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ORIGINAL ARTICLE

EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THE LEAVES OF *FRIDERICIA PLATYPHYLLA SPECIES*

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Highlights:
(1) The extract from the leaves of the species *F. platyphylla* showed the highest antioxidant activity at 250µg/mL.
(2) *F. platyphylla* extract inhibited S. aureus, *E. faecalis* and *P. aeruginosa.*
(3) *F. platyphylla* extract did not show toxicity in an in vivo model using Tenebrio molitor.

ABSTRACT

Bacterial resistance is one of the greatest threats to global health, causing difficulties in controlling infections and increasing costs in health system. The study of plant species antimicrobial activity is of foremost importance. Brazil has a wide plant species variety, offering a vast area for research new molecules to produce drugs. Among these species is *Fridericia platyphylla*, popularly known as "cervejinha do campo". This research aims to evaluate the antimicrobial and antioxidant activity of the crude extract and dichlorine fraction of *Fridericia platyphylla* species leaves. The chemical characterization of the raw plant extract was performed using HPLC-PDA, FIA-ESI-IT-MS, and LCMS techniques. The DPPH and ABTS methods were used to evaluate the antioxidant properties. The antimicrobial activity was assessed by determination of the minimum inhibitory concentration (MIC) through microdilution assay. An in vivo model using *Tenebrio molitor* was employed to evaluate the toxicity. Ten compounds were identified in the crude extract. The crude leaf extract showed the highest antioxidant activity at 250 µg/mL. The antimicrobial assays showed that *F. platyphylla* crude extract inhibited *Staphylococcus aureus* ATCC 6538 with a MIC of 64 µg/mL. The dichlorine fraction inhibited the same bacteria at a MIC of 16 µg/mL. *Enterococcus faecalis* ATCC 51299 and *Pseudomonas aeruginosa* ATCC 27856 were inhibited with MIC of 1024 µg/mL and 512 µg/mL. The results emphasize the crude extract and dichlorine fraction of *F. platyphylla* leaves potential as an alternative to prevent and combat infections and as a source of raw material in producing drugs and natural products.

Keywords: natural products; antimicrobial activity; antioxidant activity; flavonoids.

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AVALIAÇÃO DA ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DAS FOLHAS DA ESPÉCIE *FRIDERICIA PLATYPHYLLA*

RESUMO

A resistência bacteriana é uma das maiores ameaças à saúde global, causando dificuldades no controle de infecções e aumentando custos no sistema de saúde. O estudo da atividade antimicrobiana das espécies vegetais é de suma importância. O Brasil possui uma grande variedade de espécies vegetais, oferecendo um vasto espaço para pesquisa de novas moléculas para a produção de medicamentos. Dentre essas espécies está *Fridericia platyphylla*, popularmente conhecida como "cervejinha do campo". Esta pesquisa teve como objetivo avaliar a atividade antimicrobiana e antioxidante do extrato bruto e da fração diclorosa das folhas da espécie *Fridericia platyphylla*. A caracterização química do extrato vegetal bruto foi realizada utilizando técnicas de HPLC-PDA, FIA-ESI-IT-MS e LCMS. Os métodos DPPH e ABTS foram utilizados para avaliar as propriedades antioxidantes. A atividade antimicrobiana foi avaliada pela determinação da concentração inibitória mínima (CIM) por meio de ensaio de microdiluição. O modelo *in vivo* usando *Tenebrio molitor* foi empregado para avaliar a toxicidade. Dez compostos foram identificados no extrato bruto. O extrato bruto de folhas apresentou a maior atividade antioxidante a 250 µg/mL. Os ensaios antimicrobianos mostraram que o extrato bruto de *F. platyphylla* inibiu *Staphylococcus aureus* ATCC 6538 com uma CIM de 64 µg/mL. A fração dicloro inibiu a mesma bactéria com uma CIM de 16 µg/mL. *Enterococcus faecalis* ATCC 51299 e *Pseudomonas aeruginosa* ATCC 27856 foram inibidas com CIM de 1.024 µg/mL e 512 µg/mL. Os resultados enfatizam o potencial do extrato bruto e da fração dicloro de *F. platyphylla* como alternativa para prevenir e combater infecções e como fonte de matéria-prima na produção de medicamentos e produtos naturais.

Palavras-chave: Produtos naturais; atividade antimicrobiana; atividade antioxidante; flavonoides.

INTRODUCTION

Currently, bacterial resistance is one of the greatest threats to global health, causing difficulties in controlling infections and contributing to increased costs in the health system. The increase in antibiotic-resistant bacteria leads to a greater need for alternative treatments. It is observed that discovering new antibiotics occurs more slowly than the number of resistances[1].

The study of the antimicrobial activity of plant species is of great relevance since their secondary metabolites can inhibit the growth of pathogenic microorganisms. Medicinal plant extracts have demonstrated the presence of effective compounds in the control of bacterial infections[2]. The biological activities of natural plant compounds are not restricted only to antimicrobial activity; they have other properties, such as antioxidant activity[3].

The therapeutic properties of herbal products are gaining more space due to the discoveries of new plant bioactive compounds that efficiently treat various diseases[4]. Brazil stands out for having a wide variety of plant species, many still little known or unidentified. Offering a vast area for research and identifying new molecules to produce new drugs[5].

One example is *Fridericia platyphylla*, a plant native to the Brazilian cerrado, popularly known as "cervejinha do campo". Extracts from distinct parts of this plant have been investigated to identify their biological activities. Recent studies have revealed its antispasmodic, cytotoxic, antifungal, and antiproliferative action[6-7,8]. From this perspective, this study aims to search for the antimicrobial and antioxidant activity of the crude extract and dichlorine fraction of the leaves of the *Fridericia platyphylla* species.

MATERIALS AND METHODS

Material Collection

Leaves of *F. platyphylla* were collected in Brazil in April 2019 at Sant´Ana da Serra´s farm in João Pinheiro, Minas Gerais. The plant was identified at the Herbarium José Badine at the University of Ouro Preto by Dr. Maria Cristina Teixeira Braga Messias (no. 17.935). The plant was collected according to all the Brazilian laws regarding biodiversity protection (SISGEN no. A451DE4).

Hydroethanolic extract preparation

F. platyphylla leaves (600 g) were separated and extracted successively through the room´s temperature maceration with ethanol: water (7:3) and breaks between solvent changes every 48 hours, exhaustively. The crude hydroethanolic extract (70% v/v) was obtained after filtration and evaporated until dry in the vacuum; the temperature is about approximately 40°C to produce 10,34 g of leaf extract (FAB). The extract chromatogram was obtained by HPLC-PDA.

CHEMICAL COMPOUNDS IDENTIFICATION

Extract preparation for analysis by SPE and quantification by HPLC-PDA

The sample was submitted to extraction in a solid phase (SPE) using Phenomenex Strata cartridge C18 (50 mg/mL), previously activated with 2 mL of methanol and balanced with 2 mL of MeOH/H20 (1:1, v/v).

The extract (50 mg/mL) was eluted with the same mobile phase used to balance and collected in a bottle of 5 mL, with its volume fully completed at the end of the elution. The solution was filtrated in a 0.22 µm filter. Aliquots of 10 µL of this solution were injected into the high-performance liquid chromatograph. The extract was coded as FAB.

To optimize the chromatographic conditions for the quantification of rutin, experiments were done with EtOH extract 70% in a system of HPLC (Shimadzu Corp., Kyoto, Japan), composed of a solvent injection module with a binary bomb and PDA detector (Diode Arrangement). The column was a Luna 5 µm C18 100 A (150 µm x 4,6 µm). The solvents in elution used were A (water + formic acid at 0,02%) and B (methanol). The samples were eluted according to the following gradient: 5% to 100% of B in 50 minutes. The flow was 1 mL/min; the column temperature was 20 ºC. The volume of injection in the sample was 10 µL. The data was collected and processed through LC Solution software (Shimadzu).

Many conditions were assessed to get a better separation from high peaks. The better one was separated with Phenomenex RP18 column (15 cm x 4,6 mm x 5 cm) and mobile phase gradient composed of water: methanol having 0,02% of formic acid and monitoring at 254 nm. Under the conditions used, we obtained a separation from the baseline to the main components in the sample over around 50 minutes.

Preparation of the extract for analysis by FIA-ESI-IT-MSn

The hydroalcoholic extract was treated before the analysis using extraction in a solid phase (Waters Sep-Pak C18, Vac 1 cm3, 100 mg). The stationary phase was activated with 1 ml of methanol (MeOH) and balanced with 1 ml of MeOH:H2O 85:15 (v/v). The shell was filled with 5,0 mg of each extract, which was solubilized in 500 µL of MeOH:H2O 85:15 (v/v). The elution was made with 1 ml of de MeOH:H2O 85:15 (v/v) to eliminate chlorophylls and other low-polarity compounds. The eluate was dry over N2 and solubilized in 85:15 MeOH:H2O (v/v) to create 1 mg/ml of solutions for analysis by FIA-ESI-IT-MSn.

Hydroalcoholic extract analysis of Fridericia platyphylla leaves by FIA-ESI-IT-MSn

The hydroalcoholic extract of *F. platyphylla* was analyzed in a mass LCQ Fleet (Thermo Scientific) spectrometer equipped with a straight insertion device of the sample for analysis through flux injection (FIA). The ionization of the sample was done by electrospray (ESI), and fragmentations in multiple stages (MSn) were accomplished by an ion trap (IT).

The negative side was used for analysis in the spectrum of the mass first order and multiple stages. Analysis conditions: capillary tension of de –4 V and –5 kV for spray, capillary temperature of 280 °C, carrier gas (N2) with a flow rate of 60 units. Acquisition range of m/z 50-1000, with two or more full-scan to get ions in the acquisition range. Other events were the experiment MSn with the energy of induced dissociation by collisions between 20 and 35. The Xcalibur software (scientific therm) was used for data acquisition and processing.

Extract characterization by liquid chromatography coupled to mass spectrometry

The mass spectrometry tests were made in LCQ Fleet (Thermo Scientific) equipment, built with a direct sample insertion device via flux injection analysis (FIA). The studied matrix was analyzed by ionization by Electrospray (ESI), and multistage fragmentations (MSn) were achieved in an interface PRISON of ions (IT). The positive way was selected for generating and analyzing mass spectrums to the First Order (DM) and for the other multistage experiments in the following conditions: Capillary Tension, 25 V; Pulverization Tension, 5kV; and Capillary temperature, 275 °C. A carrier gas (N2) with an 8 arbitrary units flux (A.U.), and the collision gas was Helium (He). The via´s acquisition will be M/z 100-2000. The Xcalibur 1.3 software version (Thermo Finnigan, Waltham, MA) was used to acquire and process the data.

EVALUATION OF ANTIOXIDANT ACTIVITY

DDPH test

This test measured free radical scavenging activity using a radical DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich)[9].

A 250 µL aliquot of DPPH solution (1 mM) was mixed with 40 µL of different sample concentrations (5 – 100 µg/mL). The absorbance (517 nm) was measured after 30 min of incubation. The Gallic Acid (Sigma-Aldrich) was used as a reference compound. The free radical scavenging effects were determined using the following formula: [DPPH] (%) = (Ac - As) / Ac × 100, where: Ac = absorption control; As = absorbance sample.

ABTS Method

The ABTS+ method is characterized by measuring the capture of the radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), which can be generated through a chemical, electrochemical, or enzymatic reaction[9]. It was utilized 20 µL of sample and 250 µL of reagent solution ABTS (30 mg of ABTS, 379.4 mg of potassium persulfate, and 0.137 mL of persulfate solution). The spectrophotometer (734 nm) performed the reading after 6, 15, 30, 45, 60, and 120 minutes of incubation. The result was shown in inhibition percentage. The calculation percentage of inhibition was made using the following formula:

$$\text{Inhibition } (\%) = \frac{(Abs\ control - Abs\ sample)}{(Abs\ control)}\ x\ 100$$

ANTIMICROBIAL ACTIVITY IN VITRO

Microorganisms

The bacterial strains used in this study (*Staphylococcus aureus* ATCC 6538), *Escherichia coli* 042, *Salmonella enteritidis* ATCC 13076*, Pseudomonas aeruginosa* ATCC 27856 e *Enterococcus faecalis* ATCC 51299) are conserved in the Microbial Pathogenicity Laboratory of the CEUMA University.

Microbial suspensions preparation

The microorganisms were initially reactivated starting with the original culture and kept in a liquid medium BHI (Brain Heart Infusion) at 37°C for 24 hours. Right after, the samples were cultivated in Agar Nutrient plates at 37 °C for 18-24 hours. Isolated colonies were resuspended in 3 mL of saline solution (0.89% NaCl) sterile until reaching equivalent turbidity on the 0.5 Mc Farland scale (1.5 x 108 bacteria/mL).

Determination of Minimum Inhibitory Concentration (MIC)

The determination of the MIC was performed using the microdilution plate technique. For this, isolated colonies were resuspended in 3 mL of sterile saline solution (0.89% NaCl) until reaching turbidity equivalent to 0.5 Mc Farland scale (1.5 x 108 bacteria/mL) and diluted 1:10 with Mueller-Hinton broth.

Aliquots of 10 µL of the inoculum were added to each well. In each line of the plate, a test was performed, and in column 1, it was used as a negative control (Medium + Inoculum); in column 2 it was used as a positive control (Medium + Inoculum + Antibiotic); in column 3 a control of the purity of the medium (Pure Medium) and in columns 4-12 the test was carried out with the fraction at different concentrations (Medium + Serial Dilution + Inoculum).

All plates were incubated at 37ºC. After 24 h, antimicrobial effects were determined by adding 30 µL of sterile resazurin solution for each well. The test was performed in quadruplicate for each bacterium. The MIC (in µg/mL) was the lowest concentration of the crude extract and dichlorine fraction of the *Fridericia platyphylla* leaves where there was no visible bacterial growth, which in practice was the lowest concentration of the plant extract fractions where the pink color is not observed.

TOXICITY TEST USING TENEBRIO MOLITOR

The in vivo toxic effects of the crude extract and dichloride fraction were assessed using larvae of the insect *Tenebrio molitor* (Tenebrionidae). In recent years, T. molitor has been explored by the scientific community as an experimental model due to its peculiar characteristics that offer advantages over other invertebrate models used in laboratory studies[10].

Larvae weighing close to 100 mg were randomized into groups of 20 larvae per group. Before inoculating the samples at different concentrations, the cuticles of the larvae were cleaned with 70% alcohol. After the entire procedure for cleaning the larvae, 10 µL of the crude extract and dichlorine fraction were injected using a sterile insulin syringe[10]. Larvae were kept in sterile petri dishes, and survival and melanization were evaluated daily for 7 days. The survival curve was determined based on the larvae's lack of movement and complete melanization. Phosphate buffered saline (PBS; 10 µL; 1%) was injected for the negative control.

STATISTICAL ANALYSIS

The normality of variables was evaluated using the Kolmogorov–Smirnov normality test. The statistical differences between the groups were analyzed using One-way ANOVA followed by Tukey test (for the comparison of three or more groups for Gaussian data) or Kruskal-Wallis followed by Dunn method (for non-parametric data). Percentages of inhibition were calculated as the mean of the inhibitions obtained for each individual experiment. p values < 0.05 were considered statistically significant. Data are expressed as mean ± standard deviation (SD). Analyses were performed using GraphPad Prism 7.0.

RESULTS AND DISCUSSION

Chemical Characterization

Chromatographic profiles are widely used in the study of plants, allowing them to compare and suggest classes of compounds present in extracts using diode array detectors, ultraviolet or mass spectrometry, for example[11]. Figure 1 shows the chromatographic profile of the 70% hydroethanolic extract of *F. platyphylla* leaves. The result shows the presence of high and medium polarity compounds.

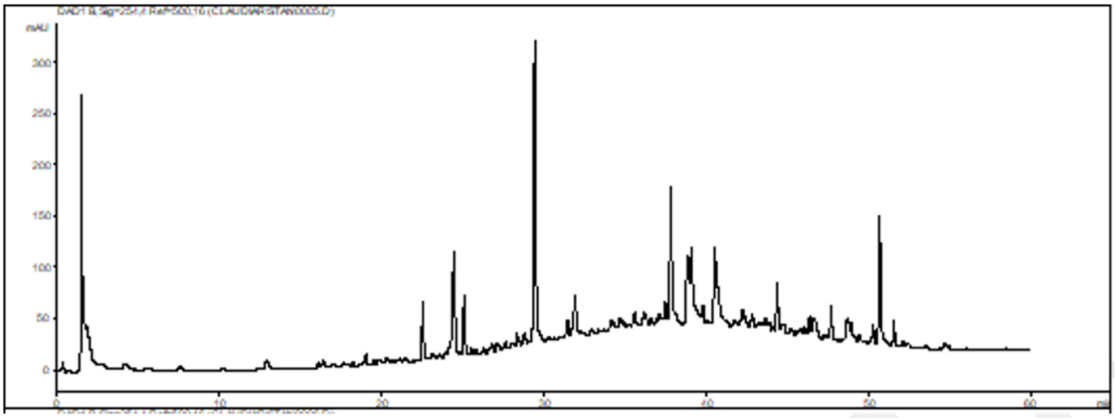

Figure 1 – Crude Extract Chromatogram obtained by HPLC-PDA.

Several peaks were observed in the chromatogram, mainly derived from phenolic compounds, and in approximately 30 minutes, a compound with a vast area was present (Figure 1). This major peak, present in the chromatogram, corresponds to rutin, which was confirmed by mass spectrometry (Figure 2).

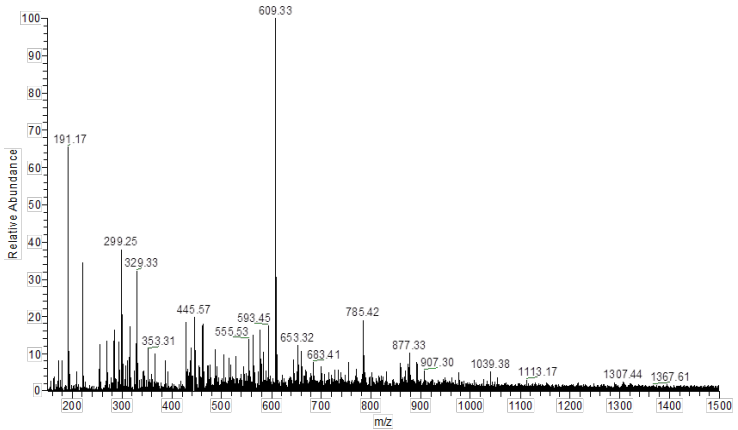

Figure 2 – Typical analysis of direct flow injection FIA-ESI-IT-MS digital fingerprint spectra obtained in negative ion mode from 70% EtOH from *Fridericia platyplylla* leaves.

Figure 2 shows a typical spectral analysis of FIA-ESI-IT-MS digital fingerprint spectra from direct flow injection analysis obtained in negative ion mode from 70% EtOH from *F. platyplylla* leaves. Ten constituents (Figure 3) were identified in the 70% EtOH extract. The data related to peak identification are shown in Table 1.

Compound	Radical					
	R_1	R_2	R_3	R_4	R_5	R_6
1	OH	H	H	OH	H	H
2	OH	OH	H	OH	H	H
3	OH	H	H	OH	OCH_3	H
4	OH	OH	H	OCH_3	OCH3	H
5	OH	H	H	OCH_3	Glc	H
6	OH	OH	H	OCH_3	Glc	H
7	OH	OH	H	OCH_3	OCH_3	H
8	OH	H	Glc	OCH_3	Glc	H
9	OH	OH	H	OH	H	OGlc-O-Rha
10	OH	OH	H	OH	H	OGlc-O-Glc-R-

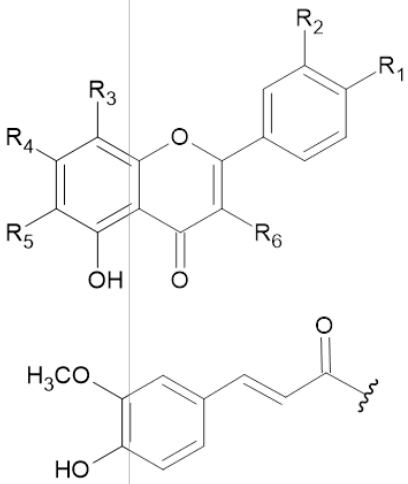

Figure 3 – The chemical structures identified by the mass spectrum of *Fridericia platyphylla* by FIA-ESI-IT/MSn.

Frame 2 shows the identification of compounds in *Fridericia platyphylla* by FIA-ESI-IT/MSn. The ESI-MS spectrum for compound 1 exhibited a deprotonated molecule at m/z 269 (C15H10O5), characteristic of apigenin. During MS/MS in negative ionization mode, apigenin showed a characteristic ion at m/z 151 produced by RDA cleavage of the C-ring.

Compound 2 was identified as luteolin after comparing its retention time and UV spectrum with standard compounds. Furthermore, the MS/MS spectrum obtained in the negative ionization mode showed the expected fragmentation patterns for luteolin and the precursor ion in m/z 285 [M - H]- (C15H10O6). During MS/MS in negative ionization mode, luteolin showed a characteristic ion at m/z 267 and 243.

In negative mode, compound 3 showed [M - H]− at m/z 299, which produced the fragmentation ion at m/z 284 by neutral loss of CH3, indicating the presence of a methoxy group. Furthermore, the characteristic ion at m/z 117 [1,3B−] was observed, indicating a hydroxyl group on the B ring. Thus, the compound was tentatively deduced as 4',5,7-trihydroxy-6-methoxyflavone or hispidulin, which must be confirmed later.

Compound 4 was identified as cirsiliol. The MS/MS spectrum obtained in the negative ionization mode showed the expected fragmentation patterns for cirsiliol and the precursor ion in m/z 329 [M - H]- (C17H14O7). During MS/MS in negative ionization mode, it showed a characteristic ion at m/z 314 due to the loss of the methyl group.

Compound 5 showed characteristics for apigenin with a substituent at the position. The negative ionization mode mass spectrum of this peak showed precursor ions at m/z 445 [M - H]- (C21H18O11)

and m/z 269, corresponding to the loss of one glucuronyl unit. Then, compound 5 was identified as 7-methoxypigenin-6-C-hexose.

In the negative ionization mode, compound 6 showed a precursor ion in m/z 461 [M-H]-(C22H22O11), and its MS/MS spectrum showed an ion in m/z 371 and 341, which corresponds to the loss of characteristic sugar fragments of the flavonoid C-glycoside. In addition, the posterior ion MS/MS spectrum produced product ions at m/z 298, characteristic of luteolin. Then compound 6 was identified as 7-methoxyluteolin-6-C-hexose.

Compound 7 showed a precursor ion at m/z 463 [M - H]-(C21H20O12), and its MS/MS spectrum showed a product ion at m/z 445 and 301 due to the loss of two xylose units. This peak was compared to the authentic standard and identified as quercetin-3-O-glycoside (isoquercetrin).

Compound 8 with precursor ion 593 [M - H]-, was assigned as 6,8-di-C-β-glucupiranosylpigenin (apigenin-6-C-hexose, 8-C-hexose), which was reinforced by its standard of fragmentation. The MS/MS spectrum in negative ionization mode showed product ions at m/z 575, corresponding to dehydration; m/z 503, m/z 473, m/z 383, m/z 353. This identification was confirmed by comparison with the authentic standard.

Compound 9 showed a precursor ion at m/z 609 [M - H]-(C27H30O15) in the negative ionization mode, and its MS/MS spectrum showed product ion characteristics for rutin at m/z 463 due to the loss of a rhamnosyl unit, and at m/z 301, formed after the loss of the hexose residue (162 u) or the direct loss of the rutinoside residue unit (ramnosyl-(α1→6)-glucose). Therefore, these data and comparisons with the authentic standard identified peak 19 as quercetin-3-O-rutinoside (rutin).

Compound 10 showed a precursor ion in m/z [M - H]- (C37H40O19), and its MS/MS spectrum showed product ions in m/z 609 and 301, which are characteristic of the loss of terminal units of two sugars (glucose + rhamnose) and ferulic acid residue, respectively. This allowed the identification of this compound as arrabidoside A.

Through chemical analysis, it was possible to identify the bioactive compounds in the crude extract of *Fridericia platyphylla* leaves. Chemical characterization is essential to determine which secondary metabolites are present in each species. This allows us to understand their ecological, pharmacological, and nutritional functions. These chemical compounds can undergo quantitative or qualitative variations influenced by the main factors: environmental, ontogenetic, and hereditary. Furthermore, the identification and quantification of these metabolites can reveal potential for the development of medicines, pesticides, and dietary supplements[12].

Most secondary metabolites in *Fridericia platyphylla* are flavonoids, emphasizing the compound rutin. Similar research conducted with the hydroalcoholic extract of the leaves of *F. platyphylla* also identified the flavonoid rutin[13]. Other studies with *F. platyphylla* have isolated rare flavonoids called Brachydins A, Brachydins B, and Brachydins C[14].

Although secondary metabolites are necessary for plant defense, they also play an essential role in human health. Flavonoids, for example, have aroused research interest due to their therapeutic properties, preventing the appearance of cardiovascular, cancerous, and immunological diseases[15]. Rutin flavonoid has shown several pharmacological activities, such as antioxidant, cytoprotective, vasoprotective, anticancer, neuroprotective, and cardioprotective effects[16].

Compound	Molecular Formula (Molecular Weight)	$[M-H]^-$	MSn
Apigenin (1)	$C_{15}H_{10}O_5$ (270)	269	151= $[M-11\ 18-H]^-$
Luteolin (2)	$C_{15}H_{10}O_6$ (286)	285	267 = $[M-18-H]^-$, 243 = $[M-42-H]^-$
Hispidulin (3)	$C_{16}H_{12}O_6$ (300)	299	284 = $[M-15-H]^-$, 117= $[M-15-167-H]^-$
Cirsiliol (4)	$C_{17}H_{14}O_7$ (330)	329	314 = $[M-15-H]^-$
7-metoxipigenina-6-C-hexose (5)	$C_{22}H_{22}O_{10}$ (446)	445	401 = $[M-44-H]^-$, 269 = $[M-132-H]^-$
7-methoxyluteolin-6-C-hexose (6)	$C_{22}H_{22}O_{11}$ (462)	461	443 = $[M-18-H]^-$, 371 = $[M-90-H]^-$341 = $[M-120-H]^-$313 = $[M-120-28-H]^-$,298 = $[M-120-28-15-H]^-$
Isoquercitrin (7)	$C_{21}H_{20}O_{12}$ (464)	463	445 = $[M-132-H]^-$, 301 = $[M-132-H]^-$
Apigenin-6-C-hexose, 8-C-hexose (8)	$C_{27}H_{30}O_{15}$ (594)	593	575 = $[M-18-H]^-$, 503 = $[M-90-H]^-$, 473 = $[M-120-H]^-$,383 = $[M-120-90-H]^-$,485 = $[M-90-18-H]^-$
Rutin (9)	$C_{27}H_{30}O_{16}$ (610)	609	463 = $[M-146-H]^-$, 301=[M-146-162-H]$^-$
Arrabidoside A (10)	$C_{37}H_{40}O_{19}$ (786)	785	609 = $[M-176-H]^-$, 301= $[M-308-H]^-$

Frame 1 – Identification of compounds in *Fridericia platyphylla* by FIA-ESI-IT/MSn.

ANTIOXIDANT ACTIVITY

The antioxidant activity investigation of the crude extract and dichloride fraction of *Fridericia platyphylla* leaves, conducted using the DPPH and ABTS methods, showed antioxidant activity. Several extracts of medicinal plants belonging to the genus *Fridericia* have already demonstrated their antioxidant capacity in vitro using different methods and fractions of the crude extract[17-18].

Figure 4 shows the values of oxidizing activity with the crude extract fraction and the dichlorine fraction of *Fridericia platyphylla* leaves at different concentrations using the DPPH method.

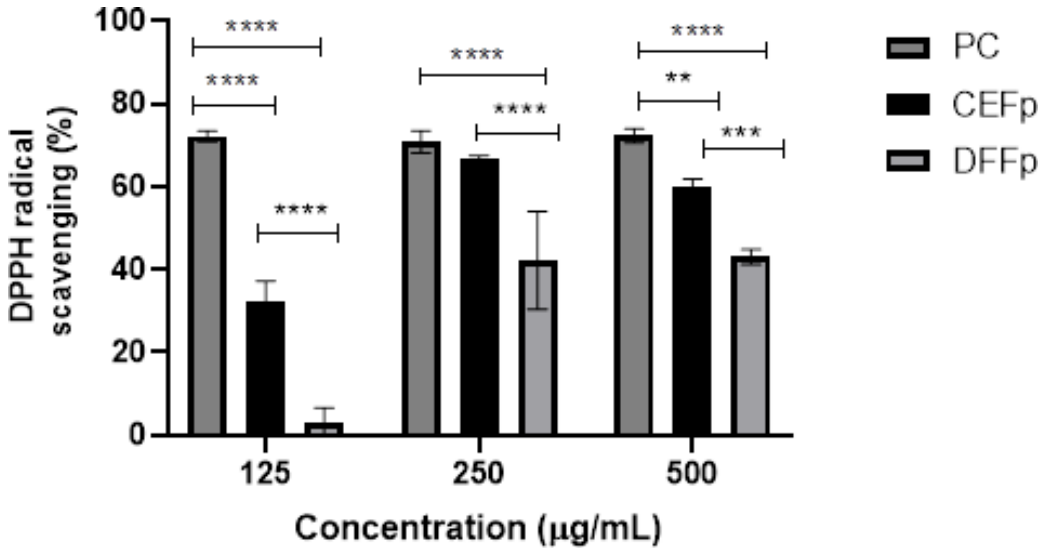

Figure 4 – Antioxidant activity of the crude extract and the dichlorine fraction of *Fridericia platyphylla* leaves at different concentrations using the DPPH method. PC: Positive Control. CE Fp: crude extract of *Fridericia platyphylla* leaves. DF Fp: dichloride fraction of *Fridericia platyphylla* leaves. (**p<0.01, ***p<0.001, ****p<0.001).

It is observed that the crude extract had a more significant antioxidant action at a concentration of 250 µg/mL. The dichloride fraction obtained approximately 40% DPPH inhibition at 250 µg/mL and 500 µg/mL. The crude extract and the dichloride fraction showed evidence of their antioxidant capacity, which indicates that the species has a promising source of natural antioxidants in a test using DPPH as a free radical. The ABTS method was also employed to evaluate the antioxidant activity of the crude extract and dichloride fraction. Figure 5 shows the values in percentage from the results found in the absorbances.

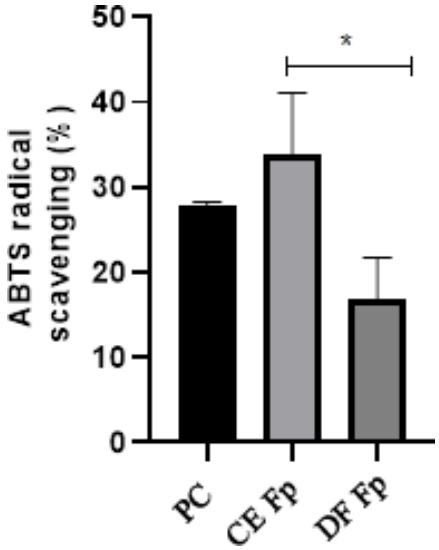

Figure 5 – Antioxidant effects of the crude extract and the dichlorine fraction of *Fridericia platyphylla* leaves at different concentrations using the ABTS method. Caption: PC: Positive control. CE Fp: crude extract of the leaves of *Fridericia platyphylla*. DF Fp: dichloride fraction of *Fridericia platyphylla* leaves. (*p<0.05).

The evaluation by the ABTS method was performed after different incubation times. According to figure 2, it is observed that the crude extract showed antioxidant activity of approximately 33%. In comparison, the dichloride fraction of the leaves showed an activity of approximately 16%. It is also observed that the crude extract showed higher antioxidant activity in both the ABTS and DPPH methods.

The antioxidant activity observed in this study can be attributed, in large part, to the flavonoids isolated from the crude extract analyzed. These flavonoids are bioactive compounds found in several medicinal plants and play a crucial role as antioxidants. They act by inhibiting the formation of reactive oxygen species (ROS), which are highly reactive molecules capable of causing cellular damage[18].

There are different mechanisms by which flavonoids exert this antioxidant activity. One of them is the inhibition of enzymes that promote the generation of free radicals. In addition, flavonoids can chelate trace elements that are involved in the production of ROS, thus reducing the availability of such elements for reactions that generate these radicals. Another important mechanism is the ability of flavonoids to directly eliminate reactive oxygen species, neutralizing their harmful effects. Furthermore, flavonoids can also act by promoting the positive regulation of the body's antioxidant defenses, or even providing protection to these already existing defenses. Thus, flavonoids play a fundamental role in protecting against oxidative stress, contributing significantly to maintaining health and well-being[19].

In vitro antimicrobial activity

Table 2 shows that the crude extract of *F. platyphylla* leaves inhibited *S. aureus* at a concentration of 64 µg/mL. The dichloride fraction of *F. platyphylla* leaves inhibited the same bacteria at 16 µg/mL. Concerning enteroaggregative *E. coli* and *S. enteritidis*, there was no inhibition in any tested concentrations with the extract fractions. The MIC values for *Enterococcus* bacteria strains were 1024 µg/mL for both samples. For *P. aeruginosa*, the MIC was 512 µg/mL for both crude extract and the dichloride fraction.

Samples	*S. aureus*	*E. coli*	*S. enteritidis*	*E. faecalis*	*P. aeruginosa*
Crude extract	64 µg/mL	-	-	1024 µg/mL	512 µg/mL
Dichloride fraction	16 µg/mL	-	-	1024 µg/mL	512 µg/mL

Frame 2 – Antimicrobial effects of crude extract and dichloride fraction of *Fridericia platyphylla* leaves.

Studies using plant extracts from the same family of *F. Platyphylla* evidenced minimal inhibitory concentrations for pathogenic bacterial strains of *S. aureus*[20]. In addition, studies identified that the flavonoid Chalcona present in the dichloromethane fraction of *F. platyphylla* flowers inhibited strains of *S. aureus*[21]. Studies also showed that *F. platyphylla* compounds inhibited the *S. aureus* NorA efflux pump[22].

The inhibitions observed in this study for the bacteria *S. aureus*, *P. aeruginosa*, and *E. faecalis* can also be attributed to the flavonoids found in the crude extract of the leaves of *F. platyphylla*. The antimicrobial activity of flavonoids has been proven in several studies. It occurs through different mechanisms, such as changing the permeability of cell membranes and walls, inhibition of nucleic acid synthesis, neutralization of virulence factors, urease inhibition, and synergistic effects with antibiotics[23].

For the lack of inhibition for enteroaggregative *E. coli* and *S. enteritidis*, several factors may have contributed to these results. Among them, we can mention the origin of the plant, the time of collection, whether the extracts were prepared from fresh or dried plants, the amount of extract

evaluated, the microorganism and strain used in the test, the temperature, difficulty of propagation in the medium culture and solvent used for extraction[24-25]. Another factor that may be related is the external membrane of gram-negative bacteria, which prevents the penetration of macromolecules and hydrophobic compounds, being relatively resistant to hydrophobic compounds from plant extracts[26].

The antimicrobial evaluation of plant-derived products is of foremost importance, as the prevalence of multidrug-resistant bacteria has increased significantly in recent years, reducing the number of effective drug options to fight infections. Several secondary metabolites of medicinal plants have been investigated, as they can inhibit bacterial efflux pumps and may be a therapeutic option for treating bacterial infections[27].

TOXICITY IN AN ALTERNATIVE MODEL (TENEBRIO MOLITOR)

The in vivo toxicity was evaluated using larvae of *Tenebrio molitor*. The larvae were evaluated for 7 days. No toxicity was observed for the concentrations of FAB tested during the first 5 days. However, in the last 2 days of evaluation, there were some losses, but at different concentrations; that is, there is no pattern of death by concentration (Figure 6), thus demonstrating the *in vivo* non-toxicity of this fraction.

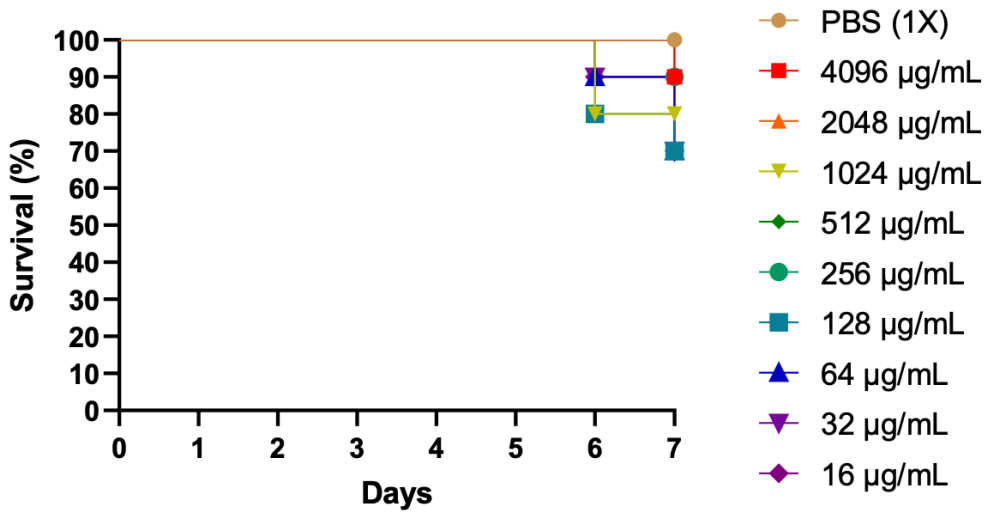

Figure 6 – Survival curve of *Tenebrio molitor* larvae treated with *F. platyphylla* Crude Extract Fraction (FAB) at different concentrations and sterile PBS1X negative control. The larvae were treated and evaluated daily for 7 days.

Regarding the dichloromethane fraction (DMF) (Figure 7), it is observed that, during the days of evaluation, the highest concentration (4096 µg/mL) showed a pattern of death from the 3rd day of evaluation, with this pattern of 1 death every 48 hours of evaluation, thus ending with 70% survival of the larvae. Despite this, it was also observed that in the other concentrations, the forgiveness occurred randomly, that is, without a justifying toxic pattern. Therefore, it is not possible to state that the compound behaves in a toxic way.

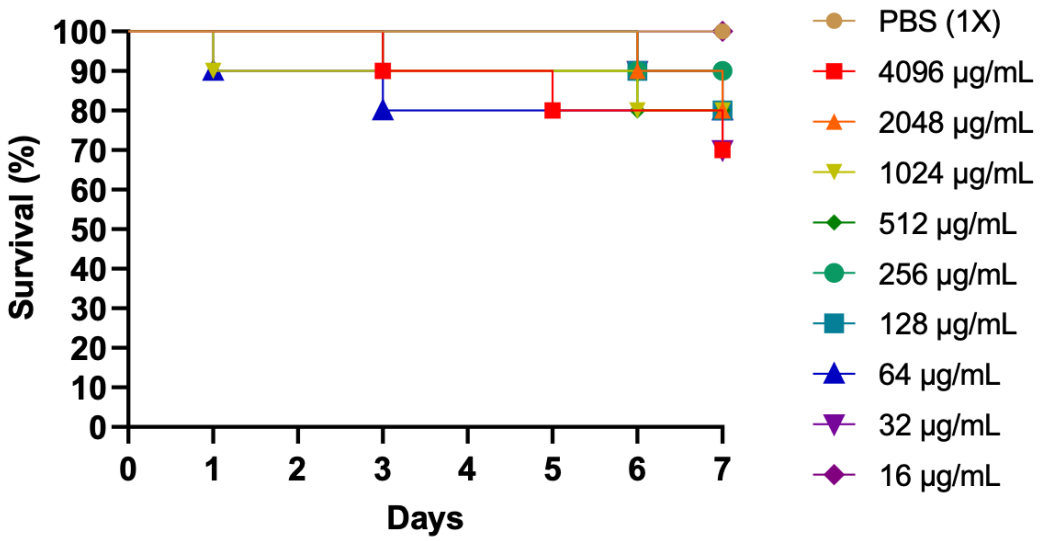

Figure 7 – Survival curve of *Tenebrio molitor* larvae treated with Dichloromethane Fraction (DMF) at different concentrations and sterile PBS1X negative control. Caption: The larvae were treated and evaluated daily for 7 days.

There are few studies in the literature about the toxicity of *F. platyphylla* in alternative models. In studies conducted with *Artemia salina*, another commonly used alternative model, it was possible to observe that the floral extract of this species presented a lethal dose between 310 and 230 µg/mL. Despite this, it was considered non-severely lethal[28]. In a study carried out using murine models, it was possible to identify that extracts from the same family can inhibit the incidence of mammary tumors, considerably reduce the levels of serum transaminases, oxidative stress, and hematological toxicity, that is, it is an excellent indication of the use of the *Bignoniaceae* family for other medicinal purposes[29].

Phytochemical assays of *F. platyphylla* extract revealed the presence of several secondary metabolites, including flavonoids. These results indicate that the antioxidant and antimicrobial activities of the crude extract and the dichloride fraction of the leaves are directly related to their chemical constituents. Thus, the crude extract and the dichloride fraction emerge as promising alternatives for the prevention and control of infections caused by *S. aureus*, *E. faecalis* and *P. aeruginosa*, in addition to serving as potential sources of raw material for the development of drugs and natural products. Regarding toxicity, the study did not show significant effects, but, according to the literature, additional cytotoxic studies are necessary to investigate the possible therapeutic and pharmacological activities of these extracts.

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