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MICROBIOLOGY

Antimicrobial activity of amazon medicinal plants

Paulo Roberto Pires de Souza Júnior[1], Geyse Souza Santos[2], Laryssa dos Santos Prado[1*], Leila Priscila Peters[1,3] and Clarice Maia Carvalho[1,4]

[1]Programa de Pós-graduação em Ciência, Inovação e Tecnologia para Amazônia, Universidade Federal do Acre, Rodovia BR-364, Km 04, Distrito Industrial, 69920-900, Rio Branco, Acre, Brazil. [2]Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Universidade Federal do Acre, Rio Branco, Acre, Brazil. [3]Centro de Ciências da Saúde e Desporto, Universidade Federal do Acre, Rio Branco, Acre, Brazil. [4]Centro de Ciências Biológicas e da Natureza, Universidade Federal do Acre, Rio Branco, Acre, Brazil. *Author for correspondence. E-mail: laryssaprado348@gmail.com

ABSTRACT. In recent decades, an increase in resistance of bacteria and fungi to antimicrobials has been observed. Therefore, have each other investigated the antimicrobial potential of plants with medicinal properties. In this context, the Amazon has a high variability of plants with potentially active compounds. Thus, the present study aimed to evaluate the antimicrobial activity of ethanol extracts from Amazonian medicinal plants. Leaves and stems of 19 plant species were dried, crushed and extracted by cold maceration with ethanol. The extracts were dried and solubilized at a concentration of 100 mg mL^{-1} to done antimicrobial activity assays using the agar diffusion method against gram-positive and gram-negative bacteria, and fungi of the genus *Candida*. Of the total of 38 extracts analyzed, 14 (36.8%) had antimicrobial activity against at least one of the microorganisms tested. The Brazilnut tree stem extract was active against the highest number of microorganisms, three bacteria (*S. aureus*, *E. coli* and *K. pneumoniae*) and three fungi (*C. tropicalis*, *C. parapsilosis* and *C. krusei*). The mulateiro leaf extract was active against three microorganisms, the saracura-mira stem extract against two, and the other extracts only against one microorganism. *K. pneumoniae* was the most sensitive microorganism, and the most resistant was *C. albicans*. The lowest values for minimum inhibitory concentration (MIC) for antibacterial activity were for stone-breaker stem extract against *K. pneumoniae* (0.048 mg mL^{-1}), and for antifungal activity for Brasilnut tree stem against *C. krusei* (3.125 mg mL^{-1}), stone-breaker leaf against *C. guillermondii* (3.125 mg mL^{-1}), and mulateiro leaf against *C. tropicalis* (3.125 mg mL^{-1}). The lowest values of minimum microbicidal concentration (MMC) were for the extracts of stone-breaker stem against *K. pneumoniae* (0.192 mg mL^{-1}) and mulateiro leaf against *C. tropicalis* (3.125 mg mL^{-1}). The tested Amazonian medicinal plants had antimicrobial activity, especially Brazilnut tree, this being the first report for this plant species.

Keywords: Brazilnut tree; saracura-mira; stone-breaker; mulateiro.

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Introduction

The use of medicinal plants is an important therapeutic option, presenting a long history of human interactions with the environment, and even today there are several native plants that continue to be used empirically by the population, due to their pharmacological activities, such as antimicrobial action (Melro et al., 2020). In this way, the selection of medicinal plants may be able to contribute to the discovery of new compounds, with effective active principles against microbial pathogens of interest to health (Fonseca et al., 2020).

The Amazon has great biological and sociocultural diversity, represented by indigenous peoples and traditional populations, who have an invaluable collection of traditional knowledge about regional biodiversity (Marques, Anjos, & Costa, 2020). Conducting studies with plants used by these ethnic groups and in traditional medicine is considered a priority in the Brazilian Amazon due to the great richness of its flora, which can be used to obtain antimicrobial substances (Pereira, Santos, Rodrigues, Andrade, & Guimarães, 2020).

In contrast to the use of plants for effective therapeutic purposes, even empirically, against various pathologies by traditional peoples, the emergence of multiresistant bacteria with high potential for dissemination, infectivity and pathogenicity is observed, which is capable of resulting in superinfections, in the which conventional antibiotic therapy may have only a limited reach (De Souza et al., 2020). With this constant increase in microbial resistance to antibiotics and antifungals, it is necessary to look for therapeutic options with active compounds that explore new pathways of action (Perfect & Ghannoum, 2020). This leads to current searches for ethnopharmacy medicinal plants and their by-products (De Sousa et al., 2019).

The use of natural adjuvants has properties that make certain compounds have antimicrobial activity, such as polyphenols, which have as their main characteristic the ability to bind to macromolecules such as glycoproteins, proteins and terpenoids, which have a high ability to penetrate the cell membrane, characteristic attributed to its high lipophilicity (Zacchino et al., 2017; Roumy et al., 2019).

Thus, the objective of this study is to evaluate the antimicrobial activity of Amazonian medicinal plants against pathogenic microorganisms.

Material and methods

Collection of Plant Material

Leaves and stems of 19 Amazonian medicinal plants were collected (Table 1) during the months of January and February 2020 at the Zoobotanical Park and Nursery from *Universidade Federal do Acre*, Rio Branco, Acre State, Brazil (09°57.429' S and 067°52.412' W) (Figures 1 and 2). Only jambu was purchased from a large local supermarket chain.

Table 1. Plant part, sample weight and extract yield of Amazonian medicinal plants.

Plant	Plant part	Dry material weight (g)	Extract yield (g)	Yield for 100 g (g)
Andiroba	leaf	15.7	0.794	5.122
	steam	34.0	1.295	3.808
Açai	leaf	23.2	1.119	4.823
	steam	42.5	0.672	1.581
Breu branco	leaf	53.2	2.178	4.093
	steam	47.8	1.275	2.667
Carapanauba	leaf	45.7	2.155	4.715
	steam	127.4	4.233	3.322
Brazilnut tree	leaf	43.1	2.356	5.466
	steam	16.8	5.321	3.672
Copaiba	leaf	110.4	6.716	6.083
	steam	84.9	4.750	5.594
Cumaru	leaf	35.1	2.879	8.202
	steam	52.4	1.923	3.669
Embauba	leaf	33.9	2.792	8.235
	steam	36.8	0.172	0.467
Jambu	leaf	32.7	1.760	5.382
	steam	29.6	0.732	2.472
Brush-pasture	leaf	39.9	1.697	4.253
	steam	33.7	1.030	3.056
Mulateiro	leaf	58.9	6.384	10.838
	steam	43.7	0.365	0.835
Murumuru	leaf	45.2	0.472	1.044
	steam	61.3	1.647	2.686
Stone-breaker	leaf	34.9	1.721	4.931
	steam	83.8	3.088	3.684
Sacaca	leaf	26.6	0.997	3.748
	steam	21.7	0.248	1.142
Dragon´s blood	leaf	29.3	2.570	8.771
	steam	52.1	1.948	3.738
Saracura-mira	leaf	40.5	1.643	4.056
	steam	55.8	3.992	7.154
Sucuuba	leaf	48.8	4.622	9.471
	steam	55.5	1.893	3.410
Cat´s claw	leaf	58.9	5.349	9.081
	steam	49.3	1.786	3.622
Urucum	leaf	27.5	1.598	5.810
	steam	36.6	0.862	2.355

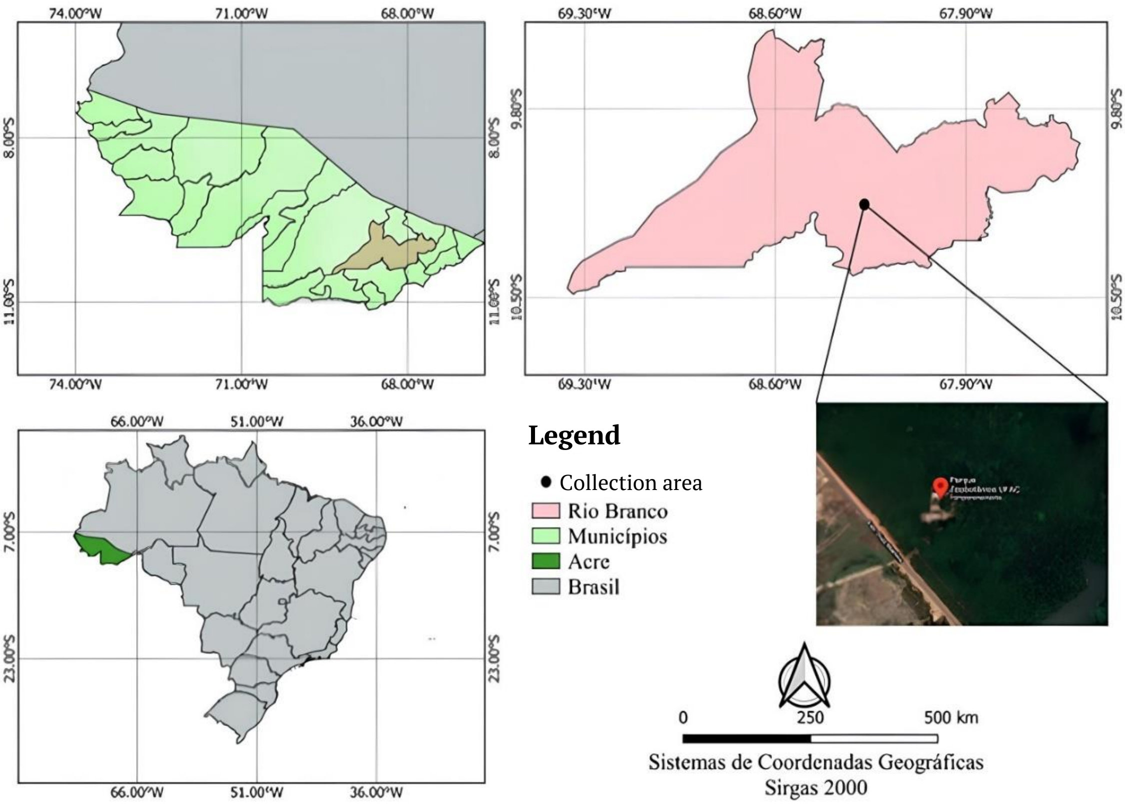

Figure 1. Collection site of Amazonian medicinal plants.

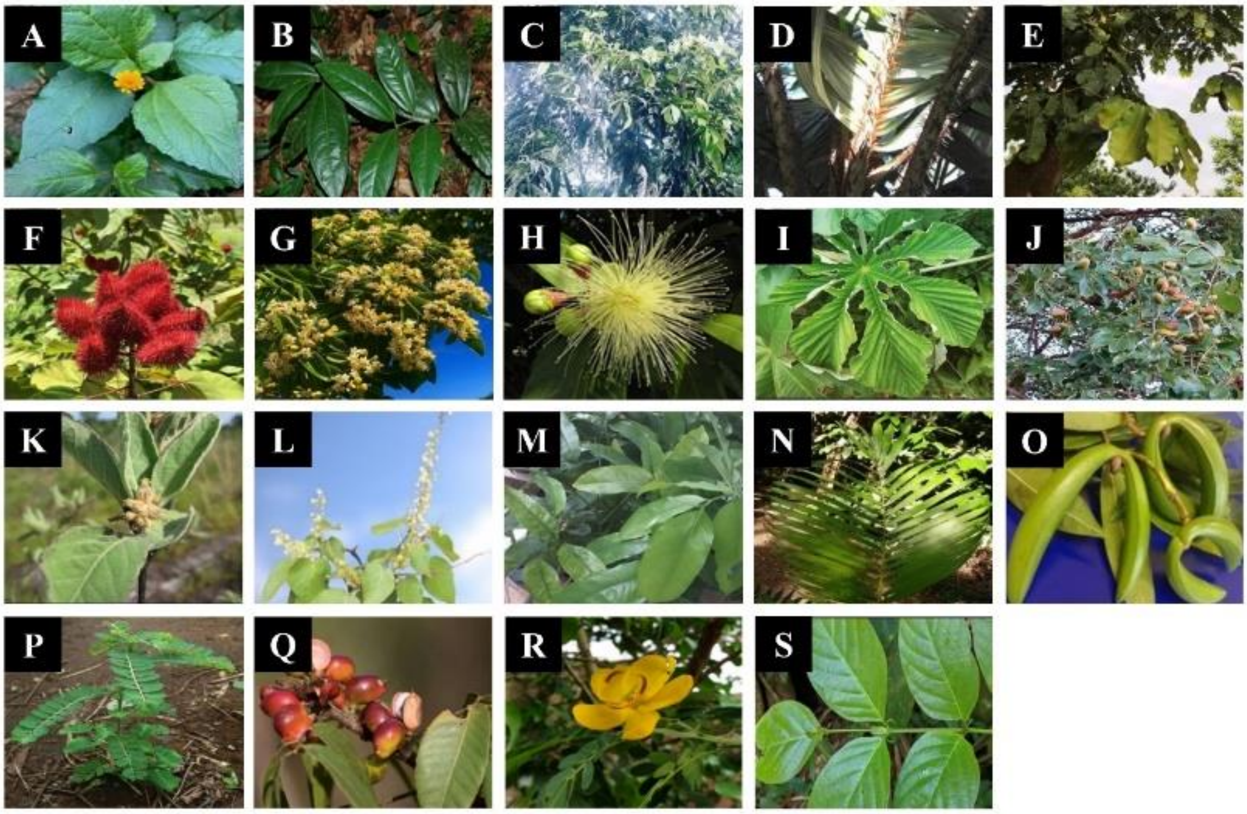

Figure 2. Image, scientific, and popular name of Amazonian medicinal plants. A) *Acmella oleracea* (Jambu); B) *Ampelozizyphus amazonicus* (Saracura-mira); C) *Aspidosperma discolor* (Carapanauba); D) *Astrocaryum murumuru* (Murumuru); E) *Bertholletia excelsa* (Brazilnut tree); F) *Bixa orellana* (Urucum); G) *Calycophyllum spruceanum* (Mulateiro); H) *Carapa guianensis* (Andiroba); I) *Cecropia pachystachya* (Embauba); J) *Copaifera multijuga* (Copaiba); K) *Croton cajucara* (Sacaca); L) *Croton lechleri* (Dragon's blood); M) *Dipteryx odorata* (Cumaru); N) *Euterpe oleracea* (Açai); O) *Himatanthus sucuuba* (Sucuuba); P) *Phyllanthus niruri* (Stone-breaker); Q) *Protium heptaphyllum* (Breu branco); R) *Senna obtusifolia* (Bush-pasture); and S) *Uncaria tomentosa* (Cat's claw).

Obtaining the crude ethanol extract (bee)

Plant leaves and stems were dried in a forced circulation oven at a temperature of 40 °C, crushed in a knife mill and weighed on a semi-analytical balance. To produce the extracts, the plant material was cold macerated in 92.8% ethanol for 24 hours, this step was repeated three times (Santi, Sanches, Silva, & Santos, 2014). The extracts were dried until complete evaporation of the solvent and constant weight in a hood with circulating air. The extracts obtained were stored in glass bottles, weighed to calculate the yield and stored at room temperature.

The extracts were solubilized in dimethylsulfoxide (DMSO) at a concentration of 100 mg mL^{-1} to done antimicrobial assays.

Antimicrobial activity by well diffusion test in solid medium

The bacterial strains used in this study were three Gram positive bacteria, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, and two Gram negative bacteria, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883. To evaluate the antifungal activity, five species of *Candida* were used, *C. albicans* ATCC 90028, *C. guillermondii* ATCC 6260, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 28707.

To prepare the microbial inocula, the bacteria were inoculated in Petri dish with Mueller Hinton agar medium (MH) and the fungi in Petri dish with Sabouraud agar medium (SDA), which were incubated for 24 hours at a temperature of 37 °C, in aerobic conditions. After incubation, isolated colonies were collected with the aid of a loop, and transferred to tubes with sterile saline NaCl 0.9% (w/v) until obtaining a turbidity corresponding to 0.5 McFarland scale, approximately 1 x 108 CFU mL^{-1} for bacterial colonies, and for fungi the turbidity corresponding to the McFarland scale 1.0, approximately 1 x 108 CFU mL^{-1} (Clinical and Laboratory Standards Institute [CLSI], 2009a).

The test described by Clinical and Laboratory Standards Institute (2009a) was used, which consists of applying a microbial inoculum with approximately 1 x 108 CFU mL^{-1} with the aid of a sterilized swab on the surface of the Petri dish with MH agar medium for bacteria and SB for fungi. After, wells measuring 5 mm in diameter were made in the inoculated media and 20 μL of plant extracts solubilized in DMSO at a concentration of 100 mg mL^{-1} were inserted, with three repetitions for each plant extract. Plates were kept at 4 °C for 24 hours for extract diffusion and then incubated for 24 hours at 37 °C. After incubation, the diameters of the microbial growth inhibition halos around each well were measured in millimeters with an antibiogram ruler. The results were expressed as mean ± standard error of the mean, and the statistical differences between the experimental groups and the control were verified using One-Way Analysis of Variance (ANOVA), followed by the Dunnett's test ($p < 0.05$). The analyses were performed using RStudio software.

Minimum inhibitory concentration and minimum microbicidal concentration

The Minimum Inhibitory Concentration (MIC) of extracts with antimicrobial activity was done using the microdilution technique, with sterile 96-well microplates. For antimicrobial activity, MH broth was used for bacteria and SDA broth for fungi. 100 μL of broth were distributed in all wells of the plate, and then 100 μL of each extract at a concentration of 200 mg mL^{-1} were added to the test well for serial dilution. Following the serial dilution process eight times, homogenizing and transferring 100 μL to the next well, and so on, resulting in final concentrations of 1.562 μg mL^{-1}. The control drug, Chloramphenicol 30 μg mL^{-1} for bacteria, and Ketoconazole 50 μg mL^{-1} for fungi, was diluted similarly to the extracts.

Five μL of the inoculum corresponding to each tested strain were added except for the negative control (Clinical and Laboratory Standards Institute [CLSI], 2009b). The negative control contained only 100 μL of broth, and the positive control 100 μL of broth and 5 μL of inoculum. The microplates were incubated at 37 °C for 24 hours, after which time 20 μL of Resazurin reagent (0.30 mg mL^{-1}) was added to each well, which indicates microbial growth when the color changes from blue to red (Riss et al., 2016).

To evaluate the Minimum Microbicidal Concentration (MMC) and confirm the microstatic/microbicidal action of the extract dilutions, the MIC dilution and the two immediately higher concentrations were inoculated in the MH agar culture medium for bacteria and SD for fungi. The plates were incubated at 37 °C for 24 hours and analyzed with observation of the presence of microbial growth (Azevedo et al., 2012).

Results

Antimicrobian activity

Of the total of 38 extracts of Amazonian medicinal plants evaluated, 14 (36.8%) extracts, 7 from leaves and 7 from stems, had antimicrobial activity (Table 2). No extract had antimicrobial activity against all microorganisms analyzed, and *C. albicans* was the only microorganism resistant to all extracts.

The extracts with activity against the greatest number of microorganisms were those of Brazilnut tree stems against *S. aureus*, *E. coli*, *K. pneumoniae*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*, and mulateiro leaf extract against *S. aureus*, *E. faecalis*, and *K. pneumoniae*. Brazilnut tree stem extract had the highest inhibition zones against *C. tropicalis*, *C. parapsilosis*, and *C. krusei*.

Klebsiella pneumoniae was the most sensitive microorganism, inhibited by 9 extracts, followed by *S. aureus* with the action of 6 extracts. The only extract with inhibitory activity against *E. coli* was from the Brazilnut tree leaf, for *E. faecalis* only from the mulateiro leaf and for *S. pneumoniae* only from the saracura-mira stem.

No extract inhibited the growth of all *Candida* species evaluated. The extract with the greatest spectrum of action was that of Brazilnut tree stem, with activity against *C. tropicalis*, *C. parapsilosis*, and *C. krusei*, with the highest inhibitory halos. The stone-breaker leaf extract also showed activity against *C. tropicalis* and *C. guillermondii* species.

Table 2. Antimicrobial activity of Amazonian medicinal plants.

Plant	Part vegetable	Microorganism halo (mm)								
		Sau	*Spn*	*Efa*	*Eco*	*Kpn*	*Ctro*	*Cpar*	*Ckru*	*Cgui*
Andiroba	Stem	-	-	-	-	10±2*	-	-	-	-
Brazilnut tree	Stem	16±2*	-	-	5±1*	12±2*	20±2*	30±2*	20±2*	-
Copaíba	Stem	-	-	-	-	12±2*	-	-	-	-
Brush-pasture	Stem	10±2*	-	-	-	-	-	-	-	-
Mulateiro	Leaf	10±1*	-	12±3*	-	14±1*	-	-	-	-
Murumuru	Leaf	-	-	-	-	10±2*	-	-	-	-
Stone-breaker	Leaf	-	-	-	-	-	10±1*	-	-	10±2*
	Stem	-	-	-	-	10±3*	-	-	-	-
Sacaca	Leaf	12±2*	-	-	-	-	-	-	-	-
Saracura-mira	Leaf	-	-	-	-	5±1*	-	-	-	-
	Stem	10±1*	10±1*	-	-	-	-	-	-	-
Sucuuba	Stem	-	-	-	-	12±2*	-	-	-	-
Cat´s claw	Leaf	12±2*	-	-	-	-	-	-	-	-
Urucum	Leaf	-	-	-	-	12±2*	-	-	-	-
chloramphenicol	30 μg mL^{-1}	38±0	48±0	43±0	46±0	48±0	-	-	-	-
ketoconazole	50 μg mL^{-1}	-	-	-	-	-	43±0	43±0	42±0	43±0

Sau: Staphyloccoccus aureus; *Spn: Streptococcus pneumoniae*; *Efa: Enterococcus faecalis*; *Eco: Escherichia coli*; *Kpn: Klebsiella pneumoniae*; *Cal: Candida albicans*; *Ctro: Candida tropicalis*; *Cpar: Candida parapsilosis*; *Ckru: Candida krusei*; and *Cgui: Candida guillermondii*. Halo in millimeters (mm). Note: *p < 0.05: significantly different from the control (vehicle). Analysis performed using One Way ANOVA followed by Dunnett's test.

Minimum inhibitory concentration (mic) and minimum microbial concentration (mmc)

Against Gram-positive bacteria, the MIC varied between 1.562 and 100 mg mL^{-1}, the lowest value was 1.562 mg mL^{-1} for the extract of saracura-mira stem against *S. pneumoniae* and the highest value was 100 mg mL^{-1} for the extracts of brush-pasture stem, saracura-mira stem and cat's claw leaf against *S. aureus*.

The lowest and highest values of MBC were for the extract of stem of saracura-mira with CMM = 6.25 mg mL^{-1} against *S. pneumoniae* and the extracts of stem from brush-pasture, stem from saracura-mira stem and leaf of cat´s claw had values of CMM 100 mg mL^{-1}.

The lowest values of MIC and MMC against Gram-negative bacteria were from the extracts of stem stone-breaker, sucuuba and copaiba, against *K. pneumoniae*. The highest MIC and MMC values were obtained from murumuru leaf extracts against *K. pneumoniae*.

For antifungal activity, the lowest MIC value was 3.125 mg mL^{-1} for the extracts of stone-breaker leaf against *C. guillermondii*, Brazilnut tree stem against *C. krusei* and mulateiro leaf against *C. tropicalis*. The highest MIC value was for the Brazilnut tree stem extract against *C. tropicalis*. The lowest MMC value was for the mulateiro leaf extract against *C. tropicalis*, and the highest value for the Brazilnut tree stem extract against *C. tropicalis* (Table 3).

Table 3. Evaluation of the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of vegetal extracts of Amazonian medicinal plants.

Plant	Part vegetable	Microorganism (mg mL^{-1})																	
		Sau		*Spn*		*Efa*		*Eco*		*Kpn*		*Cgu*		*Ckr*		*Ctr*		*Cpa*	
		MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Andiroba	Stem	-	-	-	-	-	-	-	-	6.25	25	-	-	-	-	-	-	-	-
Brazilnut tree	Stem	25	100	-	-	-	-	6.25	25	6.25	25	-	-	3.125	12.5	100	100	12.5	50
Copaiba	Stem	-	-	-	-	-	-	-	-	3.125	12.5	-	-	-	-	-	-	-	-
Brush-pasture	Stem	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mulateiro	Leaf	12.5	50	-	-	12.5	50	-	-	12.5	50	-	-	-	-	-	-	-	-
Murumuru	Leaf	-	-	-	-	-	-	-	-	25	100	-	-	-	-	-	-	-	-
Stone-breaker	Leaf	-	-	-	-	-	-	-	-	-	-	3.125	12.5	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	0.048	0.192	-	-	-	-	-	-	-	-
Sacaca	Leaf	6.25	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saracura-mira	Leaf	-	-	-	-	-	-	-	-	6.25	25	-	-	-	-	-	-	-	-
	Stem	100	100	1.562	6.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucuuba	Stem	-	-	-	-	-	-	-	-	3.125	12.5	-	-	-	-	-	-	-	-
Cat´s claw	Leaf	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urucum	Leaf	-	-	-	-	-	-	-	-	6.25	25	-	-	-	-	-	-	-	-
chloramphenicol	30 µg mL^{-1}	0.117	0.234	0.234	0.234	6.25	6.25	0.937	0.937	0.117	0.234	-	-	-	-	-	-	-	-
ketoconazole	50 µg mL^{-1}	-	-	-	-	-	-	-	-	-	-	6.25	12.5	0.097	0.390	0.390	0.780	0.195	0.195

MIC; MMC = mg mL^{-1}; *Sau: Staphyloccoccus aureus*; *Spn: Streptococcus pneumoniae*; *Efa: Enterococcus faecalis*; *Eco: Escherichia coli*; *Kpn: Klebsiella pneumoniae*; *Cgu: Candida guillermondii*; *Ckr: Candida krusei*; *Ctr: Candida tropicalis*; and *Cpa: Candida parapsilosis.*

Discussion

The plant kingdom is responsible for the largest share of chemical diversity recorded in the literature, especially in Brazil, with approximately 20% of all known plant species (Freitas et al., 2018). Thus, research with plants of consolidated ethnopharmacological use in the Amazon represents an alternative for combating pathogens, especially resistant species (Silva et al., 2017).

The ethanolic extracts of 12 evaluated Amazonian plant species had antimicrobial activity. The extracts of Brazilnut tree stem, mulateiro leaf, stone-breaker leaf and saracura-mira stem extract inhibited the growth of more than one microorganism. The extracts of leaf from cat's claw leaf, sacaca, urucum, murumuru and the stem from brush-pasture, copaiba, sucuuba and andiroba inhibited at least one microorganism tested.

The ethanolic extract of the Brasilnut tree stem inhibited the growth of Gram-positive bacteria, *S. aureus*, and Gram-negative bacteria, *K. pneumoniae* and *E. coli*, in addition to the fungi *C. krusei*, *C. tropicalis*, and *C. parapsilosis*. Other studies evaluating extracts from this plant also showed antimicrobial activity. The hydroethanolic extract form bark of Brasilnut tree had antibacterial activity against *S. aureus*, as in this work, in addition to the bacteria *Helicobacter pylori*, *Streptococcus pyogenes*, and the fungi *Aspergillus terreus*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Candida glabrata* (Silva et al., 2017). And the crude hydroalcoholic extract of Brasilnut tree bark had activity against Gram negative bacteria *K. pneumoniae* and *E. coli* (Campos, Costa, Barbosa, & Barbosa, 2011).

Other biological activities reported for Brasilnut tree *in vitro* are antiplasmodic and antiparasitic activity against *Trypanosoma cruzi* (Campos et al., 2005), and anti-inflammatory and antioxidant activity of its oil (Barata et al., 2020). These biological activities described for Brasilnut tree are related to the presence of secondary metabolites such as triterpenes, saponins, steroids, tocopherols, phenolic acids, flavonoids and derivatives, sequiterpenes, norisoprenoids, fatty acid derivatives, coumarins, xanthones and depsides (Silva et al., 2017).

The mulateiro leaf extract had antimicrobial activity against Gram-positive bacteria *S. aureus*, *E. faecalis* and Gram-negative *K. pneumoniae*. Also, about antimicrobial activity, a study was done with methanolic extract of mulateiro bark, and activity was observed against *S. aureus*, as in this work, and also against *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis*. Furthermore, the presence of chemical compounds from the class of iridoids, terpenes, phenols and alkaloids has also been reported in this extract (Dookie et al., 2021).

The stone-breaker stem extract inhibited the growth of *K. pneumoniae* and the leaf extract *C. guillermondii*. Studies were also found in the literature reporting the activity of the hydroalcoholic extract from stone-breaker (mix stem, leaf and seed) against *Candida*, having activity against *C. krusei*, *C. albicans* and *C. guilliermondii*, as well as in this work (Maia et al., 2022). For antibacterial activity, the evaluation of the activity of saponins and alkaloids extracted from stone-breaker against *E. coli*, *S. aureus*, *Salmonella* spp., *Bacillus* *subtilis*, *K. pneumoniae*, and *P. aeruginosa*, a synergistic effect was observed between saponins and alkaloids to inhibit the growth of these bacteria (Ajibade, 2014).

The saracura-mira stem extract inhibited the growth of *S. aureus* and *S. pneumoniae*, and the leaf extract inhibited the growth of Gram negative bacteria *K. pneumoniae*. Study evaluating the antimicrobial activity of methanolic extract of saracura-mira leaves had activity against *C. albicans*, *Bacillus subtilis*, *Streptococcus faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella typhimurium*, and *Mycobacterium phlei* and against *K. pneumoniae*, as well as observed in this work (Lopez, Hudson, & Towers, 2001). Another study evaluated the methanolic extract of saracura-mira leaves for antimicrobial activity against the human intestinal bacteria *Bifidobacterium longum*, reported as main triterpenic compounds, lupeol, betulinic acid, betulin, melaleucic acid and phytosteroids reported with at least 23 activities, as antimalarial , anthelmintic and antiviral (Diniz et al., 2009). The presence of lapachol was also reported, a substance known to have anti-inflammatory, analgesic, antibiotic, antimalarial, antitrypanosome, antiulcerogenic, bactericidal, fungicidal activity, in addition to antitumor and anticarcinogenic activity (Araújo, Alencar, & Rolim Neto, 2002).

Extracts of cat's claw, sacaca, urucum, murumuru and matapasto, copaiba, sucuuba and andiroba stems inhibited at least one tested microorganism. Other studies have reported antimicrobial activity of stem and leaf extracts from these Amazonian plant species.

Copaiba had activity reported against the microorganisms *S. aureus* (Santos et al., 2008; Santos et al., 2013; Alencar et al., 2015; Vieira et al., 2018), *E. faecalis* (Santos et al., 2008; Abrão et al., 2015), *S. pneumoniae*, *K. pneumoniae*, *E. coli* (Abrão et al., 2015), *C. krusei* (Alencar et al., 2015), *S. mutans*, *S. sanguinis* (Vasconcelos, Veiga Junior, Rocha, & Bandeira, 2008), *Bacillus cereus* (Santos et al., 2013), *Bacillus subtilis* (Santos et al., 2008), and *Candida glabrata* (Alencar et al., 2015).

Murumuru had reports in the literature of activity against *S. aureus* (Hovorková, Laloučková, & Skřivanová, 2018), sucuuba against *C. albicans* (Morel, Graebner, Porto, & Dalcol, 2006) and *S. aureus* (Mostafa et al., 2018), sacaca against *S. aureus*, *C. albicans* (Azevedo et al., 2012), and *S. mutans* (Freires, Denny, Benso, Alencar, & Rosalen, 2015), andiroba against *S. aureus* (Brito, Brazão, Siqueira,& Santos, 2001; Meccia et al., 2013), *E. faecalis* (Santos et al., 2008; Meccia et al., 2013; Miranda et al., 2019), and *E. coli* (Brito et al., 2001), and cat's claw against *Enterobacteriaceae*, *S. mutans*, and *Staphylococcus* spp. (Ccahuana-Vasquez, Santos, Koga-Ito, & Jorge, 2007).

Gram negative bacteria were more sensitive to ethanolic extracts of the evaluated microbial species, when compared to Gram positive bacteria. Only the Gram-negative bacteria *K. pneumoniae* had its growth inhibited by the extracts of 9 species of Amazonian medicinal plants, Brazilnut tree, mulateiro, urucum, saracura-mira, murumuru, copaiba, sucuuba, andiroba and stone-breaker. All these species have lipophilic compounds such as terpenoids, which are involved in the disruption of the bacterial membrane and can be found in some botanical families such as Lecythidaceae, Meliaceae, Bixaceae, Fabaceae and Lecythidaceae (Winska et al., 2019). Species with specific activity against Gram positive bacteria, cat's claw, sacaca and brush-pasture are rich in alkaloids or lipids (Kurek, 2019). This is likely a result of differences in the cell wall structure of Gram positive and Gram negative bacteria, as the Gram negative outer membrane contains lipopolysaccharides, which act as a barrier to many environmental substances, including antibiotics (Epand, Walker, Epand, & Magarvey, 2016).

Conclusion

The Amazonian medicinal plants had activity against pathogenic microorganisms, being necessary studies of these plants as potential in the fight against resistant microorganisms. This work brings the first report of antimicrobial activity of Brasilnut tree stem and mulateiro leaf extracts. The extracts with the most promising antimicrobial activity were Brazilnut tree, mulateiro and saracura-mira stem extracts, that inhibited Gram-positive and Gram-negative bacteria and fungi of the genus *Candida*. Other studies are necessary to verify which secondary metabolites have this activity and if these extracts have activity against resistant microorganisms.

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