Journal of Ethnopharmacology 297 (2022) 115534

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Galloylquinic acid derivatives from *Byrsonima fagifolia* leaf extract and potential antifungal activity

Miriam Sannomiya [a,**], Clenilson Martins Rodrigues [b], Giovanna Castro Araújo Oliveira [a], Juliana Cajado Souza Carvalho [c], Letícia Serafim da Costa [d], Cristina de Castro Spadari [d], Marcelo José Pena Ferreira [c], Wagner Vilegas [e], Kelly Ishida [d,*]

[a] School of Arts, Sciences and Humanities, University of São Paulo, São Paulo/SP, Brazil
[b] Laboratory of Chemistry and Biomass and Biofuels, Embrapa Agroenergy, Brazilian Agricultural Research Corporation, Brasília/DF, Brazil
[c] Department of Botany, Institute of Biosciences, University of São Paulo, São Paulo/SP, Brazil
[d] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo/SP, Brazil
[e] Institute of Biosciences, São Paulo State University, Coastal Campus of São Vicente/SP, Brazil

ARTICLE INFO

Keywords:
Galloylquinic acids
Gallotannins
Quercetin derivatives
Murici
Malpighiaceae
Antifungal
Cryptococcus
Cryptococcosis

ABSTRACT

Ethnopharmacological relevance: *Byrsonima fagifolia* Niedenzu (Malpighiaceae) and other *Byrsonima* species are popularly employed in Brazilian traditional medicine in the form of preparations as cicatrizing, anti-inflammatory, and antimicrobial.

Aim of the study: To characterize the phytochemical profile of the hydromethanolic extract obtained from *B. fagifolia* leaves (BF extract) and to evaluate the toxicity and the antifungal activity.

Materials and methods: The compounds from BF extract were isolated by HPLC and the structures were elucidated based on extensive analyses of 1D and 2D NMR spectra (HMQC, HMBC and COSY) data. The antifungal effect was determined by the broth microdilution method and the toxicity was evaluated on erythrocytes from sheep's blood and *Galleria mellonella* larvae.

Results: Phytochemical investigation of the BF extract led to the isolation and characterization of pyrogallol, *n*-butyl gallate, 3,4-di-*O*-galloylquinic acid, 3,5-di-*O*-galloylquinic acid, 3,4,5-tri-*O*-galloylquinic acid, and 1,3,4,5-tetra-*O*-galloylquinic acid. The BF extract showed high content of galloylquinic acid derivatives reaching more than twenty-times the quercetin derivatives content, according to the quantification by HPLC. These galloylquinic acid derivatives, obtained during this study, and quercetin derivatives, previously isolated, were submitted to the antifungal assays. The BF extract inhibited yeast growth mainly against *Cryptococcus* spp., at concentrations of 1–16 μg/mL, comparable to isolated compounds galloylquinic acid derivatives. However, the quercetin derivatives as well as quinic acid, gallic acid, and methyl gallate showed lower antifungal effect compared with galloylquinic derivatives. In addition, the BF extract had no hemolytic effect and no toxicity on *G. mellonella*.

Conclusion: The phytochemical analysis revealed that galloylquinic acid derivatives are the major compounds in the leaves of *B. fagifolia* and they are associated to anti-cryptococcal activity and presented reduced toxicity.

1. Introduction

Byrsonima species (Malpighiaceae) are native plants of some countries of Latin America. They inhabit the phytogeographical domains of the Amazon, Cerrado, Pantanal, Atlantic Forest, and Caatinga of Brazil. According to the literature *Byrsonima fagifolia* Niedenzu has the synonyms *Byrsonima coriacea* (Sw.) DC., *Byrsonima crassifolia*, *Byrsonima crassifolia* var. *cinerea* (Poir.) Nied. and *Byrsonima crassifolia* var. *spruceana* (Nied.) Nied (Araujo et al., 2018). The leaves and barks of *B. crassifolia* and other *Byrsonima* species are used as diuretic, anti-hemorrhagic, antimicrobial, cicatrizing, and anti-inflammatory in

* Corresponding author. Instituto de Ciências Biomédicas, Universidade de São Paulo, Prof. Lineu Prestes Ave. 1374, São Paulo/SP, 05508000, Brazil.
** Corresponding author. Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, Arlindo Béttio St., 1000, São Paulo/SP, 03828-000, Brazil.
E-mail addresses: miriamsan@usp.br (M. Sannomiya), clenilson.rodrigues@embrapa.br (C.M. Rodrigues), giovannacastro@usp.br (G.C.A. Oliveira), jucajado@usp.br (J.C.S. Carvalho), leticiaserafimmdacosta@gmail.com (L.S. da Costa), spadaricris@gmail.com (C.C. Spadari), marcelopena@ib.usp.br (M.J.P. Ferreira), wagner.vilegas@unesp.br (W. Vilegas), ishidakelly@usp.br (K. Ishida).

https://doi.org/10.1016/j.jep.2022.115534
Received 1 June 2022; Received in revised form 30 June 2022; Accepted 10 July 2022
Available online 14 July 2022

Abbreviations:

BF extract	hydromethanolic extract of *Byrsonima fagifolia* leaves
CFU	colony forming unit
COSY	correlation spectroscopy
DMSO	dimethyl sulfoxide
FLC	fluconazole
GA	gallic acid
HA	hemolytic activity
HMQC	Heteronuclear multiple quantum coherence
HPLC-PAD	High-Performance Liquid Chromatography coupled to Photodiode Array Detector
HPLC/ESI-ITMS	High-Performance Liquid Chromatography coupled to Electrospray Ionization with multistage Ion Trap Mass Spectrometry fragmentation
HSQC	heteronuclear single quantum coherence
MFC	minimum fungicidal concentration
MG	methyl gallate
MIC	minimum inhibitory concentration
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
Q3-ara	quercetin-3-*O*-α-L-arabinopyranoside
Q3-gal	quercetin-3-*O*-α-L-galactopyranoside
Q3-rha	quercetin-3-*O*-α-L-rhamnopyranoside
QA	quinic acid
RPMI	Roswell Park Memorial Institute
Tetra-GQ	1,3,4,5-tetra-*O*-galloylquinic acid
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
Tri-GQ	3,4,5-tri-*O*-galloylquinic acid

Brazilian folk medicine (Almeida et al., 1998; Araujo et al., 2018).

Byrsonima fagifolia is popularly known as "murici" and the methanolic extract of *B. fagifolia* leaves displayed gastroprotective, healing, and antidiarrheal activities (Lima et al., 2008). Additionally, this extract showed antimicrobial activity against Gram-positive and Gram-negative bacteria (Lima et al., 2008; Michelin et al., 2008) and triterpenes from *B. fagifolia* leaves showed antimycobacterial activity (Higuchi et al., 2011). However, no work reported antifungal activity of extracts or isolated compounds from *B. fagifolia* leaves.

The analytical approach based on High-Performance Liquid Chromatography coupled to Electrospray Ionization (negative mode) and multistage Ion Trap Mass Spectrometry fragmentation (HPLC/ESI-ITMS^n) previously guided the detection of galloylquinic acids and flavonoid glycosides (Sannomiya et al., 2007a). The polar extract of *B. fagifolia* showed the presence of gallic acid, methyl gallate, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-xylopyranoside, quercetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-(2"-galloyl)-β-D-galactopyranoside, and quercetin-3-*O*-(2"-galloyl)-β-D-glucopyranoside (Lima et al., 2008). Herein we report the isolation of pyrogallol, *n*-butyl gallate, 3,4-di-*O*-galloylquinic acid, 3,5-di-*O*-galloylquinic acid, 3,4,5-tri-*O*-galloylquinic acid and 1,3,4,5-tetra-*O*-galloylquinic acid from hydromethanolic extract of *B. fagifolia* leaves, using a single step semi-preparative HPLC procedure. In addition, the toxicity and the *in vitro* inhibitory activity of the BF extract, galloylquinic acid derivatives, and quercetin derivatives were evaluated against the pathogenic yeasts *Cryptococcus neoformans* and *Cryptococcus gattii*.

2. Material and methods

2.1. Compounds

Quinic acid (QA), gallic acid (GA), methyl gallate (MG), and quercetin were purchased from Sigma-Merck.

2.2. Plant material

Leaves of *Byrsonima fagifolia* Niedenzu were collected at Porto Nacional (Tocantins/Brazil) according to the geographical coordinates: Lat: 10°9'041" S, Long: 48°14'011" O by Prof. Clélia A. Hiruma-Lima (Institute of Biosciences, State University of São Paulo) and identified by the Prof. Eduardo Ribeiro dos Santos (Institute of Biosciences, State University of Tocantins). A *voucher* was deposited at the Herbarium of State University of Tocantins (n°. 6398) and the botanical material was registered at Genetic Heritage Management Council (CGEN/SISGEN, Brazil) under the code A29AEE3.

2.3. Extract preparation

The leaves were dried at 40 °C for 72 h with air circulation and the compounds from powdered dry leaves (2.0 kg) were extracted by maceration using a mixture of methanol/water 8:2 (v/v) for 48 h at room temperature (three times). After filtration, the solvent was eliminated using a rotary evaporator at 60 °C under vacuum to obtain the hydromethanolic extract of *B. fagifolia* leaves (BF extract, 230.1 g, 11.5 %). The BF extract powder was obtained by freeze-drying, aliquoted in vials, and maintained at −20 °C (Park and Im, 2021).

2.4. Isolation and screening by thin-layer chromatography

The BF extract (2.0 g) was dissolved in 10.0 mL of water and 2.0 mL of methanol. Aliquots of 1.2 mL were sequentially injected in a preparative HPLC system (Varian, Walnut Creen, CA, USA) equipped with a binary solvent pump (ProStar 210), a photodiode array detector (PAD, ProStar 330) and a Rheodyne 7125 (Cotati, CA, USA) sample injection with a 2.0 mL sample loop. The preparative column was a reverse phase (RP18) Dynamax (250 × 41.1 mm d.i. x 8 μm, Cotati, CA, USA). The mobile phase composition used was water (eluent A) and methanol (eluent B), both with 0.1 % of trifluoroacetic acid (TFA). The gradient program was: 5–15% B (70 min), 15% B isocratic (10 min), 15–40% B (100 min), 40–100% B (30 min), and 100% B isocratic (20 min), 100-5% B (1 min) and 5% B isocratic (9 min). Total run time, including column rebalancing was 240 min. The flow rate of the mobile phase was 23 mL/min.

From the preparative separation, 26 chromatographic peaks were collected and grouped according to the retention time. After this procedure, TFA and methanol were removed by rotary evaporation. The aqueous samples were frozen and subjected to a lyophilization step to remove water. A few amount of each lyophilized sample was solubilized in water/methanol (8/2, v/v) and spotted on silica gel thin-layer chromatography (TLC) plates (20 cm × 20 cm, Aldrich). The spots were eluted using the solvent mixture composed of ethyl acetate/*n*-propanol/*n*-butanol/water (5:6:1:4 (v/v)) and the eluted spots were revealed in a dark grey color after spraying with sulfuric anisaldehyde reagent followed by heating on a hot plate. This screening step revealed that 6 compounds were individually eluted as a single spot during the TLC analyses and isolated directly from the HPLC procedure (Fig. 1): 3.0 mg of **1** (Peak 1), 2.0 mg of **2** (Peak 5), 3.0 mg of **3** (Peak 8), 13 mg of **4** (Peak 14), 6.0 mg of **5** (Peak 15) and 3.0 mg of **6** (Peak 25).

2.5. Chemical characterization

Isolated compounds were analyzed through NMR spectra for structural elucidation. The NMR experiments in deuterated dimethyl

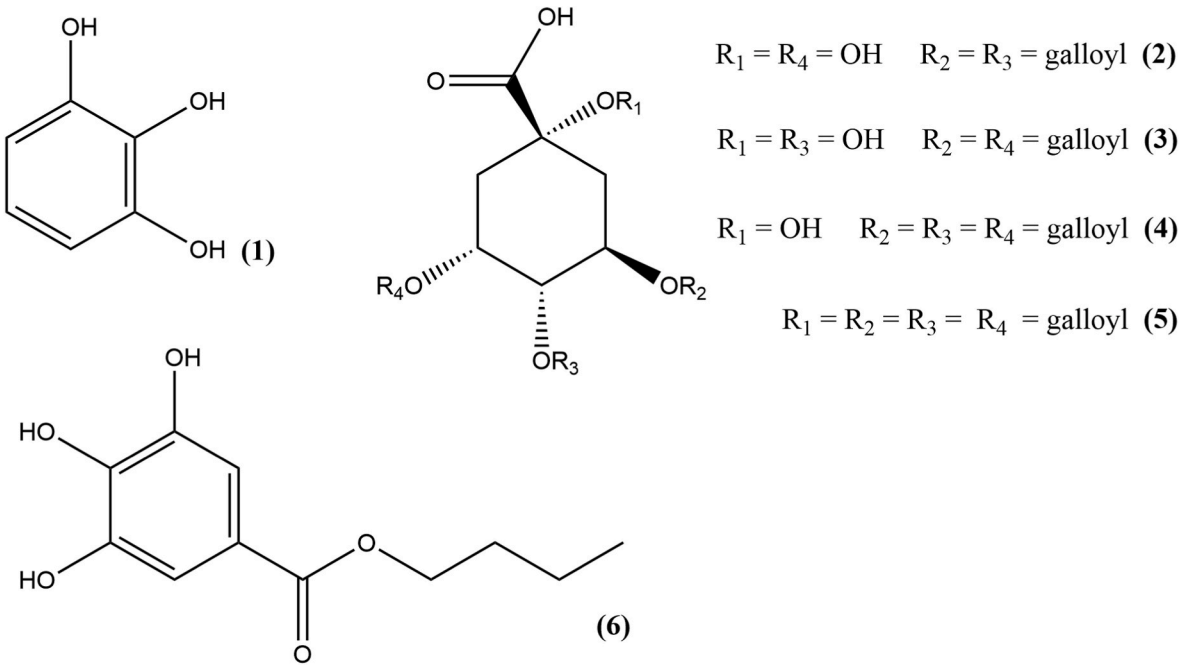

Fig. 1. Structures of compounds isolated from hydromethanolic extract of *Byrsonima fagifolia* Niedenzu leaves. (**1**) pyrogallol, 3,4-di-*O*-galloylquinic acid, (**3**) 3,5-di-*O*-galloylquinic acid, (**4**) 3,4,5-tri-*O*-galloylquinic acid, (**5**) 1,3,4,5-tetra-*O*-galloylquinic acid, and (**6**) *n*-butyl gallate.

sulfoxide (DMSO-d_6) were achieved using a Varian INOVA 500 spectrometer, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C and 2D NMR (1H–1H COSY, g-HMQC, TOCSY and g-HMBC). The chemical shifts are given as δ (ppm) using TMS as an internal standard. The isolated compounds were identified by comparing ^{1}H NMR data (Table S1) with literature data (Gottlieb et al., 1991; Motta et al., 2017; Nishimura et al., 1984; Souza et al., 2014; Nishizawa et al., 1989; Neszmélyi et al., 1993; Srinivasan et al., 2008).

Additionally, the quercetin derivatives quercetin-3-*O*-α-L-arabinopyranoside (Q3-ara), quercetin-3-*O*-α-L-rhamnopyranoside (Q3-rha), and quercetin-3-*O*-α-L-galactopyranoside (Q3-gal) were isolated from the BF extract, according to the methodology described by Lima and collaborators (2008), for the antifungal testing assay.

2.6. Quantification of galloylquinic acids and flavonoids

For calibration curves elaboration, both compounds (gallic acid and quercetin) were solubilized in HPLC-grade methanol (2 mg/mL) and filtered using a 0.45 μm syringe filter. The obtained solutions were diluted in a concentration range of 2–1600 μg/mL, resulting in ten standard solutions for each compound. These samples were analyzed in triplicate by HPLC–PAD (model 1260, Agilent), with an injection volume of 3 μL. The temperature of C18 column (Zorbax Eclipse Plus, 4.6 × 150 mm, 3.5 μm) was set at 45 °C and the chromatographic method was constituted by a gradient of mixtures of solvents A (0.1 % acetic acid in water) and B (acetonitrile) of: 0–6 min (10 % B); 6–7 min (10–15 % B); 7–22 min (15 % B); 22–23 min (15–20 % B); 23–33 min (20 % B); 33–34 min (20–25 % B); 34–44 min (25 % B); 44–54 min (25–50 % B); 54–60 min (50–100 % B). Absorbance detection was performed at λ = 280 and 352 nm for gallic acid and quercetin, respectively. The average peak areas vs. the concentration of each analyte were used to construct the calibration curve for each compound. The limits of detection (LOD) and quantification (LOQ) were calculated, respectively, for gallic acid and quercetin as 0.083 and 0.252 μg/mL, and 0.061 and 0.185 μg/mL.

In order to quantify galloyl and quercetin derivatives, BF extract was solubilized in HPLC-grade methanol (2 mg/mL) and filtered using a 0.45 μm syringe filter. The resulting sample was analyzed in triplicate by HPLC–PAD with the same parameters used to elaborate the calibration curve.

2.7. Microorganisms

Aspergillus fumigatus ATCC 16913, *Fusarium oxysporum* ATCC 48112, *Candida albicans* SC 5314, *Cryptococcus neoformans* stricto sensu ATCC 208821 (or H99), *Cryptococcus gattii* lato sensu ATCC 56990 were used in this work. The clinical isolates of *C. neoformans* (541, 542, 543, 544A, 545A) and *C. gattii* (525, 527, 551A, 616A, 652A) were also included in this study and were previously obtained from patients diagnosed with neurocryptococcosis (Spadari et al., 2020). The strains were stored in brain heart infusion broth and 20 % glycerol at −80 °C and recovered and subcultured on Sabouraud dextrose agar for 72 h at 35 °C prior to assays.

2.8. In vitro antifungal susceptibility assay

The antifungal activity of BF extract and compounds was evaluated by the broth microdilution technique (CLSI, 2017). BF extract, QA, GA, MG, and galloylquinic acid derivatives and quercetin derivatives were dissolved in dimethyl sulfoxide (DMSO) and diluted in Roswell Park Memorial Institute 1640 medium buffered with 0.165 M 3-(N-morpholino)propane sulfonic acid (or simply RPMI) to obtain the work concentration of 4096 μg/mL (10 % DMSO).

The BF extract and compounds were serially diluted (1:2) in RPMI into 96-well flat-bottom plates and 100 μL of fungal suspension added to the wells, obtaining the final fungal concentration at 0.5–2.5 × 10^3 CFU/mL and final concentrations of BF extract and compounds ranging from 0.125 to 64 μg/mL. The plates were incubated at 35 °C for 24 h to visually determine the minimum inhibitory concentration (MIC) defined as the lowest concentration that inhibits 50 % of fungal growth.

Subsequently, 10 μL aliquots from each well in which there was no fungal growth were transferred onto Sabouraud dextrose agar and incubated at 35 °C for 48 h to determine the minimum fungicidal concentration (MFC), which was defined as the lowest concentration of BF extract or compounds which reduces >99.9% of the viability of the initial inoculum (Spadari et al., 2020).

2.9. Hemolytic activity

The hemolytic activity of BF extract was evaluated on a 4 % suspension of erythrocytes from sheep's blood (red blood cells) (v/v, in a sterile 5 % PBS-glucose solution) at concentrations ranging from 16 to 1024 μg/mL for 2 h in a water bath at 37 °C. Negative (untreated) and positive (0.1 % triton X100) controls were included in the test. After incubation, the samples were centrifuged at 1500 rpm. for 5 min and the supernatant analyzed in a spectrophotometer at 540 nm. The hemolytic activity (HA) was calculated as follows: HA (%) = 100 – [(CP –AT)/(CP–CN) x 100]; where CP – absorbance of the positive control, AT – absorbance of the test sample and CN – absorbance of the negative control (Li et al., 2002).

2.10. Toxicity on Galleria mellonella larvae

A volume of 10 μL of BF extract, at 100 mg/kg, previously diluted in PBS was inoculated in the last pro-leg of *G. mellonella* larvae (~200 mg of body weight) using a Hamilton syringe. A larvae group that received only PBS was included in the assay for the control of mechanical trauma by injections. A total of 20 larvae were used for each group and incubated at 37 °C. Larvae survival and health status (features such as movement, cocoon formation and melanization) were monitored every 24 h for up to 5 days after treatment for construction of the survival and morbidity curves, respectively (Loh et al., 2013).

2.11. Statistical analysis

The data were analyzed using the GraphPad Prism v8.0 software (GraphPad Software Inc., La Jolla, CA), and a *P-value* < 0.05 was considered significant.

3. Results and discussion

Medicinal plants act as an interesting source of bioactive phenolic compounds such as flavonoids and tannins against pathogenic microorganisms (Aboody MSA, Mickymaray S, 2020; Górniak et al., 2019; Brighenti et al., 2021). The phenolic compounds had been also detected in many species of *Byrsonima*, but few phytochemical studies were carried out, besides the scarcity of studies that scientifically prove the pharmacological activity of traditional use. Here, we described a phytochemical profile of the hydromethanolic extract obtained from B. *fagifolia* leaves as well as the antifungal properties and toxicity.

3.1. Isolation, characterization, and quantification of compounds

Six compounds were isolated on a preparative HPLC-PAD procedure from BF extract. The ^{1}H NMR spectrum of substance **1** exhibited the presence of a doublet at δ 6.23 (2H, $J = 8.5$ Hz) and a triplet at δ 6.4 (1H, $J = 8.5$ Hz), which indicated the presence of a 1,2,3-trisubstituted aromatic ring. Analysis of ^{13}C, HMQC, and HMBC NMR spectra allowed the identification of (**1**) as pyrogallol (Gottlieb et al., 1991). Compounds **2** and **3** showed in their spectra two singlets at δ 6.9 (2H) and δ 6.8 (2H), and δ 7.0 (2H) and δ 6.9 (2H), respectively, which were characteristic of the presence of two galloyl groups in each compound. Additionally, both spectra showed multiplets in the region of δ 1.98–2.20 (4H) corresponding to four aliphatic hydrogens, together with multiplets at δ 4.51 (H-5), δ 5.15 (H-4), and δ 5.58 (H-3) for (2), and at δ 4.26 (H-4) and δ 5.3 (H-3 and H-5) for (3), which were assigned to hydrogens from methine groups bearing oxygen atoms. These signals and the ^{13}C NMR data confirmed the presence of a quinic acid moiety in the structures. Through HMBC correlations the positions of galloyl groups were determined in the quinic acid moieties. Therefore, by comparison with literature data, these compounds were identified as 3,4-digalloylquinic acid (**2**) (Motta et al., 2017) and 3,5-digalloylquinic acid (**3**) (Nishimura et al., 1984; Souza et al., 2014). Similarly, the presence of quinic acid moiety was identified in the compounds (**4**) and (**5**). However, the signals of three singlets at δ 6.93 (2H), δ 6.88 (2H) and δ 6.85 (2H) in the compound (**4**), and four singlets at δ 6.94 (2H), δ 6.92 (2H), δ 6.84 (2H) and δ 6.79 (2H) in the compound (5) suggested the presence of three and four galloyl groups in the structures. From the 2D NMR spectra, compounds (**4**) and (**5**) were identified as 3,4,5-trigalloylquinic acid and 1,3,4,5-tetragalloylquinic acid, respectively. These structures were confirmed by comparison of data with those reported in the literature (Nishimura et al., 1984; Nishizawa et al., 1989; Neszmélyi et al., 1993). Finally, the spectrum of compound **6** showed an aromatic singlet at δ 6.9 (2H) attributed to two symmetrical protons (H-2 and H-6) of a galloyl group. Four additional signals were observed: two triplets at δ 4.15 (2H) and δ 0.95 (3H, $J = 7.5$ Hz) assigned to oxy-bearing methylene and a methyl group, respectively, and two multiplets at δ 1.40 (2H) and δ 1.52 (2H). Thus, the compound was identified as *n*-butyl-gallate (**6**). The NMR data of compounds were shown in Table S1 and the structures of pyrogallol **(1)**, 3,4-di-*O*-galloylquinic acid **(2)**, 3,5-di-*O*-galloylquinic acid **(3)**, 3,4,5-tri-*O*-galloylquinic acid **(4)**, 1,3,4,5-tetra-*O*-galloylquinic acid **(5)**, and an ester of gallic acid, the *n*-butyl gallate **(6)** were shown on Fig. 1.

It is important to highlight that all these compounds were isolated for the first time in the *B. fagifolia* leaves extract and so far, pyrogallol **(1)** and *n*-butyl gallate **(6)** were not detected before in *Byrsonima* genus. However, the galloylquinic acid derivatives were identified previously in the ethyl acetate extract of *B. coccolobifolia* stem and in the methanolic extract of *B. crassifolia* bark (Souza et al., 2014; Maldini et al., 2011). Remarkably, the content of galloylquinic derivatives is found in a higher proportion compared to the quercetin derivatives in the hydromethanolic extract from *B. fagifolia* leaves, which were quantified at 508.63 mg/g and 24.39 mg/g of galloyl derivatives and flavonoids, respectively. We highlighted that to date there are no reports in the literature on the quantification of galloylquinics in other species of *Byrsonima*.

3.2. Antifungal activity and toxicity

Previous studies had shown that extracts from *Byrsonima* spp. are composed of many phenolic compounds and have some antifungal action. *Byrsonima gardneriana* leaves extract showed fungistatic activity, with MIC of 125 μg/mL against *Candida* spp. (Souza-Melo et al., 2021). Hydroethanolic extracts of barks and leaves from *B. crassifolia* showed an inhibitory effect on dermatophytes (Cáceres et al., 1993). In addition, the ethanolic extract from *B. crassifolia* bark presented inhibitory activity on the mycelial growth of *Fusarium solani* and *Sclerotinia sclerotiorum,* at 8–24 μg/mL, and the authors attributed the antifungal potential to phenolic compounds and to triterpene derivatives (Andrade et al., 2018). Although some studies have shown antifungal activities of *Byrsonima* spp. extracts, our work demonstrated for the first time the antifungal action of hydromethanolic extract obtained from *B. fagifolia* leaves against pathogenic yeasts, emphasizing *C. neoformans* and *C. gattii*.

The high content of galloylquinic acid derivatives in *B. fagifolia* leaves had a great contribution on the inhibitory activity against *C. albicans* SC 5314 and *C. neoformans* H99 at concentrations of 16–32 μg/mL and 2–4 μg/mL, respectively. However, BF extract was not active even at the highest concentration tested (1024 μg/mL) against filamentous fungi (*A. fumigatus* and *F. oxysporum*). Therefore, we tested the BF extract, galloylquinic acid derivatives, quercetin derivatives, and other phenolic compounds (QA, GA, and MG) against *Cryptococcus* spp. clinical isolates. BF extract inhibited *C. neoformans* and *C. gattii* (1–4 μg/mL) and the inhibitory activity was similar to the reference antifungal fluconazole (0.25–16 μg/mL) (Table 1). Interestingly, all galloylquinic acid derivatives tested here had an anti-*Cryptococcus* spp. effect (0.5–4 μg/mL) similar to the BF extract. On the other hand, QA, GA, MG, and quercetin derivatives showed lower inhibitory effect (Table 1). Neither BF extract nor isolated compounds showed fungicidal action (MFC >64

Table 1
Inhibitory activity of hydromethanolic extract from *Byrsonima fagifolia* leaves, galloylquinic and quercetin derivatives, and other phenolic compounds on *Cryptococcus neoformans* and *Cryptococcus gattii* isolates.

Strains	MIC (μg/mL)[a]									
	BF extract	Tri-GQ	Tetra-GQ	Q3-ara	Q3-rha	Q3-gal	QA	GA	MG	FLC[b]
C. neoformans										
H99	2	1	1	64	≥64	32	≥64	64	16	2
541	2	1	0.5	32	16	8	≥64	32	32	0.25
542	4	1	1	64	64	16	≥64	≥64	8	16
543	1	2	1	64	32	8	≥64	32	16	0.25
544A	4	1	2	≥64	≥64	≥64	≥64	≥64	≥64	1
545A	2	2	1	32	64	32	≥64	32	16	2
C. gattii										
ATCC 56990	2	4	4	64	≥64	32	≥64	≥64	8	2
525	4	4	4	≥64	≥64	≥64	≥64	≥64	32	8
527	2	4	4	≥64	≥64	32	≥64	64	16	16
551A	2	2	2	≥64	≥64	32	≥64	64	32	2
616A	1	2	2	64	64	16	≥64	≥64	16	4
652A	4	4	4	32	32	32	≥64	64	8	4

BF extract: hydromethanolic extract from *Byrsonima fagifolia* leaves; Tri-GQ: 3,4,5-tri-O-galloylquinic acid, Tetra-GQ: 1,3,4,5-tetra-*O*-galloylquinic acid, Q3-ara: quercetin-3-*O*-α-L-arabinopyranoside, Q3-rha: quercetin-3-*O*-α-L-rhamnopyranoside, Q3-gal: quercetin-3-*O*-α-L-galactopyranoside, QA: quinic acid, GA: gallic acid, MG: methyl gallate, FLC: fluconazole.

[a] MIC, the lowest concentration that inhibits 50% of fungal growth.

[b] Data previously published (Spadari et al., 2020).

μg/mL, data not shown).

Importantly, the BF extract from *B. fagifolia* leaves did not show hemolytic activity on red blood cells up to the highest tested concentration of 1024 μg/mL (Table S2), and non-toxic effect was observed on the *G. mellonella* larvae, at dose of 100 mg/kg (Fig. 2). Previous works reported *B. fagifolia* leaves exhibited lower cytotoxic effects than other *Byrsonima* species and significant cytotoxicity was observed only at concentrations >39 μg/mL on hepatic and gastric epithelium cells (Specian et al., 2016). In addition, *B. fagifolia* showed no mutagenic properties but exhibited a relevant antimutagenic activity being a source of chemopreventive agents (Espanha et al., 2014).

Indeed, this lower cytotoxicity of *B. fagifolia* compared with the other studied species may be associated to the difference of the chemical profile (Sannomiya et al., 2005a, b, c; Sannomiya et al., 2007a, b; Lima et al., 2008). Therefore, the literature data together with our results suggest that *B. fagifolia* extract can be considered low-toxic, although more studies should be carried out to certify its safety.

Galloylquinic acid derivatives are recognized in the literature as hydrolyzable tannins. They have several pharmacological properties as antioxidant (Baratto et al., 2003), antigenotoxic (Ines et al., 2012), gastroprotective activity (Motta et al., 2017), antiprotozoal (Souza et al., 2014), antiviral (Nishizawa et al., 1989; Kamng'ona et al., 2011), antifungal (Andrade et al., 2020), and antinociceptive (Abreu et al., 2019).

The hydrolyzable tannins enriched-fractions from *Poincianella microphylla* showed anti-*Trichomonas vaginalis* activity with MIC of 70.4–142.1 μg/mL with no toxicity against *G. mellonella* (Silva et al., 2020). Some galloylquinic acid derivatives showed inhibitory activity against the susceptible and resistant strains of *Plasmodium falciparum*, with IC_{50} values of 8.0–43.0 and 16.1–93.0 μg/mL, respectively (Cao et al., 2006).

The hydrolyzable tannin, corilagin, showed an excellent antifungal activity against *Candida glabrata* with MIC of 0.8 nM (Latté and Kolodziej, 2000). The *Dittrichia viscosa* (L.) Greuter leaves extract presented mainly caffeoylquinic acid derivatives and showed the highest antifungal activities against *Malassezia* spp., *Microsporum canis,* and *A. fumigatus* strains (Rhimi et al., 2017). Interestingly, the galloylquinic acid derivatives had antiviral effects against HIV-1 and M-MLV by the inhibition of reverse transcriptase enzyme (Nishizawa et al., 1989; Kamng'ona et al., 2011).

It is important to highlight that BF extract from *B. fagifolia* as well as galloylquinic acid derivatives inhibited *Cryptococcus* spp. growth at MIC values similar or lower than FLC, emphasizing the potential use of these compounds as an alternative for antifungal development. Notably, quercetin derivatives, QA, GA, and MG did not have an important antifungal effect on *Cryptococcus* spp. as shown by the galloylquinic derivatives. Although there are few works describing the mechanisms for the different pharmacological action of galloylquinic acid derivatives, previous studies had shown that hydrolyzable tannins, hydroxyl-rich molecules, can interact with macromolecules such as proteins (enzymes) and carbohydrates through bonds with hydroxyl groups leading to the pharmacological effect, including the

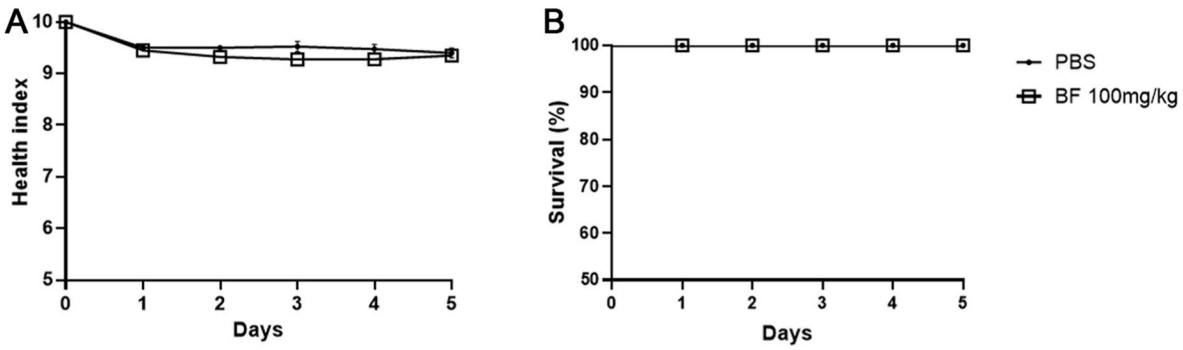

Fig. 2. Toxicity of hydromethanolic extract from *Byrsonima fogifolia* Niedenzu leaves (BF extract) on the invertebrate model of *Galleria mellonella*. **A:** morbidity curve ($P > 0.05$, two-way ANOVA); **B:** survival curve ($P > 0.05$, Log-rank tests - Mantel-Cox).

antimicrobial activities (Haslam, 1996).

4. Conclusion

The fractionation by preparative HPLC-PAD of the hydromethanolic extract of *B. fagifolia* leaves allowed the isolation of pyrogallol, *n*-butyl gallate and four galloylquinic acid derivatives. The antifungal action on *Cryptococcus* spp. of hydromethanolic extract from *Byrsonima fagifolia* leaves is attributed to the high content of the galloylquinic acid derivatives. These compounds are promising for additional *in vitro* and *in vivo* microbiological assays as well as studies of mechanisms of action for the drug development of anti-*Cryptococcus* agents.

Funding

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2021/01279-5, 2020/16554-9 and Biota program 2009/52237-9), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant 405556/2018-7), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). KI and MJPF are research fellows of the CNPq (grants 303373/2019-9 and 308952/2020-0, respectively).

Transparency declarations

The authors declare that there are no conflicts of interest.

Ethical approval

Not required.

CRediT authorship contribution statement

Miriam Sannomiya: Conceptualization, Methodology, Data curation, Writing – review & editing. **Clenilson Martins Rodrigues:** Methodology. **Giovanna Castro Araújo Oliveira:** Methodology. **Juliana Cajado Souza Carvalho:** Methodology. **Letícia Serafim da Costa:** Methodology. **Cristina de Castro Spadari:** Methodology. **Marcelo José Pena Ferreira:** Data curation, Writing – review & editing. **Wagner Vilegas:** Supervision. **Kelly Ishida:** Conceptualization, Methodology, Data curation, Writing – review & editing.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to the Especial Mycology Laboratory of the Department of Medicine, Federal University of São Paulo (São Paulo/SP, Brazil) for providing the *Cryptococcus* spp. clinical isolates.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2022.115534.

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