Biological activity and chemical composition of the ethanolic extracts of *Miconia ferruginata* DC.

Atividade biológica e composição química dos extratos etanólicos de *Miconia ferruginata* DC.

DOI:10.34117/bjdv7n4-307

Recebimento dos originais: 07/03/2021
Aceitação para publicação: 13/04/2021

Poliana Ribeiro Barroso
Farmacêutica, Mestre em Ciências Farmacêuticas
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: poliana.barroso@ufvjm.edu.br

Luiz Elídio Gregório
Farmacêutico, Doutor em Química
Universidade Federal de São Paulo, Diadema, SP, Brasil
e-mail: luizelidio@gmail.com

Kelly Cristina Kato
Farmacêutica, Doutora em Fisiologia e Farmacologia
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: kellycristinakato@gmail.com

Fernanda Fraga Campos
Bióloga, Doutora em Ciências Biológicas/Microbiologia
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: ffcmicro@gmail.com

Fabricio de Oliveira
Farmacêutico, Mestre em Ciências Farmacêuticas
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: fabriciooliveira3m@hotmail.com

Elaine Amaral Leite
Farmacêutica, Doutora em Ciências Farmacêuticas
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
e-mail: leite_elaine@hotmail.com

Gustavo Eustáquio Brito Alvim de Melo
Farmacêutico, Doutor em Ciências Biológicas
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: gbrito1998@yahoo.com

Helen Rodrigues Martins
Farmacêutica, Doutora em Ciências Biológicas
Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, MG, Brasil
e-mail: helen.martins@ufvjm.edu.br

ABSTRACT
Ethnopharmacological relevance: Miconia ferruginata DC. is a native plant from Brazilian Cerrado biome known as “pixirica” or “babatenã”, widely used in traditional medicine as an anti-inflammatory and antibiotic agent. *Aim of the study:* This study aimed to perform a preliminary analysis of the chemical profile and screening of biological activities of the ethanolic extracts of the leaves/flowers (EELF) and of the stem (EES) of this species. *Materials and methods:* The techniques Ultra-Fast Liquid Chromatography with diode-array detection (UFLC-DAD) and High-Performance Liquid Chromatography with diode-array detection and mass spectrometry (HPLC-DAD-MS) performed chemical analysis. Biological activities evaluated for the antibacterial, antitripanosamatides and antitumor effect through *in vitro* assays, by MTT and resazurin. *Results:* Although the extracts showed a negligible result for antibacterial and antitripanosamatides effect, this species showed a high cytotoxicity against tumor cells ($p< 0.001$) of 4T1, A549 and MDA-MB-231, associated with low cell toxicity against fibroblasts. High concentration of phenolic compounds detected in the extracts, especially flavonoids derivates from quercetin, catechins and phenolic acids. *Conclusion:* These phenolic compounds have a high biological potential and may be responsible for the observed cytoxicity, together the data suggest the *M. ferruginata* has a great potential for being one promising candidate for further studies against cancer.

Keywords: phytochemistry, polyphenols, cancer, biological screening, traditional medicine Meso- and Southern America.

RESUMO
Relevância etnofarmacológica: Miconia ferruginata DC. é uma planta nativa do bioma Cerrado brasileiro conhecida como “pixirica” ou “babatenã”, amplamente utilizada na medicina tradicional como agente antiinflamatório e antibiótico. Objetivo do estudo: Este estudo teve como objetivo realizar uma análise preliminar do perfil químico e rastreio das atividades biológicas dos extratos etanólicos das folhas / flores (EELF) e do caule (EES) desta espécie. Materiais e métodos: As técnicas de Cromatografia Líquida Ultra-Rápida com detecção de arranjo de diodos (UFLC-DAD) e Cromatografia Líquida de Alta Performance com detecção de arranjo de diodos e espectrometria de massa (HPLC-DAD-MS) realizaram análises químicas. Atividades biológicas avaliadas quanto ao efeito antibacteriano, antitripanosamatídeo e antitumoral por meio de ensaios in vitro, por MTT e resazurina.
Resultados: Embora os extratos tenham apresentado resultado desprezível para efeito antibacteriano e antitripanosamatídeo, esta espécie apresentou alta citotoxicidade contra células tumorais ($p <0{,}001$) de 4T1, A549 e MDA-MB-231, associada à baixa toxicidade celular contra fibroblastos. Alta concentração de compostos fenólicos detectados nos extratos, principalmente flavonóides derivados da quercetina, catequinas e ácidos fenólicos. Conclusão: Esses compostos fenólicos apresentam alto potencial biológico e podem ser responsáveis pela citotoxicidade observada, em conjunto os dados sugerem que M. ferruginata tem grande potencial para ser um candidato promissor para futuros estudos contra o câncer.

Palavras-chave: fitoquímica, polifenóis, câncer, triagem biológica, medicina tradicional Mesoamérica e América do Sul.

1 INTRODUCTION

Screening of natural products has been a successful tool in biological research to reveal candidate the prototype drugs and other therapeutic substances (Newman and Cragg, 2012; WHO, 2014). Several human diseases as *Leishmania*sis, Chagas disease, and cancer are an important cause of death worldwide (WHO, 2010; Figueredo et al., 2014; Siegel et al., 2016). Another serious health problem is the bacterial and antifungal resistance, especially in nosocomial patients (Talbot et al., 2006; Liu et al., 2014). The treatment of those diseases has several problems such as serious side effects, difficult management, drug resistance or low efficiency and expensive price (Howlader et al., 2012; Calixto Júnior et al., 2016). The development of drugs more effective and less toxic to treat those diseases is of great importance and interest.

Several studies show that natural products are an important source for the discovery of new drugs, and especially in cancer treatment, they have primarily target proliferating tumor cells. Some examples of drugs got from natural products are vinblastine and vincristine isolated from *Catharanthus roseus* (L.) G. Don, paclitaxel from *Taxus brevifolia* Nutt and docetaxel from *Taxus baccata* L. (Mann, 2002; Altmann and Gertsch, 2007). Other studies have showed the therapeutic potential of bioactive compounds and plants extracts (Lee et al., 2016; Alam et al., 2017; Basholli-Salihu et al., 2017; Mfotie-Njoya et al., 2017; Murata et al., 2017). In Brazil, the Cerrado region represents about 25% of national territory and has high biodiversity diversity of species (Ratter et al., 1997; Brazil, 2007). Many plants may have significant pharmacological activities; however, few studies have been carried out (Simon et al., 2009; de Menezes et al., 2021).

Among these, the *Miconia* genus the greatest representative of the Melastomataceae family, show with wide distribution in American continent, concentrated in the cerrado and caatinga biome of the Brazilian territory (Baumgratz et al., 2006). Several plant species of this genus are commonly used in Brazilian folk medicine as anti-inflammatory agents and to treat infectious diseases (Alves et al., 2000; Rodrigues et al., 2008; Rodrigues et al., 2011; Lima et al., 2018), but still poorly studied. The few studies found showed that crude extracts and their isolated compounds from several species of genus *Miconia* sp. have shown biological activities with promising results, such as antioxidant (Gontijo et al., 2019), anti-*Trypanosoma* (Cunha et al., 2006); anti-*Leishmania* (Peixoto et al., 2011; Viegas et al., 2019); antibiotic (Alves et al., 2008; Rodrigues et al., 2008; Gontijo et al., 2019; Viegas et al., 2019), analgesic, anti-inflammatory (Vasconcelos et

al., 2006; Gatis-Carrazzoni et al., 2018) and antitumor (Li et al., 2002; Cunha et al., 2008; Serpeloni et al., 2008).

However, different species shown that that often known for the same popular name (pixirica and/or pixiricão) and have similar medicinal use (Ortíz-Martinez et al., 2016; Gatis-Carrazzoni et al., 2018). In this context, species *Miconia ferruginata* DC. fits perfectly (Almeida and Bandeira, 2010), needing adequate investigations on its true pharmacological potential. This species is part of our continuous research on plants with popular use for toxicity investigations and screening of biological activities. In preliminary studies by our group, on the phytochemical profile showed that sesquiterpenes β-caryophyllene (56.2%) and α-humulene (7.3%), were the classes of secondary metabolites found on fresh leaves of *M. ferruginata* collected in Minas Gerais state, Brazil (Barroso et al., 2017). These metabolites associated with different biological activities suggesting that this plant show potential pharmacological properties. Thus, the aim of the present study was to perform a phytochemical preliminary characterization by UFLC-DAD and HPLC-DAD-MS, besides perform a screening of biological activities of *M. ferruginata*.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Miconia ferruginata DC. (Melastomataceae) plants collected in the northern region of Minas Gerais in the community Ribeirão da Areia, Diamantina, Brazil (18°11'08.0"S 43°42'11.0"W), in May 2013. The botanical identification was performed by Dr. Evandro Luiz Mendonça Machado, from Jeanine Felfili Dendrologic Herbarium (HDJF), where one specimen deposited under voucher number HDJF2953.

2.2 PREPARATION OF PLANT EXTRACTS

First, the leaves/flowers and stem of *M. ferruginata* were dried to weight at 40ºC temperature. Then, the plant material pulverized and macerated in ethanol 96% (1:10 w/v). The extract got was dried in a rotary evaporator (Fisaton® São Paulo, Brazil) under reduced pressure, and kept in a desiccator under vacuum and protected from light. For biological assays, the extracts prepared in dimethyl sulfoxide (DMSO, 10 mg/mL) and then diluted using a culture medium to get the required concentration to assays. DMSO concentration did not exceed 0.125% in the cultures. The licenses for the collection and transport of plant material (COL: 085/12; COL; 086/12) obtained from the State Forestry

Institute. The proof of registration for botanical material collection, fungal and microbial (Registration 29793-4) obtained from the Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of Environment (MMA), and the authorization of access to genetic (Process Registration Number: AACD7CF) was obtained to National System for Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN).

2.3 DETERMINATION OF THE CHROMATOGRAPHIC PROFILE OF EELF AND EES BY ULTRA-FAST LIQUID CHROMATOGRAPHY - UFLC

The sample solutions prepared by dissolving 10 mg of previously dried *M. ferruginata* ethanolic extracts leaves/flowers (EELF) and stem (EES) in methanol or ethanol grade UFLC (J.T. Baker® Pennsylvania, USA) at a concentration of 10 mg/mL. The solution filtered through a 0.2 µm pore size syringe filter with a diameter of 13 mm. The Ultra-Fast Liquid Chromatography (UFLC) system comprised a Shimadzu Prominence UFLC system equipped with two pumps (LC-20AT), on-line degasser (DGU-20AS), column oven (CTO-20A), auto sampler (SIL-20AC HT), and PDA detector (SPD-M20A). The column used was a VP-ODS (Shimadzu® Kyoto, Japan) analytical column (150 mm x 4.6 mm, particle size 3.0 µm) with guard column GPV-ODS (5 mm x 2 mm). The mobile phase using solvent A (ultra-pure water) and solvent B (acetonitrile). In the first 45 minutes of analyses, using a gradient starting in the proportion of 90% of ultra-pure water and 10% of acetonitrile and increasing 10% the acetonitrile ratio every 5 minutes, followed by 7 minutes with 100% of acetonitrile. Finished analysis with 8 minutes using the same gradient initially described to rebalance of the chromatographic column. The analysis performed at a flow rate of 1.0 mL/min and monitored at 190-600 nm. For EELF also realized with a High-Performance Liquid Chromatography with diode-array detection and mass spectrometry (HPLC-DAD-MS) analysis. The HPLC system comprised a Shimadzu coupled with a mass spectrometer (Burker Daltonics® Massachusetts, USA) and equipped with a source of electrospray ionization and time-of-flight analyzed. The sample was injected using an injector (Rheodyne® Salt Lake City, USA), equipped with a loop of 20 µL.

2.4 BIOLOGICAL ASSAYS

2.4.1 Cell culture

The cell lines got from American Type Culture Collection (ATCC, LGC Promochem, Rockville®, MD, USA). Mouse fibroblast (L929), murine breast carcinoma (4T1), human lung carcinoma (A549), and human breast cancer MDA-MB-231 were used. L929 and 4T1 cells cultured in Roswell Park Memorial Institute 1640 medium (RPMI1640). A549 and MDA-MB-231 maintained in Dulbecco's changed Eagle medium (DMEM). Both media supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco® Invitrogen Corp., USA) and antibiotics (100 mg/ml of streptomycin and 100 units/ml of G penicillin, Sigma® St. Louis, USA). Cells kept at 37°C in a balanced air humidified incubator with an atmosphere of 5% CO_2, in the exponential growth phase to confluence and harvested with trypsin-EDTA (Sigma® St. Louis, USA) dissociation.

2.4.2 MTT dye reduction assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay, which measures mitochondrial respiratory function (Mosmann, 1983). The cell line exponentially grown and 1×10^4 cells/well, seeded in 96-well flat-bottom plates and allowed to adhere for 24 hours. The cadmium chloride 2 μM (Próquimios® Rio de Janeiro, Brazil) and paclitaxel (Quiral Química® Juiz de Fora, Brazil) positive controls, respectively, in the cytotoxicity and antitumor assays. Cells maintained in culture medium without a test compound a viability control and only the medium culture used as the blank. After incubation for 72 hours with the crude extracts of *M. ferruginata* at concentrations ranging from 1000 to 7.8 μg/mL, cells incubated with MTT dye (1 mg/mL) for 4 hours. Then, the medium removed and 100 μL of DMSO was added to solubilize the reduced formazan deposit. The optical density (OD) value was measured on a spectrophotometer at the wavelength of 570 nm (Molecular Devices, Sunnyvale, USA). The relative growth rate (RGR) evaluates the cytotoxicity of extracts. The method of logarithmic regression analysis of data determined inhibitory concentration 50% (IC_{50}). All analyzes were in triplicate and the average data reported as results.

2.4.3 Antitripanosomatides activity of EELF

For tripanocidal activity, two *Trypanosoma cruzi* strains, Y and Colombian strains were used. Considered prototypes of partial susceptibility and resistance to benznidazole

as defined by Filardi and Brener (1997). Assays to determine the anti-*Leishmania*l activity performed with *Leishmania (L.) amazonensis* (strain MHOM/BR/73/M2269) and *Leishmania (Leishmania) infantum* (MHOM/BR/1070/BH46) species which causes cutaneous and visceral *Leishmania*sis, respectively. Epimastigotes and promastigotes were harvested axenically in liver infusion tryptose medium (LIT - Camargo, 1977) at 26 °C, being supplemented with 10% fetal bovine serum and used in all experiments from stationary phase. The parasites were counted in a Neubauer hemocytometer and 1×10^7 parasites /mL was added into each well. It was treated with different concentrations of extract EELF of *M. ferruginata* (500-125 µg /mL) for 72 hours. Medium culture without parasites was the blank, and parasites maintained in the medium culture without treatment used as viability control. Commercial drug amphotericin B 50 µg /mL (Sigma® St. Louis, USA) and benznidazole 75 µg /mL (Lafepe® Pernambuco, Brazil) were used as standard drug (positive controls) in the trypanocidal and anti-*Leishmania*l assay, respectively. After the treatment, MTT colorimetric modified method (Mosmann, 1983) evaluated the viability of parasites as described above. All analyzes performed in triplicate from three independent trials. The average data reported as results and the calculated IC_{50}.

2.4.4 Antibacterial Activity

Minimum inhibitory concentration (MIC) was determined by the broth microdilution method on 96-well plates, according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2012). All tests conducted in triplicate in three different experiments and involved fifteen reference strains of the following bacteria: *Staphylococcus aureus* (ATCC 29313), *Salmonella typhimurium* (ATCC 14028), *Bacillus cereus* (ATCC 11778), *Shigella sonnei* (ATCC), *Escherichia coli* (ATCC 25922), *Salmonella enteriditis* (ATCC 13076), *Klebsiella oxytoca* (ATCC 49131), *Streptococcus agalactiae* (ATCC 29313), *Listeria monocytogenes* (ATCC 19115), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25931), *Streptococcus agalactiae* (ATCC 29313), *Micrococcus* sp. (ATCC 49732), *Enterobacter aerogenes* (ATCC 13048) and *Enterococcus faecalis* (ATCC 19433). The inoculum prepared by suspending several bacterial colonies in Mueller-Hinton broth and adjusting to an OD 625 nm of 0.08 a 0.1, corresponding to approximately 1×10^7 cells /mL. 50 µL bacterial suspension standardized (approximately 5×10^5 cells /mL) and 50 µL of extracts of *M. ferruginata* at concentrations ranging from 500 to 125 µg /mL added to each well. The commercial antibiotic chloramphenicol 30 µg /mL (Sigma® St. Louis, USA) was used

as positive controls in the assay plate. The plates kept in an incubator at 37°C for 24 hours. Resazurin reduction assay analyzed the minimum inhibitory concentration (MIC) of the extracts against pathogens as described by Sarker et al. (2007). After 24 hours incubation with treatment, 30 µL of 0.01% resazurin dye (Sigma® St. Louis, USA) solution added, and the plates incubated at 37°C for 2 hours and the color development was observed. The color change from blue to pink is recorded as a dye reduction by viable bacteria. The MIC value was the record of the lowest concentration in which there was no color change.

2.5 STATISTICAL ANALYSIS

To perform the statistical analysis, GraphPad Prism 5.0 (GraphPad Software®, San Diego California USA) was used, and p-values ≤0.05 considered significantly different. Data are reported as the mean ± standard deviation (SD). The IC_{50} value was calculated using a sigmoid dose-response curve. The Shapiro-Wilk test was used to evaluate the normality of the data. The differences between variables with normal distribution were tested using Analysis of variance (one-way ANOVA) followed by *Bonferroni* Post Hoc. For variables with asymmetric distribution, the *Kruskall-Wallis* test followed by *Dunns* Post Hoc.

3 RESULTS

A complex chemical profile for EELF and EES was observed in the UFLC-DAD analysis, showing substances of high and medium polarity (Figure 1). In the analysis, the retention time parameters and the molecular absorption spectrum got through the diode detector, and the data got in the MS, and its mass fragmentation pattern and comparison with other articles of its kind. Flavonoids have two absorption bands in the ultraviolet-visible region: band II with a maximum absorption between 240-285 nm, corresponding to the benzoyl group, and the band I with a maximum absorption region from 300 to 550 nm, which corresponds to the cinnamoyl group. The analysis of the bands I and II (absorption maximum and intensity) contribute to the identification of the flavonoid type (Marston and Hostettmann, 2006). The UFLC-DAD spectra revealed an absorption characteristic of flavonoids quercetin derivatives in 348-354 nm, with glycosylation at position 3 of ring C. Thus, the absorption and intensity analysis of the UV/VIS spectra suggest catechins and the flavonoids of the subclass of flavonol, flavone and glycosylated quercetin derivatives in EELF and catechins and flavonol in EES (Table 1).

Figure 1 – Chromatograms and spectra in the UV-Visible region (UV-Vis) of the analysis Ultra-Fast Liquid Chromatography with diode array detection (UFLC-DAD) of ethanolic extracts of the leaves/flowers (EELF) and stem (EES) of *Miconia ferruginata* DC. (λ 280 nm).

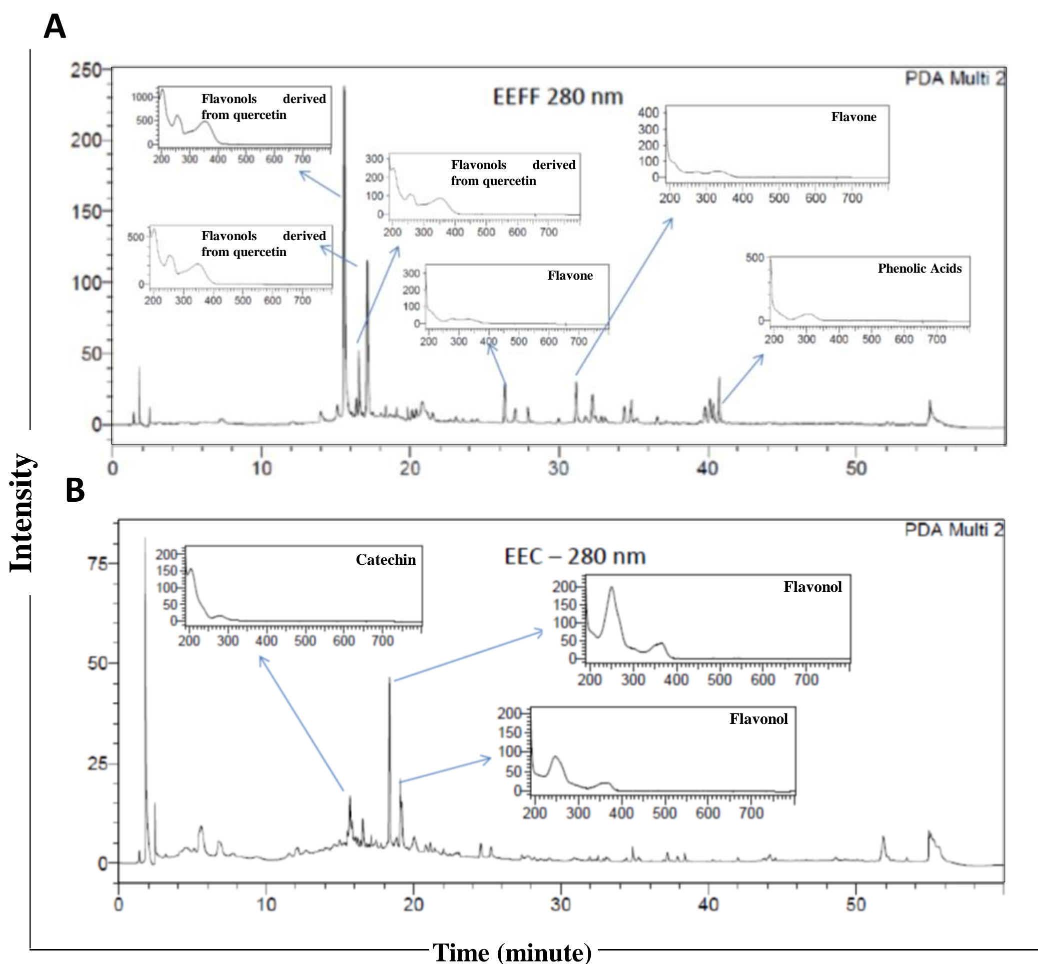


Table 1 – Dates obtained from the analysis on Ultra-Fast Liquid Chromatography with diode array detection (UFLC-DAD) of ethanolic extracts of the leaves/flowers (EELF) and stem (EES) of *Miconia ferruginata* DC.

Extracts	Retention times (min)	Wavelengths UV-Vis (nm)	Chemical class proposal
EELF	15.562	255; 354	Flavonols derived from quercetin
	16.540	256; 352	Flavonols derived from quercetin
	17.048	255; 346	Flavonols derived from quercetin
	26.341	278; 330	Flavone
	31.142	274; 331	Flavone
	40.747	290; 310	Fenolic Acids
EES	15.674	204; 281	Catechin
	18.351	250; 350; 365	Flavonol
	19.082	247; 351; 367	Flavonol

EELF: Ethanolic extracts leaves/flowers of *M. ferruginata*; EES: Ethanolic extracts stem of *M. ferruginata*

The chromatographic profile used in the UPLC-DAD of the EELF and the EES is in accordance with the literature data on the genus *Miconia* (Gatis-Carrazzoni et al., 2018). We also obtained the EELF total ion (TIC) and ultraviolet (UV) chromatograms and are represented in Figure 2. Table 2 shows the results for HPLC-DAD-MS of the EELF extract, identifying quinic acid and monocaffeylquininic compounds, acid phenolic compounds with antioxidant action.

Figure 2 – Total ion chromatograms (TIC) and ultraviolet (UV) of the analysis high-performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD-MS) of ethanolic extract of the leaves/flowers (EELF) of *Miconia ferruginata* DC.

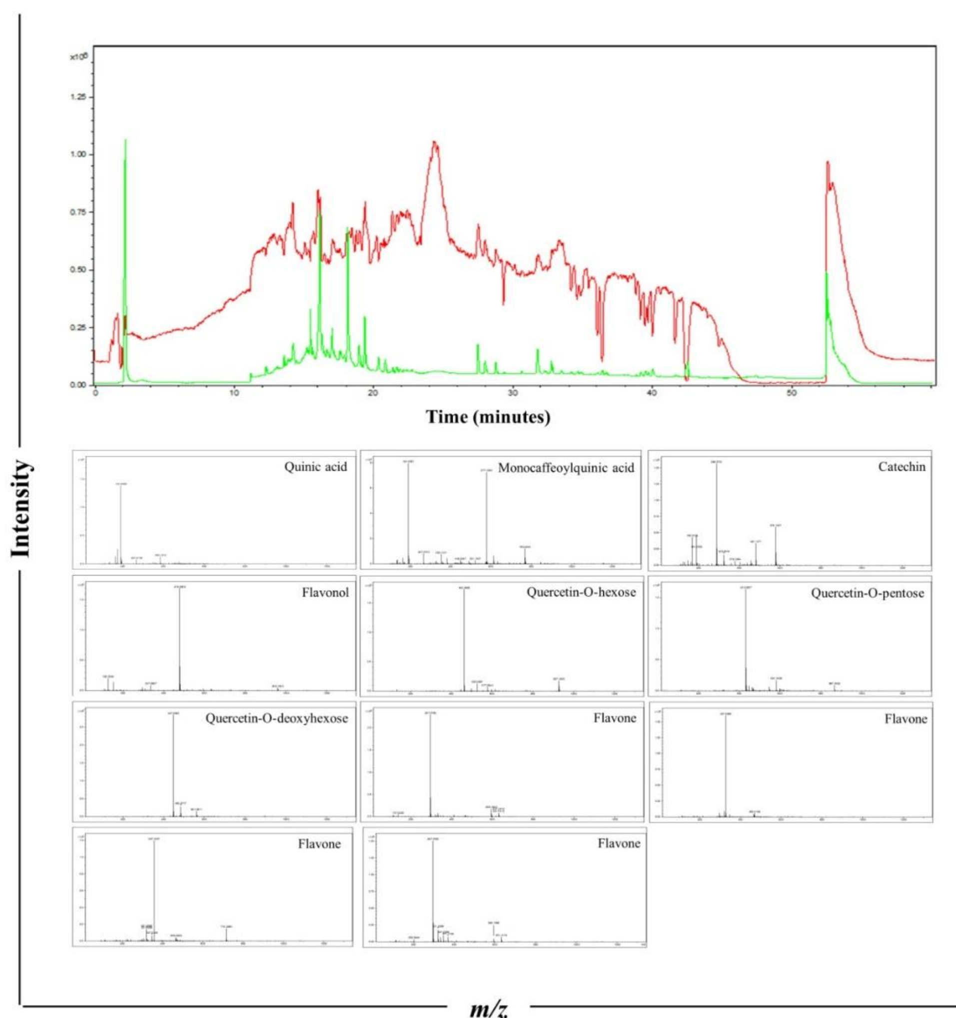


Table 2 – Dates obtained from the analysis on high-performance liquid chromatography with detection of extracts diode array and mass spectrometry (HPLC-DAD-MS) of ethanolic extract of the leaves/flowers (EELF) of *Miconia ferruginata* DC.

Retention times (min)	Mass spectrometry (-)	Maximum absorption peaks (nm)	Chemical class proposal
11.3	383.1212 191.0563	ND	Quinic acid
15.5	353.1101 191.0567	ND	Monocaffeoylquinic acid
16.2	579.1530 289.0730	278	Catechin
17.1	479.0850 337.0800 128.0330	261; 356	Flavonol
18.2	463.0900	255; 354	Quercetin-O-hexose
19.0	433.0800	254; 354	Quercetin-O-pentose
19.4	447.0960	254; 348	Quercetin-O-deoxyhexose
27.5	595.1640 297.0780	278; 330	Flavone
28.0	327.0890	277; 339	Flavone
28.8	357.1000	280; 328	Flavone
31.8	297.0780	274; 331	Flavone

ND: not determined; UV: ultraviolet

The extracts did not show antibacterial activities against all bacteria species tested, where the MIC > 500 µg/mL (Figure 3). Both showed negligible result against *Leishmania Leishmania* sp and *Trypanosma cruzi* (Figure 4), with inhibition below 15 percent of cell growth, at the highest concentration tested compared to the viability control group.

Figure 3 – Test for Minimum Inibitory Concntration (MIC) of ethanolic extracts of *Miconia ferruginata* against bacteria by resazurin reduction assay in microplate.

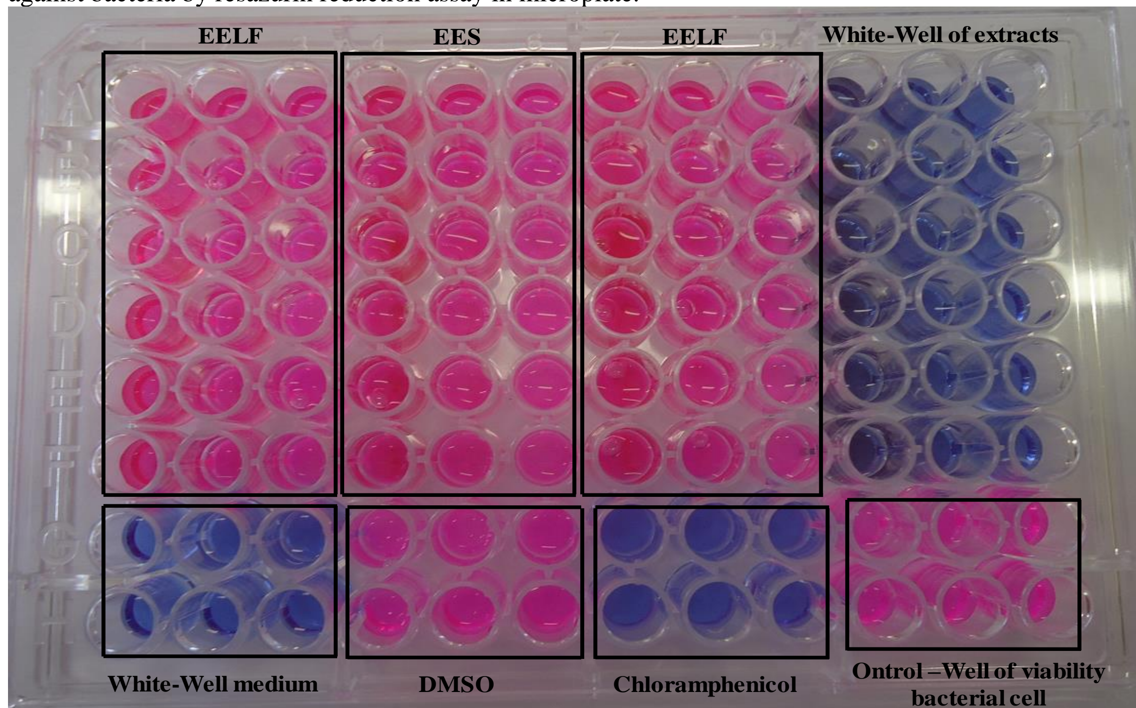
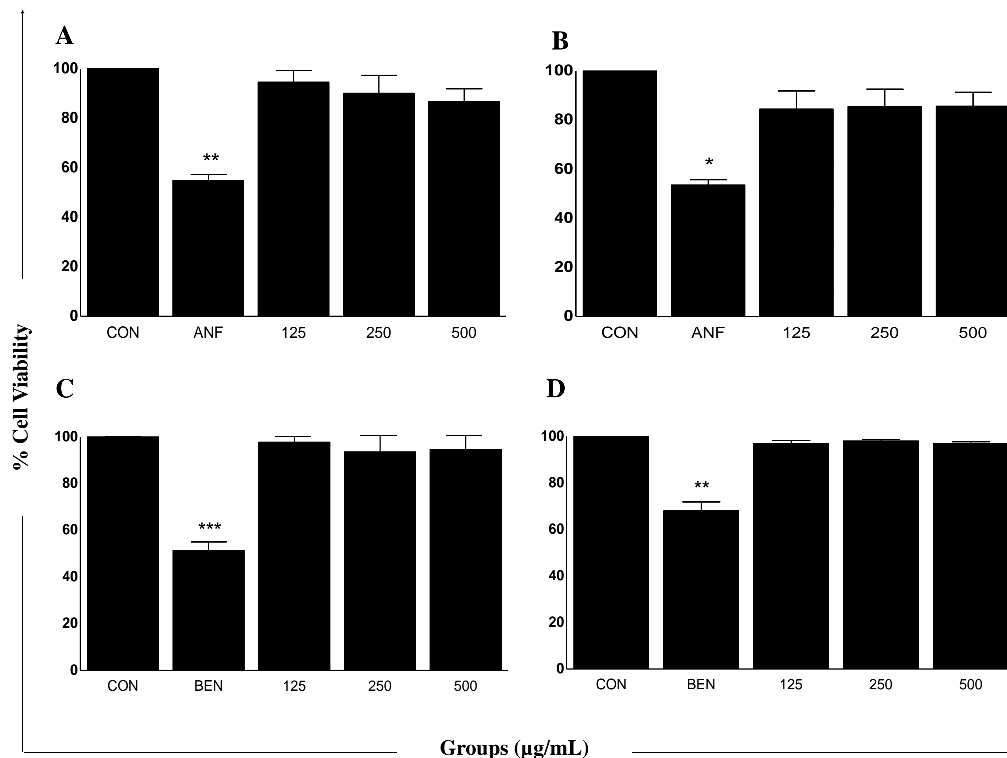
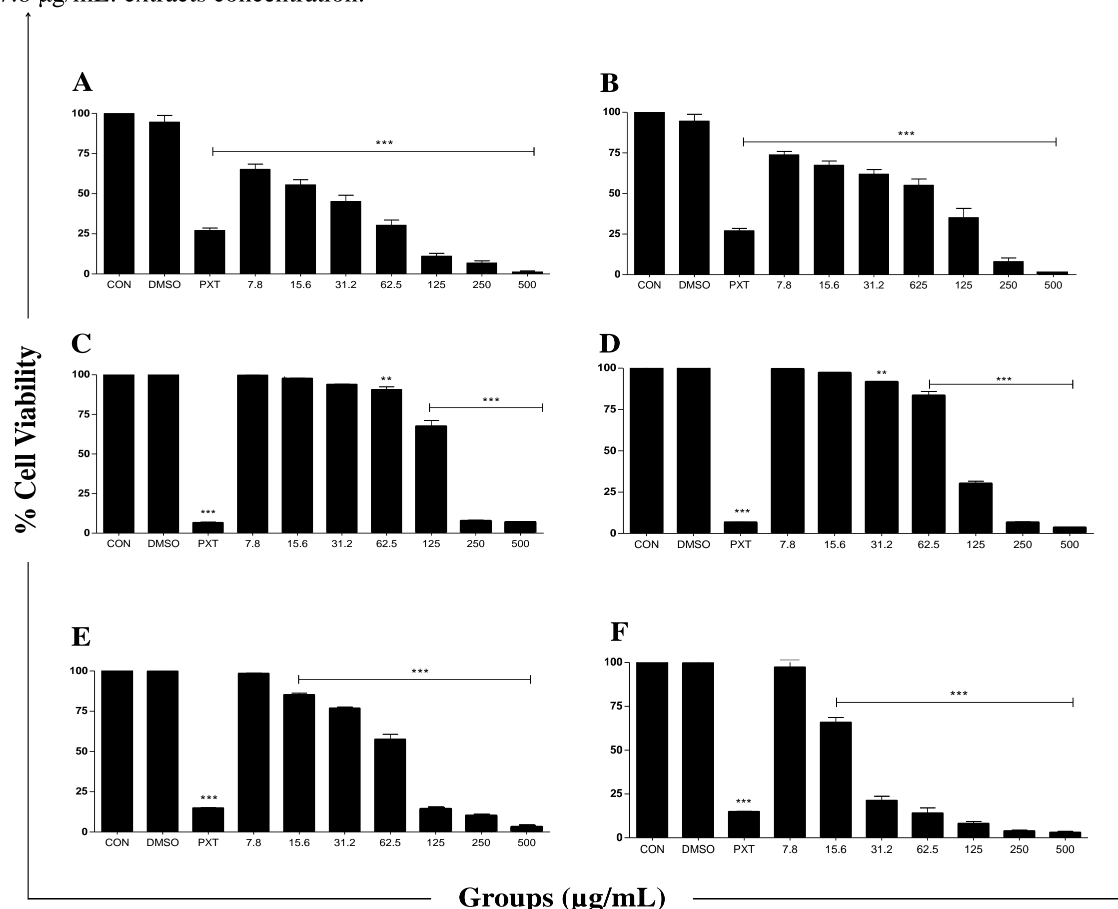


Figure 4 – Percentage of viability of *T. cruzi* and *Leishmania sp.* strains exposed the EELF extract after 72 hours. Data represent the mean ± SEM of triplicate determination of three distinct experiments (n=9). * p<0.05; ** p<0.01; ***p<0.001 ANOVA, with *Bonferroni* post hoc. A – BH46 strain of *Leishmania (Leishmania) infantum* strain; B – M2269 strain of *L. (Leishmania) amazonensis* strain; C – Y of *T. cruzi* strain; D – Colombiana of *T. cruzi* strain. CON: control group of viability; ANF: drug standard amphotericin B 50 µg/mL; BEN – drug standard benznidazole 75 µg/mL; 500 to 125 µg/mL: extracts concentration.



The results of cytoxicity on tumor cells by MTT assays were summarized in Table 3 and Figure 5. There was an important dose-dependent inhibition of tumor cell proliferation (MDA-MB-231, 4T1 and A549) when exposed to ethanolic extracts of *M. ferruginata*. Ethanolic extracts had a high inhibitory effect on all cells after 72 hours of exposure. EELF and EES promoted inhibition above 93%, showing high cytotoxicity on all tumor lines, with IC_{50} ranged from 18.5 to 141.8 µg /mL. When compared to paclitaxel (standard drug) effectiveness in reducing cell viability, the extracts showed greater cellular cytotoxicity, in which the ESS showed an efficiency of 135.4% about MDA and 113.9% about A549. Therefore, the data showed that both extracts of *M. ferruginata* has a possible potent antitumor effect, achieving a 100-fold efficacy above the standard drug, even as crude extract.

Figure 5 – Percentage of viability of different tumour cell lines exposed the EELF and EES extracts. Data represent the mean ± SEM of triplicate determination of three distinct experiments (n=9). ** p<0.01; ***p<0.001, ANOVA, with *Bonferroni* post hoc. A – EELF against MDA-MB-231; B – EES against MDA-MB-231; C – EELF against 4T1; D – EES against 4T1; E – EELF against A549 and F – EES against A549. CON: control group of viability; PXT: drug standard paclitaxel; DMSO: control group of solvent; 500 to 7.8 µg/mL: extracts concentration.



Both extracts showed low toxicity in murine fibroblast lineage presenting CC_{50} above of active concentrations of extracts with EELF = 448.6 µg/mL and EES = 286.6 µg/mL (Figure 6). From these data the selectivity index (SI) was calculated (Table 3). SI is a ratio that measures the window between cytotoxicity and biological activity. Thus, higher SI ratio suggests that a compound might be more effective and safer during *in vivo* treatment. The selectivity index of a compound is a widely accepted parameter and used to express your *in vitro* efficacy in that SI values above 2.0, for natural products, are accepted as a compound with biological potential (Badisa et al., 2009; Sebaugh, 2011). Thus, the EELF and EES extracts showed a strong selective cytotoxicity for all cell lines analyzed, showing SI above 3.0, with special emphasis was of the EELF against MDA (SI= 14.6) and EES against A549 (SI= 15.5).

Figure 6 – Percentage of viability of fibroblasts exposed the EELF and EES extracts. Data represent the mean ± SEM of triplicate determination of three distinct experiments (n=9). * $p<0.05$; ** $p<0.01$; ***$p<0.001$ ANOVA, with *Bonferroni* post hoc. A – EELF and B – EES. CON: control group of viability; CM: control group of death with cadmium chloride 2 µM; 1000 to 7.8 µg/mL: extracts concentration.

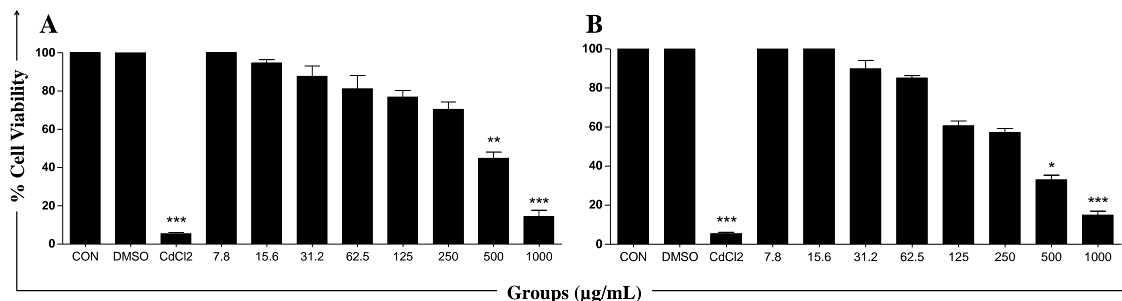


Table 3 – Results of the evaluation of antitumoractivity of EELF and EES on tumoral cells lines (MDA-MB-231, 4T1 and, A549) after 72 hours extract exposure.

Tumor cell lines	Extracts	Cell inhibition (%)	IC_{50} (µg/mL)	CC_{50} (µg/mL)	Efficiency (%)	Selectivity index (SI)
	EELF	98.7 to 34.8	30.7	448.6	135.4	14.6
MDA	EES	99.7 to 26.1	77.7	286.6	136.8	3.7
	PXT	93.2 a 12.9	15.5	36.4	-	2.3
	EELF	93.1 to 9.4	141.8	448.6	99.7	3.2
4T1	EES	96.5 to 16.6	96.0	286.6	103.3	3.0
	PXT	94.8 a 4.9	9.4	36.4	-	3.9
	EELF	96.9 to 42.5	66.0	448.6	113.9	6.8
A549	EES	96.9 to 2.72	18.5	286.6	113.9	15.5
	PXT	85.6 a 14.4	22.5	36.4	-	1.6

EELF: Ethanolic extracts leaves/flowers of *M. ferruginata*; EES: Ethanolic extracts stem of *M. ferruginata;* PXT: paclitaxel.

4 DISCUSSION

The investigation of medicinal plants can be the first step in the discovery of new drugs. Although several plant-derived drugs (vinca alkaloids, taxanes, podophyllotoxin

derivatives, camptothecins and others) are widely used in pharmacological therapies, especially in cancer therapy, the pharmacological potential of the plants remains largely unexplored. In this context, this research consisted of phytochemistry and biological study of ethanolic extracts of *M. ferruginata* from Brazilian Cerrado. This species has a large use in the popular medicine, however, there is not the scientific evidence that prove its activities and safety.

The chemical profile of the ethanolic extracts of *M. ferruginata* revealed a high concentration of phenolic compounds, especially of catechins and flavonoids subclass. The EES extract showed less complexity of phenolic compounds than EELF, however both showed to contain phenolics belonging to the tannin (catechin) and flavonoid (flavone and flavonol) classes. The EELF, also presented the compounds quinic acid, monocaffeoylquinic acid and derivatives of glycosylated quercetin. Similar profile is described in other species of this genus (Rodrigues et al., 2007; Rodrigues et al., 2011). The chemical identification of compounds presents in a biologically evaluated extract is of great relevance, for investigating the correlation between the chemical compost and the biological activity (Gontijo et al., 2019).

Several studies have associated high biological activity, especially to phenolic compounds (Lee et al., 2008). Phenolic compounds of plant origin present outstanding action as complementary to the cellular enzymatic antioxidant system and might reduce the effects of different Reactive Oxygen Species (ROS), responsible for triggering and/or promoting the progression of a variety of diseases, such as atherosclerosis, diabetes mellitus, cardiovascular, neurodegenerative, infectious, chronic inflammatory diseases, and cancer (Schieber and Chandel, 2014; El-Kenawi and Ruffell, 2017). Thus, the high concentration of phenolic compounds present in *M. ferruginata* extracts must be responsible for the cytotoxic effect observed against tumor cells.

In addition, other studies have shown promising antitumor activity, described for other plants to *Miconia* genus (Li et al., 2002; Cunha et al., 2008; Kuete et al., 2016; Farhan et al., 2016; Zubair et al., 2016). In the present study, the results of antitumor activity showed that both extracts were highly cytotoxic for the different tumoral cell lines evaluated, with an average growth inhibition of 93% vs the control. The cytotoxic effect of the extracts was more effective than paclitaxel, the drug of choice to treat cancer, showing results with effectiveness above 100%, except for the EELF about 4T1 cells (99.7%). EELF presented an extraordinary effect on breast cancer with an efficacy of

135.4% and SI of 14,6, while the EES showed an efficacy of 113.9% and SI of 15.5 against A549 cells, suggesting a great antitumor potential of both extracts.

The present study associated the potential antitumor with phenolic compounds, such as the quercetin derivatives flavonoids, flavone and catechin. Studies have showed that the flavonoid present in species of this genus are free aglycone and O-glycosylated derivatives forms, especially quercetin (Rodrigues et al., 2007; Mancini et al., 2008; Pieroni et al., 2011) as the founds at the present study. Evidence suggests that the such polyphenols present antitumor properties (Khan et al., 2014; Farhan et al., 2016; Kuete et al., 2016; Szychowski et al., 2018). Considering that crude extracts consist of a complex mixture of substances, and it requires more studies to clarify the active substances and their antitumor effect.

For the antibacterial evaluation, there was no activity in the different species evaluated. Similar results described by Queiroz et al. (2011) testing the ethanolic/dichloromethane extracts and isolated compounds of *M. rubiginosa*. The antitripanosomatides assay did also not show activity of EELF extract against the species evaluated. However, in previous studies, the isolate compounds of species of the genus *Miconia* sp., such as ursolic acid and oleanoic acid, showed potent activities tripanocidal (Cunha et al., 2006; Paduch et al., 2007; da Silva Ferreira et al., 2013), leishmanicidal (Peixoto et al., 2011; Viegas et al., 2019) and antibacterial (Cunha et al., 2010; Gontijo et al., 2019). Thus, the lack of observation of activity at the crude extracts does not exclude the hypothesis of the isolation compounds of this species present such activity. It is possible, that low concentration which may have affected the visualization of the final effect.

The evaluation cytotoxic of extracts against murine fibroblasts revealed low toxicity, leading to high values of CC50, above 280 μg /mL, being much larger than the active concentrations on the different cell lines. While tumoral cells IC50 varying 18.5 to 141.8 μg /mL. Thus, a selective cytotoxicity to tumoral cells was observed, in which both extracts showed high SI values, mainly about A549 and MDA cells for the EES and EELF extracts, respectively. The SI values found, of both extracts for all cell lines evaluated were from higher than those observed for PXT, the standard drug used, which SI values were 1.6 for A529, 2.3 for MDA-MB-231 and 3.9 for 4T1 cells.

Together the data suggest a great potential and selective effect of secondaries metabolites, for the anti-tumor effect, even associated with crude extracts. Thus, considering the several problems present in cancer treatments, mainly serious side effects

and failure therapeutic (Jaehde et al., 2008; Yeoh et al., 2015), the obtained results stimulate further investigations about *M. ferruginata*. Further investigations are necessary for a more detailed evaluation of the antitumor mechanisms involved and identification and standardization of an extract with the active compounds, and using in later studies by our group, intending to make a future herbal medicine.

5 CONCLUSION

In this study, we performed the chemical and biological profile of the aerial part and the stem of *M. ferruginata*, the first work described in the literature for the species. The UFLC-DAD analysis suggests phenolic compounds presence: flavone, flavonol and flavonoids derivates from quercetin in EELF and catechins and flavonol in EES. By EELF analysis by HPLC-DAD-MS, quininic acid and monocaffeoilquinic compounds were also identified. Although antibacterial and antitriphanosamatid activities (*T. cruzi* and *Leishmania* sp.) were not detected for ethanolic extracts, a highly selective cytotoxic effect shown for different tumor cells. This effect may be associated with the high concentration of phenolic compounds, especially flavonoids derivates from quercetin and catechins. Thus, these and other substances found in this species deserve more investigations to validate this potential of the *M. ferruginata* as a prototype to develop novel antitumor drugs and/or herbal medicine.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was supported by the State of Minas Gerais Research Support Foundation (FAPEMIG) (Process APQ-01626-13) and National Council for Scientific and Technological Development (CNPq) (Process 449401/2014-6). We are also grateful and Integrated Center for Postgraduate Studies and Research in Health at UFVJM, Diamantina, MG and Pharmacy Department for providing equipment's and technical support for experiments.

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