

## Full Length Research Paper

## Crude extracts and semi-fractions from *Myracrodruon urundeuva* with antibacterial activity against American type culture collection (ATCC) strains of clinical relevance

Carvalho Michelle da Silva<sup>1\*</sup>, Ferreira Bárbara Caroline Mota<sup>1</sup>, Magalhães Daniel Rodrigues<sup>1</sup>, Oliveira Dario Alves<sup>1</sup> and Valério Henrique Maia<sup>2</sup>

<sup>1</sup>Bioprospecting and Genetic Resources Laboratory, State University of Montes Claros, Brazil.

<sup>2</sup>Environmental Microbiology Laboratory, State University of Montes Claros, Brazil.

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The objective of this study was to investigate the antibacterial activity of crude and semi-pure extracts obtained from *Myracrodruon urundeuva* (Aroeira do sertão) against clinically important ATCC bacterial strains by preparative thin layer chromatography (TLC). The results indicate potential activity as assessed by minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analysis. The crude and semi-pure extracts, obtained from the leaves and bark of *M. urundeuva* (Fr. All.), have a broad antibacterial spectrum and are capable of inhibiting the growth of different Gram-positive and Gram-negative bacterial strains. The phytochemical analysis of the leaves revealed the presence of hydrolysable tannins, flavonoids, saponins, terpenes and steroids. However, extracts from the Aroeira tree leaves showed larger inhibition zones for the species of Gram-positive bacteria than for Gram-negative species. It is possible that the active compounds present in these extracts act by inhibiting the synthesis of peptidoglycan in the cell wall, particularly in Gram-positive bacteria. The proven antibiotic action of crude and semi-pure extract from Aroeira leaves can be explored to facilitate their application as pharmaceutical adjuvants in antibiotic preparations.

**Key words:** *Myracrodruon urundeuva* (Fr. All.), antibacterial activity, extracts, extract.

### INTRODUCTION

Given the current reality of antibiotic drugs that have not proven effective against multidrug-resistant bacteria (López, 2011; Sánchez and Kouzetsou, 2010), plants and their immense variety of bioactive molecules represent a promising alternative source of possible chemical compounds that have antibacterial activity, and can serve as prototypes for the development of new drugs. This makes the metabolites of strong plants allies to pharmaceutical innovation, given their great value in applications such as medicines, cosmetics, food and agrochemicals

(Cordell and Colward, 2012; Maffei et al., 2011; Ponthi and Chawdharry, 2006).

Natural compounds from plants are usually characterized as primary metabolism compounds (proteins, lipids and carbohydrates), which are widely distributed in living organisms, or secondary metabolic compounds (such as terpenes, alkaloids, and flavonoids) of restricted occurrence, although these are essential for the organisms that produce them (Hassanpour et al., 2011; Rensheng and Weinin, 2011). The secondary

\*Corresponding author. E-mail: kambiz37diba@gmail.com, kdiba@umsu.ac.ir.

metabolites have important ecological functions for the plants, and are often associated with protection against external agents (for example, diseases, pests, and solar radiation). For humans, these compounds have been highlighted, as they present several therapeutic properties (for example, calming, antiviral, contraceptive, antibacterial, antifungal, anti-inflammatory and insecticidal) that apply to the pharmaceutical, chemical, cosmetic and food industries (Maffei et al., 2011; Cartaxo et al., 2010; Santos et al., 2010).

Brazilian plants deserve special attention because of their high level of endemism, taxonomic diversity and biomolecular richness, yet they have not been studied for their pharmacological potential (Bizerril, 2004; Cartaxo et al., 2010). Brazil, with about 55,000 catalogued species out of the 250-500 thousand floral species found in the world, has less than 10% of species that have been studied for biological relevance, and not more than 5% for chemical relevance (Cartaxo et al., 2010; Santos et al., 2010; Silva et al., 2012; Toledo et al., 2011). Considered the second largest biome in Brazil, with 22% of the land surface in the country, the Cerrado has a rich biodiversity, estimated at 160,000 species of plants, animals and fungi (Appezatto-da-Glória and Cury, 2011; Sano et al., 2008; Silva et al., 2011). Nogueira et al. (2011) indicates that the Cerrado has the richest flora among the world's savannas, with the highest levels of endemism. Sano et al. (2008) affirm that this biome contains approximately 800 arboreal species but suffers from a high degree of destruction.

Among the Brazilian species, we highlight the Aroeira tree (*Myracrodruon urundeuva* Fr. All.), which is popularly known as *Aroeira preta* and *Aroeira do sertão*. The Aroeira is a native species, belonging to the Anacardiaceae family, widely distributed in the Northeast, Southeast and Midwest regions of Brazil (Kiill and Lima, 2011). It is used in the wood and leather tanning industries, and as a medicinal plant. Widely used in construction, the wood has great strength and is virtually rot proof (Duarte et al., 2009). This species of wood is sought after in predatory exploration, and it is therefore part of the list of endangered plant species (Kiill and Lima, 2011). Aroeira can cause severe allergic reactions due to the presence of urushiol, a mixture of phenolic compounds found in this species (Reis, 2010). However, these compounds provide protection against attack by microorganisms in plants and are the precursors of other substances, such as flavonoids (Rensheng et al., 2011).

The medicinal use encompasses properties such as anti-inflammatory, antidiarrheal, healing, antiulcer, antihistamine and analgesic ones (Akin et al., 2010; Lucena et al., 2011; Lucena et al., 2008). The pharmacological properties mostly indicate use as an antibiotic for urinary and gynecological disorders (Kiill and Lima, 2011), which has stimulated studies aimed at verifying traditional uses and increasing the chances of identifying chemical constituents with antimicrobial activity. Thus, this work invest-

igates the inhibitory effects of extracts from the Aroeira against clinically relevant American Type Culture Collection (ATCC) bacterial strains by phytochemical determination using preparative thin layer chromatography (TLC) and analyses of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for each extract obtained, as well as disk diffusion tests against control strains.

## MATERIALS AND METHODS

### Collection and preparation of plant material

The plant material of *A. preta* was collected near the city of Glauclândia, north of Minas Gerais, Brazil. Leaves were obtained from individuals in the Cerrado area. The plant material was collected on May 16 between 8 am and 10 am, from fifteen apparently adult, large trees, found randomly in the study areas. Species identification was performed by comparison with the literature and voucher specimen number 32 from the Herbarium in Montes Claros (HMC), State University of Montes Claros/UNIMONTES, as well as consultations with specialists.

We dried the plant material according to previously published methods. To promote disinfection, the collected leaves were dipped in a 1% sodium hypochlorite solution (for cleaning and disinfecting) for about 30 s and rinsed in running water. Then, the leaves were deposited on newspaper sheets in a shaded place at room temperature to dry for seven days. After drying, the leaves were ground in a Willey mill (16 mm sieve), stored in paper bags and kept under refrigeration ( $\pm 5^{\circ}\text{C}$ ) for later analysis.

### Preliminary phytochemical characterization

The phytochemical characterization of the powdered leaves of plants from areas of Cerrado was performed by applying classical tests for identification, as described in the literature (Cartaxo et al., 2010). Identification of flavonoids (Shinoda reaction), tannins (ferric chloride), saponins (persistence of foam for at least 15 min that does not disappear after adding a drop of  $\text{HCl}_2 \text{ N}_2$  indicates a positive result for saponins), cardiotonics (the Liebermann-Burchard reaction, Baljet reaction, Keller-Kilian reaction), anthraquinones (Brontrager's test, the presence of a pink or red color indicated a positive reaction for anthraquinones), alkaloids (The tests were conducted with general alkaloid reagents: Dragendorff, Bertrand, Valser-Mayer and Bouchardat, after filtration and extraction with 10% HCl, the tests with the general alkaloid reagents were performed) were carried out.

### Obtaining the dry crude hydroethanol extract (CHE)

Thirty grams of powdered leaves were soaked in 1 L of 70% hydroethanol and macerated at room temperature for approximately 30 min with sporadic shaking. Subsequently, the mixture was filtered. The solvent was evaporated from the resulting filtrate in a circulating air oven ( $40 \pm 5^{\circ}\text{C}$ ). The mass of the dry extract obtained was scraped to collect the CHE, in powder form. Then, 250 mg of powder was dissolved in 1 ml of 0.95% saline solution containing 5% Tween to obtain extracts at a concentration of 250 mg/ml. The CHE was used in the screening of antibacterial activity.

### Obtaining semi-pure extracts

The semi-pure extracts were obtained from the fractionation of CHE

through the partition of organic solvents of increasing polarity. To obtain a semi-purification of the substances, we adopted the protocol described by Othman et al. (2011) in which the CHE is partitioned successively using solvents of increasing polarity. For this purpose, we used hexane, dichloromethane, ethyl acetate and butanol, respectively. Five grams of CHE were weighed and transferred to a large test tube. Then, 50 ml of hexane were added. The tube was shaken vigorously for 5 min. Next, the entire contents were centrifuged for 3 min at 1500 rpm, and the supernatant was transferred to a beaker. To increase the yield, the procedure was performed five times, yielding 250 ml of semi-pure extract. The same procedure was performed sequentially with dichloromethane, ethyl acetate, and butanol. The extracts were dried in a circulating air oven ( $40 \pm 5^\circ\text{C}$ ) until the solvent was completely evaporated. The dried content was completely dissolved in 1 ml of 5% Tween for use in the antimicrobial testing. We obtained fractions of semi-pure hexane extract (SPE-HEX), semi-pure dichloromethane extract (SPE-DC), semi-pure ethyl acetate extract (SPE-EA), and semi-pure butanolic extract (SPE-BUT).

### Preparative thin layer chromatography (PTLC)

We used PTLC for the isolation of compounds. The PTLC was applied to the semi-pure butanolic extract, which showed the best performance in the biological test. The SPE-BUT was further subjected to phytochemical screening by the conventional methods described above. We determined the inhibitory activities of the isolated compounds and mixtures made from combinations of the compounds against Gram-negative (*Proteus mirabilis* and *Proteus vulgaris*) and Gram-positive (*Staphylococcus aureus* and MRSP coagulase-negative *Staphylococcus* sp. MRSP) strains obtained from ATCC. The combinations were obtained by mixing equal parts of the isolated compounds.

### Preparation of bacterial inoculum

To evaluate the antibacterial activity of the plant extracts from *M. urundeuva* (Fr. All.), the disc diffusion method and determination of MIC and MBC were carried out. To determine the antibacterial action of the analyzed substances against microorganisms type, the Gram-negative strains (*P. mirabilis* and *P. vulgaris*) used were obtained from the clinical specimen maintained on the bacterial library from Central Microbiology Laboratory in the Hospital Universitário Clemente Faria (HUCH) of UNIMONTES. Moreover, strains of *S. aureus* (ATCC 6538) and *P. mirabilis* (ATCC 15922) were also used as reference strains to achieve good reproducibility in the tests. The bacterial inoculums used in susceptibility testing were prepared from a direct suspension of 0.95% saline solution containing isolated colonies of the microorganisms of interest. The suspension was adjusted to coincide with the optical turbidity of the 0.5 McFarland standard solutions of bacteria. All tests for antibacterial activity were performed in quintuplicate, and the averages analyzed by analysis of variance (ANOVA), with 5% significance levels.

### Agar diffusion method

The agar diffusion method was performed according to CLSI (2009). The filter paper discs (Whatman N. 2 of 6.35 mm diameter) were saturated with different concentrations of crude (250 mg/mL) and semi-pure (SPE-HEX - 220 mg/mL; SPE-DC - 190 mg/mL; SPE-AE - 140 mg/mL and SPE-BUT - 120 mg/mL) extracts. Equal volumes of 10  $\mu\text{L}$  were, in triplicate, added on the surface of Mueller Hinton agar (Difco) that was previously seeded with a standardized

suspension of the microorganism as previously described. The seeded Petri dishes were incubated at  $37^\circ\text{C}$  for 24 h. The presence of a growth inhibition halo around the paper discs on the surface of the plates indicated antibacterial activity. Discs containing 5% Tween were used as a negative control. Discs with 10  $\mu\text{g}$  of LB containing the gentamicin antibiotic (Laborclin®) that is commonly used in antibacterial susceptibility testing as recommended by CLSI (2009) were used as positive controls.

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests

To determine the MIC, we used the broth microdilution method described by CLSI (2009), with some modifications. Briefly, 0.1 ml of the BHI (Brain Heart Infusion) broth medium was added to each well of a 96-well microdilution plate with a micropipette. Next, we added 0.05 ml of the respective extracts (raw and semi-pure) and performed a serial dilution. One well of the plate was left empty to serve as a negative control and another well, without the addition of the extract, served as a positive control. To avoid dehydration of the media, the plates were sealed with plastic wrap and placed in a bacteriological incubator at  $35^\circ\text{C}$  for 24 h. Subsequently, 0.01 mL of 0.01% resazurin was added. The plate was sealed again and incubated for another 60 min at  $35^\circ\text{C}$ . The plates were examined by visual interpretation of the color in each well. In general, the reduction time is inversely proportional to the number of bacteria in the sample, for example, the more bacteria in the sample, the faster the reduction of the indicator substance. Thus, a blue color indicates the absence of bacteria and a pink color indicates the presence of viable labile cells.

The MBC is the smallest amount of an antimicrobial agent that is capable of causing irreversible damage to the microbial cell and hinders the growth of the microorganism (Mahboubi et al., 2012). The test was performed by subculturing the MIC cultures from the dilution wells where there was no bacterial growth, based on the resazurin test on Muller Hinton Agar medium. After incubation (24 h at  $37^\circ\text{C}$ ), the plates were visually inspected to determine whether there was growth of microorganisms. The MBC corresponded to the amount of sample obtained from each well per inoculated plate without bacterial growth. Inhibition in the MIC test and bacterial growth in the subculture were indicators of bacteriostatic action, whereas the absence of growth indicated bactericidal action. The MBC corresponded to the lowest concentration of plant extracts that showed no cell growth on the agar surface inoculated (microbial death).

## RESULTS AND DISCUSSION

Classical phytochemical screening confirmed the presence of hydrolyzable tannins, flavonoids and terpenes in the leaves and bark samples collected in the two biomes (dry forest and Cerrado). In both areas, alkaloids and cardiotonic glycosides were not detected. Saponins were detected only in the leaf samples (Table 1). The data indicate that the Aroeira trees sampled in the Cerrado and dry forest areas showed the same class of secondary compounds because there were no qualitative differences among the secondary metabolites isolated from individuals belonging to the two biomes, except for saponin traces, which were not detected in the bark (Table 1).

**Table 1.** Partial phytochemical profile of *Myracrodruon urundeuva* leaves and barks in the Cerrado and dry forest areas.

Group of compounds	Leaves		Bark	
	Cerrado	Dry Forest	Cerrado	Dry Forest
Hydrolyzable tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	—	—
Terpenes and steroids	+	+	+	+
Alkaloids	—	—	-	-
Cardiotonic glycosides	—	—	-	-
Anthraquinones	—	—	-	-

+, Compounds present; -, compounds absent.

**Table 2.** The average values of the inhibition halos obtained in the disc diffusion test.

Microorganism	Diameters of the inhibition halos (mm) <sup>a</sup>						
	CE	E-Hex	E-DC	E-AE	E-BUT	C+	C
<i>Proteus mirabilis</i>	14	9	10	10	13	17	-
<i>Proteus vulgaris</i>	11	11	11	9	13	17	-
<i>Proteus mirabilis</i> ATCC 15922	9.5	-	8	-	8	26	-
<i>Staphylococcus aureus</i>	16	16	11	12	16	17	-
<i>Staphylococcus</i> sp. cog. neg.	16	16	12	13	16	18	-
<i>Staphylococcus aureus</i> ATCC 6538	11.5	7.5	11	8	10	21	-

-, No inhibition halo was formed. <sup>a</sup>, Average values  $\pm$  SD, n = 3 (inhibition halo (mm) including 8 mm diameter disc; C+, positive control (10  $\mu$ g of gentamicin); C-, negative control (5% Tween); CE, crude hydroalcoholic extract; E-HEX, hexane extract; E-DC, dichloromethane extract; E-EA, ethyl acetate extract; E-BUT, semi-pure butanolic extract; ATCC, American Type Culture Collection.

The phytochemical screening of SPE-BUT was also performed to direct the isolation of compounds. In this fraction, only tannins and flavonoids were found. Quantification of these compounds was not performed. Therefore, it is likely that there are quantitative differences in the metabolites from different areas. This was evidenced by the differences observed in the visual color of the crude extracts produced and differences in the chemical screening reactions. In the identification of the hydrolysable tannins, the blue color was more intense for the dry forest sample. Likewise, the intensity and persistence of foam in the saponins identification test was most prominent in this sample. In contrast, the characteristic color in the identification of flavonoids was more intense in the sample extract obtained from leaves collected in the Cerrado.

There was no consensus on the acceptable level of inhibition for natural products (Benko and Crovella, 2010). Some authors only consider results that are similar to antibiotics, whereas others also regard those with levels of inhibition above and below the standards established by CLSI as potentially good. Thus, all of the

values corresponding to the inhibition halos were considered important (Table 2). Suffredini et al. (2010) found significant results with MIC and MBC  $\leq$  200 mg/ml. This scale value was adopted in interpreting the results of MIC and MBC in this study.

The results are consistent with many studies in the literature. The extract of *Salvia cryptantha* and *Salvia heldreichiana* (Lamiaceae), endemic plants in Turkey, were able to inhibit the growth of *Escherichia coli*, *Sarcinia lutea* and *Salmonella typhimurium* (Albuquerque et al., 2011). Xia et al. (2011) demonstrated the antibacterial activity of several plants used in traditional Chinese medicine using bioassays based on the MIC of fractions and crude extracts.

Suleiman et al. (2012) found fractions of *Loxostylis alata* extract (Anacardiaceae) with antimicrobial activities against *S. aureus*, *Enterococcus faecalis*, *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans* and *Microsporum canis* also inhibits the growth of *P. mirabilis*, with an inhibition halo measuring 15 mm, and for *S. aureus*, with an inhibition halo measuring 10 mm. The organic extract obtained from *Tovomita* aff. *longifolia*

(Rich.) leaves shows significant activity against *E. faecalis* and *S. aureus*. The organic extracts obtained from the leaves of *Tovomita brasiliensis* (Mart.) and *Caraipa grandifolia* (Mart.) also shows activity against *S. aureus* (Suffredini et al., 2006).

Suleiman et al. (2006) have verified the sensitivity of *S. aureus*, *E. faecalis*, *A. fumigatus*, *C. albicans*, *C. neoformans* and *M. canis* to various types of extracts from species of the Anacardiaceae family. Silva et al. (2010) have shown that the antibacterial potential of leaf's essential oil (EO) of *Schinus terebinthifolius* (aroeira-vermelha) expresses activity against *Staphylococcus hominis*, *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*. The MIC of these EO ranged from 78.1 to 1,250 µg/ml.

Kossah et al. (2011) have demonstrated the antibacterial activity of extracts from the *Rhus coriaria* L. species, which also belong to the Anacardiaceae family. In their study, the antimicrobial activity of *R. typhina* fruit extract was tested against twelve strains including Gram-positive and Gram-negative bacteria as well as for yeasts.

The extract showed a strong antimicrobial activity with a concentration-dependence and a broad antimicrobial spectrum for all tested bacteria species. *Bacillus cereus* and *Helicobacter pylori* were found to be the most sensitive Gram-positive and Gram-negative bacteria, respectively, with a MIC of 0.10%.

The action of Aroeira tree extracts against the bacterial strains that are resistant to methicillin should be noted. The multi-resistant staphylococci (MRSA) are often isolated from hospitalized patients (Souza et al., 2010). The staphylococci recently isolated in all parts of the world, either coagulase-positive or coagulase-negative, have shown high resistance (above 70%) to benzylpenicillin (penicillin G), penicillin V, ampicillin, amoxicillin and carbenicillin (Pamplona-Zomenhan et al., 2011). The crude extract of *M. urundeuva* was also effective in inhibiting the bacteria responsible for causing urinary tract infections, such as *P. mirabilis* and *P. vulgaris*. These data reinforce the popular use of Aroeira in the treatment of gynecological diseases usually associated with this group of pathogens.

Notably, the diameter of the inhibition halos for Gram-positive bacteria are generally larger than the inhibition halos found for the Gram-negative bacteria, indicating a greater inhibitory effect against Gram-positive species (coagulase-negative *S. aureus* and *Staphylococcus* sp. MRSP). These results corroborate the findings of Suffredini et al. (2006), which show that Gram-negative bacteria are almost invulnerable to plant extracts, particularly at doses lower than 200 mg/mL. Likewise, while studying the susceptibility of Gram-positive and Gram-negative bacteria to crude extracts of plants Nepal, Panthi and Chaudhary (2006) found that 16 of the 18 extracts tested (88.88%) show activity against Gram-

positive (*S. aureus*) and no activity against the Gram-negative species tested (*P. aeruginosa* and *E. coli*).

The crude and semi-pure extracts tested in this study showed a MIC ranging from 7.5 mg for E-BUT (for coagulase-negative *Staphylococcus* sp.) to 250 mg (*P. mirabilis* and *P. vulgaris*) for CHE (Table 3). Among all of the semi-pure extracts evaluated, the butanol fraction (E-BUT) had the lowest value for the MIC. The inhibitory performance of this extract was highly significant ( $p < 0.05$ ) when compared with the other fractions tested. This result indicates that the active compounds of this fraction (E-BUT) are more efficient against the *Proteus* sp. and *Staphylococcus* sp. species when compared to other extracts because the lowest concentration was able to inhibit bacterial growth. Results from the MIC indicated that the lower concentration needed to satisfactorily inhibit bacterial growth for more effective action of an antimicrobial agent. Moreover, low values of MIC possibly have less toxic effects on living organisms (Brooks et al., 2012).

In a study by Toudert et al. (2009) the crude methanolic and butanolic extracts of the aerial parts of *Ampelodesma mauritanica* were examined for *in vitro* antibacterial and antifungal activity using the disc diffusion method. Liasu and Ayandele (2008) argue that the low MIC values exhibited by the plant extracts represent a possible replacement of the usual antibiotics to treat infections because the development of resistance to known antibiotics by selection pressure mechanisms in bacteria is a reality. In this study, we demonstrated MIC values of  $> 100$  mg/ml for *S. aureus* and *P. vulgaris*.

Several studies have indicated the sensitivity of *S. aureus* to plant extracts. In a study by Chomnawang et al. (2009) the hydroalcoholic extracts of *Garcinia mangostana* showed significant activity against *S. aureus*, with MIC and MBC values of 1.95 and 3.91 µg/ml, respectively. Suffredini et al. (2006) have shown that the organic extract obtained from *T. aff. longifolia* leaves and *Haploclathra paniculata* seeds possesses significant activity against *S. aureus* in the broth microdilution test, with an MIC of  $\leq 200$  mg/mL. The experiments with ethanol extract of *Synclisa scabrida* used by Okoli and Iroegbu (2005) indicate an MIC of 6 mg/ml for *S. aureus* and antimicrobial activity investigation of roots of *Murraya koenigii* L. Spreng. (Rutaceae), an endemic plant in Turkey by Vats et al. (2011) showed that the same bacteria are sensitive to the chloroform and ethyl acetate extracts from *M. koenigii*, with an MIC  $\geq 0.625$  µg/ml and inhibitions halos  $< 15$  mm for chloroform extract.

The values of MBC obtained were equal to or lower than the concentration of MIC. This fact indicates the bactericidal action of the crude and semi-pure extracts tested. If the bacterial strains survive in numbers higher than those found for the MIC, they are tolerant to the tested antibacterial agent. This is an important concept to be considered for the treatment of infections, because

**Table 3.** The Minimum Inhibitory Concentration-MIC and Minimum Bactericidal Concentration-MBC values of the crude and semi-pure extracts of *Myracrodruon urundeuva*.

Microorganism	Inhibition (mg/ml) <sup>ab</sup>				
	CE	HEX-E	DC-E	EA-E	BUT-E
Minimal inhibitory concentration (MIC)					
<i>Proteus mirabilis</i>	≥250	≥220	≥190	≥140	≥120
<i>Proteus vulgaris</i>	≥250	≥220	≥ 70	≥30	≥ 30
<i>Proteus mirabilis</i> ATCC 25933	≥250	≥220	≥190	≥140	≥120
<i>Staphylococcus aureus</i>	≥125	≥110	≥190	≥140	≥30
<i>Staphylococcus</i> sp. cog. neg.	≥125	≥ 55	≥47.5	≥35	≥7. 5
<i>Staphylococcus aureus</i> ATCC 14458	≥125	≥110	≥190	≥140	≥30
Minimum bactericidal concentration (MBC)					
<i>Proteus mirabilis</i>	≥250	≥220	≥190	≥140	≥120
<i>Proteus vulgaris</i>	≥250	≥220	≥ 70	≥30	≥ 30
<i>Proteus mirabilis</i> ATCC 25933	≥250	≥220	≥190	≥140	≥120
<i>Staphylococcus aureus</i>	≥125	≥110	≥190	≥140	≥30
<i>Staphylococcus</i> sp. cog. neg.	≥125	≥ 55	≥47.5	≥35	≥7. 5
<i>Staphylococcus aureus</i> ATCC 14458	≥125	≥110	≥190	≥140	≥30

<sup>a</sup>, Average values  $\pm$  SD; n = 3 (the CIM and CBM were evaluated in triplicate for each of the extracts and fractions tested); C+, positive control (10  $\mu$ g of gentamicin); C-, negative control (5% Tween); CE, crude hydroalcoholic extract; E-HEX, hexane extract; E-DC, dichloromethane extract; E-EA, ethyl acetate extract; E-BUT, semi-pure butanolic extract; ATCC, American Type Culture Collection. <sup>b</sup>, Statistical analysis of the values by variance analysis (ANOVA).

antibiotic therapy should encompass a synergistic combination of antibiotics once tolerance is observed. In clinical practice, this phenomenon is often observed, for example, in cases of bacterial endocarditis, whose treatment combines gentamicin and amoxicillin (Frank and Taccone, 2012).

The bactericidal and/or bacteriostatic characteristics of a substance are considered important in the action of an antimicrobial agent. However, for strains with a multi-resistant profile, such as those that are often isolated in the hospital environment, like in these cases of study, the bactericidal activity of these preparations is a desirable characteristic (Tessier and Scheld, 2010).

The World Health Organization has encouraged the investigation of natural products that can act as antimicrobial agents, particularly against microorganisms having resistance profiles to commonly used antibiotics in clinical therapy, and the valorization and conservation of native Brazilian flora as a tool for independence in the production and distribution of low cost drugs (WHO, 2002).

## Conclusion

The crude and semi-pure extracts obtained from the leaves and bark of *M. urundeuva* (Fr. All.) showed a broad antibacterial spectrum, being capable of inhibiting the growth of different Gram-positive and Gram-negative bacterial strains. However, the extracts of Aroeira leaves

showed larger inhibition halos for Gram-positive than for Gram-negative bacteria species evaluated. It is possible that the active compounds present in these extracts act by inhibiting the synthesis of peptidoglycan of the cell wall of these microorganisms, especially in the Gram-positive bacteria tested.

The proven antibiotic action of the crude and semi-pure extracts from Aroeira leaves can be exploited to facilitate their applications as pharmaceutical adjuvants in antibiotic preparations. However, chromatographic studies that are more specific are needed for the characterization of antimicrobial molecules. Moreover, the feasibility of the antimicrobial application of the Aroeira extracts used in this study contributes to the preservation and recovery of this endangered species.

## ABBREVIATIONS

**MIC**, Minimal inhibitory concentration; **MBC**, minimum bactericidal concentration; **ATCC**, American Type Culture Collection; **CHE**, crude hydroethanol extract; **SPE-HEX**, semi-pure hexane extract; **SPE-DC**, semi-pure dichloromethane extract; **SPE-EA**, semi-pure ethyl acetate extract; **SPE-BUT**, semi-pure butanolic extract; **PTLC**, preparative thin layer chromatography; **EO**, essential oil.

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