Anais da Academia Brasileira de Ciências (2017) 89(3 Suppl.): 2423-2432
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201720170254
www.scielo.br/aabc | www.fb.com/aabcjournal

In vitro antifungal activity of *Myracrodruon urundeuva* Allemão against human vaginal *Candida* species

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Manuscript received on April 3, 2017; accepted for publication on May 4, 2017

ABSTRACT

Myracrodruon urundeuva is a plant native to Brazil, which is used by the indigenous population for the treatment of candidiasis. The aims of this study were to evaluate the antifungal activity of extract against human vaginal *Candida* species and evaluate the possible toxicological activities of *M. urundeuva*. Initially, ethanol extracts, ethyl acetate fractions, and hydroalcoholic fractions of the bark and leaf of *M. urundeuva* were used to determine the minimum inhibitory concentration. The extracts that showed antifungal activity were characterized by liquid chromatography and subjected to toxicity assessment. Toxic, cytotoxic, genotoxic, and mutagenic testing were performed using *Allium cepa* and Ames assays with the ethanol extracts of the bark and leaves. Hemolytic activity was evaluated in erythrocytes and acute toxicity in rats. The ethanol bark extracts showed best activity against *Candida albicans, C. krusei,* and *C. tropicalis* ATCC (4-512 μg/mL). Chemical characterization indicated the presence of flavonoids and tannins in the extracts. Hemolytic activity, genotoxicity, and mutagenicity were not observed. The results of the Ames and *A. cepa* tests were also in agreement, ethanol bark extracts and ethanol leaf extracts of *M. urundeuva* showed absence of mutagenic activity. Similar results were observed in the *A. cepa* assay and acute toxicity test in rats. *M. urundeuva* bark extracts showed potential for the treatment of vaginal infections caused *Candida* species, as a topical.

Key words: Anacardiaceae, candidiasis, *Myracrodruon urundeuva*, traditional medicine.

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INTRODUCTION

Vulvovaginal candidiasis is a common fungal infection among women causes physical and psychological discomfort relevant in women's health. The infection is caused by opportunistic *Candida* yeasts that are commensal vaginal mucosa (Álvares et al. 2007). *C. albicans* is responsible for over 90% of cases of infection vulvovaginal (Ilkit and Guzel 2011). Normally, due to the characteristic symptoms of infection, women perform self-diagnosis and seek treatment alternatives as sitz bath with natural products.

Myracrodruon urundeuva Allemão (Anacardiaceae) is popularly known as aroeira, and can be found in Brazil (northeast, southeast, and mid-west regions), Bolivia, Paraguay, and Argentina (Lorenzi and Matos 2008). It the bark infusion is used by the indigenous population of Brazil for the treatment of vaginal infections. According to reports of the indigenous women of the village Jaguapiru, the infusion of the bark as sitz bath promotes symptom relief (Lopes 2011). Studies have reported that extracts of this plant have an antifungal activity (Jandú et al. 2013).

The study of toxicity, genotoxicity and mutagenicity of plants popularly used in traditional medicine are important to avoid adverse reaction. Several studies have focused on the discovery of new derived bioactive agents from vegetable extracts and other natural products that have demonstrated effectiveness in the treatment of diseases and limited toxicity (Bagiu et al. 2012, Eren and Özata 2014, Gehrke et al. 2013). Toxicological evaluation with several different methodologies is recommended to ensure accurate results (Brasil 2015, WHO 2005). The tests that have been used for evaluation are: Ames test (Maron and Ames 1983), *Allium cepa* test (Fiskesjö 1994), hemolytic activity assessment (Khalil and El-Adawy 1994) and acute toxicity tests in rats (OECD 2015).

The World Health Organization (WHO 2005) estimates that the plant extracts or their active ingredients are used in folk medicine in traditional therapies 80% of the world population. The evaluation of the effectiveness of antimicrobial action and possible toxicity are relevant for proper and safe therapy. In this sense, the aims of the study were to evaluate the antifungal activity of extract against yeast from vaginal secretions from women and evaluate the possible toxicological activities of *M. urundeuva*.

MATERIALS AND METHODS

PLANT MATERIAL

The bark and leaves of *M. urundeuva* were collected in a farm of the Universidade Federal da Grande Dourados (UFGD), Dourados - Mato Grosso do Sul, Brazil (S 22°14′′ 877 W 54′ 59′′ 615). The voucher specimen was identified by Dr. Zefa Valdivina Pereira and deposited in the herbarium of the Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, under registration number 534.

PREPARATION OF EXTRACTS

Dried plant materials (100 g) were extracted with 900 mL of absolute ethanol for 48 h at 25°C, with occasional agitation. After filtration, the ethanol extract was evaporated at 35°C on a rotary evaporator and lyophilized. The crude extract was partitioned with ethyl acetate and chloroform, based on increasing polarity of the solvent. Extraction using different solvents is based on the chemical properties of each, in this case according to their polarity. This causes each substance present in the plant to interact differently with each solvent, which makes it possible to extract the compounds according to their polarity, in different fractions. The ethanol extracts, hydroalcoholic fractions, and ethyl acetate fractions of the bark and leaves of

M. urundeuva were dried and stored at 4 °C until analysis.

ANALYSIS OF THE EXTRACTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The ethanol extracts of the bark and leaves were analyzed by HPLC (VARIAN 210), with a diode array detector (DAD), scanning between 200–800 nm. The chromatographic column used was a Phenomenex C18 column (Ø 4.6 mm × ||250 mm, particle diameter 10 µm) and a guard column (25 mm × 3 mm) of the same phase was used. Elution of solvents was performed by the gradient method: MeOH/H_2O 5 to 100% methanol, taking 15 min to reach 100% methanol, 100% methanol for 5 min, 5 min to return to the initial condition, 25 min in total, with flow rate of 1 mL/min, and injection volume of 5 µL. Prior to injection, the samples were filtered through a 0.45 µm microfilter.

ANTIFUNGAL ACTIVITY ASSAYS

Microorganisms

We used 15 yeast isolates from vaginal secretions from women and American Type Culture Collection (ATCC, Rockville, MD, USA) strains *Candida albicans* 90028, *C. krusei* 6258, *C. tropicalis* 750, and *C. glabrata* 2001. Microorganisms are part of yeast bank Applied Microbiology Laboratory / UFGD.

Determination of the minimum inhibitory concentration (MIC) of the extracts

The MIC was determined by the microdilution broth technique, with some adaptations for natural products (CLSI 2008). The ethanol extracts, ethyl acetate fractions, and hydroalcoholic fractions of the bark and leaves were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich®, St. Louis, MO, USA), and serial diluted (1:2) in sterile microplates to obtain concentrations of 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256, 0.512, 1.024 and 2.048 mg/mL. The suspension of each microorganism tested was at concentration of 0.5 McFarland, resulting in 0.5 x 10^3 to 2,5 x 10^3 CFU/mL. RPMI-1640 (Sigma-Aldrich®) for yeast was used as the culture medium. The microplates were incubated for 48 h at 37 °C. The MIC was defined as the lowest concentration that inhibited fungal growth. The test was performed in triplicate at two different occasions.

Minimum fungicide concentration (MFC)

Aliquots from each well of the microplate were transferred with sterile toothpicks to a Petri plate containing Sabouraud dextrose agar. Plates were incubated for 48 h at 37 °C. The MFC were defined as the lowest concentration that did not show fungal growth (Bagiu et al. 2012).

Allium cepa TEST

The concentrations of the extracts (bark and leaves) were 0.2; 0.3; 0.4; 0.5, and 1 mg/mL. Each concentration was prepared in three plates (100 × 15 mm). One hundred *A. cepa* (Isla®, Porto Alegre, RS, BR) seeds were distributed on each plate that contained filter paper moistened with 3 mL of ethanol extracts of the bark or leaves of *M. urundeuva*. Distilled water was used as the negative control and trifluralin (Nortox®, Arapongas, PR, BR) (84 mg/mL) and 3% formalin were employed as the positive control (Fernandes et al. 2007).

The roots of the germinated seeds were measured using a millimeter ruler over five days to calculate the germination index (GI) and evaluate toxicity. On the fifth day, the roots were collected, pre-treated, and fixed in Carnoy solution (acetic acid and ethanol in the ratio of 3:1, v/v) for 24 h. Subsequently, they were washed three times with distilled water, hydrolyzed in HCl (5 M) for 10 min in a water bath at 60 °C, then washed again, and stained with Schiff reagent for 45 min (in the

absence of light). After, a section of the root apical meristem was obtained, placed on a slide, and 1 drop of acetic carmine (45%) was added. This was covered with a cover slip and the slide was observed under a light microscope (100× immersion). One thousand cells were analyzed per slide under a microscope and five slides of each treatment were evaluated, with a total of five thousand cells analyzed per treatment.

Toxicity, cytotoxicity, genotoxicity, and mutagenicity

Germination index (GI) of the seeds and medium-sized (MS) roots were analyzed to assess toxicity. The chromosomal abnormalities index (CAI) and mutagenicity index (MtI) were used to evaluate genotoxicity and mutagenicity, respectively (Fernandes et al. 2007).

Germination index was determined by the equation: GI = NG/TS × 100, where NG = number of seeds that germinated and TS = total exposed seed treatment. The average size of the roots was determined by measurements over the five treatment days.

Mitotic index (MI) was determined by the following equation: MI = NCM/TC × 100, where NCM = number of cells in mitosis and TC = total number of cells analyzed. We analyzed 1000 cells per slide under light microscope (100×) and evaluated five slides of each treatment, with a total of 5000 cells analyzed per treatment.

The rate of chromosomal abnormalities (loss of chromosomes, c-metaphase, chromosome bridge, polyploidy, and multipolarity) was established by the following equation: CAI = NCA/TC × 100, where NCA = number of altered cells and TC = total observed cells. The mutagenicity index (MtI) was determined according to the following equation: MtI = NCMn + NCB/TC × 100, where NCMn = number of cells that had micronuclei, NCB = number of cells that showed chromosomal break, and TC = total number of observed cells. As with MI assessment, 1000 cells per treatment were analyzed, with five slides for each treatment.

AMES TEST

The test was conducted according to the pre-incubation method developed by Maron and Ames (1983). Toxicity is indicated by a reduction in the number of his+ revertants or as background growth plates in minimal glucose agar (Mortelmans and Zeiger 2000). The *Salmonella* Typhimurium strains used were TA97a, TA98, TA100, and TA102.

The concentrations of ethanol bark extracts and ethanol leaf extracts were 5^{-5}, 9^{-5}, 19^{-3}, 28^{-3}, and 38^{-3} mg/plate. To each concentration of the extract, 0.5 mL of phosphate buffer (0.2 M) and 0.1 mL of bacterial suspension were added, and then incubated at 37 °C for 30 min. Next, 2 mL of top agar supplemented with histidine and biotin traits were added to the mixture. It was then slightly homogenized and plated on glucose minimal medium. After the top agar solidified, the plates were incubated at 37 °C for 48 h. and the revertant colonies were counted. The assay was performed in triplicate.

The mutagenic standards used to confirm the reversion of properties and specificity of each strain were sodium azide (0.00125 mg/plate), 4-nitro-o-phenylenediamine (0.01 mg/plate), and mitomycin C (0.0005 mg/plate). DMSO (0,1mL/plate) was used as the negative control.

The concentration of the extract was expressed as units of mass/plate. The results were evaluated by the mutagenicity ratio (MR):

MR = number of revertants in test sample/ number of revertants in the negative control

The sample was considered to be mutagenic when there was a significant increase in the number of revertants and MR is greater than or equal to two, in at least one of the concentrations tested (Mortelmans and Zeiger 2000).

ASSESSMENT OF HEMOLYTIC ACTIVITY *In vitro*

Blood (5–10 mL) was obtained by venipuncture from non-smoking and healthy volunteer with informed consent. The erythrocytes were separated by centrifugation at 1500 rpm for 10 min. After the plasma was removed, the erythrocytes were washed three times with phosphate buffered saline (PBS, pH 7.4) and 1% erythrocyte suspension was prepared using the same buffer (adapted Khalil and El-Adawy 1994).

The hemolytic activity of ethanol bark extracts and ethanol leaf extracts were evaluated *in vitro*. Each tube received 1.1 mL of erythrocyte suspension and 0.4 ml of the extracts (0.05, 0.1, and 0.5 mg/ml). PBS (solvent) and *Quillaja saponina* (0.0025%) were the negative and positive control, respectively (Jandú et al. 2013). After 60 min of incubation at 37 °C, the cells were centrifuged, and the absorbance of the supernatant was measured using a spectrophotometer (540 nm), which indicates hemoglobin released.

Hemolytic activity was determined by the following formula:

Hemolytic activity (%) = (Ae - An)/ (Ap - An) × 100, where Ae = absorbance of the extract, An = absorbance of the negative control, and Ap = absorbance of the positive control. The average value (n = 3) was calculated.

ACUTE TOXICITY TEST

The acute toxicity of the extracts was investigated in 25 adult Wistar rats (65 days old, weighing approximately 250 g) from the animal facility of the Universidade Federal da Grande Dourados (UFGD). The animals were kept at controlled conditions (12-hour light/12-hour dark cycle and temperature of 23 °C), and received water and commercial feed *ad libitum*. The acute toxicity test was performed in accordance with the protocol of Organization for Economic Co-operation and Development (OECD 2015).

The animals were divided into five groups (5 animals/group) and treated with ethanol extracts (0–2000 mg/kg body weight) of the bark and leaves of *M. urundeuva*. The animals were fasted for 12 h before administration of the extracts and the treatments were administered as single oral dose (gavage). The animals were observed within the first hour and every 24 h for 14 days. Behavioral parameters were analyzed, such as irritability, twitching, righting reflex, tremors, convulsions, piloerection, breathing, and death. The body weight, amount of water, and food consumed by the group during the 14 days were also evaluated. On the 15th day, all animals were weighed, anesthetized with ketamine (25 mg/kg) and xylazine (10 mg/kg), and euthanized.

The Ethics Committee of Animal Experimentation of UFGD approved the experimental procedures (Protocol 003/2012).

Histological analysis

All animals were autopsied at the end of the experiment to analyze the macroscopic characteristics of the liver, lung, and kidney. The organs were removed carefully and then individually weighed, fixed in 10% buffered formalin, and embedded in paraffin. Sections were obtained using a microtome. The sections were stained with hematoxylin-eosin and the slides were analyzed using an optical light microscope (40× magnification).

STATISTICAL ANALYSIS

Data analysis was performed using descriptive statistics, including Mann-Whitney and ANOVA tests. Differences were considered significant at $p < 0.05$. The data were stored and analyzed using the Software BioEstat 5.0.

RESULTS AND DISCUSSION

M. urundeuva of bark extracts showed best antifungal activity against *C. albicans*, major yeast associated with vulvovaginal candidiasis (Ilkit and Guzel 2011), however the leaves extract showed no activity for this species (Table I). This information confirms the popular use of the bark and no leaves.

Ethanol extracts bark showed the best antifungal activity compared to its fractions, Jandú et al. (2013) reported the bark methanol extract of *M. urundeuva* showed inhibitory effects against bacteria and fungi at concentrations 390-3190 mg/L. In this study, the ethanol bark extracts showed best activity (0.004-0.512 mg/mL), which was possible attributed the type of extraction employed, to place and collection period. Environmental variations can affect plant development and consequently influence the production of various chemical compounds, especially secondary metabolites, therefore, induce different response in biological tests.

The antifungal activity of *M. urundeuva* extracts can be attributed to the presence of flavonoids and tannins. Studies have confirmed the significance of flavonoids and tannins with the antifungal activity of *M. urundeuva* (Jandú et al. 2013, Siqueira et al. 2012, Araújo et al. 2008). In addition to their antifungal activity, it has been shown that these compounds possess antioxidant activity, anti-inflammatory, and anticancer properties (Jandú et al. 2013, Siqueira et al. 2012, Araújo et al. 2008, Dourado and Ladeira 2008).

The ethanol extracts of the bark and leaves of *M. urundeuva* were analyzed by HPLC, which indicated the presence of substances such as flavonoids (flavanols and chalcones), phenols, and tannins. Sá et al. (2009) analyzed the chemical components of *M. urundeuva* ethanol extract using thin layer chromatography and noted the presence of flavonoids and tannins. Mota et al. (2015) evaluated the similarities between the phytochemical profiles of the leaves and bark of *M. urundeuva* and observed that the chromatographic profiles of the leaves and bark were similar however, the concentrations of some compounds differed, with higher concentration of functional compounds found in the bark compared to that of the leaves. Our results showed that the antimicrobial activity was greater in the bark extracts than that in the leaf extracts. Plants that have flavonoids and tannins with antifungal activity may also exhibit toxicity (Resende et al. 2012, Silva et al. 2014). Therefore, antimicrobial activity studies must be accompanied by toxicology studies.

All concentrations of ethanol bark extracts and ethanol leaf extracts influenced seed germination and root growth of *A. cepa*, in a dose-response manner. Increasing concentration of the extracts resulted in a decrease in the number of seeds that germinated and size of the roots ($p < 0.05$) (Table II). These extracts reduced the mitotic activity of meristematic cells and did not show significant mutagenic activity to cells, as confirmed by the values of the CAI and MTI at the concentrations tested ($p > 0.05$). The results of the germination rate, root growth, and mitotic index indicated that the extracts reduced the number of cells in the plant tissue, suggesting antiproliferative activity. The reduction in the mitotic index of meristematic cells of *A. cepa* implies cytotoxic related cell death (Ping et al. 2012). In this study, concentration of 0.2 mg/mL showed toxicity and significantly reduced the germination of seeds and growth of roots of *A. cepa* ($p < 0.05$).

Several species of the Anacardiaceae family, in which *M. urundeuva* belongs to, are considered allelopathic (Donnelly et al. 2008), due to secondary metabolites derived from the metabolism of plants, and include flavonoids, tannins, steroids, and terpenoids and may be responsible for reducing or even inhibiting the germination of seeds and growth of roots (Li et al. 2010, Rodrigues et al. 2012).

TABLE I

Minimum inhibitory concentration (MIC) and minimum fungicide concentration (MFC) of ethanol extracts, ethyl acetate fractions, and hydroalcoholic fractions of the bark and leaves of *M. urundeuva* against different yeast isolates.

Microrganism	Bark (mg/mL)						Leaves (mg/mL)						Fluconazole (mg/mL)	
	Ethanol		Ethyl acetate		Hydroalcoholic		Ethanol		Ethyl acetate		Hydroalcoholic			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans ATCC 90028	0.064	0.128	0.064	0.064	0.032	0.128	-	-	-	-	-	-	0.004	0.004
C. glabrata ATCC 2001	-	-	-	-	-	-	1.024	2.048	-	-	-	-	0.032	0.032
C. krusei ATCC 6258	0.032	1.024	0.064	0.512	0.064	-	0.064	2.048	-	-	0.064	1.024	0.016	0.032
C. tropicalis ATCC 750	0.064	0.128	0.064	0.128	0.032	0.256	0.512	1.024	0.512	0.512	1.024	2.048	0.004	0.008
C. albicans 01	0.032	0.032	0.064	0.064	0.256	0.512	-	-	-	-	-	-	0.004	0.064
C. albicans 02	0.016	0.064	0.064	0.128	0.512	1.024	-	-	-	-	-	-	0.004	0.004
C. albicans 03	0.032	0.064	0.256	0.256	0.512	1.024	-	-	-	-	-	-	0.004	0.064
C. albicans 04	0.032	0.064	0.064	0.512	0.256	0.512	-	-	-	-	-	-	0.004	0.004
C. albicans 05	0.016	0.032	0.016	0.064	0.256	1.024	-	-	-	-	-	-	0.004	0.008
C. albicans 06	0.004	0.008	0.008	0.008	0.512	0.512	-	-	-	-	-	-	0.004	0.064
C. albicans 07	0.512	-	0.016	0.128	0.256	0.512	-	-	-	-	-	-	0.004	0.064
C. albicans 08	0.256	-	0.512	-	-	-	-	-	-	-	-	-	0.004	0.008
C. albicans 09	0.256	-	0.512	-	0.512	-	-	-	-	-	-	-	0.004	0.064
C. albicans 10	0.256	-	0.256	-	1.024	1.024	-	-	-	-	-	-	0.016	0.064
C. glabrata 01	-	-	0.512	-	-	-	-	-	1.024	-	-	-	0.016	0.064
C. glabrata 02	-	-	1.024	-	-	-	-	-	1.024	-	-	-	0.016	0.032
C. glabrata 03	-	-	-	-	-	-	-	-	1.024	-	-	-	0.016	0.032
C. glabrata 04	-	-	0.512	-	-	-	-	-	1.024	-	-	-	0.004	0.064
C. glabrata 05	-	-	-	-	-	-	-	-	1.024	-	-	-	0.004	0.016

(-) no activity.

M/SD: mean ± standard deviation. The a and b letters in the table indicate the type of positive control used. PC_A: positive control (GI; MT) -formaldehyde 3%; PC_B: positive control (MI;CAI; MtI) -trifluralin (0.84 ppm); negative control *Mann-Whitney test ($p < 0.05$).

TABLE II
Germination index (GI) of seeds, medium-sized root (MS), mitotic index (MI), chromosomal alterations index (CAI), and mutagenicity index (MtI) for *A. cepa* after treatment with different concentrations of ethanol bark extracts and ethanol leaves extracts of *M. urundeuva*.

Extracts	Concentrations (mg/mL)	GI (%) (M/SD)	MS (M/SD)	MI (M/SD)	CAI (M/SD)	MtI (M/SD)
Leaves	Positive control	0_a	0_a	$1.12\pm0.97_b$*	$1.8\pm0.55_b$*	$1.98\pm0.31_b$*
	Negative control	63±2.52	1.95±0.10	3.53±0.79	0.01±0.02	0.06±0.05
	0.2	48±2.00*	1.16±0.20*	3.34±1.20	0.02±0.01	0.09±0.07
	0.3	40±4.73*	0.80±0.34*	2.87±0.80*	0.02±0.01	0.10±0.05
	0.4	47±1.53*	0.88±0.29*	2.79±1.41*	0.03±0.01	0.10±0.12
	0.5	32±2.52*	0.58±0.23*	1.46±0.69*	0.02±0.02	0.10±0.12
	1.0	17±3.06*	0.24±0.27*	0.83±0.47*	0.01±0.03	0.10±0.10
Bark	Positive control	0_a	0_a	$0.91\pm0.88_b$*	$1.8\pm0.55_b$*	$1.68\pm0.31_b$*
	Negative control	69±3.61	1.97±0.12	3.00±0.60	0.01±0.02	0.06±0.05
	0.2	42±1.53*	1.09±0.40*	1.78±0.32*	0.01±0.01	0.02±0.05
	0.3	47±3.06*	1.14±0.31*	1.60±0.77*	0.01±0.02	0.04±0.05
	0.4	56±1.53*	0.98±0.21*	1.83±0.49*	0.03±0.01	0.04±0.05
	0.5	57±1.53*	0.91±0.18*	1.79±0.84*	0.01±0.01	0.02±0.04
	1.0	45±2.65*	0.63±0.14*	1.12±0.13*	0.01±0.01	0.02±0.04

Despite its cytotoxic activity, *M. urundeuva* extracts did not show mutagenic activity in the *A. cepa* test and AMES test did not show mutagenic activity of direct action at all concentrations tested in the four strains of *S.* Typhimurium (TA97a, TA98, TA100, and TA102), as shown by the mutagenicity ratio (less than 2) (Table III) (Maron and Ames 1983). The fact the extracts did not present direct mutagenicity to is relevant is to ensure the safe use of this plant, as it was not observed mutagenic changes in the *A. cepa* test and AMES test.

The extracts showed hemolytic activity of less than 25% at the concentrations tested, indicating that the bark and leaf extracts of *M. urundeuva* were not cytotoxic to erythrocytes. Other studies (Jandú et al. 2013, Carvalho and Oliveira 2012) evaluated the toxicity of methanol extract did not show hemolytic activity in erythrocytes.

In the acute toxicity test some symptoms such as lethargy, increased respiratory rate, and absence of movement they were soon observed after administration of the extracts. After 15 min, movements were observed but high respiratory rate remained. On the first day, two rats (one from each group) had nasal bleeding and difficulty in breathing, and died on the following day. On gross examination, swollen abdomen was observed and after dissection, yellow structures and formation of internal voids were observed in the small intestine. Histopathological evaluation of the liver, lung, and kidney did not indicate abnormality in these organs.

In conclusion, the bark extracts of *M. urundeuva* showed best antifungal activity against *C. albicans*, major yeast associated with vulvovaginal candidiasis. Chemical analysis of the extracts confirmed the presence of flavonoids (flavanols and chalcones) and tannins. The ethanol extracts of the bark and leaves did not show mutagenic and genotoxic effects, but demonstrated cytotoxic and toxic properties. *M. urundeuva* bark extracts

TABLE III
The number of revertant colonies and mutagenicity ratio of the different treatments of ethanol bark extract and ethanol leaves extract of *M. urundeuva* in *S.* Typhimurium strains (TA97a, TA98, TA100, and TA102).

Extracts	Treatment (mg/plate)	TA97a		TA98		TA100		**TA102**	
		M/SD	**MR**	**M/SD**	**MR**	**M/SD**	**MR**	**M/SD**	**MR**
Leaves	5^{-5}	121.67±6.64	1.26	14.67±2.08	1.05	54.67±2.58	1.12	214.67±4.69	1.05
	9^{-5}	100.33±8.5	1.04	14.67±3.54	1.05	66.67±0.71	1.37	329.33±4.73	1.61
	19^{-3}	97.33±5.22	1.01	12.67±2.52	0.90	53.00±4.36	1.09	285.67±5.73	1.39
	28^{-3}	118.67±4.32	1.23	13.00±4.24	0.93	47.00±3.9	0.97	255.00±6.19	1.24
	38^{-3}	103.33±3.44	1.07	13.67±2.12	0.98	43.67±1.41	0.90	273.00±4.95	1.33
	Negative control	96.33±2.44	1	14.00±1.3	1	48.67±2.2	1	205.00±10.45	1
	Positive control	729.00±5.5*	7.57	569.33±8.85*	40.67	512.33±4.3*	10.53	1143.00±25.67*	5.58
Bark	5^{-5}	154.67±6.29	1.61	12.67±1.15	0.90	69.00±8.89	1.42	260.00±2.83	1.27
	9^{-5}	152.67±2.03	1.58	14.00±3.36	1.00	60.67±4.85	1.25	154.67±6.71	0.75
	19^{-3}	93.33±9.29	0.97	15.00±2.65	1.07	59.00±7.78	1.21	165.67±4.33	0.81
	28^{-3}	110.00±8.42	1.14	14.33±2.12	1.02	65.67±8.49	1.35	267.67±9.19	1.31
	38^{-3}	97.67±8.99	1.01	15.33±4.24	1.10	78.00±9.09	1.60	192.67±3.21	0.94
	Negative control	96.33±2.44	1	14.00±1.3	1	48.67±2.2	1	205.00±10.45	1
	Positive control	729.00±5.5*	7.57	569.33±8.85*	40.67	512.33±4.3*	10.53	1143.00±25.67*	5.58

M/SD: Mean revertant colonies ± standard deviation; MR: mutagenicity ratio. * Significant ($p < 0.05$).

showed potential for the treatment of vaginal infections caused *Candida* species, however, it is more suitable as a topical formulation.

ACKNOWLEDGMENTS

The authors are grateful to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for the financial support and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for funding a scholarship for postgraduate studies. All authors declare that they have no conflict of interest.

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