}O_6$	286.05 (100) 258.05 (10) 165.05 (3)	Chrysoeriol	MassBank	881
G10	18.5	229.0860	$C_{14}H_{13}O_3$	107.05 (100) 165.07 (52) 135.04 (33) 155.09 (21)	Resveratrol	MassBank	754
	20.8	289.0710	$C_{15}H_{12}O_6$	153.02 (100) 163.04 (47) 145.03 (6) 135.04 (6)	Eriodictyol	MassBank	693

Table 3. Contd.

G11	19.0	303.0500 435.0922	$C_{15}H_{11}O_7$ $C_{20}H_{18}O_{11}$	303.05 (100) 153.01 (10) 257.04 (8) 229.04 (7)	Quercetin pentosyl derivative	Standard/ in-house DB	990*
	20.8	289.0703	$C_{15}H_{12}O_6$	153.02 (100) 163.04 (62) 145.03 (10) 135.04 (9)	Tetrahydroxy flavanone derivative	MassBank	749
	26.9	317.0654	$C_{16}H_{12}O_7$	302.04 (59) 285.04 (15) 318.07 (14) 274.05 (12)	Tetrahydroxy-methoxyflavone derivative	MassBank	849

*Similarity index to aglycon moiety confirmed by standard co-elution of quercetin.

standard).

Phenolic compounds, mainly flavonoids, are a class of substances that has been widely studied due to their bioactivity. They are compounds that present antioxidant activity due to their ability to donate hydrogen (Shekhar and Arobindo, 2014) and promote protection against several diseases that are caused by radical species. These compounds have also been found to have anti-inflammatory (Cuong et al., 2012; Hung et al., 2010), immunomodulatory (Chen et al., 2009), antitumor (Gao et al., 2006; Jayaprakasha et al., 2007) and hepatoprotective (Liu et al., 2014) properties.

According to our results, it can be inferred that the biological activity observed in these fractions could be attributed to the great proportion of phenolic compounds identified in the active groups from G7 to G11.

Further investigations of the compounds responsible for the biological activities of *M. flexuosa* leaves and stems are being carried out.

Conclusions

There are few studies on the biological activity of *M. flexuosa*. This research comparatively evaluated the antibacterial and cytotoxicity activity of *M. flexuosa* extracts and partitioned fractions from fruits, leaves and stems. The DCM fraction from stems and some of its groups have significant antibacterial properties against *S. aureus* strains, and also have differential antiproliferative activity on human tumor cell lines. In addition, even though the extracts and partitioned fractions did not present biological activity at the same level as the therapeutic drugs, they had low toxicity. Characterization of the active groups by LC-MS resulted in the identification of phenolic compounds.

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Conflict of interest

All authors declare that they have no conflict of interest.

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