Article

Journal of the Brazilian Chemical Society

https://dx.doi.org/10.21577/0103-5053.20240132

J. Braz. Chem. Soc. **2025**, *36*, 2, e-20240132, 1-13

Chemical Characterization and Evaluation of the Anti-Cancer Potential of Flowers from *Fridericia platyphylla* (Bignoniaceae)

Marcelino S. do Rosário,[a,b] Helena Mannochio-Russo,[c] Ana L. P. dos Santos,[b] Aglaete A. Pinheiro,[a] Luna N. Vasconcelos,[d] Ana Paula S. A. Santos,[e] Lila T. de Oliveira,[f] Monique M. Martins,[f] Marcelo S. de Andrade,[f] Maria D. S. B. Nascimento,[f] Vanderlan S. Bolzani,[b] Josélia A. Lima[f] and Cláudia Q. da Rocha*[a]

[a]*Laboratório de Química de Produtos Naturais (LQPN), Departamento de Química, Centro de Ciências Exatas e Tecnologia, Universidade Federal do Maranhão, 65080-805 São Luís-MA, Brazil*

[b]*Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais (NuBBE), Departamento de Bioquímica e Química Orgânica, Instituto de Química, Universidade Estadual Paulista Julio de Mesquita Filho (UNESP), 14800-900 Araraquara-SP, Brazil*

[c]*Skaggs School of Pharmacy and Pharmaceutical Sciences, Universidade da Califórnia, San Diego, CA, United States*

[d]*Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal do Maranhão, 65080-805 São Luís-MA, Brazil*

[e]*Departamento de Ciências Fisiológicas, Universidade Federal do Maranhão, 65080-805 São Luís-MA, Brazil*

[f]*Programa de Pós-Graduação em Saúde do Adulto, Laboratório de Cultura de Células, Universidade Federal do Maranhão, 65080-805 São Luís-MA, Brazil*

In this study, we aimed to investigate the anticancer activity of the extract, fractions, and isolated compounds of the flowers of *Fridericia platyphylla*, and to characterize the bioactive compounds. The chemical diversity of the extracts and fractions was investigated using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS). We were able to annotate 26 compounds from the classes of flavones, flavonols, flavanones, isoflavones, and cinnamic acid and its derivatives. The dichloromethane fraction showed greater cytotoxicity at a concentration of 100 μg mL^{-1}. In addition, the inhibitory concentrations of the dichloromethane fraction were 22.14 and 30.9 μg mL^{-1} for MCF-7 and MDA-MB-231 cell lines, respectively, and were capable of inhibiting tumor cell migration. Brachydins A and C were isolated from the dichloromethane fraction and showed the greatest cytotoxicity. The results obtained from this study show the potential biological effect of *F. platyphylla* flowers as a possible antitumor pharmacological agent.

Keywords: Bignoniaceae, spectral annotation, flavonoids, brachydins, cancer

Introduction

The Bignoniaceae family comprises 80 genera and 860 species, representing an important component of neotropical forests.[1] Within this family, the *Fridericia* genus includes about 170 species with shared taxonomic characteristics. *Fridericia platyphylla* (*F. platyphylla*), popularly known as "*cervejinha do campo*", is a branched shrub with purple-pink terminal inflorescence flowers native to the Brazilian Cerrado biome. This species is known for its multifaceted biological properties, such as astringent, antioxidant, anti-inflammatory, antimicrobial, antitumor, and healing agent.[2-5]

The literature[6,7] reports metabolites of several classes in the *Fridericia* genus, including *C*-glucosyl xanthones, phenylpropanoids, flavonoids, anthocyanidins, allantoin derivatives, chalcones, coumarins, tannins, cardiotonic glycosides, steroids, saponins, and triterpenes. These chemical classes have previously been documented in

*e-mail: rocha.claudia@ufma.br
Editor handled this article: Paulo Cezar Vieira

a variety of plant organs, including the leaves, stems, aerial parts, seeds, roots, and flowers. A previous study[8] of *F. platyphylla* roots have described the isolation of 19 secondary metabolites from its extracts and fractions, including the brachydins, a class of dimeric flavonoids discovered for the first time in the Bignoniaceae family.

In this context, natural products became a source of bioactive compounds to treat several diseases, including breast cancer. Breast cancer, with its frequent diagnosis in women and elevated rates of incidence, morbidity, and mortality, has emerged as a major global public health concern, claiming the position of the primary cause of death for women in Brazil in 2017.[9,10] Natural products emerged as an alternative for the treatment of cancer, especially *F. platyphylla* for its broad biological activity.

Based on research on the roots of *F. platyphylla*, this study aims to investigate the flowers of this species to characterize the hydroethanolic extract and fractions. To achieve these goals, liquid chromatography coupled with mass spectrometry was used to inspect the chemical profile using the Global Natural Product Social Molecular Networking (GNPS) platform, followed by the isolation of the bioactive compounds. The extract, fractions, and isolated compounds were subjected to anticancer assays to determine their bioactivity, showing the isolation of unusual secondary metabolites and their high bioactivity.

Experimental

General experimental procedures

Nuclear magnetic resonance (NMR) spectroscopic data were acquired on a Bruker Avance III HD 600 Hz (Karlsruhe, Germany) equipped with a QCI 5 mm cryoprobe. Chemical shifts are reported in parts *per* million (δ) using the residual deuterated methanol (CD_3OD), signal (δ_H 3.31; δ_C 49.0), as the internal standard for both ^1H and ^{13}C NMR, acquired from Sigma-Aldrich (St. Louis, USA). The coupling constants (*J*) are reported in Hz. The structural elucidation was performed based on 2D experiments (correlation spectroscopy (COSY), nuclear overhauser effect spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC)). Analytical high performance liquid chromatography (HPLC) was performed using a Shimadzu Prominence (Kyoto, Japan); pump: LC-20AT; autosampler: SIL-20A; column oven: CTO-2OA; detector: SPD-M20A; controller: CBM-20A. Semipreparative HPLC was performed using a Shimadzu LC-10AD (Kyoto, Japan) pump equipped with a UV detector (254 nm).

Plant material

The flowers of *F. platyphylla* were collected in João Pinheiro, Sant'Ana da Serra farm, Minas Gerais state, Brazil (coordinates 17°4404500 S, 46°1004400 W), in February 2021. The identification of the plant was carried out by the botanist Dr Maria Cristina Teixeira Braga Messias. The plant voucher was deposited in the Herbarium José Badine at the Universidade Federal de Ouro Preto (number 17.935). Flowers were collected following biodiversity protection laws (SisGen) under number A4551DE4.

Extraction procedure

The flowers (260.0 g) were dried in an oven at 40 °C and crushed in a knife mill. The powder obtained was extracted with ethanol/water (7:3) (FLAB-EC) through exhaustive percolation. The extract was concentrated under reduced pressure using a rotary evaporator at 40 °C. The crude extract was transferred to glasses protected from light and lyophilized for the complete removal of solvents. The crude extract was dissolved in water/methanol (7:3) and partitioned six times with dichloromethane (DCM, 98%, Êxodo Científica, Sumaré, Brazil). The aqueous fraction was partitioned with ethyl acetate (EtOAc, 98%, Êxodo Científica, Sumaré, Brazil) using the same procedure as the previous fraction. At the end of the process, it was possible to obtain three fractions with different polarities; FLAB-DCM: 15.41 g, FLAB-EtOAc: 2.05 g, and aqueous fraction (FLAB-Aq): 20.63 g.

High-performance liquid chromatography-mass spectrometry (HPLC-MS)

Chemical profile of fractions by high performance liquid chromatography with diode array detector (HPLC-DAD)

The fractions were dissolved in methanol at a concentration of 1.0 mg mL^{-1}, filtered through a 0.22 µm Millex filter, and analyzed by HPLC-DAD (Shimadzu Corp., Kyoto, Japan) with SPD-M20A DAD detector (diode array detector). A Luna Phenomenex® C18 column (C18; 250 × 4.60 mm; 5 µm; 100 Å) was used. The mobile phase A consisted of water, while phase B consisted of methanol (HPLC grade, J.T. Baker-Avantor, Radnor, USA), both acidified with formic acid (0.1%), and monitored at a wavelength of 254 nm. The gradient elution employed consisted of a linear gradient from 5 to 100% B in 40 min, and a flow rate of 1.0 mL min^{-1}, at 40 °C.

Ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis

The extract (FLAB-EC), fractions (FLAB-DCM, FLAB-AcOEt, and FLAB-Aq), and isolated compounds

were solubilized at a concentration of 250 ppm and analyzed by UHPLC-MS/MS (Waters, Milford, USA) equipped with a quaternary pump, degassing system, autosamples, PDA detector, and a Xevo G2-XS QTOF mass spectrometer (Waters, Milford, USA) containing a C18 Acquity UPLC HSST3 column (100 × 2.1 mm, 1.8 μm) with 100 Å pore diameter. Mobile phase A with ultrapure water and mobile phase B with MeOH (HPLC grade, J.T. Baker-Avantor, Radnor, USA), both acidified with 0.1% formic acid with gradient elution mode from 5 to 100% in 15 min and a flow rate of 1.0 mL min^{-1} at 45 °C. The samples were analyzed in the positive ionization mode under the following conditions: capillary voltage 2.5 kV, ionization source temperature of 120 °C, and desolvation gas flow (N_2) 800 L h^{-1} with a temperature maintained at 350 °C.

Molecular networking

The ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS/MS) raw data were initially converted to mzML files using the MSConvert from ProteoWizard software (ProteoWizard, Palo Alto, USA).[11] These converted data were uploaded to the Global Natural Products Social Molecular Networking (GNPS) platform[12] and used in the molecular networking workflow.

The data were initially filtered by removing all MS/MS fragment ions within +/–17 Da of the precursor *m/z*, and only the six most intense fragment ions in a window of +/–50 Da were selected. The MS/MS filtered data were then clustered with minimum cluster size, the precursor ion tolerance, and MS/MS fragment ion mass tolerances were set at 0.02 Da. A network was created in which edges were filtered to have a cosine value above 0.7 and more than 4 matching peaks. Finally, the maximum size of a molecular family was defined as 100. The spectra were then searched against the GNPS public spectral libraries, setting a minimum score of 0.7 and at least 4 matching peaks.[12] The molecular networks were visualized in Cytoscape.[13] The molecular networking analysis can be accessed on GNPS platform.[14]

Extraction and isolation

The dichloromethane fraction (3.0 g) was initially fractionated with silica gel 60 (0.063-0.200 mm, Merck, Darmstadt, Germany) and hexane/ethyl acetate as a mobile phase in a linear polarity gradient, resulting in 14 fractions. Fractions 2, 3, 4, 5, 6, and 8 were then further fractionated in a semipreparative high performance liquid chromatography coupled to a photodiode array detector (HPLC-PDA). The fractions were dissolved in methanol. The mobile phase A consisted of water, while phase B consisted of methanol, both acidified with formic acid (0.1%), in a gradient system of 60-100% of B in 40 min, flow rate of 4.0 mL min^{-1}, and UV absorbance was monitored at 254 nm. The column used was a Luna 5 μm C18 100 Å (250 × 10 mm).

Anticancer assays

Cell culture

MCF7 and MDA-MB-231 (Cell Bank, Rio de Janeiro, Brazil) human breast cancer cell lines were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St Louis, USA) with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, USA) (v/v) and 1% penicillin/streptomycin (50 U mL^{-1}/50 μg mL^{-1}) (Sigma-Aldrich, St Louis, USA). The culture flasks were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% humidity. Cells displaying exponential growth were detached from the culture flasks with trypsin and seeded at the density required for the experiment.

MTT cytotoxicity assays

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, Darmstadt, Germany) assay, where viable cells are detected by their ability to convert MTT into insoluble formazan crystals, as previously described. Breast cancer cells were seeded on a 96-well plate at a density of 1×10^4 cells *per* well in DMEM medium containing 10% FBS and incubated under the previous conditions. The following day, after cell adhesion, the medium was replaced with different concentrations of samples (1-100 μg mL^{-1}), and cells were incubated for 24, 48, or 72 h. Blank wells (medium + compounds without cells) were included for the highest concentration of each sample. The medium + dimethyl sulfoxide (DMSO, Thermo Fisher Scientific, Waltham, USA) was tested at the highest concentration (0.1%) used in the experiment and did not interfere with the activity of the samples. After the incubation period, the treatment was removed, the wells were washed with phosphate-buffered saline (PBS buffer, Sigma-Aldrich, Darmstadt, Germany), pH 7.2, and then a solution of medium containing 0.5 mg mL^{-1} of MTT reagent was added to each well, and the plate was incubated under the previous conditions for 3 h. Then, the MTT solution was discarded, and the dark blue formazan crystals were dissolved in ethanol. The absorbance was measured at 570 nm using a microplate spectrophotometer reader (Epoch®, BioTek Instruments, USA, Gen5 software). The results were expressed as percentages of living cells compared to control cells (cells without treatment). Data were analyzed using one-way analysis of variance

(ANOVA) followed by *post hoc* comparisons (Dunnett's test) with GraphPad Prism 7[15] software (GraphPad Software®, San Diego, USA). For each sample, the results represent the mean ± standard deviation of two independent experiments, with each experiment performed in triplicate (*p* values).

Wound-healing assay

Cells (1×10^5 cells $well^{-1}$) were plated in DMEM medium with FBS 10% in 96-well plates and incubated (atmosphere of 5% CO_2 and 95% humidity, at 37 °C) for 24 h. After confirming the formation of a complete monolayer, the cells were wounded by scratching lines with a standard 10-µl plastic tip. Then, the culture medium was removed, the wells were washed with PBS buffer (to remove debris), and 180 µL of culture medium was added. Then, the plate was covered, sealed on the sides with parafilm, and taken to photograph each well with an inverted microscope (Opticam O500i) equipped with a camera (NA0.30 WD72) and a 10× objective. Afterward, the plate was returned to the laminar flow, and 20 µL of FLAB-DCM (10× concentrated) was added to the wells to obtain final concentrations of 20, 40, 60, and 100 µg mL^{-1}, or it was simply placed in the culture medium (control), and the plate was incubated again. These photographic records correspond to time zero (t = 0). After 24 h of the treatment, the wells were photographed again (t = 24 h) to determine the migration and cell movement throughout the wound area. Each experiment was performed in triplicate.

The images were subjected to analysis using the ImageJ software[16] (National Institute of Health, Maryland, USA), which allowed the measurement of the area of each groove. The area of the grooves was analyzed at both times (t = 0 and t = 24 h). The percentage of groove closure was quantified by the percentage variation between the area t = 0 h and the area at t = 24 h, according to the formula: [t (0) – t (24 h)/ t (0)] × 100%. The means and standard deviations of the results were determined.

Results and Discussion

Metabolite annotation

The chemical diversity of *F. platyphylla* flowers crude extract and fractions (DCM, EtOAc, Aq) was investigated using the molecular networking workflow in the GNPS platform (Figure 1).[12] The MS/MS spectra were searched against the GNPS public spectral reference libraries, and molecular networks were constructed for data visualization. All the MS/MS spectral matches obtained were also manually inspected to confirm a level 2 annotation according to the Metabolomics Standards Initiative (MSI).[13] Molecular networks organize the experimental MS/MS data by the correlations detected between each compound

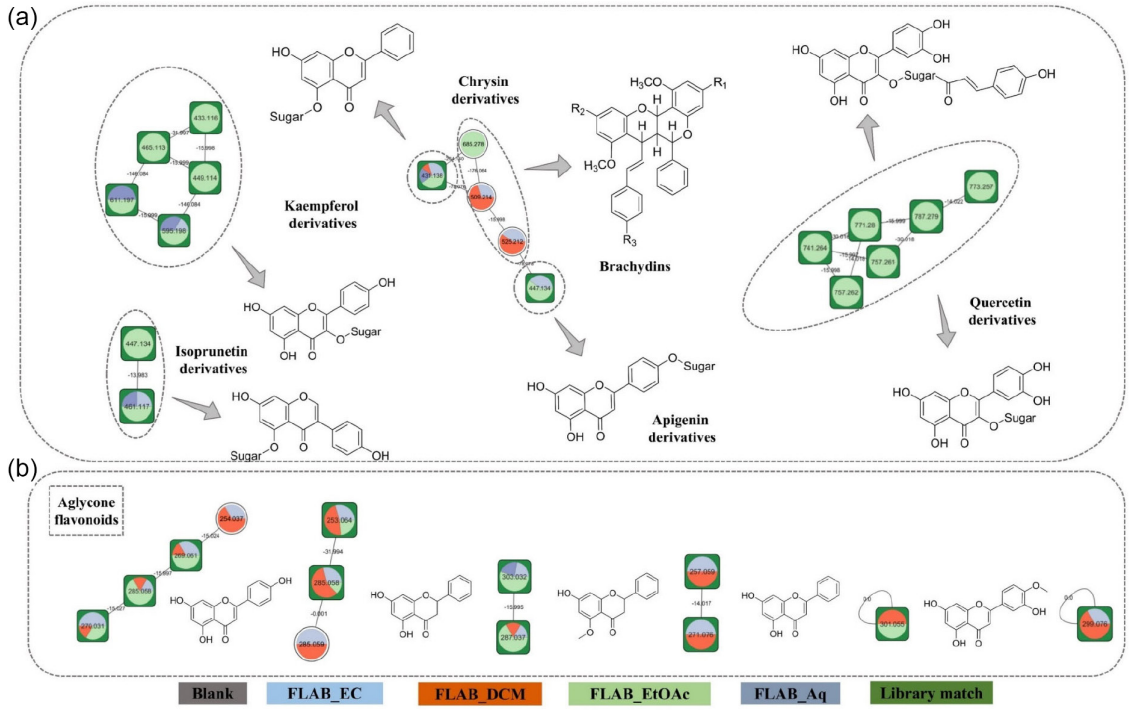

Figure 1. Molecular families obtained and annotated for the extract and fractions of *F. platyphylla*. (a) Dimeric flavonoids and flavonoids glycosylated and (b) aglicone flavonoids. Pie charts represent the relative abundance of ions in each sample. FLAB-EC: crude extract; FLAB-DCM: fraction dichloromethane; FLAB-EtOAc: fraction acetate; FLAB-Aq: aqueous fraction.

present in the mixtures, as similar molecules tend to present similar fragmentations.[17] Each node represents a fragmentation spectrum and is associated with its precursor ion. The molecular network was organized by connecting nodes with edges, grouping them by their fragmentation similarities. It is also important to emphasize that, using this workflow, isomers with different retention times but with the same fragmentation pattern and precursor ions will be grouped into the same node.

The library searches resulted in 88 correspondences with the spectral libraries and allowed the annotation of 26 compounds (see Table S1, presented in Supplementary Information (SI) section) including an important diversity of chemical structures. It is worth noting that in an untargeted analysis, it is not possible to confirm the position of double bonds or substituents or to obtain information about asymmetric centers. The nodes not annotated in the GNPS were searched in the main chemistry databases (SciFinder and PubCHEM).

The *O*-glycosylated flavonoids were predominant in the acetate fraction, in which the MS/MS with characteristic losses of 162, 146, and 132 Da were observed, corresponding to hexose, deoxyhexose, and pentose, respectively.[18] Derived from kaempferol (aglycone characterized by a fragment at *m/z* 287.0550), apigenin (aglycone at *m/z* 271.0965), and quercetin (aglycone at *m/z* 303.0510) were annotated by chemical analysis. Some of these annotated flavonoids were annotated as protonated and sodiated compounds ($[M + H]^+$ and $[M + Na]^+$); these factors contribute to having repeated nodes that represent the same compound. Therefore, the number of nodes does not necessarily reflect the number of compounds present in the samples.[19]

The molecular family characterized by *O*-glycosylated flavonoids, a neutral loss of 162 Da was observed for compounds at *m/z* 465.1033, 449.1075, 433.1123, and 447.0929. Di-glycosylated flavonoids were observed in the phenolic network and presented a neutral loss of hexose-deoxyhexose (*m/z* 308) in compounds at *m/z* 611.1601 and 595.1621. In plants, flavonoids commonly appear as glycosides, mainly because the sugar units are more soluble and mobile, making it easier for these compounds not to interfere with vital cell mechanisms in plants.[20] A neutral loss of 176 Da, relative to glucuronic acid, was also observed for compounds at *m/z* 461.0877, 447.0919, and 431.0962.

Compounds of the class of cinnamic acids and derivatives were annotated. These compounds could be determined based on the presence of *m/z* 163 fragments related to caffeic acid and *m/z* 147 fragments related to coumaric acid. These compounds were grouped in a phenolic network characterized by a long chain of sugars connected to caffeic acid at *m/z* 773.2126 and coumaric acid at *m/z* 757.2191 and 741.1629. Compounds of this class have already been reported in *Fridericia patellifera*, *Fridericia samydoides*, and *Fridericia pulchra*.[8] However, it is the first time that a subclass of these compounds containing sugar units has been reported in the genus.

Additionally, the aglycones of flavonoids quercetin (*m/z* 303.0499), kaempferol (*m/z* 287.0556), chrysin (*m/z* 255.0679), apigenin (*m/z* 271.0976) and dihydroxy-flavanone (*m/z* 257.0816) were also annotated. They are characterized by fragments (*m/z* 153) resulting from the retro Diels Alder (RDA) reaction. The aglycones diosmetin (*m/z* 301.0722), acacetin (*m/z* 285.0775), methoxy-chrysin (*m/z* 269.0797), methoxy-flavanone (*m/z* 253.0535), and dimethyl-apigenin (*m/z* 299.0944) registered neutral loss of methyl (15 Da) and fragmentation by RDA. Alpinetine is a compound that has already been found in *Fridericia triplinervia*.[5] Kaempferol is also common in the genus, as reported[19] in *Fridericia chica*. Apigenin is a compound that has already been reported[21,22] in the ethanolic extracts of the leaves of *F. platyphylla* and *F. chica*. Chrysin has already been reported[3] in *F. samydoides*. Another study[4] isolated and identified acacetin in the leaves of *F. chica*, but the substance had not yet been reported in *F. platyphylla*.

The classic workflow did not indicate spectral library hits for the brachydins already reported in the literature because they were not deposited on the libraries available in the GNPS platform. A molecular family was observed in the aglycone brachydins predominantly in the dichloromethane fractions brachydin-A (*m/z* 525.1909) and brachydin-C (*m/z* 509.1942) and glycosylated brachydin predominantly in the fraction acetate, denominated brachydin-J (*m/z* 685.2284). These compounds are characterized by the presence of fragments *m/z* 255 and 271 by RDA. As observed in a previous study,[6] brachydins have already been identified in extracts from the roots of *F. platyphylla*, thus confirming the possible presence of these compounds in the flower extract.

Then, all the extracts were used for cytotoxic screening on the MCF-7 and MDA-MB231 cell lines. The aqueous extract showed no toxicity in both breast cancer cell lineages, with viability similar to the control. The hydroalcoholic extract and the ethyl acetate had a time- and concentration-dependent cytotoxicity of 48 to 72 h. On the other hand, the dichloromethane extract showed toxicity, with around 50% of cells killed after 24 h of treatment, further reducing cell viability after 48 and 72 h (Figure 2).

The dichloromethane extract showed a higher ability to induce breast tumor cell death. Dichloromethane is a solvent with relatively low boiling point and low polarity

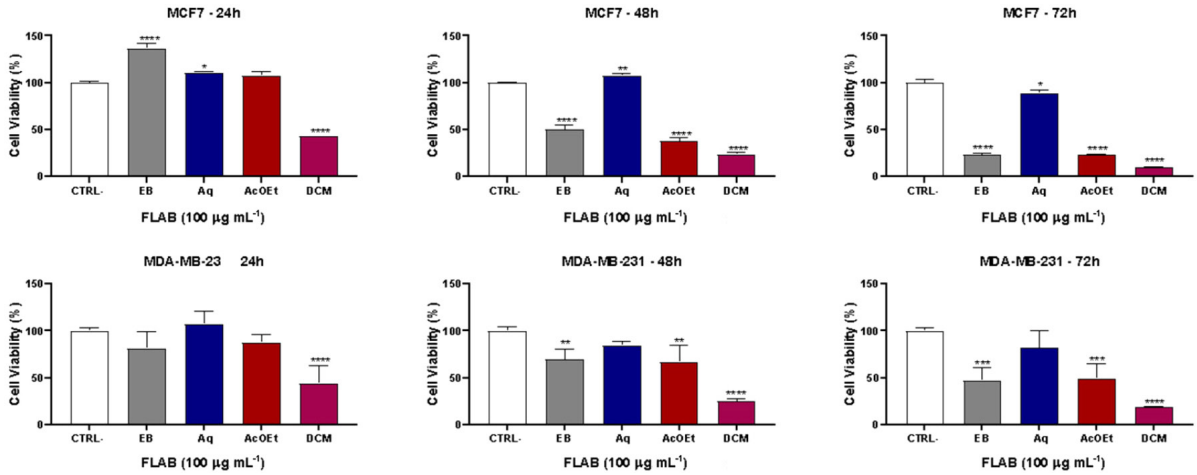

Figure 2. Cytotoxic screening. MCF-7 and MDA-MB-231 breast cancer cells were treated with 100 µg mL^{-1} of the aqueous (Aq), hydroalcoholic (EC), ethyl acetate (AcOEt), and dichloromethane extracts (DCM). Values are expressed as mean ± standard deviation of two independent experiments, each one performed in triplicate. ***$p < 0.0005$, ****$p < 0.0001$ relative to control (CTRL–).

that is safe and based on the hydro/lipophilic properties of biologically active compounds.[23,24] Flavonoids isolated from the *Kalanchoe daigremontiana* dichloromethane fraction showed cytotoxic activity in various tumor cell lines.[25] *Citrus grandis* dichloromethane extracts rich in polyethoxylated flavones presented pro-apoptotic effects in human colorectal cells by shifting the gut microbiota.[26] Anthocyanidins from methanolic extract of *F. chica* exhibited *in vitro* antiproliferative activity.[27]

The liquid chromatography-mass spectrometry (LC-MS) data further revealed the presence of brachydins within the chemical composition of DCM fraction from flowers. This newfound information holds significant relevance in the context of validating its cytotoxicity in breast cancer cells. The presence of brachydins in the DCM fraction adds a crucial layer to our understanding, particularly given that these compounds have not been previously documented in the GNPS public libraries. This highlights the importance of expanding our knowledge base and contributing novel insights into the potential therapeutic properties of the DCM fraction in the context of breast cancer treatment.

Purification of the active fraction of the flowers from *Fridericia platyphylla*

To identify the compounds present in the dichloromethane fraction obtained from *F. platyphylla*, it was fractionated using a glass column filled with silica gel as a stationary phase, and the subfractions were fractionated using semipreparative HPLC-PDA. Four flavones and two dimeric flavonoids, previously reported[6] in the roots of *F. platyphylla*, were obtained (Figure 3).

The structure elucidation of the isolated compounds was performed based on NMR spectroscopic data (see Tables S2 and S3, Figures S1-S36, SI section) and HRMS data analysis. The metabolites characterized were apigenin (**1**), 7-methoxy-chrysin (**2**), brachydin-A (**3**), chrysin (**4**), acacetin (**5**) and brachydin-C (**6**) (Figure 4).

Compound **1** (apigenin) was obtained as an amorphous solid. The electron spray ionization-mass spectrometry (ESI-HRMS) analysis showed a molecular ion at *m/z* 271.0610 $[M + H]^+$, consistent with the molecular formula $C_{15}H_{11}O_5$ (calcd. for $C_{15}H_{11}O_5$, Δppm = –0.37). In the spectrum of this compound, some fragment ions were observed: *m/z* 153.0182 and 119.0489, products of the mechanism of retro-Diels Alder and *m/z* 243.0647, from the loss of carbon monoxide (28 Da). The HMBC correlations confirmed the positions of H-6 and H-8 in the A ring and also confirmed that the compound is a flavone by the correlations in the olefinic hydrogen in H-3 with C-1', C-2, and C-10. These correlations were also observed in the flavones **2**, **4**, and **5**. In ring B, correlations were observed between δ_H 6.84 and δ_C 115.62, 121.87 and 161.85, and between δ_H 7.76 and δ_C 128.07 and 161.85. The COSY experiment also showed that H-3 is related to H-2' and H-6' and confirmed the relationship between H-6 and H-8 and between H-2'/H-6' and H-3' /H-5'.

The ESI-HRMS of **2** (7-methoxy-chrysin) revealed an $[M + H]^+$ peak at *m/z* fragment 269.0815 corresponding to $C_{15}H_{13}O_4$ (calcd. for $C_{15}H_{13}O_4$, Δppm = –1.49) and was isolated as an amorphous solid. For 7-methoxy-chrysin, an initial loss of 15 Da resulted in the fragment *m/z* 254.0577 and the loss of 28 Da, characteristic of carbonyl resulted in the fragment *m/z* 226.0627. The fragment *m/z* 152.0105 was a result of the RDA mechanism in the flavone C ring. The position of the methoxyl group (δ_H 3.81) in a C-7 position was confirmed by the nuclear Overhauser effect (NOE) experiment and by the correlating between H-6 and H-8 in the HMBC.

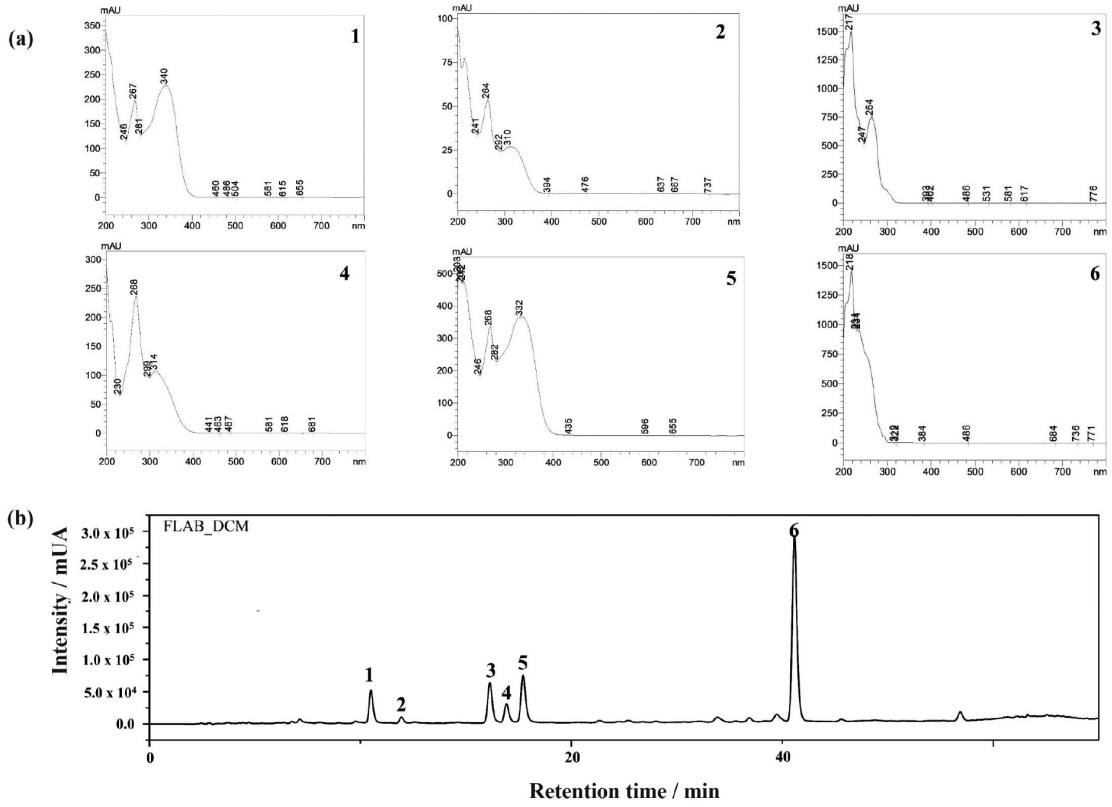

Figure 3. (a) UV data of the compounds identified. (b) HPLC-PDA metabolite profiling (UV 254 nm) of dichloromethane fraction of the flowers from *Fridericia platyphylla* (FLAB-DCM). UV: ultraviolet, HPLC-PDA: high performance liquid chromatography coupled to a photodiode array detector.

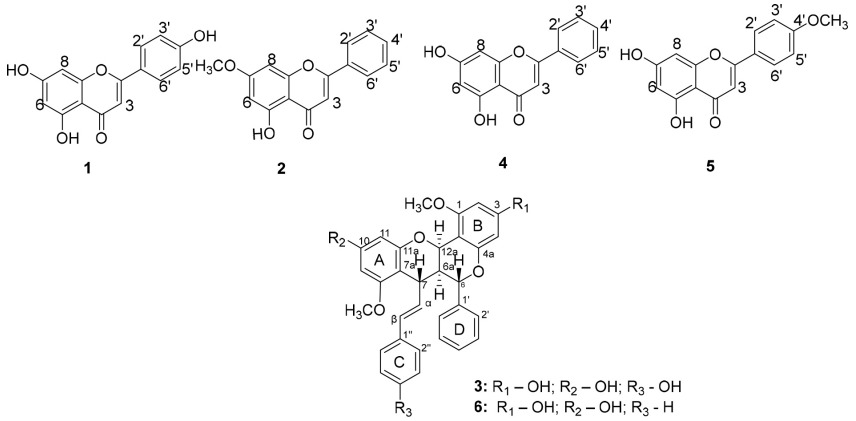

Figure 4. Isolated compounds from *Fridericia platyphylla* dichloromethane fraction of flowers.

Compound **4** (chrysin) exhibited an $[M + H]^+$ ion at *m/z* 255.0659 corresponding to $C_{15}H_{11}O_4$ (calcd. for $C_{15}H_{11}O_4$, Δppm = –1.96). This compound was identified as chrysin, an amorphous solid. This compound originated from three ionic fragments: an initial loss of 18 Da as a H_2O molecule resulted in the fragment *m/z* 237.0543; the second one was produced from the loss of 28 Da (CO), resulting in the ion *m/z* 209.0597, and the product of the mechanism of RDA with *m/z* 153.0188. The main NMR correlation for this compound is noticed by HMBC, between H-4' and C-2'/C-6' and C-3'/C-5', proving that the B ring of this compound is non-substituted, this correlation was also observed on compound **3**.

Compound **5** (acacetin) presented a molecular ion of *m/z* 285.0765 (calcd. for $C_{16}H_{29}O_7$, Δppm = –1.05) and was isolated as a yellow solid crystal. This compound also fragments by the RDA mechanism, resulting in an ion at *m/z* 153.0180. There are losses of 15 Da (CH_3) and 28 Da (CO), resulting in the ions *m/z* 270.0516 and 242.0575, respectively. The OCH_3 group (δ_C 54.66), coupled with C-4', was observed by HSQC. The NOE experiment confirmed the position of this group, showing

a correlation with aromatic protons at δ_H 6.99. For all the flavones, the COSY experiment confirmed the *meta* coupling of the protons in the A ring and the *ortho* coupling of the protons in the B ring.

Two unusual dimeric flavonoids were also isolated and identified. Compound **3** was isolated as an amorphous, white solid, denominated brachydin A. The ESI-HRMS revealed an $[M + H]^+$ ion at *m/z* 525.1910 (calcd. for $C_{32}H_{29}O_7$, Δppm = –1.71). Compound **6** (brachydin-C) was isolated as an amorphous, white solid. The ESI-HRMS revealed an ion at *m/z* 509.1898 (calcd. for $C_{32}H_{29}O_6$, Δppm = –9.91). The presence of the ions *m/z* 271.0963 for brachydin-A and *m/z* 255.1024 for brachydin-C by RDA, a result of the cleavage of the bond between C-6a and C-12a, resulting in the loss of 254 Da, is the basis for the mechanism of brachydin fragmentation. The presence of these compounds was confirmed by the NMR spectroscopic data, which noticed the presence of two fused benzopyran rings, which was also confirmed by the various HMBC correlations observed, the positions of the methoxyl groups were confirmed by the NOESY experiment and already reported in the literature.[6]

It was possible to notice the presence of two brachydins in the flowers of *F. platyphylla*, compounds already reported in the roots of this plant.[6] Apigenin is a compound that has already been reported[22,28] in the ethanolic extracts of the leaves of *F. platyphylla* and *F. chica*. Chrysin has already been reported[3] in *F. samydoides*, but this is the first report in *F. platyphylla,* there are no reports of methoxylated chrysin in the genus. Acacetin was isolated and identified in the leaves of *F. chica*,[4] but the substance had not been reported in *F. platyphylla*.

Anticancer assays

In the preliminary screening (Figure 5), the dichloromethane fraction showed the best cytotoxic activity. Thus, the dichloromethane fraction was tested and showed toxicity in breast cancer lines with an IC_{50} (half maximal inhibitory concentration) of 22.14 and 30.9 μg mL^{-1} for MCF-7 and MDA-MB-231, respectively.

The dichloromethane fraction contains non-usual dimeric flavonoids, which have been described to have antitumoral, antinociceptive, anti-inflammatory, antitrypanosomal, antileishmanial, and antimicrobial activities.[6,7,29-33] Brachydins isolated from the dichloromethane fraction exhibited cytotoxic and antitumor activity in the human prostate tumor cell line for the pro-apoptotic pathway.[34,35] Another study[29] carried out by our group, with cervical (HeLa), breast (MCF-7), and prostate (DU-145) tumor cell lines, showed that the dichloromethane fraction rich in brachydins presented cytotoxicity and caused ultrastructural nuclear membrane surface changes in the DU-145 tumor cell, with low toxicity in normal cell lines (PNT2). The results obtained in the present study with breast cancer cells also showed that the dichloromethane fraction has antitumor properties, suggesting that its major compounds may act on common pathways of carcinogenesis, which broadens the therapeutic potential with applicability in different types of cancer.

For analyzing the migration test, the results showed that the concentration of 60 μg mL^{-1} (Table 1) had the lowest percentage of breast cancer cell migration. The images (Figure 6) of the scratched areas illustrate the inhibitory capacity at the highest concentrations of the dichloromethane fraction in the tumor cell lines.

The process of cell migration is important in cancer research because it is associated not only with cell viability but also with their ability to expand, proliferate, and metastasize, a malignant aspect of the disease.[36] The scrape test is an accessible model that allows us to monitor the ability of cells to migrate, repair the scrape, and evaluate drugs with anti-metastatic potential.[37] The formation of a wound by scratches triggers a process of collective migration

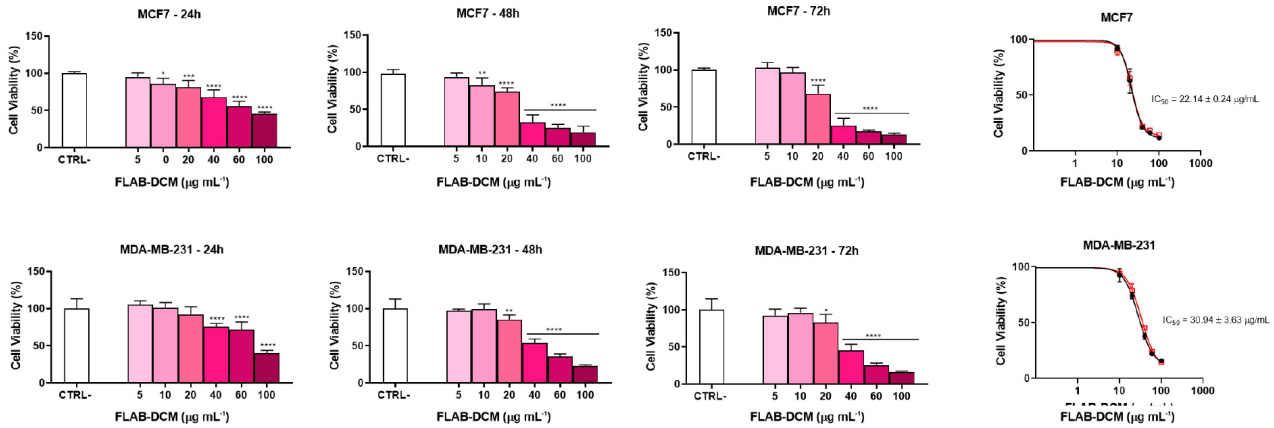

Figure 5. Analysis of cell viability and IC_{50} (half maximal inhibitory concentration) of the dichloromethane fraction in breast cancer cells. Values expressed as mean ± standard deviation of two independent experiments, each one performed in triplicate. ***$p < 0.0005$, ****$p < 0.0001$ relative to control (CTRL–).

Table 1. Inhibitory effects of the dichloromethane fraction on human breast tumor cell lines

		MCF-7	MDA-MB-231
	CRTL–	52.42 ± 9.6	70.77 ± 5.37
	10 μg mL^{-1}	59.05 ± 2.53	85.03 ± 7.97
FLAB-DCM	20 μg mL^{-1}	48.01 ± 2.06	78.42 ± 8.38
	40 μg mL^{-1}	29.73 ± 4.63	57.06 ± 3.29
	60 μg mL^{-1}	12.83 ± 1.56	51.70 ± 4.94

Values expressed as mean ± standard deviation of three independent experiments.

of neighboring cells in an attempt to cover the gap.[38] Thus, the higher concentrations of the dichloromethane fraction showed less migration, maintaining the wound area, when compared to the control. This result may be related to reduced cell viability as well as the reduction in the ability of the cells to move and, consequently, to invade.

In this study, two breast cancer line cells were used to analyze the dichloromethane fractions effects on the same line cell origins, but they have specific molecular profiles. MCF7 and MDA-MB-231 cells present the same identity as invasive ductal breast carcinoma cells, yet they exhibit numerous disparities in terms of phenotype and genotype. MCF7 cells are hormone-dependent, bearing both estrogen and progesterone receptors (ER and PR), whereas MDA-MB-231 cells are characterized as triple negative. The absence of ER renders MDA-MB-231 cells unresponsive to treatments involving antiestrogens. In normoxic metabolism, MCF7 cells rely on oxidative phosphorylation, however, MDA-MB-231 relies on glycolysis for adenosine triphosphate (ATP) production.[39] Furthermore, MCF7 cells exhibit an epithelial phenotype in contrast to the more mesenchymal nature of MDA-MB-231 cells.[40] The results suggest that the compounds present in the dichloromethane fraction act on one or more pathways common not only to the breast lineage but also to other types of tumors.

In identifying the molecules isolated from the dichloromethane fraction, brachydins A and C showed greater toxicity than the other secondary metabolites, with a reduction in viability of more than 50% after 24 h of treatment and in the two breast cancer line cells tested (Figure 7).

Brachydins are flavonoids composed of four independent rings (A, B, C, and D) and two fused benzopyran rings, with

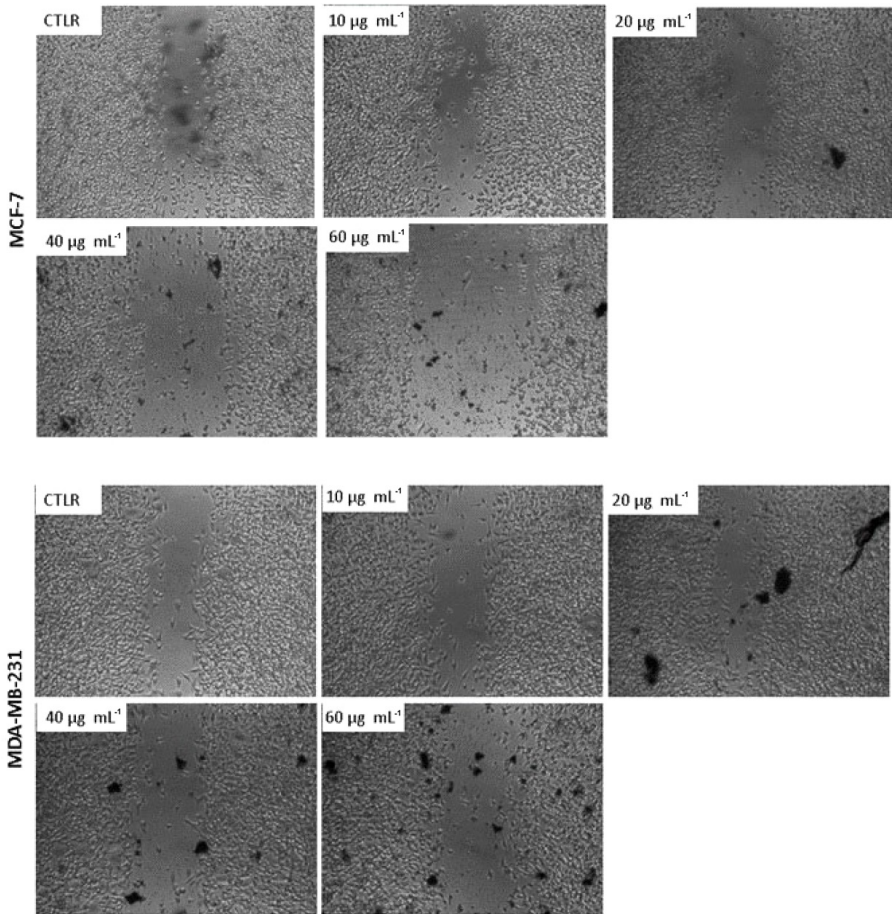

Figure 6. Representative images of inhibition of breast tumor cell migration treated with the dichloromethane fraction (10; 20; 40 and 60 μg mL^{-1}) relative to control (CTRL–).

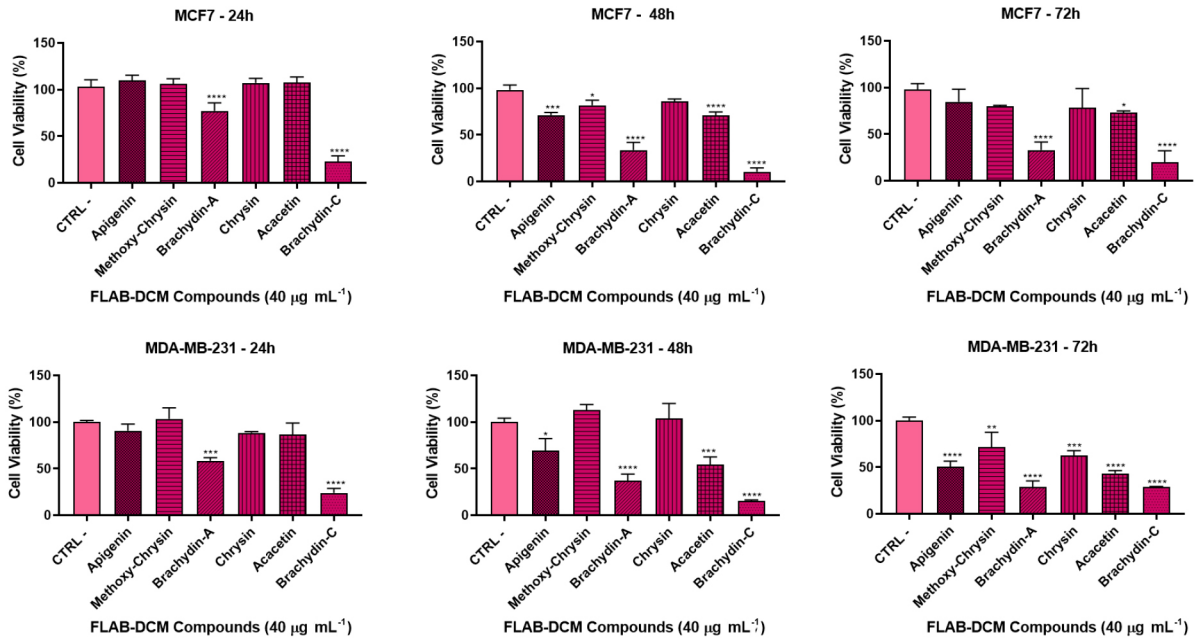

Figure 7. Cytotoxic effect of dichloromethane fraction isolated in breast cancer line cells. Values expressed as mean ± standard deviation of two independent experiments performed in triplicate. ***$p < 0.0005$, ****$p < 0.0001$ relative to control (CTRL–).

different substituent groups on the C ring. The activities of brachydin A and C isolates were evaluated by the cytotoxicity assay. The results showed that the brachydins were cytotoxic in both breast lines. Brachydin C showed a significant reduction in viability at a concentration of 20 μg mL^{-1}, while brachydin A was cytotoxic at a concentration of 40 μg mL^{-1} (Figure 8).

The structural diversity of the brachydin family is directly linked to the biological potential of its constituents.[8] In this work, dimers A and C differed only in terms of the R3 substituent on the C ring, a hydroxyl group and a hydrogen, respectively. These radicals may explain the greater cytotoxicity of brachydin C compared to brachydin A, since the presence of the hydrogen in the C ring gives the molecule apolarity. In contrast, brachydin A has a hydroxyl which, despite being a neutral group, gives the structure more polarity. In another study[6,7] by the group, it was observed that brachydins have a similar structure to steroids, targeting nuclear receptors. The non-polarity conferred by brachydin C's R3 may improve its penetration through the lipid membrane, favoring its action on the intracellular target receptor.

This study used the MCF-7 cell line, which expresses high levels of estrogen receptors, and MDA-MB-231, which is a hormone-independent cell line. Our data suggest that brachydin A and C were not specific for the estrogen receptor, as the cytotoxic effect was similar in both cell lines. Studies comparing the pattern of proteins showed that both two cell lines have a common protein pattern associated with substrate recognition, ATPase cycle, and chaperones function. Thus, although other studies,[6,7] including that of our group, have shown that steroid receptors could be possible targets for flavonoids, including brachydins, the results suggest that the non-polarity of the molecule could determine its bioavailability for intracellular targets and that other proteins, such as enzymes and chaperones, could be molecular targets.

Conclusions

The flowers from *Fridericia platyphylla* can be a significant source of naturally occurring cancer-fighting compounds because of their high biological activity. We were able to recognize substances from the classes of flavones, flavonols, flavanones, isoflavones, cinnamic acid, and derivatives by examining the chemical diversity of the extract and fractions from this species. These results are consistent with previous studies related to the chemical composition of *Fridericia* genus. The biological analysis of human breast cancer cells showed that the dichloromethane fraction was the most efficient, with death-inducing and antimigratory effects. Brachydins A and C, as the most active compounds, could influence the activity of the extract.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

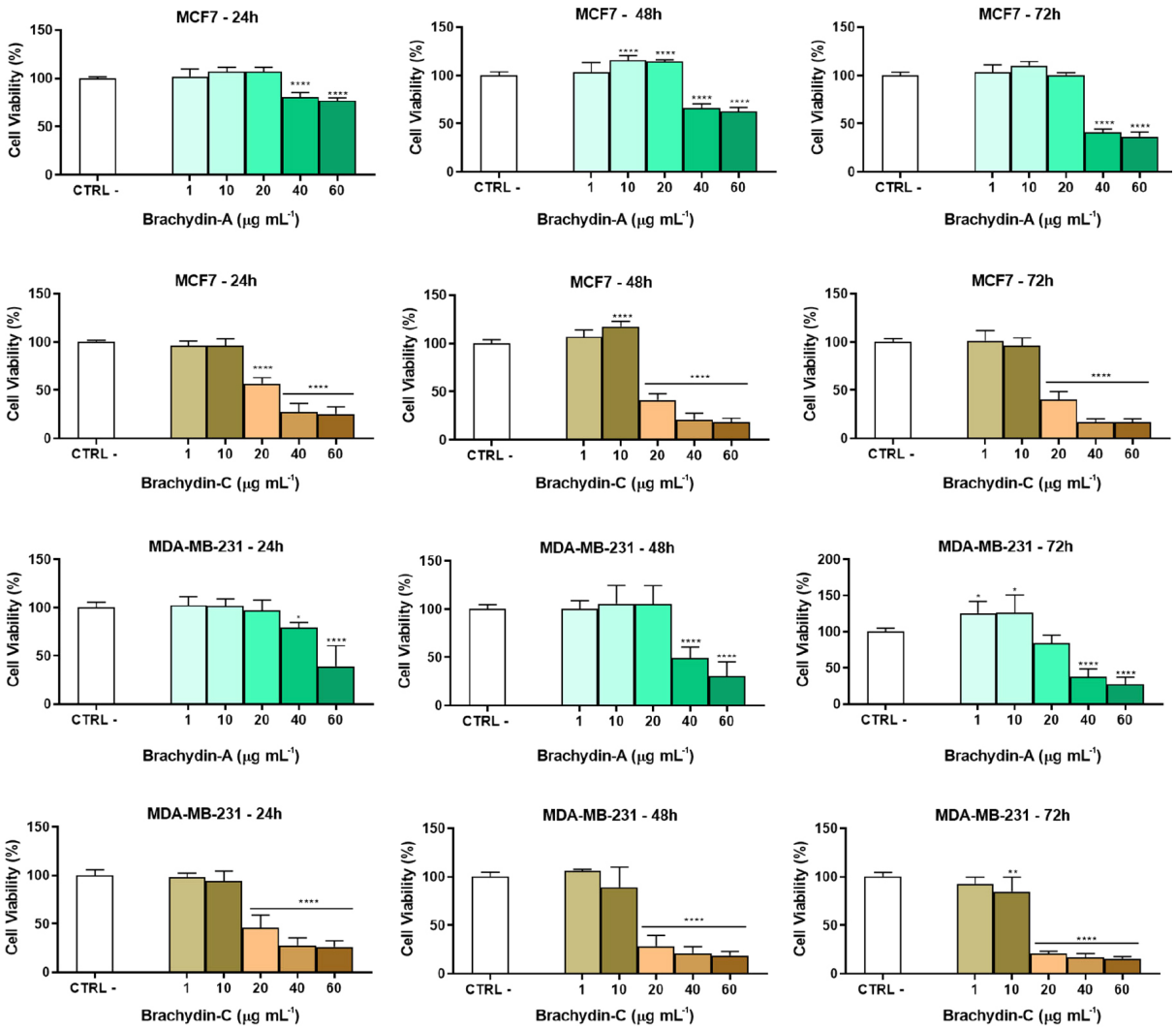

Figure 8. Cytotoxic effect of brachydins A and C isolated in breast cancer line cells. Values expressed as mean ± standard deviation of two independent experiments, each one performed in triplicate. *$p < 0.05$, ****$p < 0.0001$ relative to control (CTRL–).

Acknowledgments

The authors acknowledge Dr Juliana Rodrigues and Dr João Bronzel for technical support. This research was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grants No. 409680/2021-4; 311090/2021-4; 403668/2022-0, CNPq-INCT, grant No. 2014/465637-0 and 163796/2021-1), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-CEPID, project No. 2013/07600-3; 2016/16970-7; 2017/19702-6, and FAPESP-INCT, project No. 2014/50926-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brazil (CAPES) (Finance Code 001; grants No. 88887.472618/2019-00-PROCAD-AM and 23038015702/2018-01-PROCAD-AM) and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA; grant No. UNIVERSAL-01183/19).

Author Contributions

Marcelino S. do Rosário was responsible for performing the extraction, and chemical analysis, inspecting the results, and writing the original draft; Helena Mannochio-Russo, Ana L. P. dos Santos and Aglaete de A. Pinheiro for chemical analysis and writing (original draft and review); Vanderlan S. Bolzani and Cláudia Q. da Rocha for conceptualization, funding acquisition, and review; Maria D. S. B. Nascimento for funding acquisition; Luna N. Vasconcelos for analyzing and writing biological data; Josélia A. Lima, Marcelo S. de Andrade and Ana Paula S. A. Santos for planning and analyzing biological assays and writing (original draft and review); Luna N. Vasconcelos, Lila T. de Oliveira and Monique M. Martins for performing biological assays.

References

1. Olmstead, R. G.; Zjhra, M. L.; Lohmann, L. G.; Grose, S. O.; Eckert, A. J.; *Am. J. Bot.* **2009**, *96*, 1731. [Crossref]

2. Martin, F.; Hay, A. E.; Cressend, D.; Reist, M.; Vivas, L.; Gupta, M. P.; Carrupt, P. A.; Hostettmann, K.; *J. Nat. Prod.* **2008**, *71*, 1887. [Crossref]
3. Bolzani, V. S.; Pauletti, P. M.; Young, M. C. M.; *Quim. Nova* **2003**, *26*, 641. [Crossref]
4. Zorn, B.; García-Pieres, A. J.; Castro, V.; Murillo, R.; Mora, G.; Merfort, I.; *Phytochemistry* **2001**, *56*, 831. [Crossref]
5. Leite, J. P. V.; Oliveira, A. B.; Lombardi, J. A.; Filho, J. D. S.; Chiari, E.; *Biol. Pharm. Bull.* **2006**, *29*, 2307. [Crossref]
6. da Rocha, C. Q.; Queiroz, E. F.; Meira, C. S.; Moreira, D. R. M.; Soares, M. B. P.; Marcourt, L.; Vilegas, W.; Wolfender, J. L.; *J. Nat. Prod.* **2014**, *77*, 1345. [Crossref]
7. da Rocha, C. Q.; Vilela, F. C.; Cavalcante, G. P.; Santa-Cecília, F. V.; Silva, L. S.; dos Santos, M. H.; Giusti-Paiva, A.; *J. Ethnopharmacol.* **2011**, *133*, 396. [Crossref]
8. do Nascimento, J. R.; Miranda, A. J. A.; Vieira, F. C.; Rodrigues, C. D. P.; Vasconcelos, L. N.; Pereira Filho, J. L.; Lopes, A. C. C. B.; Tangerina, M. M. P.; Vilegas, W.; da Rocha, C. Q.; *Pharmaceuticals* **2022**, *15*, 658. [Crossref]
9. International Agency for Research on Cancer, https://gco.iarc.fr/today/home, accessed in July 2024.
10. Instituto Nacional de Câncer, *Estimativa 2020 - Incidência de Câncer no Brasil*, https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2020-incidencia-de-cancer-no-brasil.pdf, accessed in July 2024.
11. Chambers, M. C.; MacLean, B.; Burke, R.; Amodei, D.; Ruderman, D. L.; Neumann, S.; Gatto, L.; Fischer, B.; Pratt, B.; Egertson, J.; Hoff, K.; Kessner, D.; Tasman, N.; Shulman, N.; Frewen, B.; Baker, T. A.; Brusniak, M. Y.; Paulse, C.; Creasy, D.; Flashner, L.; Kani, K.; Moulding, C.; Seymour, S. L.; Nuwaysir, L. M.; Lefebvre, B.; Kuhlmann, F.; Roark, J.; Rainer, P.; Detlev, S.; Hemenway, T.; Huhmer, A.; Langridge, J.; Connolly, B.; Chadick, T.; Holly, K.; Eckels, J.; Deutsch, E. W.; Moritz, R. L.; Katz, J. E.; Agus, D. B.; MacCoss, M.; Tabb, D. L.; Mallick, P.; *Nat. Biotechnol.* **2012**, *30*, 918. [Crossref]
12. Wang, M.; Carver, J. J.; Phelan, V. V.; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D.; Watrous, J.; Kapono, C. A.; Luzzatto-Knaan, T.; Porto, C.; Bouslimani, A.; Melnik, A. V.; Meehan, M. J.; Liu, W. T.; Crüsemann, M.; Boudreau, P. D.; Esquenazi, E.; Sandoval-Calderón, M.; Kersten, R. D.; Pace, L. A.; Quinn, R. A.; Duncan, K. R.; Hsu, C. C.; Floros, D. J.; Gavilan, R. G.; Kleigrewe, K.; Northen, T.; Dutton, R. J.; Parrot, D.; Carlson, E. E.; Aigle, B.; Michelsen, C. F.; Jelsbak, L.; Sohlenkamp, C.; Pevzner, P.; Edlund, A.; McLean, J.; Piel, J.; Murphy, B. T.; Gerwick, L.; Liaw, C. C.; Yang, Y. L.; Humpf, H. U.; Maansson, M.; Keyzers, R. A.; Sims, A. C.; Johnson, A. R.; Sidebottom, A. M.; Sedio, B. E.; Klitgaard, A.; Larson, C. B.; Boya, C. A. P.; Torres-Mendoza, D.; Gonzalez, D. J.; Silva, D. B.; Marques, L. M.; Demarque, D. P.; Pociute, E.; O'Neill, E. C.; Briand, E.; Helfrich, E. J. N.; Granatosky, E. A.; Glukhov, E.; Ryffel, F.; Houson, H.; Mohimani, H.; Kharbush, J. J.; Zeng, Y.; Vorholt, J. A.; Kurita, K. L.; Charusanti, P.; McPhail, K. L.; Nielsen, K. F.; Vuong, L.; Elfeki, M.; Traxler, M. F.; Engene, N.; Koyama, N.; Vining, O. B.; Baric, R.; Silva, R. R.; Mascuch, S. J.; Tomasi, S.; Jenkins, S.; Macherla, V.; Hoffman, T.; Agarwal, V.; Williams, P. G.; Dai, J.; Neupane, R.; Gurr, J.; Rodríguez, A. M. C.; Lamsa, A.; Zhang, C.; Dorrestein, K.; Duggan, B. M.; Almaliti, J.; Allard, P. M.; Phapale, P.; Nothias, L. F.; Alexandrov, T.; Litaudon, M.; Wolfender, J. L.; Kyle, J. E.; Metz, T. O.; Peryea, T.; Nguyen, D. T.; VanLeer, D.; Shinn, P.; Jadhav, A.; Müller, R.; Waters, K. M.; Shi, W.; Liu, X.; Zhang, L.; Knight, R.; Jensen, P. R.; Palsson, B.; Pogliano, K.; Linington, R. G.; Gutiérrez, M.; Lopes, N. P.; Gerwick, W. H.; Moore, B. S.; Dorrestein, P. C.; Bandeira, N.; *Nat. Biotechnol.* **2016**, *34*, 828. [Crossref]
13. Sumner, L. W.; Amberg, A.; Barrett, D.; Beale, M. H.; Beger, R.; Daykin, C. A.; Fan, T. W. M.; Fiehn, O.; Goodacre, R.; Griffin, J. L.; Hankemeier, T.; Hardy, N.; Harnly, J.; Higashi, R.; Kopka, J.; Lane, A. N.; Lindon, J. C.; Marriott, P.; Nicholls, A. W.; Reily, M. D.; Thaden, J. J.; Viant, M. R.; *Metabolomics* **2007**, *3*, 211. [Crossref]
14. Global Natural Product Social Molecular Networking (GNPS), https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=4ec9e2d1761a4e6fb2244ce6aa3b99a1, accessed in July 2024.
15. *GraphPad Prism*, version 7.0; GraphPad Software Inc., San Diego, CA, USA, 2018.
16. Rasband, W. S.; *ImageJ*, version 1.50f; National Institutes of Health, Bethesda, MD, USA, 2016.
17. Moura, M. S.; Bellete, B. S.; Vieira, L. C. C.; Sampaio, O. M.; *Rev. Virtual Quim.* **2022**, *14*, 214. [Crossref]
18. Mannochio-Russo, H.; Nunes, W. D. G.; Almeida, R. F.; Albernaz, L. C.; Espindola, L. S.; Bolzani, V. S.; *J. Nat. Prod.* **2023**, *86*, 621. [Crossref]
19. Mannochio-Russo, H.; de Almeida, R. F.; Nunes, W. D. G.; Bueno, P. C. P.; Caraballo-Rodríguez, A. M.; Bauermeister, A.; Dorrestein, P. C.; Bolzani, V. S.; *Front. Plant Sci.* **2022**, *13*, 854842. [Crossref]
20. Ververidis, F.; Trantas, E.; Douglas, C.; Vollmer, G.; Kretzschmar, G.; Panopoulos, N.; *Biotechnol. J.* **2007**, *2*, 1214. [Crossref]
21. Barbosa, W. L. R.; Pinto, L. D. N.; Quignard, E.; Vieira, J. M. D. S.; Silva Junior, J. O. C.; Albuquerque, S.; *Rev. Bras. Farmacogn.* **2008**, *18*, 544. [Crossref]
22. Monteiro, F. S.; Costa, J. R. S.; Martins, L. J. A.; da Rocha, C. Q.; Borges, A. C. R.; Borges, M. O. R.; *Rev. Cienc. Farm. Basica Apl.* **2020**, *41*, e667. [Crossref]
23. Jones, W. P.; Kinghorn, A. D.; *Methods Mol. Biol.* **2012**, *864*, 341. [Crossref]
24. Brusotti, G.; Cesari, I.; Dentamaro, A.; Caccialanza, G.; Massolini, G.; *J. Pharm. Biomed. Anal.* **2014**, *87*, 218. [Crossref]
25. Stefanowicz-Hajduk, J.; Asztemborska, M.; Krauze-Baranowska, M.; Godlewska, S.; Gucwa, M.; Moniuszko-

Szajwaj, B.; Stochmal, A.; Ochocka, J. R.; *Planta Med.* **2020**, *86*, 239. [Crossref]

26. Eom, T.; Choi, J. H.; Kim, J.; Kim, J.; Unno, T.; *J. Funct. Foods* **2022**, *88*, 104903. [Crossref]
27. Taffarello, D.; Jorge, M. P.; Sousa, I. M. O.; Duarte, M. C. T.; Figueira, G. M.; Queiroz, N. D. C. A.; Rodrigues, R. A. F.; de Carvalho, J. E.; Goes, A. L. T. R.; Foglio, M. A.; Riveros, J. M.; Eberlin, M. N.; Cabral, E. C.; *Quim. Nova* **2013**, *36*, 431. [Crossref]
28. do Amaral, R. R.; Santos, A. A. D.; Saraiva, A.; Botas, G.; Cruz, R. A. S.; Fernandes, C. P.; Rocha, L.; Boylan, F.; *Lat. Am. J. Pharm.* **2012**, *31*, 451. [Link] accessed in July 2024
29. Maciel-Silva, V. L.; da Rocha, C. Q.; Alencar, L. M. R.; Castelo-Branco, P. V.; de Sousa, I. H.; Azevedo-Santos, A. P.; Vale, A. A. M.; Monteiro, S. G.; Soares, R. E. P.; Guimarães, S. J. A.; do Nascimento, J. R.; Pereira, S. R. F.; *Drug Chem. Toxicol.* **2023**, *46*, 665. [Crossref]
30. de Lima, C. A.; Cubero, M. C. Z.; Franco, Y. E. M.; Rodrigues, C. D. P.; do Nascimento, J. R.; Vendramini-Costa, D. B.; Sciani, J. M.; da Rocha, C. Q.; Longato, G. B.; *BioMed Res. Int.* **2022**, *2022*, 3319203. [Crossref]
31. Salgado, C.; Morin, H.; de Aquino, N. C.; Neff, L.; da Rocha, C. Q.; Vilegas, W.; Marcourt, L.; Wolfender, J. L.; Jordan, O.; Queiroz, E. F.; Allémann, E.; *Molecules* **2020**, *25*, 5219. [Crossref]
32. da Rocha, C. Q.; de Faria, F. M.; Marcourt, L.; Ebrahimi, S. N.; Kitano, B. T.; Ghilardi, A. F.; Ferreira, A. L.; de Almeida, A. C. A.; Dunder, R. J.; Souza-Brito, A. R. M.; Hamburger, M.; Vilegas, W.; Queiroz, E. F.; Wolfender, J. L.; *Phytochemistry* **2017**, *135*, 93. [Crossref]
33. Andrade, L. M. S.; de Oliveira, A. B. M.; Leal, A. L. A. B.; Oliveira, F. A. A.; Portela, A. L.; de Sousa Lima Neto, J.; de Siqueira-Júnior, J. P.; Kaatz, G. W.; da Rocha, C. Q.; Barreto, H. M.; *Microb. Pathog.* **2020**, *140*, 103935. [Crossref]
34. Nunes, H. L.; Tuttis, K.; Serpeloni, J. M.; do Nascimento, J. R.; da Rocha, C. Q.; Silva, V. A. O.; Lengert, A. H.; Reis, R. M.; de Syllos Cólus, I. M.; *J. Toxicol. Environ. Health, Part A* **2020**, *83*, 547. [Crossref]
35. de Oliveira, L. C. B.; Ribeiro, D. L.; do Nascimento, J. R.; da Rocha, C. Q.; Cólus, I. M. S.; Serpeloni, J. M.; *Chem. Biol. Drug Des.* **2022**, *100*, 747. [Crossref]
36. Tang, L.; Wu, J. J.; Ma, Q.; Cui, T.; Andreopoulos, F. M.; Gil, J.; Valdes, J.; Davis, S. C.; Li, J.; *Br. J. Dermatol.* **2010**, *163*, 38. [Crossref]
37. Ashby, W. J.; Zijlstra, A.; *Integr. Biol.* **2012**, *4*, 1338. [Crossref]
38. Poujade, M.; Grasland-Mongrain, E.; Hertzog, A.; Jouanneau, J.; Chavrier, P.; Ladoux, B.; Buguin, A.; Silberzan, P.; *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 15988. [Crossref]
39. Sakamoto, T.; Niiya, D.; Seiki, M.; *J. Biol. Chem.* **2011**, *286*, 14691. [Crossref]
40. Gjerdrum, C.; Tiron, C.; Høiby, T.; Stefansson, I.; Haugen, H.; Sandal, T.; Collett, K.; Li, S.; McCormack, E.; Gjertsen, B. T.; Micklem, D. R.; Akslen, L. A.; Glackin, C.; Lorens, J. B.; *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 1124. [Crossref]

Submitted: February 4, 2024
Published online: July 22, 2024