PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL POTENTIAL OF EXTRACTS AND FRACTIONS OF BROSIMUM GAUDICHAUDII TRÉCUL AGAINST BACTERIA OF CLINICAL IMPORTANCE

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ABSTRACT: Medicinal plants constitute an arsenal of products with different potentials to be explored. Therefore, the objective of this paper was to assess the antimicrobial potential of the ethanolic extract from Brosimum gaudichaudii leaves and fractions against clinically important bacteria. The crude extracts and fractions from the leaves and stem bark were used against Escherichia coli, Klesbsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus strains. The crude extracts and the fractions were obtained by means of maceration in ethanol and chemically characterized. In the results of the phytochemical screening, the presence of a variety of secondary metabolites was verified, such as flavonoids, steroids, saponins, alkaloids, tannins and coumarins. The extracts and their fractions showed inhibitory activity for all three bacteria tested. The inhibition halo varied from 8±0.00 to 14±0.00 mm fir K. pneumoniae, from 8±0.00 to 10±0.00 mm for P. aeruginosa and from 8±0.00 to 9±0.00 mm for S. aureus. Among the fractions tested, the ethyl acetate fraction from both the stem and the leaves presented the best inhibition potential. This indicates that the Brosimum gaudichaudii Trécul vegetable extracts present antimicrobial potential. Such being the case, it is suggested to isolate the metabolites present in this fraction to delimit the main compounds responsible for the antimicrobial action.

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KEYWORDS: Antibacterial Potential; Brosimun; Moraceae; Plant Compounds.

ANÁLISE FITOQUÍMICA E POTENCIAL ANTIMICROBIANO DE EXTRATOS E FRAÇÕES DE BROSIMUM GAUDICHAUDII TRÉCUL CONTRA BACTÉRIAS DE IMPORTÂNCIA CLÍNICA

RESUMO: As plantas medicinais constituem um arsenal de produtos com diferentes potenciais a serem explorados. Portanto, o objetivo deste trabalho foi avaliar o potencial antimicrobiano do extrato etanólico das folhas e frações de Brosimum gaudichaudii contra bactérias de importância clínica. Os extratos brutos e as frações das folhas e da casca do caule foram usados contra cepas de Escherichia coli, Klesbsiella pneumoniae, Pseudomonas aeruginosa e Staphylococcus aureus. Os extratos brutos e as frações foram obtidos por meio de maceração em etanol e caracterizados quimicamente. Nos resultados da triagem fitoquímica, foi verificada a presença de uma variedade de metabólitos secundários, como flavonoides, esteroides, saponinas, alcaloides, taninos e cumarinas. Os extratos e suas frações apresentaram atividade inibitória para todas as três bactérias testadas. O halo de inibição variou de 8±0,00 a 14±0,00 mm para K. pneumoniae, de 8±0,00 a 10±0,00 mm para P. aeruginosa e de 8±0,00 a 9±0,00 mm para S. aureus. Entre as frações testadas, a fração de acetato de etila do caule e das folhas apresentou o melhor potencial de inibição. Isso indica que os extratos vegetais de Brosimum gaudichaudii Trécul apresentam potencial antimicrobiano. Sendo assim, sugere-se o isolamento dos metabólitos presentes nessa fração para delimitar os principais compostos responsáveis pela ação antimicrobiana.
PALAVRAS-CHAVE: Potencial Antibacteriano; Brosimun; Moraceae; Compostos Vegetais.

ANÁLISIS FITOQUÍMICO Y POTENCIAL ANTIMICROBIANO DE EXTRACTOS Y FRACCIONES DE BROSIMUM GAUDICHAUDII TRÉCUL CONTRA BACTERIAS DE IMPORTANCIA CLÍNICA

RESUMEN: Las plantas medicinales constituyen un arsenal de productos con diferentes potenciales por explorar. Por lo tanto, el objetivo de este estudio fue evaluar el potencial antimicrobiano del extracto etanólico de hojas de Brosimum gaudichaudii y fracciones contra bacterias clínicamente importantes. Los extractos crudos y las fracciones de las hojas y la corteza del tallo se utilizaron contra cepas de Escherichia coli, Klesbsiella pneumoniae, Pseudomonas aeruginosa y Staphylococcus aureus. Los extractos crudos y las fracciones se obtuvieron por maceración en etanol y se caracterizaron químicamente. Los resultados del cribado fitoquímico verificaron la presencia de diversos metabolitos secundarios, como flavonoides, esteroides, saponinas, alcaloides, taninos y cumarinas. Los extractos y sus fracciones presentaron actividad inhibitoria para las tres bacterias ensayadas. El halo de inhibición varió de 8±0,00 a 14±0,00 mm para K. pneumoniae, de 8±0,00 a 10±0,00 mm para P. aeruginosa y de 8±0,00 a 9±0,00 mm para S. aureus. Entre las fracciones probadas, la fracción de acetato de etilo del tallo y las hojas mostró el mejor potencial de inhibición. Esto indica que los extractos vegetales de Brosimum gaudichaudii Trécul tienen potencial antimicrobiano. Así, se sugiere el aislamiento de los metabolitos presentes en esta fracción para determinar los principales compuestos responsables de la acción antimicrobiana.
PALABRAS CLAVE: Potencial Antibacteriano; Brosimun; Moraceae; Compuestos Vegetales.

1. INTRODUCTION

Medicinal plants constitute an arsenal of products with different potentials to be explored, being used as an alternative and complementary therapy for several health problems, also becoming the first option for health care, as the population believes in the benefits of treatments based on medicinal plants and, as they are easily accessible, they becomes a more accessible cure and prevention modality (SILVA et al., 2012; JUTEE et al., 2017).

With this, several studies are being focused on medicinal plants with the purpose of knowing their characteristics and biological potential for the development of new therapeutic products due to the increasing resistance of fungi and bacteria to conventional treatments (Sadiq et al. 2016). This bacterial resistance to antibiotics expands the need for new drugs, both for the treatment of infections acquired in the community and for in-hospital infections (PADILHA et al., 2010).

Bacterial resistance poses serious risks to the quality of life achieved over the years with the advancement of microbiology, engineering, pharmacy and medicine, further compromising the budget of healthcare systems, either public or private, and intensifying in-hospital infections (COSTA, 2017).

It is necessary to develop new therapeutic alternatives for the treatment of these pathologies due to some limitations presented by the conventional treatments, such as prolonged treatment period, toxicity, drug interactions and development of resistance (Ghuman et al. 2019). Consequently, researching new antimicrobial agents is a continuous need, and medicinal plants represent an important option with therapeutic potential targeted at resistant bacteria (SCORZONI et al., 2016; LEE; LEE, 2018).

The Brazilian savanna presents a diverse fauna and flora rich in natural resources with bioactive compounds that have significant importance for the development of new medications, as well as for the food and cosmetics industries (ROESLER et al., 2007; RAMOS et al., 2008; MALTA, 2011; ALVES et al., 2019).

The Moraceae family stands out among the varied plant species of the savanna. Representatives of this can be found as shrubs, trees, herbs or subshrubs, presenting woody stems, alternated leaves, unisexual flowers and fruits in the form of small nuts. In Brazil there are 19 genera and 203 species registered, with the Northeast region having 81 valid names and Maranhão with 36 valid names for the species (ROMANIUC et al., 2015). Many species of this family stand out in popular medicine for their expectorant

and bronchodilating effects in the treatment of Chagas disease, as anthelmintics, and in the treatment of skin diseases, vitiligo among them (LUZ et al., 2015).

The Brosimum Gaudichaudii Trécul species belongs to this family and is popularly known as mama-cadela, inharé or algodãozinho. It is economically important in certain regions of the country for both the sale and consumption of its fruit in natura; however, its most common application is in popular medicine, being empirically used by the population as an alternative for the treatment of fungal and bacterial infections (SILVA et al., 2011).

Given the above, the general objective of this paper was to assess the antibacterial potential of the crude extracts and fractions from Brosimum gaudichaudii (Moraceae) against clinically important bacteria.

2. MATERIALS AND METHODS

2.1 Selection and Acquisition of the Plant Material

The process to select the material is characterized as of utmost importance for the development of a study with vegetables, when referring to the possibility of them presenting some biological activity. For this purpose, Brosimun gaudichaudii Trécul fresh leaves and the stem bark were collected. Collection was performed in a private property located in the Luiza Queiroz neighborhood, municipality of Caxias, Maranhão: 4°54'06.6"S and 43°20'49.3"W.

The species was collected in August 2021, during the morning period between 08h00 and 11h00. All the parts of the vegetables were collected in amounts of approximately 2 kg, a value estimated as sufficient to obtain the extracts to be tested. After collection, exsiccatae of the plant material were made and identified under voucher number HABIT 4141 and deposited in the Professor Aluízio Bittencourt Herbarium (HABIT), belonging to the State University of Maranhão (Universidade Estadual do Maranhão, UEMA). The project and use are registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado, SisGen) under number AFC779E.

2.2 Preparation of the Plant Material

After collection, the material was sanitized with running water, and then dried in a drying oven with air circulation/renewal (Solab® Model SL-102), at a temperature of 37ºC. Grinding was performed in a Willye-type knife mill (Tecnal® Model R-TE-650/1), immediately before the extraction process, to mechanically reduce the plant material to small fragments.

2.3 Acquisition of the Ethanolic Extracts

To obtain the ethanolic extract, 1 L of the ethanol solvent (C2H5OH, 99%, Dinâmica®) was added to 100 grams of the leaves and 100 grams of the stem bark of the plant material separately. The extract was left to rest for 15 days, being exposed to daily agitation for 30 minutes in a mechanical agitator (Fisatom® Model 711). After this period, the material was filtered through a 9 mm filter paper (Química Moderna®) and placed into a rotary evaporator (Solab® Model SL-126) at 180 rpm with 45ºC, coupled to a vacuum pump (Prismatec® Model 132), for complete evaporation of the solvent, thus obtaining the crude ethanolic extract of the leaves and stem bark.

To perform this stage, the methodology described in the Brazilian Pharmacopoeia, 6th edition, published in 2019 by Resolution of the Collegiate Board of Directors (Resolução de Diretoria Colegiada) - RDC No. 298 of August 12th, 2019, was followed, and all the stages to obtain the extracts were performed in the Laboratory of Research in Nature Sciences (Laboratório de Pesquisa em Ciências da Natureza, LAPECNA) of the Federal Institute of Maranhão (Instituto Federal do Maranhão, IFMA), Caxias Campus.

2.4 Chemical Fractionation of the Extracts

For chemical fractionation, the methodology followed was the one described by Ûchoa et al., (2016), with modifications. The crude ethanolic extract was suspended in a Methanol/Water (CH3OH, Dinâmica®/H2O) mixture; the respective mixture was prepared by adding 10 mL of Methanol and 20 mL of distilled water- It was then subjected to a liquid-liquid partition process using Hexane (C6H14, Dinâmica®) (3 x 4 mL), Chloroform (CHCl3, Dinâmica®) (3 x 4 mL) and Ethyl Acetate (C4H8O2, Dinâmica®) (3 x 4 mL). The solvent was then removed in a rotary evaporator and the hexanic, chloroform, ethyl acetate and hydromethanolic fractions were obtained. Subsequently,

the crude ethanolic extract and the fractions were subjected to in vitro antimicrobial activity tests.

2.5 Calculation of the Extracts' Yields

Yield of the extracts was calculated according to the formula used by Rodrigues et al. (2011):

$$Y = (Wext/Wplant) \times 100.$$

Where:

Y=Total extract yield (%); Wext=Weight of the dry extract (g); Wplant=Weight of the fresh or dried leaves/stem bark (g).

2.6 Phytochemical Prospection of the Extracts

The phytochemical prospection of the crude ethanolic extracts and fractions of B. gaudichaudii was carried out at the Research in Nature Sciences Laboratory (LAPECNA) at IFMA-Caxias, following the methodology proposed by Matos (1997), and the assays were performed through colorimetric and precipitation reactions. This is a qualitative method for identifying the main metabolites present in plant material; therefore, the presence of tannins, flavonoids, steroids and terpenoids, saponins, alkaloids and coumarins was investigated. It is noted that all the phytochemical prospection tests were performed in triplicate and that a control test with distilled water was conducted.

2.7 Preparation and Maintenance of the Bacterial Strains

The Brazilian Microbiology Association recommends using standard strains from the American Type Culture Collection (ATCC) to perform quality control while working with bacteria. The following strains were used to such end: Escherichia coli ATCC-25922, Klesbsiella pneumoniae ATCC-13883, Pseudomonas aeruginosa ATCC-27853 and Staphylococcus aureus ATCC-25923, all commercially acquired.

Activation of the microorganisms was initiated with hydration of the freeze-dried strains, following the manufacturer's recommendations, adding 1 mL of 0.85% saline solution in each vial containing the strains, and leaving them at rest for one hour. Immediately after that, activation in BHI (Brain Heart Infusion, Kasvi®) broth was conducted. In this stage, test tubes containing 3 mL of BHI broth were prepared and a

100 µL aliquot of each strain was added and incubated at 36ºC for 24 hours in a bacteriological incubator.

After growth of the microorganisms, the inoculation procedure was performed in petri plates containing TSA (Tryptone Soy Agar, Kasvi®) culture medium, forming streaks and incubated at 36ºC for 24 hours. After this period, microbiological tests were initiated and the suspension prepared in test tubes containing BHI broth was stored in a refrigerator as stock.

2.8 Susceptibility Tests

2.8.1 Preparation of the extracts

The crude extracts and fractions were weighed on an analytical scale in the amounts of 10 mg, 25 mg, 50 mg and 100 mg, and were then diluted in DMSO (Dimethylsulfoxide, Dinâmica®) (100µL) and sterile distilled water (900µL) and vortexed, (Oleman® Model 22615) until complete dilution.

2.8.2 Preparation and inoculation of the microbial suspension

The susceptibility tests were performed following the diffusion disk method. This method consists in impregnating the disks with the substances at the concentrations to be tested. For this purpose, the plates were prepared containing Mueller Hinton Agar culture medium (Kasvi®), and the bacterial suspension was made by adding small fragments of the microorganisms' colonies in 3 mL of saline solution, adjusting the concentration to 0.5 on the MacFarland scale, obtaining approximately 1.5 x 108 colony forming units (CFUs)/mL.

After adjusting the inoculum, seeding was performed using a sterile swab. The swab was introduced into the suspension of colonies and, when removed, a slight compression was made against the inner wall of the test tube to remove the excess of inoculum, and then seeded onto the previously prepared plates. After seeding, a 10-minute interval was adopted between absorption of the inoculum by the agar and application of the disks; this time is necessary to prevent the disks from slipping on the agar.

2.9 Application of the Disks

Sterile disks (Kaj-Lab®, 6 mm) impregnated with 15 µL of the extracts at different concentrations (10, 25, 50 and 100 mg/mL) were used. The ciprofloxacin antibiotic was used as positive control, and DMSO 10% was employed as negative control, with certain distance between one disk and the other to avoid overlapping halos. After applying the discs, the plates were incubated in an oven at 36ºC for 24 hours, after which the growth halos were read.

The diameter of the inhibition halos was measured using a halometer on the bottom of the plate, and inhibition halos of bacterial growth ≥ 8 mm were considered active. This entire process was performed in triplicate (BAUER et al., 1966). The microbiological tests were performed in the Microbiology Laboratory of the Federal Institute of Maranhão – IFMA, Caxias Campus.

2.10 Statistical Analyses

The usual descriptive statistics procedures were used for data analysis. The microbial growth inhibition halos (measures in mm) of the analysis were expressed as mean ± standard deviation of the values obtained in the experiments that were performed in triplicate.

The data referring to the inhibition halos corresponding to the antimicrobial activity were subjected to the normality (Shapiro-Wilk) and homogeneity (Levene) tests. As they did not present normal distribution or variance equality, non-parametric tests were applied.

To analyze the data from the extracts and fractions of the leaves and stem bark, both in isolation and together, the Kruskal-Wallis test was performed and, when found to be different, Bonferroni's post hoc multiple comparisons test was used. The data were typed into Excel and analyzed in the Statistical Package for the Social Sciences (SPSS) software, version 20.0. The GraphPad Prism software, version 8.0.1, was used for the illustrations. The significance level adopted was 5% ($p<0.05$).

3. RESULTS

From the data obtained it can be seen that there was a difference between the yield of the leaves and stem bark of the ethanolic extracts, being 1.228% for the leaves and

2.41% for the stem bark, in both parts of the plant. Crude fractions, followed by the hydromethanolic ones, had higher percentages, as shown in Table 1.

Table 1: Total yield and yield per fraction of the ethanolic extract from *Brosimun gaudichaudii Trécul* leaves and stem bark.

Fractions	Leaves (g)	Yield (%)	Stem Bark (g)	Yield (%)
Fresh	1,500	-	1,052	-
Dry	832	-	408.5	-
Crude	6.21 (60.64%)	0.746	5.53 (56.13%)	1.353
Hexanic	0.395 (3.86%)	0.047	0.565 (5.74%)	0.138
Chloroform	1.015 (9.91%)	0.121	1.005 (10.20%)	0.246
Ethyl Acetate	0.495 (4.84%)	0.059	0.267 (2.71%)	0.065
Hydromethanolic	2.125 (20.75%)	0.255	2.485 (25.22%)	0.608
Total	**10.24 (100%)**	**1.228**	**9.852 (100%)**	**2.41**

Data expressed in grams (g) for the weight of fresh and dry leaves and stem bark, and in percentage (%) for total yields. (-): Data not applicable.
Source: Elaborated by the authors (2023).

3.1 Phytochemical Screening

Through the phytochemical screening of the crude extracts and leaf fractions it was possible to identify the presence of a diversity of secondary metabolites, with hydrolyzable tannins, condensed tannins, flavonoids, saponins, free steroids, triterpenoids and alkaloids among them, whereas the crude extract and fractions of the stem bark did not show presence of hydrolyzable tannins, phenols or saponins, in any of its analyzed fractions, as described in Table 2.

Table 2: Phytochemical screening of crude extracts and fractions of *Brosimun gaudichaudii Trécul* leaves and stem bark.
Key: Reaction: present (+); absent (-); FCr: Crude Fraction; FHex: Hexanic Fraction; FChl: Chloroform Fraction; FEA: Ethyl Acetate Fraction; FHy: Hydromethanolic Fraction.

Compounds	***Brosimun gaudichaudii Trécul***									
	Leaves					**Stem Bark**				
	FCr	FHex	FChl	FEA	FHy	FCr	FHex	FChl	FEA	FHy
Hydrolyzable Tannins	-	-	+	+	-	-	-	-	-	-
Condensed Tannins	+	+	-	-	+	+	+	+	+	+
Phenols	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+	-	+	+	+
Free Steroids	+	+	-	-	-	+	+	-	-	-
Triterpenoids	-	-	+	+	+	-	-	+	+	+
Saponins	-	-	+	+	-	-	-	-	-	-
Alkaloids	-	+	+	+	+	+	+	-	+	+
Coumarins	-	-	-	-	-	-	-	+	+	-

Source: Elaborated by the authors (2023).

3.2 Microbiological Analyses

The results of the microbiological analyses are expressed as mean and standard deviation of the tests performed, in triplicate. The ethanolic extract from the leaves only presented inhibitory activity against K. pneumoniae, with an inhibition halo varying from 8±0.00 to 12±0.00 mm (Table 3).

Table 3: Mean and standard deviation of the antimicrobial activity of fractions of the ethanolic extract from Brosimun gaudichaudii Trécul leaves.

Fraction	***K. pneumoniae***			***P. aeruginosa***			***S. aureus***			***E. coli***		
	ATCC 13883			ATCC 27853			ATCC 25923			ATCC 25922		
	IC	**IH**	**C+**	**IC**	**IH**	**C+**	**IC**	**IH**	**C+**	**IC**	**IH**	**C+**
FCr	10	-	35	-	-	-	-	-	-	-	-	-
	25	-	35	-	-	-	-	-	-	-	-	-
	50	8±0.00	35	-	-	-	-	-	-	-	-	-
	100	10±0.00	35	-	-	-	-	-	-	-	-	-
FChl	10	-	35	-	-	-	-	-	-	-	-	-
	25	8±0.00	35	-	-	-	-	-	-	-	-	-
	50	-	35	-	-	-	-	-	-	-	-	-
	100	10±0.00	35	-	-	-	-	-	-	-	-	-
FEA	10	-	35	-	-	-	-	-	-	-	-	-
	25	12±0.00	35	-	-	-	-	-	-	-	-	-
	50	12±0.00	35	-	-	-	-	-	-	-	-	-
	100	12±0.00	35			-	-	-		-	-	-
FHy	10	-	35	-	-	-	-	-	-	-	-	-
	25	-	35	-	-	-	-	-	-	-	-	-
	50	10±0.00	35	-	-	-	-	-	-	-	-	-
	100	10±0.00	35	-	-	-	-	-	-	-	-	-

p-value=0.000*

Key: *p-value=Kruskal-Wallis. FCr: Crude Fraction; FHex: Hexanic Fraction; FChl: Chloroform Fraction; FEA: Ethyl Acetate Fraction; FHy: Hydromethanolic Fraction; IC: Inhibitory Concentration (mg/mL); IH: Inhibition Halo (mm); C+: Positive Control.

Source: Elaborated by the authors (2023).

Among the fractions obtained from the leaf ethanolic extract, only the hexanic fraction was able to inhibit K. pneumoniae. The ethyl acetate fraction presented the largest inhibition halo (12±0.00 mm) at all concentrations tested (25, 50 and 100 mg/mL). Thus, the approximate Minimum Inhibitory Concentration (MIC) for the ethanolic extract from the leaves against K. pneumoniae was 25 mg/mL for the chloroform (8±0.00 mm) and ethyl acetate (12±0.00 mm) fractions.

The statistical analyses showed that there were significant differences in terms of inhibition between the fractions tested ($p<0.05$). These differences are observed between the fractions when analyzing the inhibition halos, where the ethyl acetate fraction was

better than the crude and chloroform fractions (p=0.001) and the hydromethanolic (p=0.004) fraction in all their tested concentrations.

The ethanolic extract from the stem bark presented inhibitory activity against the K. pneumoniae, P. aeruginosa and S. aureus strains, with E. coli as the strain that did not show sensitivity to this extract and its fractions. The inhibition halo varied from 8±0.00 to 14±0.00 mm for K. pneumoniae, from 8±0.00 to 10±0.00 mm for P. aeruginosa and from 8±0.00 to 9±0.00 mm for S. aureus (Table 4).

Table 4: Mean and standard deviation of the antimicrobial activity of fractions of the ethanolic extract from *Brosimun gaudichaudii Trécul* leaves.

Fraction	***K. pneumoniae***			***P. aeruginosa***			***S. aureus***			***E. coli***		
	ATCC 13883			ATCC 27853			ATCC 25923			ATCC 25922		
	IC	**IH**	**C+**	**IC**	**IH**	**C+**	**IC**	**IH**	**C+**	**IC**	**IH**	**C+**
SFCr	10	-	35	10	8±0.00	28	10	-	24	10	-	28
	25	8±0.00	35	25	8±0.00	28	25	-	24	25	-	28
	50	8.67±1.00	35	50	8±0.00	28	50	8±0.00	24	50	-	28
	100	11±1.80	35	100	10±0.00	28	100	8±0.00	24	100	-	28
SFHex	10	-	35	10	-	28	10	-	24	10	-	28
	25	8±0.00	35	25	-	28	25	8±0.00	24	25	-	28
	50	8±0.00	35	50	-	28	50	8±0.00	24	50	-	28
	100	10±0.00	35	100	-	28	100	9±1.09	24	100	-	28
SFCHL	10	-	35	10	-	28	10	-	24	10	-	28
	25	8±0.00	35	25	-	28	25	-	24	25	-	28
	50	8±0.00	35	50	-	28	50	8±0.00	24	50	-	28
	100	10±0.00	35	100	-	28	100	8±0.00	24	100	-	28
SFEA	10	-	35	10	8±0.00	28	10	-	24	10	-	28
	25	8±0.00	35	25	8±0.00	28	25	-	24	25	-	28
	50	10±0.00	35	50	8±0.00	28	50	8±0.00	24	50	-	28
	100	14±0.00	35	100	10±0.00	28	100	8±0.00	24	100	-	28
SFHy	10	-	35	10	8±0.00	28	10	-	24	10	-	28
	25	-	35	25	8±0.00	28	25	-	24	25	-	28
	50	-	35	50	8±0.00	28	50	-	24	50	-	28
	100	10±0.00	35	100	10±0.00	28	100	8±0.00	24	100	-	28

p-value=0.001*

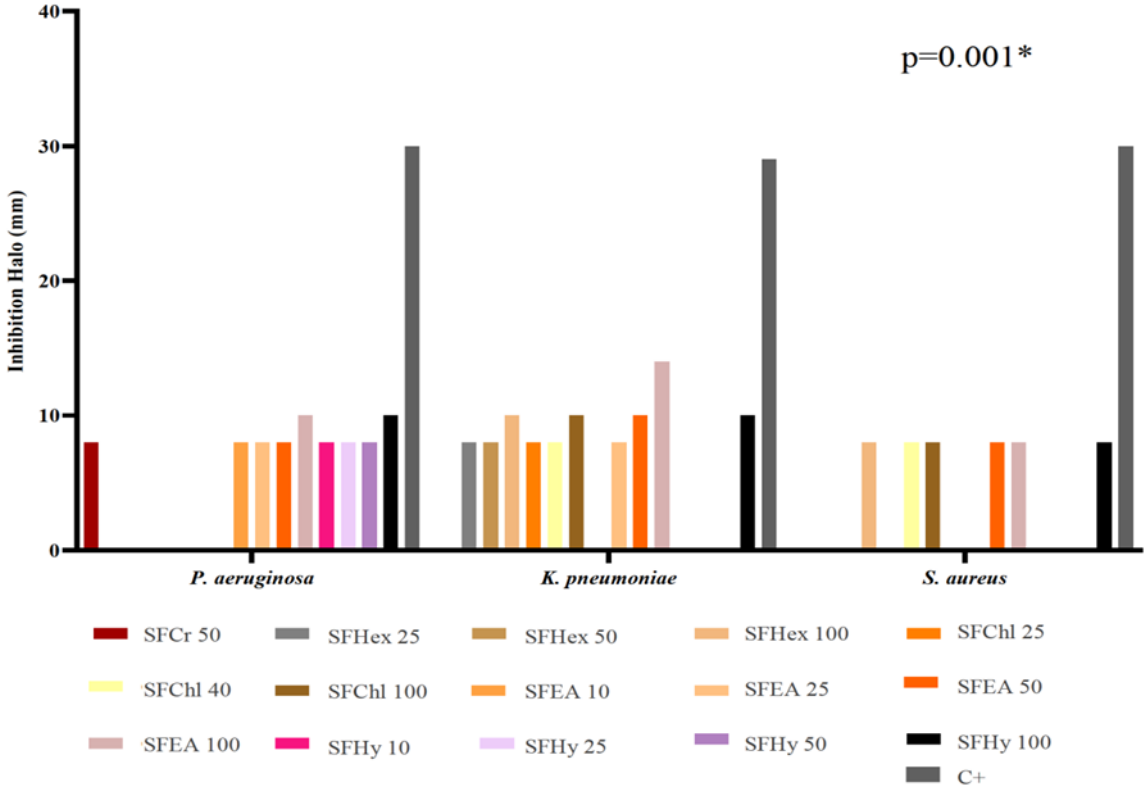
Key: *p-value=Kruskal-Wallis. SFCr: Crude Fraction; SFHex: Hexanic Fraction; SFChl: Chloroform Fraction; SFEA: Ethyl Acetate Fraction; SFHy: Hydromethanolic Fraction; IC: Inhibitory Concentration (mg/mL); IH: Inhibition Halo (mm); C+: Positive Control.
Source: Elaborated by the authors (2023).

The statistical analyses showed significant differences between the strains analyzed against the ethanolic extract from the stem (p=0.001). Among the strains that were sensitive to the extract and fractions, K. pneumoniae and S. aureus were the strains that presented sensitivity to all fractions, with K. pneumoniae as the strain with the best

sensitivity when compared to S. aureus (p=0.001) and P. aeruginosa (p=0.044). Although P. aeruginosa did not present sensitivity to the hexanic and chloroform fractions, it was sensitive to the lowest concentration tested (10 mg/mL) for the crude, ethyl acetate and hydromethanolic fractions.

When analyzing the fractions, it is possible to observe that the ethyl acetate fraction at the 100 mg/mL concentration was the one that presented the best inhibitory activity for K. pneumoniae, when compared with the other fractions and concentrations (Figure 1), showing an inhibition halo of 14±0.00 mm.

Figure I: Comparative evaluation of the inhibitory activity between the fractions of the ethanolic extract from the Brosimun gaudichaudii Trécul stem bark.

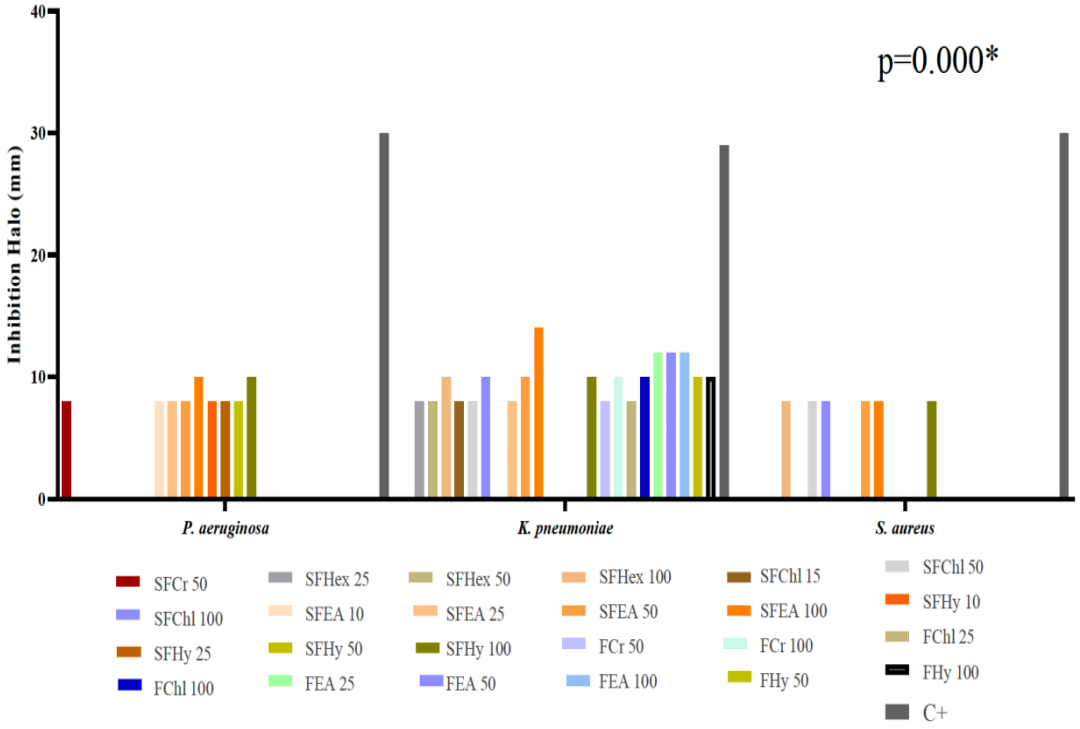


Source: Elaborated by the authors (2023).

When comparing the fractions of the leaves and stem bark extracts to each other, the statistical analyses showed evidence of significant differences (p=0.000) that are observed between the fractions. The ethyl acetate fraction from the leaves (EAFL) showed better inhibition potential when compared to the crude fraction from the stem (SFCr) (p=0.007), chloroform fraction from the stem (SFChlo), hexanic fraction from the stem (SFHex), hydromethanolic fraction from the stem (SFHy) and ethyl acetate fraction from the stem (SFEA) (p=0.000), as shown in Figure 2. There were no significant

differences (p>0.05) in the other fractions; consequently, they presented the same inhibition potential.

Figure 2: Comparative evaluation of the inhibitory activity between the fractions of the ethanolic extract from *Brosimun gaudichaudii Trécul* leaves and stem bark.



Source: Elaborated by the authors (2023).

4. DISCUSSION

4.1 Yield of the Extracts

The type of solvent and the technique used in the extraction process can influence both yield and identification of secondary metabolites. Studies conducted by Oliveira et al., (2016) showed that, in ethanolic extracts, steroid contents are higher when compared to hydroalcoholic extracts. Ethanol presents an amphiphilic character, which allows for the extraction of both apolar and polar substances; this characteristic can also influence total yield of the extracts (OLIVEIRA et al., 2016).

Given the above, knowing the yield of the extracts is of fundamental importance, both in the cultivation and harvest of medicinal plants, as this exerts direct effects on the fresh mass required to obtain the appropriate extract content, thus reducing costs and losses in the production chain of plants (RODRIGUES et al., 2011).

4.2 Phytochemical Prospection

Phytochemical screening consists in the identification of secondary metabolites present in the plant that are responsible for developing biological actions. These metabolites are characterized by varied substances and, as they have a biological effect when used by humans, they are widely studied for the development of new biological products (KABERA et al., 2014).

The contents of secondary metabolites in the composition of the plant extracts are influenced by several variables, with water availability, soil type, sunlight incidence, soil pH and collection period among them (BRUM et al., 2011). Negative results for certain classes of metabolites may not imply their total absence, as the concentration of certain classes of metabolites is not constant during the year. An example of this corresponds to phenolic compounds, which in this study obtained negative results, both for the ethanolic extract from the leaves and for the stem bark (GOBBO; NETO, 2007). Similar results regarding phenolic compounds were found in the study by Silva et al., (2023), using the same extraction methodology.

The reaction for tannins was positive in the leaf and stem bark extracts; however, there are reservations regarding the types of tannins, with hydrolyzable tannins being only present in the leaf extract, whereas condensed tannins were positive for the leaf and stem bark extract. These compounds are widely used as anti-inflammatory and cicatrizing agents, as their effect is related to their ability to form complexes, mainly with proteins (SIMÕES et al., 2016).

Flavonoids were the most abundant class of metabolites in the fractions of the extracts tested, except for the hexanic fraction, which was negative. These compounds are widely studied for presenting antioxidant, antibacterial and anti-inflammatory actions (KUMAR; PANDEY, 2013).

The reactions to the triterpenic acids, such as steroids and triterpenoids, were positive in both extracts tested. This class of compounds presents important biological and pharmaceutical activities, with their anti-inflammatory, antimicrobial, antiviral, cytotoxic and cardiovascular effects among them (SILVA et al., 2012; ABREU et al., 2013).

The saponin class was positive only in the leaf extract, in the chloroform and ethyl acetate fractions. This is a class of metabolites that is well diversified and presents the most varied effects: anti-inflammatory, antibacterial, antifungal, antioxidant and

immunomodulatory; however, their development is limited due to microheterogeneity and difficulties associated with their isolation (YANG et al., 2014).

The tests for alkaloids were positive for both extracts; these metabolites are widely known for their antimuscarinic effects, which are related to antispasmodic action; in addition, another property of alkaloids is their immunomodulatory action against respiratory disorders (VIEIRA et al., 2018; ALMEIDA, 2015).

Among the species from the Moraceae family, coumarins are the compounds with the highest frequency, especially furocoumarins, as they constitute a class of metabolites widely studied and with widespread use due to their antibacterial, antifungal, antitumor, antioxidant and anti-inflammatory actions, as well as to treat vitiligo (CHANG et al., 2005; MATOS et al., 2013; ZHENG; ZHAO, 2013; FRANCO et al., 2021). These compounds are distributed in several plant families, including Moraceae, being found in seeds, roots, stems, leaves, flowers and fruits, with the highest concentrations reported in flowers and fruits (RIBEIRO; KAPLAN, 2002; VENUGOPALA, 2013). However, in this research, the reactions for coumarins were only positive in the stem extract.

4.3 Microbiological Analyses

Microbiological tests of the ethanolic extracts from B. gaudichaudii leaves and stem bark revealed significant results for potential inhibition of the bacteria tested. Only E. coli presented resistance to the extracts and their fractions. Similar results were found in the study by Cavalcante et al., (2013), where the bark extract from Artocarpus heterophyllus, belonging to the Moraceae family, was tested against the E. coli strain and showed no inhibition potential.

This fact can be related to the characteristics observed in the cell membrane structure, since gram-negative bacteria such as E. coli, have an outer layer of lipopolysaccharides, conferring greater resistance to the treatments, which can be extended to tests with plant extracts (EL-SEEDI et al., 2020).

The K. pneumoniae, P. aeruginosa and S. aureus strains showed greater sensitivity to the stem bark fractions than the leaf fractions. Borges et al., (2017) evaluated the inhibition potential of ethanolic extracts from B. gaudichaudii leaves and stems and it was possible to observe a significant inhibitory activity for S. aureus and P. aeruginosa.

Also in the study by Borges et al., (2017), it was observed that the leaf extract had lower inhibitory activity for gram-negative bacteria when compared to the stem extract.

This data was also noticeable in the current study, as the stem extract and its fractions showed higher activity on gram-negative bacteria.

Among the fractions analyzed, both in the leave and in the stem bark, the ethyl acetate fraction showed the best inhibition potential. Through this solvent it is possible to extract several metabolites, with flavonoids, tannins, xanthones, saponins, triterpenic acids and phenolic compounds in general among them (CECHINEL FILHO; YUNES, 1998).

Among these metabolites, in the current study it was possible to identify the presence of tannins in all the stem bark fractions, while in the leaves their presence was only identified in the crude, hexanic and hydromethanolic fractions. In turn, for the flavonoids, only in the hexanic fraction it was not possible to identify them, both for the leaf extract and the stem bark.

According to Soares et al., (2008), to determine the antimicrobial activity of plant specimens, it is preferable to use the bark, due to the higher concentration of tannins present in this part of the plant.

Pozetti et al., (1972) and Goulart et al., (1975) conducted a study in order to evaluate the antimicrobial activity of the benzoic and hydroalcoholic extract from different parts of the plant against Escherichia coli, Shigella sonnei, Klebsiela sp., Enterobacter sp., Serratia marcescens, Proteus morganii, Providence sp., Pseudomonas aeruginosa and Staphylococcus aureus and showed that there was no inhibitory activity at the 25mg/mL and 50mg/mL concentrations.

The ethanolic and hexanic extracts from Brosimum gaudichaudii Trécul were also employed to determine the anthelmintic activity for Strongyloides stercoralis and Ancilostomideos and, according to the results obtained, only the extracts prepared from the roots showed anthelmintic activity (GOULART et al., 1975).

In the study by Araújo et al., (2015) significant diversity of secondary metabolites was shown, just as in the current study; this suggests that the bacterial inhibitory activity can be related to these compounds. Such being the case, it is necessary to isolate these metabolites to elucidate the antimicrobial activity of this plant.

5. CONCLUSION

The qualitative phytochemical screening of the extracts and fractions from Brosimum gaudichaudii Trécul found presence of a variety of secondary metabolites,

such as flavonoids, steroids, saponins, alkaloids, tannins and coumarins, revealing potential inhibitory action *for K. pneumoniae, P. aeruginosa* and *S. aureus*.

It was possible to verify that the tested extracts and fractions showed antimicrobial activity. Among the microorganisms tested, E. coli was the strain that presented the highest resistance to the extracts and their fractions, while *K. pneumoniae* showed the highest sensitivity. Of all the fractions, the ethyl acetate one showed the best inhibitory activity.

Finally, during the execution, the present study presented limitations related to the execution of the crude extract and its fractions, since the yield was low in both parts of the vegetable, which requires a greater amount of vegetable necessary for obtaining.

It is necessary to carry out further studies for the isolation and characterization of the metabolites present in the remaining fractions, in order to delimit the main compounds responsible for the antimicrobial action, as well as their toxicity.

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