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Phytochemical analysis and antimicrobial and antifungal potential of extracts and fractions of *Poincianella bracteosa*

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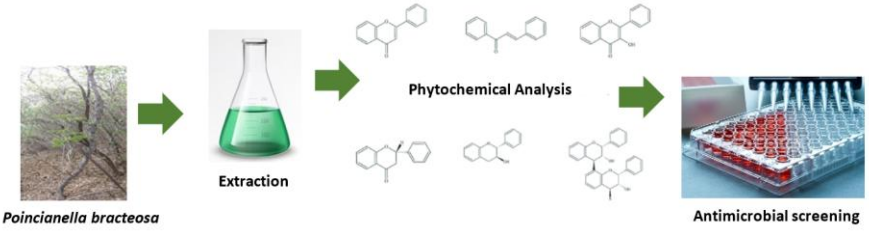
Abstract

The Caatinga is a Brazilian biome with great plant diversity and significant economic and pharmacological potential. It is a valuable source of medicinal resources, with many species used in folk medicine. The study on *Poincianella bracteosa* highlighted its medicinal properties, such as anti-inflammatory and antimicrobial action. The antimicrobial activities of crude extracts and fractions obtained from different plant parts were evaluated against pathogenic microorganisms. The extracts were obtained through exhaustive percolation with a 70% hydroethanolic solution and fractionated using solvents of lower polarity, resulting in hexane, dichloromethane, and ethyl acetate fractions. The fractions showed greater effectiveness than the crude extracts, with the dichloromethane fraction from the root exhibiting the highest inhibitory activity, with a minimum inhibitory concentration of 0.03 mg/mL against Enterococcus faecalis, Staphylococcus aureus, and Pseudomonas aeruginosa. These results indicate the antibacterial potential of *Poincianella bracteosa*.



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Section Editors

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Highlights

- Caatinga biome holds vast pharmacological potential with medicinal plant resources.
- *Poincianella bracteosa* exhibits anti-inflammatory and antimicrobial properties.
- Dichloromethane root fraction showed the highest MIC = 0.03 mg/mL.
- Effective against *E. faecalis*, *S. aureus*, and *P. aeruginosa* pathogens.
- Findings support its potential for antibacterial applications in medicine.

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1. Introduction

Medicinal plants serve as valuable sources of traditional medicines, containing a wide variety of bioactive molecules effective against various conditions (Bruning *et al.*, 2011; Felhi *et al.*, 2016; Sousa *et al.*, 2019; Tuttis *et al.*, 2018). The Caatinga, an exclusively Brazilian biome, is recognized for its high economic and pharmacological potential, especially in the Northeast region of Brazil (IBAMA, 2010; Yang *et al.*, 2018). The therapeutic efficacy of plants depends on the proper selection of plant parts used and their pharmacological activities, influenced by the presence and nature of secondary metabolites (Felhi *et al.*, 2016; Yang *et al.*, 2018).

These plants are a rich source of substances with diverse therapeutic properties, including antioxidants, anti-inflammatory, antibacterial, antiviral, diuretic, anticonvulsant, and anticancer properties (Majolo *et al.*, 2020; Nunes *et al.*, 2020; Parvaiz *et al.*, 2014; Szopa *et al.*, 2017). Flavonoids, alkaloids, tannins, and phenolic compounds are some of the chemical constituents that significantly contribute to the medicinal value of plants (Davoodi *et al.*, 2013; Majolo *et al.*, 2020; Tuttis *et al.*, 2018).

According to the World Health Organization (WHO), approximately 80% of the population in developing countries seek traditional health methods, with 85% of this percentage using medicinal plants (Souza *et al.*, 2019; Starfield, 2012; Teixeira *et al.*, 2011). In the Brazilian context, various factors, such as high costs of conventional medicines, limited access to healthcare services, and consumers' inclination towards natural products, contribute to the preference for medicinal plants (Figueredo *et al.*, 2014; Marmitt *et al.*, 2018).

An emblematic example is *Poincianella bracteosa* (Tul.) L. P. Queiroz, known as Catingueira, Catinga de Porco Preta, or Pau de Rato, belongs to the Fabaceae family. Species of this genus are common in Caatinga areas and widely used for medicinal purposes, reflecting not only a necessity due to socioeconomic factors but also a valorization of ancestral knowledge and local biodiversity (Coradin *et al.*, 2018). Its leaves and barks are widely used in traditional medicine to treat several conditions, such as kidney infections, hypertension, and digestive problems (Castro and Cavalcante, 2010; Karina *et al.*, 2016; Monteiro *et al.*, 2014). Phytochemical studies have revealed the presence of alkaloids, flavonoids, and tannins in different parts of the plant (Couto *et al.*, 2019; Pereira *et al.*, 2020; Santos *et al.*, 2015).

The metabolites present in *P. bracteosa*, such as flavonoids and tannins, possess antioxidants and antimutagenic properties, protecting DNA against genetic damage (Majolo *et al.*, 2020; Oyenihi and Smith, 2019). These substances act as chemoprotective agents and play a crucial role in reducing the incidence of diseases, including cancer (Słoczyńska *et al.*, 2014).

Despite the benefits associated with medicinal plants, it is crucial to recognize that all plants have some toxicity at specific dosages. The misconception that natural products are risk-free can lead to adverse reactions or toxic effects. This study evaluated crude extracts' antibacterial and antifungal activity and different fractions obtained from *Poincianella bracteosa*. This was achieved by determining the Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC). Additionally, chemical prospecting of the crude extracts obtained from *P. bracteosa* was performed through *in vitro* assays to assess their main secondary metabolites.

2. Experimental

2.1. Collection and identification of botanical material

Different parts of *Poincianella bracteosa* (leaves, stems, and roots) were collected in the Contendas do Sincorá National Forest (FLONA), located between the municipalities of Contendas do Sincorá-Ba and Tanhaçu-Ba. Exsiccates of the botanical material were sent to the Herbarium of the State University of Southwest Bahia (HUESB) for analysis and identification and are recorded in the collection under number HUESB 5894. The collected plant material was taken to the Laboratory of Natural Product Research (LAPRON) at UESB for separation, weighing, and drying.

2.2. Ethanol extract (EE) obtaining

The crushed plant material was subjected to a percolation process in a 2-L separatory funnel using a 70% (v/v) hydroethanolic solution. The solvent remained in contact with the plant material for approximately 24 h at a temperature of 20–25 °C. After this period, the extract was collected and concentrated using a rotary evaporator at 45 °C and 80 rpm, yielding the ethanolic extract. This procedure was repeated until the collected liquid exhibited a clear yellow coloration. The resulting ethanolic extract was stored in an amber bottle and refrigerated at 10 °C until fractionation and analysis.

2.3. Fractionation process

The ethanolic extracts (EEs) obtained from different plant parts were fractionated by liquid-liquid partitioning using solvents of increasing polarity. Initially, 50 g of EEs were diluted in a 70% (v/v) hydroethanolic solution. The fractionation was carried out sequentially using hexane, dichloromethane, and ethyl acetate, yielding the Hexane Fraction (FH), Dichloromethane Fraction (FD), Ethyl Acetate Fraction (FAE), and the remaining hydroethanolic phase, referred to as the Hydroalcoholic Fraction (FHA).

2.3.1. Hexane fraction (FH) obtaining

To the hydro-ethanolic solutions of the previously obtained extracts, portions of 200 mL of hexane were added, and the mixture was carefully agitated along the funnel walls. The process was repeated until the complete extraction, using approximately 2 liters of hexane (Synth). The hexane extracts were collected in an Erlenmeyer flask and evaporated in a rotary evaporator at 40 °C to obtain the FH. The lower portions of the hydro-ethanolic solutions were not extracted with hexane and reused for FD.

2.3.2. Dichloromethane fraction (FD) obtaining

The reserved hydro-ethanolic solutions from the previous partitioning were then fractionated with portions of 100 mL of dichloromethane until complete extraction, using approximately 700 mL of dichloromethane. To facilitate extraction, saturated NaCl solutions (Synth) were added to the mixtures in the separating funnels. After phase separation, the lower portions (dichloromethane) were collected, while the upper hydroalcoholic fractions remained in the funnels for the final fractionation stage. The combined dichloromethane extracts were evaporated in a rotary evaporator at 40 °C.

2.3.3. Ethyl acetate fraction (FAE) obtaining

In the reserved solutions from the dichloromethane extraction (upper parts), portions of 100 mL of ethyl acetate were added. The hydroalcoholic solutions were extracted from exhaustion, corresponding to 500 mL of ethyl acetate. The upper extracts were combined and evaporated in a rotary evaporator at 40 °C to obtain the FAE.

2.4. Test microorganisms

The standard strains of microorganisms used in this study were: *Enterococcus faecalis* (ATCC 31299), *Staphylococcus aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (INCOS 006), *Proteus vulgaris* (CBAM 0169), and *Enterococcus faecalis* (CBAM 0278). Microorganisms were cultured on Mueller-Hinton Agar (MHA) and incubated in a bacteriological incubator at 37 °C for 18 to 24 h.

2.4.1 Evaluation of antibacterial activity by determining the MIC

To determine the MIC of the extracts, the susceptibility assay was conducted on sterile polystyrene plates with 96 wells for microdilution. The ethanolic extracts of stems and leaves, along with their respective fractions, were diluted in 10% Tween 80 solutions, while the ethanolic extract of the root and its fractions were diluted in a 20% (v/v) ethanol–water solution and sterilized by filtration through a cellulose acetate bacteriological membrane (0.22 µm).

Microorganism inocula were prepared by diluting them in saline solution to a concentration equivalent to 1 x 10^8 cells per mL, according to a turbidity of 0.5 on the McFarland scale, then further diluted to 1 x 10^6 cells per mL for use.

Ninety microliters of Mueller Hinton broth (MHB) were added to the micro-wells, followed by an additional 90 µL of the extracts at the initial concentration of 5.0 mg/mL in wells from column A to column 9. This was followed by eight serial dilutions of the extracts in MHB, transferring 90 µL from the most concentrated well to its successor, resulting in decreasing concentrations (5.0; 2.5; 1.25; 0.62; 0.31; 0.15; 0.07; 0.03 mg/mL).

After the serial dilutions, 10 µL of bacterial suspension was inoculated into each micro-well, and the plates were incubated at 37 °C in a bacteriological incubator for 24 hours. The tests were conducted in triplicate, with columns 10, 11, and 12 used for positive and negative controls of microorganisms, broth, and extract, respectively (**Fig. 1**).

To ensure experiment reliability, five controls were performed: **1)** to evaluate the sterility of the culture medium; **2)** to assess the sterility of the test extract; **3)** to evaluate the activity of the antibiotic chloramphenicol (20 mg/mL); **4)** to certify the viability of the tested microorganisms; **5)** to check for interference from extract diluents on the tested microorganisms. All tests were conducted in triplicate.

After the incubation period, 50 µL of resazurin (0.01%) was added as an indicator of bacterial growth in the assay wells, allowing the detection of cell viability. Pink and red colorations indicated the presence of viable cells in growth while blue coloration indicated absence. To determine if the extracts showing positive results had a bactericidal or bacteriostatic effect, the MMC test was conducted.

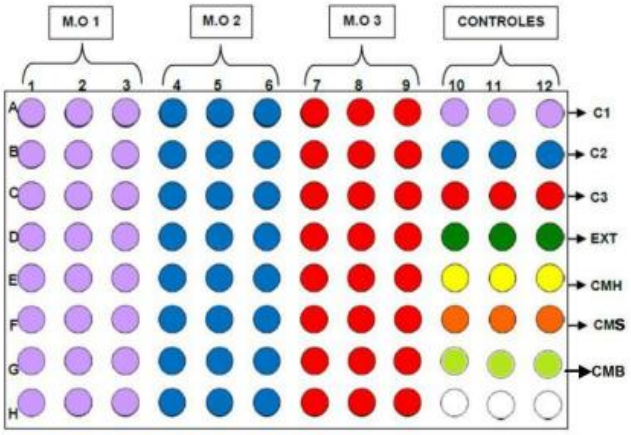

Figure 1. Scheme of the antibacterial assay conducted for determining the MIC.
Note: **C1, C2, and C3:** microorganism controls; **EXT:** extract control; **CMH:** culture medium control; **CMS:** diluent control; **CMB:** antibiotic control; **M.O:** microorganisms.
Source: Elaborated by the authors.

2.4.2. Evaluation of antifungal activity of extracts

The extracts' inhibitory effect on filamentous fungi's growth was determined using the disc diffusion test, accepted by the Food and Drug Administration (FDA) and established by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The Mycology Laboratory of the Federal University of Lavras (UFLA) provided the fungal colonies used in the experiments. Fungi were subcultured on Malt Extract Agar (MEA) and incubated at 30 °C. The fungi used in the test were: *Aspergillus flavus*, *Aspergillus carbonarius*, *Aspergillus niger*, *Penicillium comune*, *Penicillium clodosporoides*.

2.5. Chemical prospecting tests

2.5.1. *In vitro* tests for identification of chemical constituents

The ethanol extracts underwent preliminary phytochemical screening to identify the main classes of secondary metabolites in the samples through *in vitro* chemical reactions (Matos, 2009; Simões *et al.*, 2004). For the tests, 2 g of the EE was solubilized in 40 mL of a 70% (v/v) ethanol–water solution. Portions of 5 mL of this solution were transferred to different numbered test tubes and subjected to analyses according to the methodology described by Matos (2009). The following tests were performed: alkaloid identification, tannin identification, saponin identification, flavonoid identification, coumarin identification, cardiotonic glycosides identification, catechin identification, anthocyanin heterosides identification, and gums and mucilage identification.

2.5.2. Identification of chemical constituents in extracts

For all qualitative phytochemical assays, the term "hydroethanolic solution" refers to the extract previously solubilized in an ethanol–water mixture at the concentration described above and does not imply the use of absolute ethanol.

Alkaloid identification: in the alkaloid identification test, 2.0 mL of the hydroethanolic solution was mixed with 2.0 mL of 0.1 mol L^{-1} HCl and heated for 10 min. After cooling, the mixture

was filtered, the filtrate was divided into three test tubes, and a few drops of the recognition reagents Dragendorff, Mayer, and Wagner were added. The formation of slight turbidity or precipitate (purple to orange, white to cream, and brown, respectively) indicates the potential presence of alkaloids.

Tannins identification: the methodology involved a hydroethanolic solution of 2.0 mL, to which 5.0 mL of distilled water was added. After filtration, 1 or 2 drops of 10%(w/v) ferric chloride solution were introduced. A blue coloration indicates the possible presence of hydrolysable tannins, while a green coloration suggests the presence of condensed tannins.

Saponins identification: 2.0 mL of the hydroethanolic solution was used to add 5.0 mL of boiling water. After cooling, the mixture was vigorously agitated and allowed to stand for 20 min. The formation of foam classifies the presence of saponins.

Flavonoid identification: in a tube, 2.0 mL of hydroethanolic solution, some fragments of metallic magnesium, and a few drops of diluted HCl were added along the tube walls. The production of coloration was observed, varying for different structures.

Coumarin identification: 2.0 mL of hydroethanolic solution was placed in a test tube, covered with filter paper impregnated with 10%(w/v) NaOH solution, and heated in a water bath for 10 min. After removing the filter paper, it was examined under ultraviolet light. Yellow or green fluorescence indicates the presence of coumarins.

Glycoside Identification: 2.0 mL of the extract solution was used to add 3.0 mL of 10%(w/v) lead acetate solution and 2.0 mL of distilled water. The mixture was heated in a water bath for 10 min, filtered, and the filtrate was shaken with 10.0 mL of chloroform. The chloroform phase was separated, and the solvent was evaporated. 1.0 mL of Baljet reagent was added. Purple, orange-reddish, or violet coloration indicates the presence of cardiotonic glycosides.

Catechin Identification: The wood of a matchstick was moistened with the aqueous extract and subsequently with concentrated hydrochloric acid. It was dried by flaming in a strong flame. The color formation of the wood was evaluated. The appearance of red indicates the presence of catechins.

Anthocyanin heterosides identification: 1 mL of the aqueous extract was added to 4 test tubes. One of them served as a standard, and the others were treated as follows: the first one was alkalinized with a 5%(w/v) NaOH solution, the second was acidified with 10%(v/v) hydrochloric acid, and the third was kept at a neutral pH. The change or appearance of coloration in the different tubes indicates the presence of heterosides.

Gums and mucilage's identification: 1 mL of the aqueous extract was placed in a test tube, and gradually, drops of neutral lead acetate were added until the precipitate ceased to appear. The mixture was filtered on filter paper, and 0.5 mL of acidic lead acetate was added. The precipitate's appearance indicates a positive reaction.

2.5.3. Determination of total flavonoid content

The total flavonoid concentration was determined through a colorimetric assay using aluminum chloride as a chromophore agent, following the methodology outlined by Marinova *et al.* (2005) with some modifications. The ethanolic extract was diluted in methanol to a 250 µg/mL concentration. 0.5 mL aliquots were mixed with 4.0 mL of deionized water in test tubes. Subsequently, 0.3 mL of a 5% aqueous solution of sodium nitrite ($NaNO_2$) was added, and the mixture was stirred. After 5 min, 0.3 mL of a 10% aqueous solution of aluminum chloride ($AlCl_3$) was added, and the solution was homogenized. After 6 min, 2.0 mL of a 1.0 mol L^{-1} aqueous solution of sodium hydroxide (NaOH) was added, and the volume was adjusted to 10.0 mL with deionized water. The solution was stirred, and absorbances were measured at 510 nm using a Shimadzu UV 1800 spectrophotometer, with methanol as the "blank". To calculate the flavonoid content, an analytical curve was constructed using standard quercetin solutions, and the absorbances of the samples were interpolated. The results were expressed as mg of Quercetin Equivalent (QE) per g of extract, with a linear regression equation $y = 0.00095x - 0.00530$ and a correlation coefficient $R^2 = 0.99784$. The analyses were performed in triplicate under dark conditions.

3. Results and discussion

3.1. Microbial activity

Table 1 presents the results of antimicrobial activity for samples obtained from the leaves of *Poincianella bracteosa*. MIC found for the FD was 2.5 mg/mL for the strains of *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* (CBAM 0278). The ethanol extracts inhibited *E. cloacae* and *P. vulgaris* strains at 1.25 mg/mL.

Hexane fraction proved effective at a concentration of 0.15 mg/mL for *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* (CBAM 0278), 1.25 mg/mL for *E. cloacae* and *P. vulgaris*, and 0.62 mg/mL for the remaining bacteria.

The results for samples obtained from the stems of *P. bracteosa* are described in Table 1. The fractions obtained from the stem of *P. bracteosa* inhibited strains of *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* (CBAM 0278) at a concentration of 2.5 mg/mL and strains of *E. cloacae* and *P. vulgaris* at a concentration of 5.0 mg/mL. Except for the *E. cloacae* strain, the FAE inhibited all other microorganisms at a 5.0 mg/mL concentration.

The samples obtained from the stems of *P. bracteosa* exhibited higher MIC than those obtained from the plant's leaves.

The samples obtained from the root of *P. bracteosa* exhibited superior results in evaluating antibacterial activity compared to other parts of the plant. Ethanol extract proved effective at 0.62 mg/mL concentration against the *E. faecalis* strain. However, for *S. aureus*, *P. aeruginosa*, *P. vulgaris*, and *E. faecalis* (CBAM 0278), it was active at a concentration of 1.25 mg/mL and unable to inhibit the *E. cloacae* strain at any of the tested concentrations.

Dichloromethane fraction from the root of *P. bracteosa* showed the best results in bacterial growth inhibition tests, inhibiting the strains of *E. faecalis*, *S. aureus*, and *P. aeruginosa* at a concentration of 0.03 mg/mL, the strains of *P. vulgaris* and *E. faecalis* (CBAM 0278) at a concentration of 0.15 mg/mL, and *E. cloacae* at a concentration of 5.0 mg/mL.

Ethyl acetate fraction from the root inhibited the *E. faecalis* strain at 0.15 mg/mL and the *E. cloacae* strain at a concentration of 2.5 mg/mL. The FH inhibited the strains of *E. faecalis* and *E. cloacae* at a concentration of 0.62 mg/mL and the other strains at a concentration of 1.25 mg/mL (Table 1).

Table 1. MIC of extracts from *Poincianella bracteosa* against different bacterial strains: leaf extracts, stem extracts, and root extracts.

Microorganisms MIC (µg/mL)	*E. faecalis* (ATCC 31299)	*S. aureus* (ATCC 43300)	*P. aeruginosa* (ATCC 278553)	*E. cloacae* (INCOS 006)	*P. vulgaris* (CBAM 0169)	*E. faecalis* (CBAM 0278)
Leaf EE	NI	NI	NI	NI	NI	NI
Leaf FH	0.15	0.31	0.31	0.62	0.62	0.62
Leaf FD	2.50	2.50	2.50	1.25	1.25	2.50
Leaf FAE	NI	NI	NI	NI	NI	NI
Stem EE	NI	NI	NI	NI	NI	NI
Stem FH	NI	NI	NI	NI	NI	NI
Stem FD	2.5	2.5	2.5	5.0	5.0	2.5
Stem FAE	5.0	5.0	5.0	NI	5.0	5.0
Root EE	0.62	1.25	1.25	NI	1.25	1.25
Root FH	0.62	1.25	1.25	1.25	1.25	0.62
Root FD	0.03	0.03	0.03	5.00	0.15	0.15
Root FAE	0.15	0.31	0.31	2.50	0.62	0.31

Note: **EE**, **FH**, **FD**, and **FAE** correspond, respectively, to the ethanol extract, hexane fraction, dichloromethane fraction, and ethyl acetate fraction. "**NI**": No inhibition occurred.
Source: Elaborated by the authors.

In a study conducted by Saraiva *et al.* (2012), the tests evaluating the antimicrobial activity of the methanolic extract from the leaves of *Caesalpinia pyramidalis* (*Poincianella pyramidalis*) against *P. aeruginosa* showed MIC values of 0.125 mg/mL. The root methanolic extract exhibited moderate activity when tested against the strain *E. faecalis* (AM128). For the FAE of the root of the same species, the MIC values were 0.50 and 0.25 mg/mL, respectively, against the strains of *E. coli* and *S. aureus*, with results like those obtained in the present study.

Comparative studies involving medicinal plants are challenging due to the diversity of abiotic factors impacting plant growth, ranging from climatic conditions to the developmental stage during collection, the plant part analyzed, and the experimental procedures (Auricchio *et al.*, 2003). Analyzing the results of antimicrobial activity requires consideration of natural factors such as sunlight, UV rays, dry or rainy periods, temperature, soil, nutrients, and the season, which can influence metabolism and secondary metabolite production. Additionally, the presence of pollutants and the defense mechanisms against pathogens can affect secondary metabolite production. Regarding bacteria, their ability to develop resistance, tolerance, and persistence enables them to survive with antibiotics and chemotherapeutics. The diversity of compounds found in plant extracts and their different mechanisms of action complicates microbial adaptation and reduces the likelihood of resistance development to natural antimicrobials.

3.2. Determination of MMC

After determining the MIC, the wells where the extracts proved effective were tested to determine the MMC. The results of the bacteriostatic/bactericidal tests revealed that the FH from the leaves was the only one showing bactericidal activity against *Staphylococcus aureus* in the MMC assessment. The other samples from all parts of the studied plant did not cause the death of the tested bacteria; they only inhibited their multiplication.

3.3. Evaluation of antifungal activity of P. bracteosa extracts

The ethanolic extract from the leaves of *P. bracteosa* did not show antifungal activity against the tested microorganisms. Ethyl acetate fraction inhibited *A. carbonarius*, *P. comune*, and *P. cladosporioides* (**Table 2**)

Table 2. Results of antifungal tests of leaf extracts, stem extracts, and root extracts from *P. bracteosa* against different strains of fungi.

Fungi MIC (µg/mL)	*A. flavus*	*A. carbonarius*	*A. niger*	*P. commune*	*P. Pladosporioides*
Leaf EE	NI	NI	NI	NI	500
Leaf FH	NI	NI	NI	250	500
Leaf FD	NI	NI	NI	1000	250
Leaf FAE	NI	1000	NI	250	500
Leaf HP	100	100	100	100	100
Stem EE	NI	500	NI	62.5	250
Stem FH	NI	1000	NI	250	500
Stem FD	NI	NI	NI	125	250
Stem FAE	NI	1000	1000	125	31.25
Stem HP	100	100	100	100	100
Root EE	500	500	NI	62.5	31.25
Root FH	NI	1000	NI	250	500
Root FH	NI	500	1000	125	250
Root FAE	NI	1000	1000	125	31.25
Root HP	100	100	100	100	100

Note: **NI:** No inhibition; **HP:** 2% Hypochlorite; **EE**, **FH**, **FD**, and **FAE** correspond, respectively, to the ethanol extract, hexane fraction, dichloromethane fraction, and ethyl acetate fraction.
Source: Elaborated by the authors.

The stem extracts of *P. bracteosa* showed better results against *P. comune* and *P. cladosporioides*, ranging from 31.25 to 500 µg/mL (Table 2). For the fungus *A. flavus*, no inhibition was observed at any of the tested concentrations.

The root extracts were the most active in antifungal activity tests, which are consistent with the results of antibacterial tests.

The crude root extract was the only one to inhibit the fungus *A. flavus* at a 500 µg/mL concentration. The crude extract and the fractions effectively inhibited *P. comune* and *P. cladosporioides*.

3.4. Chemical prospecting and the relationship between antimicrobial and antifungal potentials

Tannins are important in plant–environment interactions, playing a pivotal role in herbivore inhibition and functioning as antimicrobial agents, constituting phenolic compounds of substantial economic and ecological interest (Sant'ana *et al.*, 2002). Studies on the bioactivity of tannins indicate their noteworthy antibacterial actions, as well as their influence on processes such as tissue repair, enzymatic regulation, and protein modulation, among others (Mello and Santos, 2004).

Terpenes, in turn, exhibit diverse functions within plants, ranging from attracting pollinators to safeguarding against fungi and bacteria, while also serving as precursors to plant growth hormones (Gershenzon and Dudavera, 2007). The *in vitro* chemical prospecting results of ethanol extracts obtained from different parts of Poincianella bracteosa –stem, root, and leaves– are presented in Table 3 and reveal the presence of flavonoid compounds, which are widely recognized for their antibacterial activity (Cushnie and Lamb, 2011).

Phytochemical studies on various parts of Caesalpinia pyramidalis similarly unveiled the presence of polyphenols, steroids, and sugars in all tested extracts. Metabolites such as flavonoids, condensed tannins, and alkaloids were identified in the ethanol extract obtained from the root of Poincianella bracteosa, corroborating previous findings (Cruz *et al.*, 2015). These components are associated with diverse biological activities observed in the studied species, encompassing antimicrobial action and herbivore protection. In summary, the chemical constituents identified in the studied plants, as evidenced by the chemical prospecting data (Table 3), are intricately linked to the various biological activities observed, underscoring the significance of these metabolites in plant ecology and pharmacology.

Table 3. Results of *in vitro* chemical prospecting of ethanol extracts obtained from different parts of *Poincianella bracteosa*: stem, root, and leaves.

Metabolites Leaves	Results Leaves	Metabolites Stem	Results Stem	Metabolites Root	Results Root
Coumarins	–	Anthocyanins	–	Saponins	–
Saponins	–	Flavanones	+	Cardiotonic Heterosides	–
Glycosides	+	Flavonols	+	Anthocyanins and Anthocyanidins	–
Alkaloids	–	Xanthones	+	Catechins	+
Flavonoids	+	Saponins	–	Flavones	+
Catechins	–	Pyrogallic tannins	+	Xanthones	+
Gums and mucilages	+	Anthocyanins	–	Chalcones	–
Condensed tannins	+	Alkaloids	–	Aurones	–
Anthocyanic heterosides	+	Terpenoid steroids	+	Flavonols	–
		Catechins	–	Alkaloids	–

Note: (+) Presence of metabolite (–) Absence of metabolite.
Source: Elaborated by the authors.

Determining flavonoid content in the different plant extracts demonstrated that this class of compounds is present in high concentrations in the analyzed samples. The FD showed the highest levels of flavonoids. Leaf extracts exhibited the lowest flavonoid content compared to other parts of *P. bracteosa* (Table 4). It is possible to observe that the fractions obtained from the stems of *Poincianella bracteosa* with antimicrobial activity showed higher levels of flavonoids, thus demonstrating a relationship between the presence of flavonoids in the extracts and the inhibitory effect on bacteria.

Table 4. Flavonoid content found in leaf, stem, and root extracts of *P. bracteosa.*

Extracts	Total Flavonoids (mg EQ g^{-1} of extract)		
	EEF	EEC	EER
Ethanolic	186.67	86.66	280.00
Dichloromethane Fraction	579.62	240.70	737.0
Ethyl Acetate Fraction	543.16	423.10	653.7
Hexane Fraction	–	81.90	587.0

Note: **EEF**, **EEC**, and **EER** extracts correspond, respectively, to the ethanolic extract of the leaf, stem, and root.
Source: Elaborated by the authors.

The root extracts of *P. bracteosa* showed higher levels of flavonoids than extracts from other parts of the plant (leaf and stem) and exhibited greater inhibition of the growth of the evaluated bacterial strains. Dichloromethane fraction was the most active against the evaluated bacteria. Thus, it can be inferred that the antimicrobial activity should be related to the composition of flavonoids present in the studied plant.

According to Cowan (1999), flavonoids' inhibitory effect against microorganisms may be due to their interaction with the target microorganisms' cell membrane, likely because of their ability to complex extracellular proteins and the cell wall.

The antimicrobial activity of *Poincianella bracteosa* EER extracts, compared to other parts of the plant (leaves and stems), has been associated with flavonoids, demonstrating greater inhibition of bacterial growth. This action is supported by Cushnie and Lamb (2011), who suggest that flavonoids damage the bacterial cytoplasmic membrane, leading to its perforation and/or reduced fluidity and inhibiting nucleic acid synthesis through topoisomerase inhibition.

Flavonoids are widely studied due to their antimicrobial properties and are found in various plants and derivatives, as Havsteen (2002) reported. The synthesis of these compounds occurs through the metabolic pathways of cinnamic acid and acetic acid, resulting in a diverse family of molecules, including flavones, flavonols, catechins, flavanones, anthocyanins, and isoflavones

(Hoffmann-Ribani *et al.*, 2008). Phytochemical studies on *Poincianella sp.* extracts have shown a variety of secondary metabolites, such as tannins, phenolic compounds, flavonoids, terpenes, and saponins (Tomi *et al.*, 2008; Zhumashova *et al.*, 2019).

The antioxidant activity of phenolic compounds in *P. bracteosa* extracts, such as flavonoids, has been associated with protection against oxidative damage and the neutralization of free radicals (Shukla and Meht, 2017). Although the presence of tannins and phenols in different fractions of *Poincianella* extracts has been detected, the concentration of these compounds may vary due to the polarity of the extraction solvent (Oliveira *et al.*, 2017).

Determining the antioxidant capacity of these extracts involves different methods, such as DPPH and ABTS, which yield similar findings (Denardin *et al.*, 2015). Previous studies with extracts of *P. spinosa* and *P. decapetala* have also reported significant antioxidant capacity values, demonstrating the effectiveness of these compounds in neutralizing free radicals (Gallego *et al.*, 2016; Skowyra *et al.*, 2014).

Therefore, the flavonoids present in *P. bracteosa* extracts show significant potential as antimicrobial and antioxidant agents, which are essential for protecting against diseases and oxidative damage.

4. Conclusions

This study demonstrated that *Poincianella bracteosa* exhibits antimicrobial effects against various strains of bacteria and fungi. The ethanolic extract obtained from the species' roots and its fractions showed the most promising results in assessing antibacterial activity. The FH derived from the ethanolic extract of *P. bracteosa* leaves was the only one exhibiting bactericidal activity against *Staphylococcus aureus* at concentrations ranging from 0.15 mg/mL to 0.62 mg/mL.

The antifungal activity, the ethanolic extract from the root and its fractions outperformed other extracts from different plant parts. Regarding phytochemical prospecting, flavonoids (flavones, flavonols), tannins, glycosides, and steroids, among other compounds, were found in the evaluated EEs. A positive correlation was observed between antimicrobial activity and the flavonoid content in the evaluated extracts, suggesting that this class of secondary metabolites may be linked to the antimicrobial activity of the studied species.

Authors' contribution

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request.

Artificial Intelligence usage statement

The authors declare that they did not use Artificial Intelligence tools at any stage of the preparation, correction, or evaluation of this work.

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