Taylor & Francis
Taylor & Francis Group

Journal of Toxicology and Environmental Health, Part A
Current Issues

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

Antimicrobial and antileukemic effects: in vitro activity of *Calyptranthes grandifolia* aqueous leaf extract

Fernanda Majolo, Shanna Bitencourt, Bruna Wissmann Monteiro, Gabriela Viegas Haute, Celso Alves, Joana Silva, Susete Pinteus, Roberto Christ Vianna Santos, Heron Fernandes Vieira Torquato, Edgar Julian Paredes-Gamero, Jarbas Rodrigues Oliveira, Claucia Fernanda Volken De Souza, Rui Felipe Pinto Pedrosa, Stefan Laufer & Márcia Inês Goettert

To cite this article: Fernanda Majolo, Shanna Bitencourt, Bruna Wissmann Monteiro, Gabriela Viegas Haute, Celso Alves, Joana Silva, Susete Pinteus, Roberto Christ Vianna Santos, Heron Fernandes Vieira Torquato, Edgar Julian Paredes-Gamero, Jarbas Rodrigues Oliveira, Claucia Fernanda Volken De Souza, Rui Felipe Pinto Pedrosa, Stefan Laufer & Márcia Inês Goettert (2020) Antimicrobial and antileukemic effects: in vitro activity of *Calyptranthes grandifolia* aqueous leaf extract, Journal of Toxicology and Environmental Health, Part A, 83:8, 289-301, DOI: 10.1080/15287394.2020.1753606

To link to this article: https://doi.org/10.1080/15287394.2020.1753606

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART A
2020, VOL. 83, NO. 8, 289–301
https://doi.org/10.1080/15287394.2020.1753606

Taylor & Francis
Taylor & Francis Group

Antimicrobial and antileukemic effects: in vitro activity of *Calyptranthes grandifolia* aqueous leaf extract

Fernanda Majolo [a,b*], Shanna Bitencourt [a*], Bruna Wissmann Monteiro[a], Gabriela Viegas Haute[c], Celso Alves[d], Joana Silva[d], Susete Pinteus[d], Roberto Christ Vianna Santos[e], Heron Fernandes Vieira Torquato[f,g], Edgar Julian Paredes-Gamero[f], Jarbas Rodrigues Oliveira[c], Claucia Fernanda Volken De Souza[h], Rui Felipe Pinto Pedrosa[d], Stefan Laufer[i], and Márcia Inês Goettert [a]

[a]Cell Culture Laboratory, Postgraduate Program in Biotechnology, University of Vale Do Taquari (Univates), Lajeado, Brazil; [b]Brain Institute of Rio Grande Do Sul (Brains), Pontifical Catholic University of Rio Grande Do Sul, Porto Alegre, Brazil; [c]Cellular Biophysics and Inflammation Laboratory, Pontifical Catholic University of Rio Grande Do Sul (PUCRS), Porto Alegre, Brazil; [d]MARE – Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Peniche, Portugal; [e]Oral Microbiology Research Laboratory, Department of Microbiology and Parasitology, Federal University of Santa Maria, Santa Maria, Brazil; [f]Department of Biochemistry, Universidade Federal De São Paulo, São Paulo, Brazil; [g]Faculty of Pharmacy, Braz Cubas University Center, Mogi das Cruzes, Brazil; [h]Laboratory of Food Biotechnology, University of Vale Do Taquari (Univates), Lajeado, Brazil; [i]Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, University of Tuebingen, Tuebingen, Germany

ABSTRACT

Natural products are still a promising source of bioactive molecules. Food and Drug Administration data showed that approximately 49% of the approved molecules originate naturally or chemically-resemble these substances, of which more than 70% are being used in anticancer therapy. It is noteworthy that at present there are no scientific studies to prove the effectiveness and safety of a number of plants used in folk medicine such as in the case of *Calyptranthes grandifolia* O. Berg (Myrtaceae) originally from South America. The aim of the present study was to determine the biological potential and toxicological effects of the aqueous leaf extract of *C. grandifolia*. The main detected phytoconstituents were condensed tannins and flavonoids and a high quantity of polyphenols. Regarding the antimicrobial potential, the extract exerted inhibitory activity against *Pseudomonas aeruginosa*. The results also revealed the extract induced DNA damage in a concentration-dependent manner in RAW 264.7 cells. In addition, *C. grandifolia* produced cytotoxicity in leukemia cell lines (HL60 and Kasumi-1) without affecting isolated human lymphocytes but significantly inhibited JAK3 and p38α enzyme activity. Taken together, these findings add important information on the biological and toxicological effects of *C. grandifolia*, indicating that aqueous extract may be a source of natural antimicrobial and antileukemic constituents.

KEYWORDS

Antibacterial; bioactive natural products; cytotoxicity; leukemia; Myrtaceae

Introduction

Approximately 3000 plants reported to possess anticancer properties due to the presence of constituents with important antiproliferative activity (Alves-Silva et al. 2017; Rody et al. 2018; Tariq et al. 2017; Tuttis et al. 2018), plants may serve as an alternative in cancer treatment. Naturally derived anticancer agents may be considered as the best choice. Between 1940 and 2014, approximately 49% of the approved molecules for use were derived from or resembled chemically natural products (Newman and Cragg 2016). Food and Drug Administration data showed that more than 70% of these approved agents were used in anticancer therapy (Seca and Pinto 2018). Due to this, the utilization and investigation of medicinal plants have been increasing, but only a small portion of the plants employed in traditional medicine provide scientific evidence of their constituents and pharmacological effects (Adebayo et al. 2015). Thus, there is still a lack of studies to prove the effectiveness and safety of these products considering the importance of natural products in the discovery of new molecules for therapeutic uses (Cordell and Colvard 2012; Fridlender, Kapulnik, and Koltai 2015; Rayan, Raiyn, and Falah 2017; Seca and Pinto 2018). In addition, investigators

CONTACT Márcia Inês Goettert marcia.goettert@univates.br Post-graduate Program in Biotechnology, Univates, Av. Avelino Talini, 171, Lajeado 95914-014, Brazil

*These authors contributed equally to this work.

reported that some natural products contain toxic compounds that may be potentially harmful to human health (Araújo et al. 2015; Kich et al. 2017; Maistro et al. 2019; Majolo et al. 2019). The evaluation of cytotoxic or genotoxic effects of natural compounds might minimize the risk of adverse consequences of these agents for human health. Alternatively, these bioactive compounds might be a source for the development of novel antimicrobial or antineoplastic drugs since infectious diseases and cancer continue to be major public health problems (de Souza Filho et al. 2013; Machado et al. 2016). Usually, natural product-derived drugs present important therapeutic potential and a broad structural diversity (Lima et al. 2016).

Myrtaceae is one of the dominant plant families found in Brazilian biomes and of great economic importance, mostly due to some species that produce edible fruits used in the food industry (Cascaes et al. 2015). *Calyptranthes grandifolia* O. Berg is a Southern Brazilian native plant member of this family (Limberger et al. 2002). The essential oil of *C. grandifolia* was found to be a potent antileishmanial agent with cytotoxic activity on RAW 264.7 and CHO-K1 cell lines (Faleiro et al. 2017; Kauffmann et al. 2016). Regarding the antitumor effects, Dexheimer et al. (2017) demonstrated that the ethanolic extract of *C. grandifolia* inhibited TNF-α gene expression and cytokine release. The ethanol and hexane extracts of *C. grandifolia* also possess some suppressive properties against neurotoxicity induced by 6-OHDA (Kich et al. 2016). Nevertheless, the lack of more information regarding therapeutic activity and safety suggests that more data are necessary to ensure the safe use of this plant. As there are no apparent reports of ethnopharmacological studies regarding *C. grandifolia*, the aim of the present investigation was to evaluate the biological potential of the aqueous leaf extract of *C. grandifolia* on tumor and bacterial cell viability.

Materials and methods

Chemicals

All chemicals – if not otherwise stated – were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, phosphoric acid, gallic acid, chlorogenic acid, *p*-coumaric acid, and caffeic acid were purchased from Merck (Darmstadt, Germany).

Plant material and extraction procedure

Leaves of *C. grandifolia* O. Berg were collected in September 2013 in Teutônia (29° 26′ 52″ S, 51° 48′ 21″ W), Southern Brazil. The material was authenticated by Prof. Dr. Elisete M. de Freitas, a botanist from the University of Vale do Taquari – Univates. For further analysis, leaves of *C. grandifolia* were dehydrated in a circulating air oven at 38°C for 24 hr. After drying, plant material was crushed with pestle and ground with a blender. An aqueous extract was then prepared by decoction in water at 70°C for 120 min. This process was repeated twice, and resulting biomass combined, filtered using a vacuum system (Kitasato + vacuum pump), and dried in a rotary evaporator at 40°C. The resultant dried material was then solubilized in ultrapure water at a concentration of 20 mg/ml.

Phytochemical screenings

Phytochemical analysis was carried out following standard procedures. The qualitative phytochemical analysis was based upon the methodologies described by Simões et al. (2003). The phytoconstituents of the aqueous extract were examined through qualitative tests, involving precipitation reactions, color, and fluorescence development characteristic to detect different compounds. Samples of aqueous *C. grandifolia* extract were screened for the following phytoconstituents: alkaloids, steroids/triterpenoids, tannins, flavonoids, coumarins, and quinones.

Analysis of total phenolic content (TPC)

To determine the amount of the extract's total phenolic compounds (TPC), the Folin-Ciocalteu colorimetric method (Bonoli et al. 2004) was applied using a standard curve prepared with gallic acid. Briefly, 2 µl extract was added to 158 µl distilled water in a 96-well microplate, followed by 10 µl Folin-Ciocalteu reagent. The reaction

mixture was pre-incubated for 2 min at room temperature and then 30 µl 20% Na_2CO_3 (w/v) was added and mixed. After one hr reaction in the dark, the absorbance was measured at 765 nm (SpectraMax 190, Molecular Devices Co., Sunnyvale, CA, USA) against blank solution (prepared by the same procedure described above but replacing Folin-Ciocalteu reagent for the same amount of water) and used to calculate the phenolic content. The TPC is expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Antioxidant activity

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Based on Brand-Williams, Cuvelier, and Berset (1995) with slight modifications the DPPH radical scavenging activity was tested. DPPH radical was dissolved in absolute ethanol. From 198 µl DPPH radical solution (0.1 mM), different concentrations (10–1000 µg/ml) of 2 µl sample solution were produced and subsequently vortexed and incubated for 30 min in the dark at room temperature. The absorbance was read at 517 nm. Butylated hydroxytoluene (BHT) was used as a reference standard. The equation was set up to verify the ability of samples to scavenge the DPPH radical:

$$DPPH\ radical\ scavenging\ activity\ (\%\ of\ control) = \left[1 - \left(-\frac{A_{Sample} - A_{Blank}}{A_{control}}\right)\right] \times 100$$

$A_{control}$ means the absorbance of the control (DPPH solution with dimethyl sulfoxide); A_{sample} means the absorbance of the test sample (DPPH solution plus test sample); and A_{Blank} is the absorbance of the sample in ethanol (sample without DPPH solution). The IC_{50} values were determined in µg/ml.

High-performance liquid chromatography-diode array detector (HPLC-DAD)

HPLC-DAD analysis was conducted using a SIL-20A Shimadzu Prominence Auto Sampler HPLC system (Shimadzu, Kyoto, Japan) equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator and SPD-M20A diode array detector. For the chemical characterization of *C. grandifolia*, the method described by da Silva Brum et al. (2016) was followed with slight modifications. Briefly, a reverse phase chromatography was conducted using Phenomenex C18 column (4.6 mm x 250 mm) packed with 5 µm diameter particles. For solvent A, the mobile phase was water with 1% phosphoric acid (v/v) and for solvent B, acetonitrile. The composition gradient was: 5% of solvent B reaching 15% at 20 min; 20% solvent B at 30 min, 45% solvent B at 40 min, 60% solvent B at 50 min and 98% solvent B at 60 min, followed by 70 min at isocratic elution until 75 min when at 80 min the gradient reached the initial conditions again. For maximum resolution, *C. grandifolia* was analyzed at 10 mg/ml and the concentration of stock solutions used as a standard reference ranged from 0.025 to 0.5 mg/ml. Identification of the presence of phenolic compounds was performed by integration of the peaks using the external standard method at 254 nm for gallic acid, 327 nm for chlorogenic, *p*-coumaric, and caffeic acid, and 366 nm for luteolin, apigenin, and rutin. The compounds were identified comparing their retention time with those of the commercial standards and by DAD spectra (200 to 600 nm).

Antimicrobial activity

The antimicrobial activity of plant extract was assessed against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 10231), which were the five microorganisms tested against the antimicrobial activity of the plant extract. The culture media were: Luria broth medium for *B. subtilis, E. coli*, and *P. aeruginosa*; trypticase soy yeast extract medium for *S. aureus*, and yeast extract peptone dextrose medium for *C. albicans*. The microorganisms were cultured at 37°C in 96-well plates in the presence or absence of plant extracts (300 µg/ml), and growth monitored at 600 nm by optical density. Microorganisms where growth was inhibited by more than 50%, a concentration-response analysis was conducted and IC_{50} determined (Horta et al. 2014).

Cell lines and culture

RAW 264.7 murine macrophage, epithelial cells from CHO-K1 Chinese hamster ovary and human colon adenocarcinoma Caco-2 cell lines were obtained from the Banco de Células do Rio de Janeiro (BCRJ), and leukemia cell lines (HL60, K562, and Kasumi-1) were obtained from the American Type Culture Collection (ATCC, USA). RAW 264.7 and Caco-2 cells were cultured in DMEM medium and CHO-K1 cells in DMEM + Nutrient Mixture F-10 Ham medium (Ham's F-10) (Sigma-Aldrich). All cell lines were supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/ml penicillin G and 100 µg/ml streptomycin). HL60, K562, and Kasumi-1 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotics. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Preliminary cell viability was determined by the exclusion method with trypan blue.

Cell-free kinase assay

The *C. grandifolia* aqueous extract was screened for inhibition of p38 mitogen-activated protein kinase (MAPK) and Janus kinase 3 (JAK3). The inhibitory potency was assessed by previously established ELISA assays measuring the inhibition of p38α-mediated ATF-2 phosphorylation and JAK3-mediated ATP phosphorylation (Anastassiadis et al. 2011; Dörr et al. 2018; Goettert et al. 2011, 2012). The half-maximal inhibitory concentration (IC_{50}) of the extract was calculated.

Human PBMC isolation

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers after they were informed and consent was obtained. This study was approved by the Ethics Research Committee of Univates. Isolation of PBMCs was performed by Ficoll gradient centrifugation as previously described (Haute et al. 2015). Bordignon et al. (2003) were the first to use this technique, it is known that approximately 85-90% of the PBMCs are lymphocytes, the majority being T type.

Alkaline comet assay

Normally, the results obtained from the comet assay indicate early or immediate DNA responses and are essential for safety and efficacy evaluation of the compounds present in medicinal plants (Araldi et al. 2015). DNA damage may be transient and prone to repair (Avishai, Rabinowitz, and Rinkevich 2003; Kich et al. 2017). The comet assay was performed under alkaline conditions as previously described (Singh et al. 1988). Briefly, RAW 264.7 cells were plated (2 x 10^4 cells/ml) in a 12-well microplate and challenged with increasing concentrations (25, 50, 100, and 200 µg/ml) of *C. grandifolia* extract for 3 hr. Ethyl methanesulfonate (EMS) was used as a positive control. Samples were analyzed at 400x magnification under a light microscope. DNA damage in the cells was assessed by quantification of the amount of DNA released from the core of the nucleus. Extension and distribution of DNA damage were evaluated by analysis of 100 cells/sample randomly selected and non-overlapping. Comets were visually scored into five classes according to tail length: (Class 0) undamaged, without a tail; (Class 1) short tail, smaller than the diameter of the head (nucleus); (Class 2) medium tail, up to twice the diameter of the head; (Class 3) long tail, more than twice the diameter of the head; (Class 4) very wide tail, comet without head, maximum DNA damage. The damage to DNA was presented as DNA damage index (DI) and calculated as follows: $DI = n_1 + 2n_2 + 3n_3 + 4n_4$; where n1-n4 represents the number of cells with level 1–4 of damage.

Cytotoxicity assays

RAW 264.7, CHO-K1, and Caco-2 cell viability were performed according to the MTT colorimetric assay (Mosmann 1983). Cells were seeded at a density of 3.5×10^3 cells/well in 96-well microplates, and then challenged with increasing concentrations of aqueous extract for 48 hr. Absorbance was read using a SpectraMax i3 microplate reader. Leukemia cell lines were stained with fluorescein calcein-AM (CAM) according to the flow cytometry protocol for a viability assay. Cells were seeded at a density of 2×10^4 cells/well in 96-well plates. Cells were then treated with the extract at a concentration of100 µg/ml for 48 hr and subsequently incubated with CAM

for 15 min at room temperature while protected from light. The stained cells were analyzed by flow cytometer (BD Accuri C6; BD Biosciences, Franklin Lakes, NJ). The % distribution of viable and dead cells was determined by FlowJo software (Tree Star, Inc, Ashland, OR).

PBMCs (1.6×10^6 cell/ml) were plated and cultured with different concentrations of the extract (50, 100, or 200 µg/ml) in 96-well microtiter plates for 96 hr. Cell viability was determined by trypan blue dye exclusion.

Statistical analyses

All experiments were performed at least in triplicate. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, Inc). All data are expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated using analysis of variance (ANOVA) followed by Tukey's test. A p – value <0.05 was considered statistically significant. The IC_{50} concentration was calculated from nonlinear regression analysis using GraphPad Prism software with the equation: $Y = 100/(1 + 10^{(X-LogIC50)})$.

Results

Phytoconstituents of C. grandifolia extract

The main phytoconstituents of the aqueous leaf extract of *C. grandifolia* were identified by qualitative screening revealing that the aqueous extract contained condensed tannins and flavonoids with no traces of alkaloids, coumarins, quinones, or steroids/triterpenoids. Regarding the assessment of total phenolic compounds, *C. grandifolia* extract possessed 265.4 mg GAE/g, indicating a high polyphenol content. The individual phenolic compounds of *C. grandifolia* were identified and quantified by HPLC. As illustrated in Figure 1, chromatographic separation of phenolic compounds detected gallic acid (peak 1, retention time [Rt]: 9.83 min); chlorogenic acid (peak 2, Rt: 21.59 min); caffeic acid (peak 3, Rt: 25.04 min); rutin (peak 5, Rt: 37.16 min); luteolin (peak 6, Rt: 56.72 min); and apigenin (peak 7, Rt: 64.09 min). Table 1 presents the concentration of the six identified polyphenols and demonstrates that the concentration of chlorogenic acid (3.07 mg/g), luteolin (3.27 mg/g), and apigenin (3.35 mg/g) appeared equal, and consequently the major compounds present in the aqueous extract. Apigenin is the one with the highest concentration value.

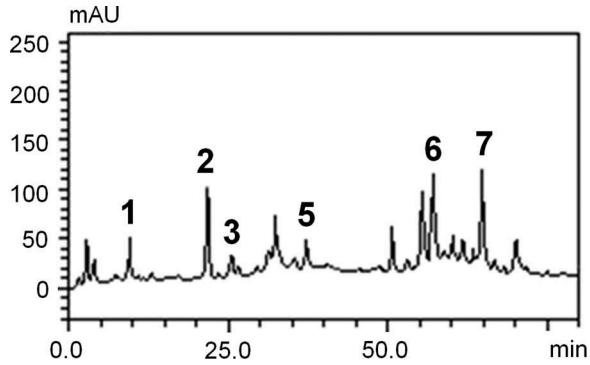

Figure 1. Phytoconstituents of the aqueous leaf extract of *C. grandifolia*. Representative high-performance liquid chromatography profile. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 5), luteolin (peak 6), and apigenin (peak 7).

C. grandifolia antioxidant activity

In DPPH assay, the extract was noted to be a potent natural antioxidant with effects were similar to standard ascorbic acid. Our results in the DPPH assay demonstrated that *C. grandifolia* aqueous leaf extract exhibited antioxidant activity, with an IC_{50} of 13.11 µg/ml, while the standard ascorbic acid showed an IC_{50} of 7.95 µg/ml (Figure 2).

C. grandifolia antimicrobial activity

In order to explore the biological potential of *C. grandifolia*, the antimicrobial activity of the aqueous extract was determined against five different microorganisms. The results are presented in Figure 3a illustrating the capacity of the extract

Table 1. HPLC quantitative determination of some components of *Calyptrathes grandifolia* aqueous leaf extract.

Compounds	Extract (mg/g)
Gallic acid	0.95 ± 0.04b
Chlorogenic acid	3.07 ± 0.01a
Caffeic acid	0.48 ± 0.02c
p-Coumaric acid	-
Rutin	0.51 ± 0.01c
Luteolin	3.27 ± 0.01a
Apigenin	3.35 ± 0.03a

Mean ± SEM is shown (n = 3). Different letters differ by ANOVA, Tukey test at $p < 0.05$.

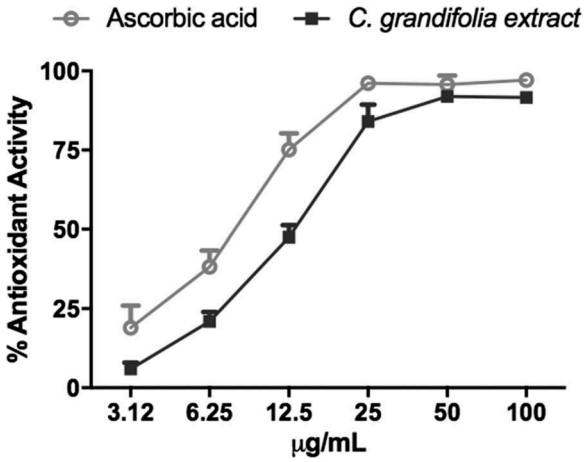

Figure 2. Antioxidant activity from DPPH radical scavenging of *C. grandifolia* aqueous leaf extract. Determination of half-maximal inhibitory concentration (IC_{50}) of the extract activity expressed in µg/mL.

(300 µg/ml) to inhibit the target microorganisms' growth. In addition, the extract displayed inhibitory activity against *P. aeruginosa*, and *S. aureus*. However, only *P. aeruginosa* was inhibited by more than 50% and therefore a concentration-response of the extract against this microorganism was examined. Antimicrobial activities were not observed against *E. coli, B. subtilis*, and *C. albicans*. As shown in Figure 3b, the extract exhibited a significant antibacterial activity with an IC_{50} = 273.6 µg/ml.

C. grandifolia *extract as a potent JAK3 and p38α kinase inhibitor in vitro*

Since modulation of the immune system has been an emerging concept in the control of tumor cell proliferation, targeting protein kinases may be a useful strategy to generate antitumor drugs (Kauffmann et al. 2016; Limberger et al. 2002). In order to investigate the specificity of *C. grandifolia* as a kinase inhibitor, the aqueous extract was tested for its ability to inhibit JAK3 and p38α *in vitro*. The inhibitory potency (IC_{50}) of the extract was assessed by a direct ELISA assay. Figure 4 demonstrates *C. grandifolia* extract markedly inhibited JAK3 and p38α activity with an IC_{50} value in low concentration (JAK3 = 20.09 ng/ml; p38α = 5,9 µg/ml). CP-690550 (Tofacitinib), a commercial pan-JAK inhibitor, and SB203580, a commercial p38-inhibitor, were used as positive controls, presenting, respectively, the following values IC_{50} 0.57 ng/ml and not detectable (0 µg/ml).

Genotoxic effects of C. grandifolia *extract on RAW 264.7 cells*

To investigate whether the extract induced genotoxic effects, the comet assay was performed under alkaline conditions with RAW 264.7 cells. Supplementary material displays the five classes of comets in RAW 264.7 cells, and the DNA damage induction (DI) is shown in Table 2. Based upon the score, the extract produced a concentration-dependent DI after 3 hr with moderate genotoxicity at lower concentrations (25 and 50 µg/ml). The positive control EMS induced significant DNA fragmentation; however, this rise was lower than the score of the highest concentration (200 µg/ml) of

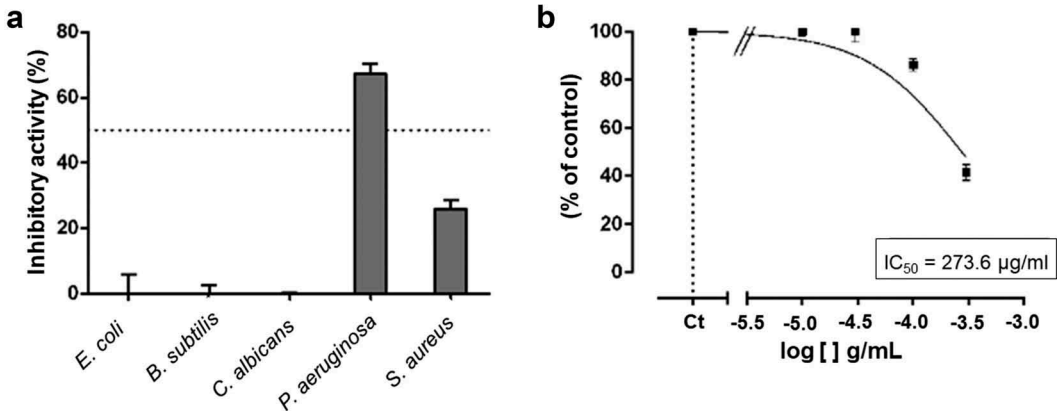

Figure 3. Antimicrobial activity of *C. grandifolia* aqueous leaf extract. (a) Antimicrobial activity of the extract (300 µg/mL) against five different microorganisms (*Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*). (b) Determination of half-maximal inhibitory concentration (IC_{50}) of the extract activity against *P. aeruginosa*.

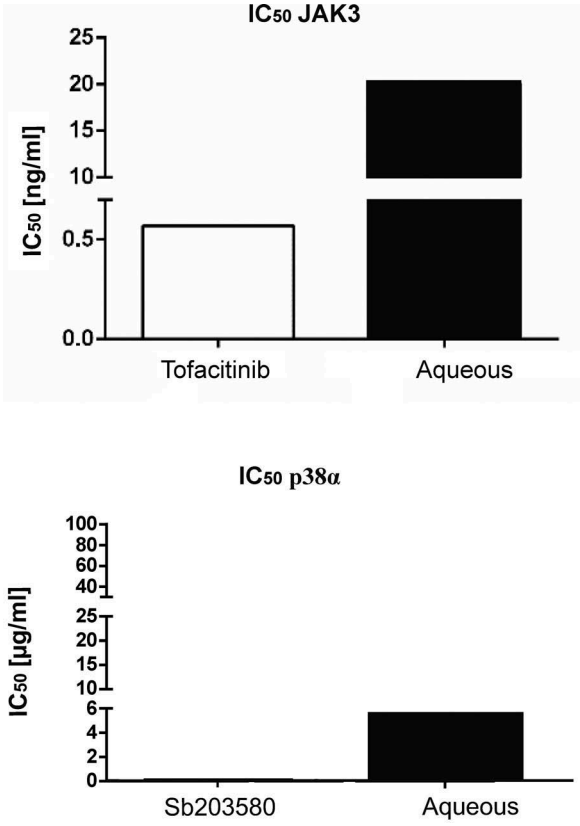

Figure 4. JAK3 and p38α inhibitory activity of *C. grandifolia* leaf extracts. Half-maximal inhibitory concentration (IC_{50}) of aqueous extract. CP-690550 (Tofacitinib) was used as standard JAK inhibitor, SB203580 was used to inhibit p38α.

the extract. The frequency and intensity of damage were proportional to the concentration of the extracts, and no or minimal damage was seen in samples exposed to lower concentrations.

Cytotoxicity of C. grandifolia *extracts on cell lines*

The MTT colorimetric assay was used to assess the viability of different cell lines challenged with aqueous extract of *C. grandifolia*. According to our results, no significant change in cell viability was observed after 48 hr treatment with Caco-2, RAW 264.7, and CHO-K1 cell lines (Figure 5) at 100 µg/ml, where values ranged 90, 91–95, 53% between 25 and 200 µg/ml concentrations (Figure 6). The extract produced cytotoxicity in leukemic cell lines and HL-60 e Kasumi cell lines. No marked effect was observed in the leukemic K562 cell line. The cytotoxic effect was also tested on human lymphocytes. Surprisingly, the extracts did not produce any significant effect on cell viability after 48 hr, maintaining it similar to control cells but initiating activity only in leukemia cell lines where the cells were challenged with 100 µg/ml extract. The extract's cytotoxicity on human lymphocytes was examined in different concentrations of the extract ranging from 50 to 200 µg/ml on freshly isolated PBMC for 96 hr, and cellular viability measured by trypan blue exclusion. As reported in Figure 7b, *C.grandifolia* extract did not produce any cytotoxicity on PBMCs after 96 hr incubation. Collectively, these findings indicate that *C. grandifolia* extract may exert a cytotoxic selective action toward leukemia cell lines.

Discussion

Calyptranthes grandifolia belongs to the neotropical Myrtaceae family frequently found in Latin America, mainly in Southern Brazil (Limberger et al. 2002). It is noteworthy that the species from the Myrtaceae family was found to possess steroids, terpenoids, flavonoids, and tannins as the commonly detected organic compounds (Figueirôa Ede et al. 2013; Takao, Imatomi, and Gualtieri 2015; Wen-Hung et al. 2014). Faleiro et al. (2017) reported that the essential oil composition of *C. grandifolia* contained predominantly of β-pinene (38.3%) and E-caryophyllene (20.1%). These data are in agreement with our findings which demonstrated a high amount of

Table 2. Comet assay analysis of DNA fragmentation in RAW 264.7 cells treated with aqueous leaf extract of *C. grandifolia* for 3 hr.

Treatment	Class 0	Class 1	Class 2	Class 3	Class 4	Score
Control	83.00 ± 3.06	15.00 ± 2.	3.66 ± 1.2	ND	ND	22.34 ± 3.58
EMS (200 µg/ml)	32.±1.53	25.33 ± 2.03	27.±0.58	10.33 ± 1.2	6.33 ± 0.88	135.64 ± 6.83
C. grandifolia extract						
25 µg/ml	65.±0.58	27.±1.15	5.67 ± 1.20	2.±0.58	0.33 ± 0.33	45.66 ± 5.58
50 µg/ml	49.67 ± 2.33	28.±1.00	10.00 ± 1.15	6.5 ± 1.22	6.67 ± 0.33	94.14 ± 2.21
100 µg/ml	38.67 ± 2.33	27.33 ± 2.91	18.33 ± 0.88	11.±0.88	5.±1.15	118.99 ± 3.83
200 µg/ml	16.67 ± 1.76	39.5 ± 2.86	19.67 ± 3.71	11.5 ± 1.20	11.67 ± 0.67	160.01 ± 2.51

EMS (ethyl methanesulfonate), positive control. Mean values ± SEM are shown (n = 3).
ND, non-detected.

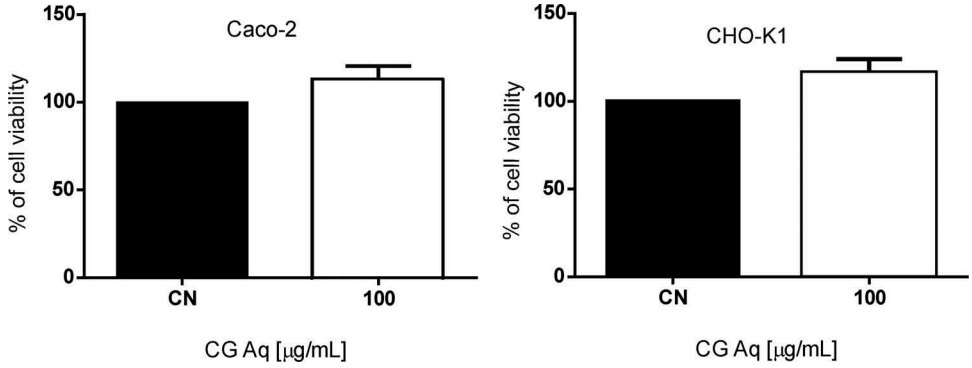

Figure 5. Cytotoxicity of *C. grandifolia* aqueous leaf extract on Caco-2 and CHO-K1 cell lines. Cell viability was assessed using MTT assay after 48 hr. Mean values ± SEM are shown (n = 2).

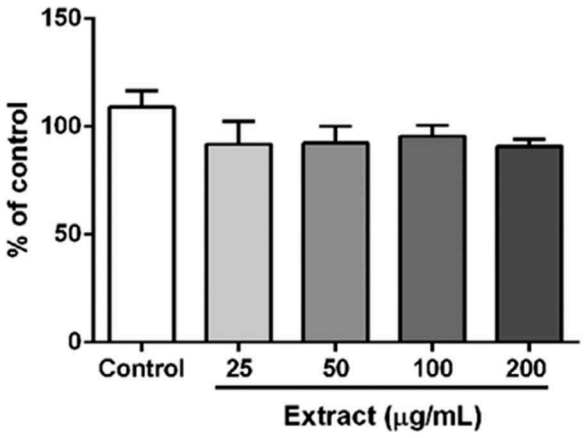

Figure 6. Cytotoxicity and genotoxicity of *C. grandifolia* aqueous leaf extract on RAW 264.7 cell lines. Cell viability was assessed using MTT assay after 48 hr. Mean values ± SEM are shown (n = 2).

polyphenols in the aqueous extract of *C. grandifolia*. Since there are only few data concerning the chemical composition of *C. grandifolia*, our study is of importance and our identified molecules need to be added to the known molecules previously identified in this species which may help to elucidate some attributed biological activities such as neuromodulatory effects (Kich et al. 2016) and potent antileishmanial actions (Kauffmann et al. 2016).

Several members of the Myrtaceae family are known to exhibit antimicrobial activity including the essential oils of other species of the genus *Calyptranthes* (Anago et al. 2011; Cascaes et al. 2015; Stefanello, Pascoal, and Salvador 2011). In contrast to Anago et al. (2011) work on *Psidium guajava* (Myrtaceae), our data showed *C. grandifolia* to display potent antibacterial activity against *P. aeruginosa*. This microorganism, a Gram-negative bacillus, presents the great capacity to adapt and survive in unfavorable environmental conditions with minimal physiological requirements for survival. It is one of the main pathogens associated with nosocomial infections. Despite the progress of antimicrobial therapies,

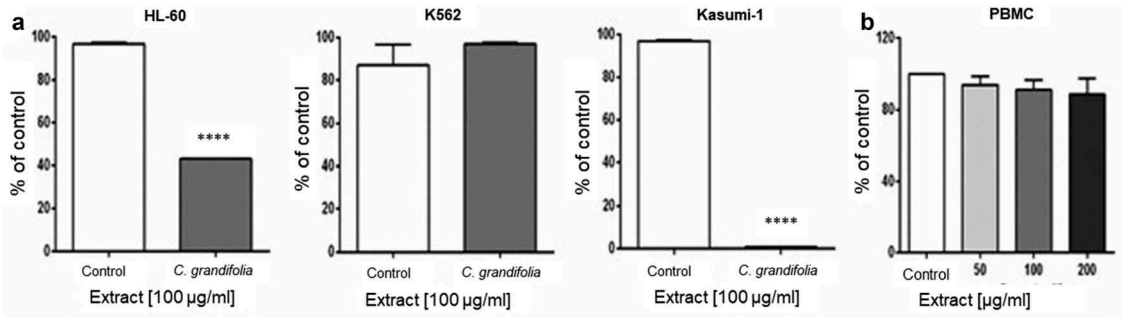

Figure 7. Cytotoxicity of *C. grandifolia* aqueous leaf extract on human leukemia cell lines (HL60, K562, and Kasumi-1) and on peripheral blood mononuclear cells (PBMCs). (a) Flow cytometer was used to assess calcein-stained leukemia cells after 24 hr of treatment with the extract. (b) PBMCs were challenged with the extract for 96 hr and cell viability was assessed using trypan blue exclusion. Mean values ± SEM are shown (n = 3). *p ≤ 0.05.

P. aeruginosa infections are still the major cause of mortality with rates between 18% and 61% (Comin et al. 2016). Our findings indicated that *C. grandifolia* may serve as an interesting source of bioactive compounds with therapeutic potential against *P. aeruginosa*. The constituents might be the organic acids caffeic and gallic acid which are known antimicrobial activity (Lima et al. 2016). In addition, previously Kim et al. (2018) noted that the antibacterial action of caffeic acid was due to the inhibition of *P. aeruginosa* RNA polymerase enzyme. Further, some antimicrobial agents such as quinolones were investigated for their application as anticancer drugs with some advantages to the attributed topo II drugs such as etoposide and doxorubicin, without any significant cardiotoxicity (Aldred, Kerns, and Osheroff 2014; Andriole 2005; Lavorgna et al. 2019; Sissi and Palumbo 2003).

As cellular toxicity includes genotoxic effects, and because these effects were previously reported in other species of *Calyptranthes*, the evaluation of cytotoxic and genotoxic effects of *C. grandifolia* is necessary to minimize possible risks to human health (Kich et al. 2017). Macrophages are important innate immune cells with key roles in the primary response to pathogens and presentation of foreign and self-antigens following infection or injury (Hao et al. 2012). Cell culture systems including mouse macrophage RAW 264.7 cell lines are widely used to screen and study the effects of natural products. In addition, acute toxicity tests are the initial assessment of adverse effects of new substances for therapeutic purposes providing preliminary data of target organs as well as concentration-specific toxic effects (Catelan et al. 2018; Rodríguez-Chávez et al. 2015). Our results revealed that the extract induced DNA damage in a concentration-dependent manner in RAW 264.7 cells suggesting that the damage noted herein may be liable to repair, since the comet assay is considered only indicative of mutagenicity due to its detection of primary DNA damage. Thus, this damage after 3 hr treatment may be reversible, and not all DNA fragments are related to cell death processes (Araldi et al. 2015). This reversal might even be attributed to antioxidant action and the damage may have occurred due to the presence of some compound, tannins, and/or other secondary metabolites. In addition to its antibacterial potential, *C. grandifolia* might exhibit an effective activity against tumor cells similar to doxorubicin, an anthracycline antibiotic that induces antineoplastic activity against hematological and solid malignancies (Szwed et al. 2014). Using essential oil from *C. grandifolia*, Faleiro et al. (2017) reported moderate activity in RAW 264.7 and CHOK1 cell lines. The primary intention of cancer chemotherapy is to target cancer cells without affecting normal cells. While the extract showed low activity in Caco-2, Raw 264.7, K562, and CHO-K1 reaching approximately 100% viability in the current study, it is important to emphasize that cytotoxicity was selective in comparison to acute myeloid leukemia cell lines, HL-60 and Kasumi-1 where cell death was significantly increased as evidenced by a decrease in cell viability after 24 hr. Further, the cytotoxicity of the extract on human normal peripheral blood mononuclear cells (PBMC) was also not significant from control. These findings suggest a high selective killing ability of these extracts for tumor cell lines without impacting normal cells. It should be noted that PBMCs are the first normal cell populations that come into contact with antitumor drugs used in conventional chemotherapy where destruction of PBMC occurs in the first week of intravenous treatment of patients resulting in significant immune deficiency and increased side effects reaffirming that the extract may be effective in cancer therapy without an associated damage to the immune cells. This is the first investigation addressing the effect of the aqueous extract on these cell lines, demonstrating cytotoxic selectivity toward leukemia cell lines. Clinical data demonstrated that anticancer drugs or cytotoxic agents are more effective in leukemia cells since they are more susceptible to oxidative stress than other cancer cells (Lindholm et al. 2002). The anticancer activity of flavonoids was observed in many different types of leukemia cell lines. Apigenin, for example, is known to initiate cytotoxic activity against several leukemia cell lines with IC_{50} ranging from 15 to 55 μM (Liu et al. 2015; Mahbub et al. 2013). However, different leukemia cell lines exhibit relative sensitivity/resistance toward apigenin which induced variable effects on the cell cycle depending on the cell line. Phenolic compounds are also known to produce antileukemic activity (Viktorsson et al. 2017). Previously Chiang et al. (2003) showed caffeic acid to display

antileukemic effects in HL-60 and U937 cells. Our results might be attributed to a synergism between the above-mentioned compounds and other compounds not yet identified.

The human body has protection mechanisms against free radicals and other oxidants that benefit the health of the individual (Alam, Zafar, and Sharmin 2013). Although several members of the Myrtaceae family were previously investigated and showed antioxidant activities (Mosmann 1983), the aqueous extract from *C. grandifolia* demonstrated significant antioxidant potential similar to that of standard ascorbic acid. Dexheimer et al. (2017) also reported a concentration-dependent antioxidant activity of the *C. grandifolia* ethanolic extract and no activity by using the hexanic extract.

In order to investigate the specificity of *C. grandifolia* as a source of molecules able to inhibit kinases, the aqueous extract was tested for its ability to inhibit JAK3 and p38α *in vitro*, which are important enzymes in cellular functions involved in the progression of diverse pathologies such as neurodegenerative disorders and inflammation. According to the results, *C. grandifolia* extract markedly inhibited p38α and especially JAK3 activity. JAK3 belongs to the Janus family of kinases. It is primarily expressed in myeloid and lymphoid cell lines and – unlike other JAKs – is required for immune cell development. Therefore, JAK dysregulation may result in several hematological disorders. In addition, previous investigators showed JAK3 mutations to be associated with myeloid leukemia (Klusmann et al. 2007; Marjanovic et al. 2016). Due to its unique expression in cells of the hematopoietic lineage, JAK3 is considered a highly appealing therapeutic target. Thus, inhibition of JAK3 might be expected to display high specificity and a low amount of cross-reactivity to other non-target cells (Dymock et al. 2014; Goedken et al. 2015). This is the first study that demonstrated the inhibitory effect of *C. grandifolia* extract on p38α. Between the identified polyphenols from our study, apigenin was the main compound followed by luteolin in the aqueous extract. These two compounds are associated with suppressing the JNK and p38-MAPK pathways and exhibit an affinity for proteins involved in cancer, especially JAK3 (Kim and Lee 2018; Pu et al. 2018). Thus, the effect of *C. grandifolia* extract may be related to the presence of this substance.

In conclusion, the potential of the aqueous extract of *C. grandifolia* was noted at low concentrations as an inhibitor of JAK3 and p38α in low concentration. Thus, these findings suggest that *C. grandifolia* contains active compounds that might be used in the development of antileukemic drugs using JAK3 as a target. Collectively, the results of the current study demonstrate that *C. grandifolia* might serve as a source for a variety of active compounds with promising therapeutic potential including antimicrobial and antileukemic biomolecules. Further studies need to be conducted to isolate, characterize, and understand the mechanism of action of these active compounds.

Acknowledgments

RP and MIG conceived and designed the experiments. SB, BWM, SMI, DF, GVH, HFVT performed the mammalian cell experiments. CA, JS, and SP performed the microbiological studies. SMI, DF, MIG performed the cell-free experiments. AAB and RCVS designed and performed the HPLC analysis. SB, FM, CFVS, RP, and MIG analyzed the data. SB, FM, BWM, and MIG wrote the paper. RCVS, CFVS, EJP-G, JRO, RP, JAPH, and SL critically revised the manuscript and contributed reagents/materials/analysis tools.

Funding

This work was supported by Universidade do Vale do Taquari (Univates); the National Counsel of Technological and Scientific Development (CNPq-Brazil) [grant number 445645/2014-8 and 311655/2017-3] and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) [Finance Code 001].Language proof-reading was carried out by Kristine Schmidt.

Declaration of interest

No potential conflict of interest was reported by the authors.

Ethical approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

ORCID

Fernanda Majolo http://orcid.org/0000-0002-7955-078X
Shanna Bitencourt http://orcid.org/0000-0002-5854-1550
Márcia Inês Goettert http://orcid.org/0000-0002-3648-5033

References

Adebayo, S. A., J. P. Dzoyem, L. J. Shai, and J. N. Eloff. 2015. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in Southern African. *BMC Complement. Altern. Med.* 15 (1):159. doi:10.1186/s12906-015-0669-5.

Alam, M., F. Zafar, and E. Sharmin. 2013. Polyetheramide-birth of a new coating material. In *advanced functional polymers and composites: Materials, devices and allied applications*, ed.. Inamuddin, India, 123–36. USA: Nova Publishers.

Aldred, K. J., R. J. Kerns, and N. Osheroff. 2014. Mechanism of quinolone action and resistance. *Biochemistry* 53 (10):1565–74. doi:10.1021/bi5000564.

Alves-Silva, J. M., A. Romane, T. Efferth, and L. Salgueiro. 2017. North African medicinal plants traditionally used in cancer therapy. *Front. Pharmacol.* 8:383. doi:10.3389/fphar.2017.00383.

Anago, E., L. Lagnika, J. Gbenou, F. Loko, M. Moudachirou, and A. Sanni. 2011. Antibacterial activity and phytochemical study of six medicinal plants used in Benin. *Pakistan J. Biol. Sci.* 14 (7):449–55. doi:10.3923/pjbs.2011.449.455.

Anastassiadis, T., S. W. Deacon, K. Devarajan, H. Ma, and J. R. Peterson. 2011. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. *Nat. Biotechnol* 29 (11):1039. doi:10.1038/nbt.2017.

Andriole, V. T. 2005. The quinolones: Past, present, and future. *Clin. Infect. Dis.* 41 (Supplement_2):S113–S119. doi:10.1086/428051.

Araldi, R. P., T. C. de Melo, T. B. Mendes, P. L. de Sá Júnior, B. H. Nozima, E. T. Ito, R. F. de Carvalho, E. B. de Souza, and R. de Cassia Stocco. 2015. Using the comet and micronucleus assays for genotoxicity studies: A review. *Biomed. Pharmacother.* 72:74–82. doi:10.1016/j.biopha.2015.04.004.

Araújo, S. D. S., T. C. Fernandes, Y. T. Cardona, P. M. Almeida, M. A. Marin-Morales, A. V. Dos Santos, K. P. Randau, A. M. Benko-Iseppon, and A. C. Brasileiro-Vidal. 2015. Cytotoxic and genotoxic effects of ethanolic extract of *Euphorbia hyssopifolia* l. on HEPG2 cells. *J. Ethnopharmacol.* 170:16–19. doi:10.1016/j.jep.2015.04.044.

Avishai, N., C. Rabinowitz, and B. Rinkevich. 2003. Use of the comet assay for studying environmental genotoxicity: Comparisons between visual and image analyses. *Environ. Mol. Mutagen* 42 (3):155–65. doi:10.1002/em.10189.

Bonoli, M., V. Verardo, E. Marconi, and M. F. Caboni. 2004. Antioxidant phenols in Barley (*Hordeum vulgare* L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *J. Agric. Food Chem.* 52 (16):5195–200. doi:10.1021/jf040075c.

Bordignon, N. F., G. C. Meier, F. J. C. Alves, A. Lunardelli, E. Caberlon, A. Peres, and J. Rodrigues De Oliveira. 2003. Immunomodulatory effect of fructose-1,6-bisphosphate on T-lymphocytes. *Int. Immunopharmacol.* 3 (2):267–72. doi:10.1016/S1567-5769(02)00295-3.

Brand-Williams, W., M. E. Cuvelier, and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* 28 (1):25–30. doi:10.1016/S0023-6438(95)80008-5.

Cascaes, M. M., G. M. Guilhon, E. H. Andrade, M. Zoghbi, and L. S. Santos. 2015. Constituents and pharmacological activities of Myrcia (Myrtaceae): A review of an aromatic and medicinal group of plants. *Int. J. Mol. Sci.* 16 (10):23881–904. doi:10.3390/ijms161023881.

Catelan, T. B. S., J. A. Santos Radai, M. M. Leitão, L. S. Branquinho, P. C. P. Vasconcelos, S. C. Heredia-Vieira, C. A. L. Kassuya, and C. A. L. Cardoso. 2018. Evaluation of the toxicity and anti-inflammatory activities of the infusion of leaves of *Campomanesia guazumifolia* (Cambess.) O. Berg. *J. Ethnopharmacol.* 226:132–42. doi:10.1016/j.jep.2018.08.015.

Chiang, L. C., W. Chiang, M. Y. Chang, L. T. Ng, and C. C. Lin. 2003. Antileukemic activity of selected natural products in Taiwan. *Am. J. Chin. Med.* 31 (1):37–46. doi:10.1142/S0192415X03000825.

Comin, V. M., L. Q. Lopes, P. M. Quatrin, M. E. de Souza, P. C. Bonez, F. G. Pintos, R. P. Raffin, R. D. A. Vaucher, D. S. Martinez, and R. C. Santos. 2016. Influence of *Melaleuca alternifolia* oil nanoparticles on aspects of pseudomonas aeruginosa biofilm. *Microb. Pathol.* 93:120–25. doi:10.1016/j.micpath.2016.01.019.

Cordell, G. A., and M. D. Colvard. 2012. Natural products and traditional medicine: Turning on a paradigm. *J. Nat. Prod.* 75 (3):514–25. doi:10.1021/np200803m.

da Silva Brum, E., L. da Rosa Moreira, A. R. da Silva, A. A. Boligon, F. B. Carvalho, M. L. Athayde, R. Brandao, and S. M. Oliveira. 2016. *Tabernaemontana catharinensis* ethyl acetate fraction presents antinociceptive activity without causing toxicological effects in mice. *J. Ethnopharmacol.* 191:115–24. doi:10.1016/j.jep.2016.06.036.

de Souza Filho, O. C., M. R. Sagrillo, L. F. Garcia, A. K. Machado, F. Cadoná, E. E. Ribeiro, M. M. Duarte, A. F. Morel, and I. B. da Cruz. 2013. The in vitro genotoxic effect of Tucuma (Astrocaryum aculeatum), an Amazonian fruit rich in carotenoids. *J. Med. Food* 16 (11):1013–21. doi:10.1089/jmf.2012.0287.

Dexheimer, G. M., L. K. O. Becker Delving, H. S. de Oliveira, V. Biolchi, M. I. Goettert, and A. Pozzobon. 2017. *Calyptranthes grandifolia* O.Berg (Myrtaceae) ethanolic extract inhibits TNF-α gene expression and cytokine release in vitro. *In Vitro. Mol. Med. Rep* 15 (5):2873–80. doi:10.3892/mmr.2017.6319.

Dörr, J. A., S. Bitencourt, L. Bortoluzzi, C. Alves, J. Silva, S. Stoll, S. Pinteus, A. A. Boligon, R. C. V. Santos,

S. Laufer, et al. 2018. *In vitro* activities of *Ceiba speciosa* (a. st.-hil) ravenna aqueous stem bark extract. *Nat. Prod. Res.* 24:1–4.

Dymock, B. W., E. G. Yang, Y. Chu-Farseeva, and L. Yao. 2014. Selective JAK inhibitors. *Future Med. Chem* 6 (12):1439–71. doi:10.4155/fmc.14.92.

Faleiro, D., S. Immich, F. Majolo, L. Mayer, E. M. Ethur, and M. I. Goettert. 2017. GC/MS Analysis and potential cytotoxic activity of *Calyptranthes grandifolia* (O. BERG), *Calyptranthes tricona* (D. Legrand) and *Myrciaria plinioides* (D. Legrand) essential oil in RAW264.7 and CHO-K1 cells. *Biomed. Pharmacother.* 89:1431–41. doi:10.1016/j.biopha.2017.03.040.

Figueirôa Ede, O., L. C. Nascimento da Silva, C. M. de Melo, J. K. Neves, N. H. da Silva, V. R. Pereira, and M. T. Correia. 2013. Evaluation of antioxidant, immunomodulatory, and cytotoxic action of fractions from *Eugenia uniflora* l. and *Eugenia malaccensis* l.: Correlation with polyphenol and flavanoid content. *Sci. World J.* 2013:125027.

Fridlender, M., Y. Kapulnik, and H. Koltai. 2015. Plant derived substances with anti-cancer activity: From folklore to practice. *Front. Plant Sci.* 6:799. doi:10.3389/fpls.2015.00799.

Goedken, E. R., M. A. Argiriadi, D. L. Banach, B. A. Fiamengo, S. E. Foley, K. E. Frank, J. S. George, C. M. Harris, A. D. Hobson, D. C. Ihle, et al. 2015. Tricyclic covalent inhibitors selectively target JAK3 through an active site thiol. *J. Biol. Chem.* 290 (8):4573–89. doi:10.1074/jbc.M114.595181.

Goettert, M., S. Luik, R. Graeser, and S. A. Laufer. 2011. A direct ELISA assay for quantitative determination of the inhibitory potency of small molecules inhibitors for JNK3. *J. Pharm. Biomed. Anal.* 55 (1):236–40. doi:10.1016/j.jpba.2011.01.014.

Goettert, M., S. Luik, R. Graeser, and S. A. Laufer. 2012. Development of a p38delta mitogen activated protein kinase ELISA assay for the quantitative determination of inhibitor activity. *J. Pharm. Biomed. Anal.* 66:349–51. doi:10.1016/j.jpba.2012.03.008.

Hao, N. B., M. H. Lü, Y. H. Fan, Y. L. Cao, Z. R. Zhang, and S. M. Yang. 2012. Macrophages in tumor microenvironments and the progression of tumors. *Clin. Dev. Immunol.* 2012:1–11. doi:10.1155/2012/948098.

Haute, G. V., E. Caberlon, E. Squizani, F. C. de Mesquita, L. Pedrazza, B. A. Martha, D. A. da Silva Melo, E. Cassel, R. S. Czepielewski, S. Bitencourt, et al. 2015. Gallic acid reduces the effect of LPS on apoptosis and inhibits the formation of neutrophil extracellular traps. *Toxicol. In Vitro* 30 (1):309–17. doi:10.1016/j.tiv.2015.10.005.

Horta, A., S. Pinteus, C. Alves, N. Fino, J. Silva, S. Fernandez, A. Rodrigues, and R. Pedrosa. 2014. Antioxidant and antimicrobial potential of the *Bifurcaria bifurcata* epiphytic bacteria. *Mar. Drugs* 12 (3):1676–89. doi:10.3390/md12031676.

Kauffmann, C., E. M. Ethur, B. Buhl, T. Scheibel, G. M. C. Machado, and M. M. C. Cavalheiro. 2016. Potential antileishmanial activity of essential oils of native species from southern Brazil. *Environ. Nat. Resour. J.* 6:18–25.

Kich, D. M., S. Bitencourt, C. Alves, J. Silva, S. Pinteus, R. Pedrosa, S. Laufer, C. F. V. de Souza, and M. I. Goettert. 2016. Neuromodulatory effects of *Calyptranthes grandifolia* extracts against 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y cells. *Biomed. Pharmacother.* 84:382–86. doi:10.1016/j.biopha.2016.09.063.

Kich, D. M., S. Bitencourt, B. Caye, D. Faleiro, C. Alves, J. Silva, S. Pinteus, M. Mergener, F. Majolo, A. A. Boligon, et al. 2017. Lymphocyte genotoxicity and protective effect of *Calyptranthes tricona* (Myrtaceae) against h2o2-induced cell death in MCF-7 cells. *Mol. Cell Biochem.* 424 (1–2):35–43. doi:10.1007/s11010-016-2840-9.

Kim, A. K., and C. S. Lee. 2018. Apigenin reduces the Toll-like receptor-4-dependent activation of NF-KAPPAB by suppressing the Akt, mTOR, JNK, and P38-MAPK. *Naunyn-Schmiedeberg's Arch. Pharmacol* 391 (3):271–83. doi:10.1007/s00210-017-1454-4.

Kim, G., C. Dasagrandhi, E. H. Kang, S. H. Eom, and Y. M. Kim. 2018. *In* vitro antibacterial and early stage biofilm inhibitory potential of an edible chitosan and its phenolic conjugates against *Pseudomonas aeruginosa* and *Listeria monocytogenes*. *3 Biotechnol.* 8:439.

Klusmann, J. H., D. Reinhardt, H. Hasle, G. J. Kaspers, U. Creutzig, K. Hahlen, M. M. van den Heuvel-eibrink, and C. M. Zwaan. 2007. Janus kinase mutations in the development of acute megakaryoblastic leukemia in children with and without Down's syndrome. *Leukemia* 21 (7):1584–87. doi:10.1038/sj.leu.2404694.

Lavorgna, M., R. Iacovino, C. Russo, C. Di Donato, C. Piscitelli, and M. Isidori. 2019. A new approach for improving the antibacterial and tumor cytotoxic activities of pipemidic acid by including it in trimethyl-beta-cyclodextrin. *Int. J. Mol. Sci.* 20 (2):1–15. doi:10.3390/ijms20020416.

Lima, V. N., C. D. Oliveira-Tintino, E. S. Santos, L. P. Morais, S. R. Tintino, T. S. Freitas, Y. S. Geraldo, R. L. Pereira, R. P. Cruz, I. R. Menezes, et al. 2016. Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol. *Microb. Pathol.* 99:56–61. doi:10.1016/j.micpath.2016.08.004.

Limberger, R. P., C. A. Simões-Pires, M. Sobral, C. Menut, J. M. Bessiere, and A. T. Henriques. 2002. Essential oils from *Calyptranthes concinna, C. lucida* and *C. rubella* (Myrtaceae). *Rev. Bras. Cienc. Solo* 38 (3):355–60. doi:10.1590/S1516-93322002000300011.

Lindholm, P., J. Gullbo, P. Claeson, U. Göransson, S. Johansson, A. Backlund, R. Larsson, and L. Bohlin. 2002. Selective cytotoxicity evaluation in anticancer drug screening of fractionated plant extracts. *J. Biomol. Screen* 7 (4):333–40. doi:10.1177/108705710200700405.

Liu, X., F. Ye, J. Wu, B. How, W. Li, and D. Y. Zhang. 2015. Signaling proteins and pathways affected by flavonoids in leukemia cells. *Nutr. Cancer* 67 (2):238–49. doi:10.1080/01635581.2015.989372.

Machado, M. M., L. F. S. Oliveira, L. De, Zuravski, R. O. Souza, P. De, Fischer, J. A. Duarte, M. O. Rocha, C. M. Güez, A. A. Boligon, M. L. R. Athayde et al. 2016. Evaluation of genotoxic and cytotoxic effects of hydroalcoholic extract of *Euphorbia tirucalli* (Euphorbiaceae) in cell cultures of human leukocytes. *An. Acad Bras. Cienc* 88 (1):17–28. doi:10.1590/0001-3765201520140076.

Mahbub, A. A., C. L. Le Maitre, S. L. Haywood-Small, G. J. McDougall, N. A. Cross, and N. Jordan-Mahy. 2013. Differential effects of polyphenols on proliferation and apoptosis in human myeloid and lymphoid leukemia cell lines. *Anti-Cancer Agents Med.* 13 (10):1601–13. doi:10.2174/18715206113139990303.

Maistro, E. L., P. M. Terrazzas, F. F. Perazzo, I. M. M. Gaivao, A. C. H. F. Sawaya, and P. C. P. Rosa. 2019. *Salix alba* (white willow) medicinal plant presents genotoxic effects in human cultured leukocytes. *J. Toxicol. Environ. Health Part A* 82 (23–24):1223–34. doi:10.1080/15287394.2019.1711476.

Majolo, F., L. K. O. B. Delwing, D. J. Marmitt, I. C. Bustamante-Filho, and M. I. Goettert. 2019. Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. *Phytochem. Lett.* 31:196–207. doi:10.1016/j.phytol.2019.04.003.

Marjanovic, I., J. Kostic, B. Stanic, N. Pejanovic, B. Lucic, T. Karan-Djurasevic, D. Janic, L. Dokmanovic, S. Jankovic, N. S. Vukovic, et al. 2016. Parallel targeted next generation sequencing of childhood and adult acute myeloid leukemia patients reveals uniform genomic profile of the disease. *Tumour Biol.* 37 (10):13391–401. doi:10.1007/s13277-016-5142-7.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Meth.* 65 (1–2):55–63. doi:10.1016/0022-1759(83)90303-4.

Newman, D. J., and G. M. Cragg. 2016. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* 79 (3):629–61. doi:10.1021/acs.jnatprod.5b01055.

Pu, Y., T. Zhang, J. Wang, Z. Mao, B. Duan, Y. Long, F. Xue, D. Liu, S. Liu, and Z. Gao. 2018. Luteolin exerts an anticancer effect on gastric cancer cells through multiple signaling pathways and regulating miRNAs. *J. Cancer* 9 (20):3669–75. doi:10.7150/jca.27183.

Rayan, A., J. Raiyn, and M. Falah. 2017. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. *PLoS ONE* 12 (11):e0187925. doi:10.1371/journal.pone.0187925.

Rodríguez-Chávez, J. L., E. Coballase-Urrutia, G. Sicilia-Argumedo, T. Ramírez-Apan, and G. Delgado. 2015. Toxicological evaluation of the natural products and some semisynthetic derivatives of *Heterotheca inuloides* Cass. (Asteraceae). *J. Ethnopharmacol.* 175:256–65. doi:10.1016/j.jep.2015.08.055.

Rody, H. V. S., D. C. Gontijo, V. P. M. Coelho, M. C. Ventrella, R. M. Padua, L. G. Fietto, and J. P. V. Leite. 2018. Mutagenic activity and chemical composition of phenolic-rich extracts of leaves from two species of Ficus medicinal plants. *J. Toxicol. Environ. Health Part A* 81 (17):861–72. doi:10.1080/15287394.2018.1498420.

Seca, A. M. L., and D. C. G. A. Pinto. 2018. Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. *Int. J. Mol. Sci.* 19 (1):263. doi:10.3390/ijms19010263.

Simões, C. M. O., E. P. Schenkel, G. Gosmann, J. C. P. Mello, L. A. Mentz, and P. R. Petrovick. 2003. *Farmacognosia: Da planta ao medicamento*. 5 ed. Porto Alegre/Florianópolis: Editora da UFRGS/Editora da UFSC.

Singh, N. P., M. T. McCoy, R. R. Tice, and E. L. Schneider. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res* 175 (1):184–91. doi:10.1016/0014-4827(88)90265-0.

Sissi, C., and M. Palumbo. 2003. The quinolone family: From antibacterial to anticancer agents. *Curr. Med. Chem. Anticancer Agents* 3 (6):439–50. doi:10.2174/1568011033482279.

Stefanello, M. É., A. C. Pascoal, and M. J. Salvador. 2011. Essential oils from neotropical Myrtaceae: Chemical diversity and biological properties. *Chem. Biodivers.* 8 (1):73–94. doi:10.1002/cbdv.201000098.

Szwed, M., A. Matusiak, A. Laroche-Clary, J. Robert, I. Marszalek, and Z. Jozwiak. 2014. Transferrin as a drug carrier: Cytotoxicity, cellular uptake and transport kinetics of doxorubicin transferrin conjugate in the human leukemia cells. *Toxicol. In Vitro* 28 (2):187–97. doi:10.1016/j.tiv.2013.09.013.

Takao, L. K., M. Imatomi, and S. C. J. Gualtieri. 2015. Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from cerrado (Brazilian savanna). *Braz. J. Biol* 75 (4):948–52. doi:10.1590/1519-6984.03314.

Tariq, A., S. Sadia, K. Pan, I. Ullah, S. Mussarat, F. Sun, O. O. Abiodun, A. Batbaatar, Z. Li, D. Song, et al. 2017. A systematic review on ethnomedicines of anti-cancer plants. *Phytother. Res.* 31 (2):202–64. doi:10.1002/ptr.5751.

Tuttis, K., D. L. M. G. da Costa, H. L. Nunes, A. F. L. Specian, J. M. Sepeloni, L. M. Dos Santos, E. A. Varanda, W. Vilegas, W. Martinez-Lopez, and I. M. S. Colus. 2018. Pouteria ramiflora (Mart.) Radlk. extract: Flavonoids quantification and chemopreventive effect on HepG2 cells. *J. Toxicol. Environ. Health Part A* 81 (16):792–804. doi:10.1080/15287394.2018.1491911.

Viktorsson, E. Ö., B. Melling Grøthe, R. Aesoy, M. Sabir, S. Snellingen, A. Prandina, O. A. Høgmoen Åstrand, T. Bonge-Hansen, S. O. Døskeland, L. Herfindal, et al. 2017. Total synthesis and antileukemic evaluations of the phenazine 5,10-dioxide natural products iodinin, myxin and their derivatives. *Bioorg. Med. Chem. Lett.* 25 (7):2285–93. doi:10.1016/j.bmc.2017.02.058.

Wen-Hung, W., T. Yu-Chang, C. Zong-Shiow, L. Ching-Gong, Y. Ming-Hui, Y. Shyng-Shiou, and T. Wan-Chi. 2014. Evaluation of the antioxidant activity and antiproliferative effect of the jaboticaba (*Myrciaria cauliflora*) seed extracts in oral carcinoma cells. *Biomed. Res. Int.* United States: 185946. doi:10.1155/2014/185946