



Antiviral Activity and Chemical Characterization of *Ficus citrifolia* Leaf Extract Against Emerging Arboviruses

Horrana Acácio Mardegan^{1,2} · Allana Martins dos Santos Ataíde¹ · Loueny Celine Ribeiro Andrade¹ · Abraão de Jesus Barbosa Muribeca³ · Johan Carlos Costa Santiago³ · Sônia das Graças Santa Rosa Pamplona¹ · Ariane Coelho Ferraz⁵ · Marília Bueno da Silva Menegatto⁵ · Cíntia Lopes de Brito Magalhães^{4,5} · Consuelo Yumiko Yoshioka e Silva^{1,2} · Milton Nascimento da Silva^{1,2}

Received: 8 October 2025 / Accepted: 3 March 2026
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Abstract

Arboviruses represent a serious public health problem, especially in tropical regions, where environmental conditions favor the proliferation of mosquito vectors, facilitating viral transmission and leading to debilitating clinical manifestations ranging from fever and joint pain to severe neurological complications. This study evaluated the *in vitro* antiviral activity and chemical characterization of the ethanolic extract of *Ficus citrifolia* Mill. leaves, Moraceae. Using high-resolution LC–MS/MS analysis, 20 compounds were annotated, including phenolic acids, flavonoids, lignans, terpenes, and steroids—many of which are known for their antiviral and anti-inflammatory properties. The crude ethanolic extract exhibited significant antiviral activity against Mayaro virus (EC₅₀ 61.24 µg/ml, SI 12.96), Chikungunya virus (EC₅₀ 117.92 µg/ml, SI 6.73), and Zika virus (EC₅₀ 117.29 µg/ml, SI 6.77), with no observed activity against Oropouche virus. The findings demonstrate *in vitro* antiviral activity of a crude plant extract and support its traditional use in treating inflammatory and infectious diseases. Further studies, including bioassay-guided fractionation and mechanistic evaluations, are recommended to identify the active constituents and validate these findings.

Keywords LC–MS/MS dereplication · Secondary metabolites · Viral inhibition · Cytotoxicity assay · Selectivity index · Natural products

Introduction

Arboviruses stand out as infectious agents transmitted to humans by the bite of infected blood-feeding arthropods. Tropical and subtropical climates greatly facilitate the reproduction of these vectors, especially in countries such as Brazil, where climatic conditions favor the spread of arboviruses such as dengue, yellow fever, Chikungunya, Zika, Mayaro, and Oropouche. Although these diseases are endemic, they continue to be neglected, with 1,645,678 cases reported in the Americas in 2020 based on PAHO (2020).

In developing countries such as Brazil, unorganized urbanization and poor sanitation infrastructure increase the vulnerability of the population. In addition, the lack of effective vaccines and antivirals aggravates the situation, overburdening health systems and favoring outbreaks in the world (Girard et al. 2020). The resurgence of Zika (ZIKV), Oropouche (OROV), Mayaro (MAYV), and Chikungunya (CHIKV) in urban centers has intensified these challenges.

✉ Milton Nascimento da Silva
miltonsilva@ufpa.br

¹ Central Integrada em Metabolômica da Amazônia, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, Belém, PA 66075-110, Brazil

² Programa de Pós-graduação em Ciências Farmacêuticas, Instituto de Ciências da Saúde, Universidade Federal do Pará, Belém, PA, Brazil

³ Departamento de Ciências Naturais, Centro de Ciências Sociais e Educação, Universidade do Estado do Pará, Belém, PA 66050-540, Brazil

⁴ Programa de Pós-graduação em Biotecnologia, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

⁵ Programa de Pós-graduação em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

These viruses produce similar symptoms, such as fever, rashes, and severe joint pain, sometimes progressing to chronic arthritis. ZIKV is known to cause congenital malformations and vertical transmission (Pielnaa et al. 2020), and OROV has recently been linked to similar physical changes and vertical transmission (Schwartz et al. 2024). Mayaro virus can cause complications such as myocarditis, hemorrhagic syndromes, and neurological manifestations.

Faced with these clinical challenges, the search for new therapeutic sources has increasingly turned to the ancestral knowledge of traditional communities about medicinal plants. The biodiversity of the Amazon offers several species with therapeutic potential, supporting traditional healing practices and providing an accessible alternative.

Among these species, those of the genus *Ficus* are widely used in traditional medicine for their anti-inflammatory, analgesic, and healing properties (Lansky et al. 2008). These plants are known for their rich chemiodiversity, including organic acids, phenolic compounds, and terpenes (Cruz et al. 2022). Previous studies have demonstrated the traditional use and therapeutic effects of *Ficus* species such as *F. iteophylla* (syn. of *Ficus thonningii* Blume), which reduced pain and inflammation in rodents (Abdulmalik et al. 2011), and *F. amplissima* Sm., which exhibited *in vivo* anti-inflammatory, antioxidant, and wound-healing properties (Arunachalam and Parimelazhagan 2012).

Although these effects are not directly antiviral, viral pathogens induce inflammatory responses in the host as part of the infection process (Pinggen et al. 2016). Ethnopharmacological applications may therefore be linked to antiviral potential; however, this relationship remains hypothetical and requires experimental validation. Nevertheless, antiviral activity has been reported for other *Ficus* species, such as *F. sur* Forssk. and *F. rubiginosa* Desf. ex Vent. inhibiting herpes simplex virus type 1 (HSV-1) (Sieniawska et al. 2022; Dell'Annunziata et al. 2022), *F. deltoidea* Jack showing antiviral activity against dengue virus type 2 (Wu et al. 2024), and *F. carica* L. inhibiting SARS-CoV-2 replication (Hamed et al. 2023).

Although several *Ficus* species have been studied, no research has evaluated the antiviral potential of *F. citrifolia*, commonly known as “fig tree” or “black gameleira”. Previous studies have highlighted the antimycobacterial effects of its ethanolic leaf extract against *Mycobacterium tuberculosis*, as well as the antiproliferative effect of three lignans isolated from the leaf extract with dichloromethane (Antoun et al. 2001; Baetas et al. 2018). Furthermore, the isolated lignans have demonstrated antiviral activity in previous studies (Carvalho et al. 2023).

Based on these findings and the ongoing search for new antiviral agents, we conducted a study to evaluate the *in vitro* antiviral properties of the ethanolic extract of *F. citrifolia* leaves against the MAYV, CHIKV, ZIKV, and OROV

strains, selected due to their recurrent circulation and health relevance in the Amazon region. In addition, we performed chemical characterization using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) to support future phytopharmacological investigations of this species.

Material and Methods

Plant Collection, Identification, and Extraction

Leaves of *Ficus citrifolia* Mill., Moraceae, were collected in Belém (Pará, Brazil) under the coordinates 01°02'28" latitude and 048°27'00" longitude, in the reserve of the Brazilian Agricultural Research Corporation (Embrapa-PA) and cataloged under voucher No. IAN202214. Access to genetic heritage was legalized before the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under the registration AF01766 (available at <https://sisgen.gov.br>).

The botanical material was washed and oven-dried at 45 ± 1 °C. The dried material was ground in a knife mill and sieved through in mesh screens with an average nominal opening of 180 µm.

Extraction was performed with ethanol (99.5%, v/v, TEDIA®; 1:10, m/v), using 300 g of dried and crushed material in two cycles of 24 h each, at room temperature. The extract was vacuum filtered and dried in an oven (45 ± 1 °C) until constant weight. The crude extract yielded 14 g. The sample (crude extract) was cleaned by solid phase extraction (SPE) using a 50 mg C18 cartridge (Phenomenex, Torrance, CA, USA). The cartridge was conditioned according to the manufacturer. The sample (10 mg) was dissolved in 1 ml of H₂O:ACN (2:8, v/v) and eluted through the cartridge. The volume was collected, and a second volume was passed through the cartridge. The volumes were pooled and dried, yielding 2.3 mg, which was stored away from light until analysis.

High-Resolution LC–MS Analysis

High-resolution LC–MS/MS analyses were performed on a Xevo G2-S QToF (Waters Corp., Milford, MA, USA) equipped with an Electrospray Ionization (ESI) source. Leucine enkephalin was used as lock-mass for accurate mass calibration. The system and data acquisition were controlled by MassLynx 4.1 software. The extract (2 mg/ml) was injected (2 µl) into a BEH C18 column (50 × 2.1 mm, 1.7 µm) at 40 °C. Ultrapure water (A) and acetonitrile (B) were used as mobile phases at a flow rate of 300 µl/min. The gradient elution program was as follows: 0–18 min (10–100% B), 18–20 min (100% B), 20–21 min (100–10% B), and

21–24 min (10% B). Data were acquired in both positive and negative ionization modes over an m/z range of 50–1200. Data Dependent Acquisition (DDA) was applied with a centroid shape and the selection of five ions (Top 5 experiment). The collision energy was set from 15 to 65 eV. The scan time was 0.1 s and charge states were +2, with a tolerance window of ± 0.2 Da and a peak extraction tolerance of 2 Da. Deisotope tolerance was ± 3 Da and deisotope extraction tolerance of 6 Da. Source and desolvation temperatures were 150 °C and 300 °C, respectively. Cone and desolvation gas flow rates were 20 l/h and 600 l/h, respectively. The capillary voltage was 3.0 kV, and cone voltage was 40 V.

Data Processing and Annotation of Chemical Compounds

Mass spectrometry data files obtained from the Xevo G2-S QToF instrument for all extracts were converted to mzML format using MSConvert 3.0.2 (ProteoWizard, Palo Alto, CA, USA) (Chambers et al. 2012). Data processing was performed with MZmine 4.2.0 (Schmid et al. 2023). Ion detection thresholds were set at 1×10^3 for MS¹ and 8×10^1 for MS². Chromatogram deconvolution was performed using Local Minimum Resolver with a chromatographic threshold of 1.0×10^3 . The retention time intervals were set at 0.05 min, with a minimum absolute height of 1000 and peak-to-peak ratio of 1.7. Isotopes were detected using a peak window with a tolerance of 0.002 m/z or 10 ppm, a retention time tolerance of 0.2 min, and a maximum charge of 2. Peak alignment was performed using an m/z tolerance of 0.002 m/z (10 ppm) and a retention time tolerance of 0.2 min. The resulting list was filtered to remove duplicates and lines without an associated MS² spectrum. The mgf and CSV files were exported for library searching and molecular network analysis on the GNPS platform (Wang et al. 2016). The detailed analyses can be accessed via the IDs: for the negative mode, ID=57c3e0b4cedf4f698ee422b761de598d, and for the positive mode, ID=7f6942fd82484d0d9f2fd0a-b8af95450. Compound annotations were performed according to the Metabolomics Standards Initiative (MSI) criteria and correspond to level 2 (putatively annotated compounds based on MS/MS spectral similarity with GNPS libraries) and level 3 (putatively characterized compound classes based on spectral features and literature data).

Antiviral Activity of the Extract

Cell Lines

In the *in vitro* assays, Vero continuous lineage cells (CCL-81), which are fibroblast-like cells derived from the kidney of the African green monkey (*Cercopithecus aethiops*) obtained from the American Type Culture Collection

(ATCC; USA) were used. The cells were cultured in Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM; Sigma-Aldrich, USA), which was supplemented with 5% fetal bovine serum (FBS; Gibco, USA), penicillin/streptomycin (200 U/ml), and amphotericin B (2.5 g/ml) from Sigma-Aldrich (USA). Cell culture was conducted according to the guidelines established by the ATCC (2022), and the environment was maintained at 37 °C with 5% CO₂ and humidity.

The arboviruses Mayaro (*Alphavirus*), Chikungunya (*Alphavirus*), Zika (*Orthoflavivirus*), and Oropouche (*Orthobunyavirus*) were used in the antiviral trials. The Mayaro virus, strain Acre27 (GenBank KM400591), was initially isolated during an epidemiological survey from a blood sample of a febrile, malaria-negative patient collected in Acrelândia, in the Amazon Basin, Acre, Brazil (Terzian et al. 2015). The Chikungunya virus strain S27-African (GenBank AF369024) was initially isolated from a febrile patient and serves as a prototype for the African genotype (Khan et al. 2002). Zika virus strain PE243/2015 (GenBank KX197192.1) was initially recovered from a patient in the state of Pernambuco, Brazil, who presented with the classic symptoms of Zika fever, but did not have any neurological complications during the 2015 outbreak (Donald et al. 2016). The Oropouche virus, strain BeAn19991 (GenBank KP052850-KP052852), which represents the prototype of the Brazilian genotype, was initially isolated from a "sloth" (*Bradypus tridactylus*) in Pará (Brazil) in 1960, made by Saeed et al. (2000).

Cytotoxicity Assays

Vero cells were seeded in 96-well microplates at a density of 5×10^4 cells/well and incubated at 37 °C overnight. After this period, the culture medium was replaced with *F. citrifolia* samples at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml obtained by twofold serial dilutions in DMEM supplemented with 1% FBS. All assays were performed in technical duplicates and repeated in three independent biological experiments. DMSO was used to solubilize the extracts, maintaining the same proportion as the vehicle control. The final DMSO concentration did not exceed 1% (v/v) at the highest extract concentration evaluated (1000 µg/ml). The plates were incubated at 37 °C for 48 h. After the incubation period, the cells were evaluated under optical microscopy, and then, 25 µl MTT (methylthiazolyl diphenyl tetrazolium bromide, 1 mg/ml) was added to determine cell viability by colorimetric assay, and absorbance was measured at 490 nm using a spectrophotometer. Cell viability values, calculated from the absorbance of groups, were plotted against extract concentrations. The 50% cytotoxic concentration (CC₅₀) was determined using GraphPad Prism software (version 8.0) through non-linear regression analysis based on dose–response curves.

Antiviral Activity Assays

Vero cells were seeded in 96-well plates at a density of 5×10^4 cells/well and infected with MAYV, CHIKV, ZIKV, or OROV at a multiplicity of infection (MOI) of 1. Infected cells were then treated with *F. citrifolia* at non-cytotoxic concentrations of 250, 125, 62.5, 31.25, 15.6, and 7.8 $\mu\text{g/ml}$, obtained by twofold serial dilutions in DMEM supplemented with 1% FBS. The control groups, which include uninfected and untreated cells, received only the culture medium. In contrast, the virus control groups were infected without any treatment. All tests were carried out in triplicate and incubated for 48 h at 37 °C for MAYV, CHIKV, and OROV, and 72 h for ZIKV. All assays were performed in technical duplicates and repeated in three independent biological experiments. After the incubation period, the cells were evaluated under optical microscopy and then 25 μl MTT (1 mg/ml) were added to determine cell viability by colorimetric assay, and absorbance was measured at 490 nm, using a spectrophotometer. Cell viability values, calculated from the absorbance of groups, were plotted against extract concentrations. The effective concentration for 50% of the cells (EC_{50}) values were determined using GraphPad Prism 8.0 software through non-linear regression analysis based on dose–response curves and represented the concentration that protected 50% of the infected cells compared to the virus control. The antiviral selectivity index of the extract was assessed using the selectivity index (SI), which is defined as the ratio of CC_{50} to EC_{50} .

Results and Discussion

Putative Identification of Compounds

High-resolution LC–MS/MS analysis in the negative $[\text{M}-\text{H}]^-$ and positive $[\text{M}+\text{H}]^+$ modes enabled the annotation of 20 chemical compounds belonging to different classes, such as phenolic acids, flavonoids (flavones, flavanones, flavonols, isoflavones), lignans, fatty acids, and terpenes. Of these, 10 were annotated through an integrated approach, which combined high-precision experimental data in comparison with the fragments found in the literature (MSI level 3) and 10 through analysis carried out using the GNPS platform (MSI level 2). The results obtained so far provide a solid basis for understanding the chemical composition of the species.

Table 1. shows the compounds annotated with their respective experimental m/z values compared to the theoretical values calculated from the molecular formulas. The experimental error remained below 5 ppm for all the compounds, indicating high precision in the identification. Compounds such as caffeic acid (1), identified on the basis of its

characteristic fragment m/z 135 (Sieniawska et al. 2022), pinoresinol (13) with fragments m/z 342, 327, 311, 151, and 136 (Patyra et al. 2022). Naringenin (14), with m/z 151, 119, and 107 (Zeng et al. 2018), (iso)orientin (5) with m/z 327, 297, and 285 and (iso)vitexin (8) m/z 311, 283, 269, and 134 (Amaral et al. 2019), proved to be consistent with data previously reported in the literature, giving greater reliability to the annotation carried out.

Among the flavonoids, the isoflavones isowigtheone (17) and alpinumisoflavone (18) were observed. Their characteristic fragmentations corroborate the proposed structures, including the loss of the prenyl group, resulting in the ion of m/z 283 and 271, common to all of them. Isowigtheone (17) showed fragments at m/z 283, 271, 255, and 153 (Hostettmann et al. 2005), and alpinumisoflavone (18) showed fragments at m/z 319, 295, 283, 271, and 247 (Chen et al. 2024). The triterpene lupenone (19) with ions at m/z 407, 231, 191, 177, and 109 (Wang et al. 2020) and the steroid stigmasterol (20) with m/z at 311, 297, 283, and 255 were also identified, reinforcing the chemical complexity of the species (Rozenberg et al. 2003). The compounds orientin (2), vitexin (3), (*epi*)loliolide (4), loliolide (6), narigerin-7-*O*-glucoside (7), eriodictiol (9), luteolin (10), quercetin (11), ethyl caffeate (12), apigenin (15), and pinocembrin (16) were identified using the GNPS platform. This tool made it possible to correlate the experimental mass spectra with data libraries of previously described compounds.

Cytotoxicity and Antiviral Activity of the Extract

The ethanolic extract of *F. citrifolia* leaves exhibited low cytotoxicity with a CC_{50} value of 793.92 $\mu\text{g/ml}$ (Fig. 1a). The effective concentration that protects 50% of cells (EC_{50}) infected with MAYV was 61.24 $\mu\text{g/ml}$ (Fig. 1b), while EC_{50} values of 117.92 $\mu\text{g/ml}$ and 117.29 $\mu\text{g/ml}$ were obtained for CHIKV (Fig. 1c) and ZIKV (Fig. 1d), respectively. The selectivity index (SI) was highest for MAYV (12.96), followed by CHIKV (6.73) and ZIKV (6.77), as presented in Table 2. Although viral load was not directly quantified, the observed protection against virus-induced cytopathic effects supports the presence of bioactive compounds with antiviral potential, warranting further validation using direct viral quantification assays.

No antiviral activity was observed against OROV. The selectivity of antiviral compounds is often determined by the stages of the viral replication cycle and by the mechanism of action of the compounds present in the extract (Mazzon et al. 2019; Geraghty et al. 2021). MAYV and CHIKV are both Alphaviruses from *Togaviridae* with positive-sense single-stranded RNA genomes (Figueiredo et al. 2014), while ZIKV, a member of the family *Flaviviridae*, also possesses a positive-sense RNA genome (Pielnaa et al. 2020). Therefore, the lack of effect against

Table 1 Chemical compounds annotated for the ethanolic extract of *Ficus citrifolia* leaves

Compound	RT (min)	Molecular formula	Experimental <i>m/z</i>	Theoretical <i>m/z</i>	Error (ppm)	Fragments (MS/MS)	Putative name	Chemical class	Ionization mode
1	0.51	C ₉ H ₈ O ₄	179.0346	179.0344	1.1	135	caffeic acid*	Phenolic acid	[M–H] [–]
2	2.43	C ₂₁ H ₂₀ O ₁₁	447.0920	447.0927	1.5	357, 327, 297, 285	orientin	Flavone	[M–H] [–]
3	2.76	C ₂₁ H ₂₀ O ₁₀	431.0973	431.0978	1.1	311, 284, 283, 269, 239, 161, 135	vitexin	Flavone	[M–H] [–]
4	2.89	C ₁₁ H ₁₆ O ₃	197.1172	197.1178	3.0	197, 179, 161, 135, 107	(epi)loliolide	Monoterpene	[M+H] ⁺
5	3.01	C ₂₁ H ₂₀ O ₁₁	447.0920	447.0927	1.5	327, 297, 285	(iso)orientin*	Flavone	[M–H] [–]
6	3.29	C ₁₁ H ₁₆ O ₃	197.1178	197.1178	0	197, 179, 161, 135, 107	loliolide	Monoterpene	[M+H] ⁺
7	3.55	C ₂₁ H ₂₂ O ₁₀	433.1122	433.1135	3.0	433, 313, 295, 272, 271	naringenin-7- <i>O</i> -glucoside	Flavanone	[M–H] [–]
8	3.56	C ₂₁ H ₂₀ O ₁₀	431.0968	431.0978	2.3	311, 283, 269, 134	(iso)vitexin*	Flavone	[M–H] [–]
9	4.59	C ₁₅ H ₁₂ O ₆	287.0552	287.0556	1.3	287, 151, 135, 125, 107	eriodictyol	Flavanone	[M–H] [–]
10	4.78	C ₁₅ H ₁₀ O ₆	285.0398	285.0399	0.3	285, 151, 133, 107	luteolin	Flavone	[M–H] [–]
11	4.88	C ₁₅ H ₁₀ O ₇	301.0345	301.0348	0.9	178, 159, 151, 139, 121, 107	quercetin	Flavonol	[M–H] [–]
12	5.05	C ₁₁ H ₁₂ O ₄	207.0654	207.0657	1.4	207, 179, 161, 135, 134	ethyl caffeate	Phenolic acid	[M–H] [–]
13	5.09	C ₂₀ H ₂₂ O ₆	357.1335	357.1338	0.8	342, 327, 311, 151, 136	pinoresinol*	Lignan	[M–H] [–]
14	5.50	C ₁₅ H ₁₂ O ₅	271.0607	271.0606	0.3	151, 119, 107	naringenin*	Flavanone	[M–H] [–]
15	5.60	C ₁₅ H ₁₀ O ₅	269.0453	269.0450	1.1	159, 151, 117, 107	apigenin	Flavone	[M–H] [–]
16	7.71	C ₁₅ H ₁₂ O ₄	255.0656	255.0657	0.3	255, 213, 187, 151, 125, 107	pinocembrin	Flavanone	[M–H] [–]
17	9.41	C ₂₀ H ₁₈ O ₅	339.1222	339.1232	2.9	283, 271, 255, 153	isowighteone*	Isoflavone	[M+H] ⁺
18	10.29	C ₂₀ H ₁₆ O ₅	337.1070	337.1076	1.7	319, 295, 283, 271, 247	alpinumisoflavone*	Isoflavone	[M+H] ⁺
19	17.94	C ₃₀ H ₄₈ O	425.3763	425.3783	4.7	407, 231, 191, 177, 109	lupenone*	Triterpene	[M+H] ⁺
20	19.04	C ₂₉ H ₄₈ O	413.3764	413.3783	4.5	311, 297, 283, 255	stigmastrol*	Steroid	[M+H] ⁺

*Notes made based on chemical prospecting of previous studies reported for the genus and comparative analysis of spectra available in the literature

OROV may be attributed to its segmented negative-sense RNA genome, which involves replication mechanisms distinct from those of unsegmented arboviruses (Emmanuel et al. 2021; Files et al. 2022). Nevertheless, further studies investigating antiviral mechanisms of action are required to better characterize the exact action of the *F. citrifolia* extract. Still, the lack of a universal positive antiviral control across all tested arboviruses and the use of a cell viability-based assay represent limitations of this study;

however, these choices were justified by the exploratory nature of the screening and the absence of compounds with broad activity against all viruses evaluated.

Ficus species are rich in diverse secondary metabolites such as flavonoids, terpenes, phenols, sterols, and lignans (Chandra et al. 2023), which may contribute to their reported biological activities. In this study, the high selectivity index (SI) values, particularly against MAYV (12.96), suggest a favorable antiviral profile with low cytotoxicity, supporting

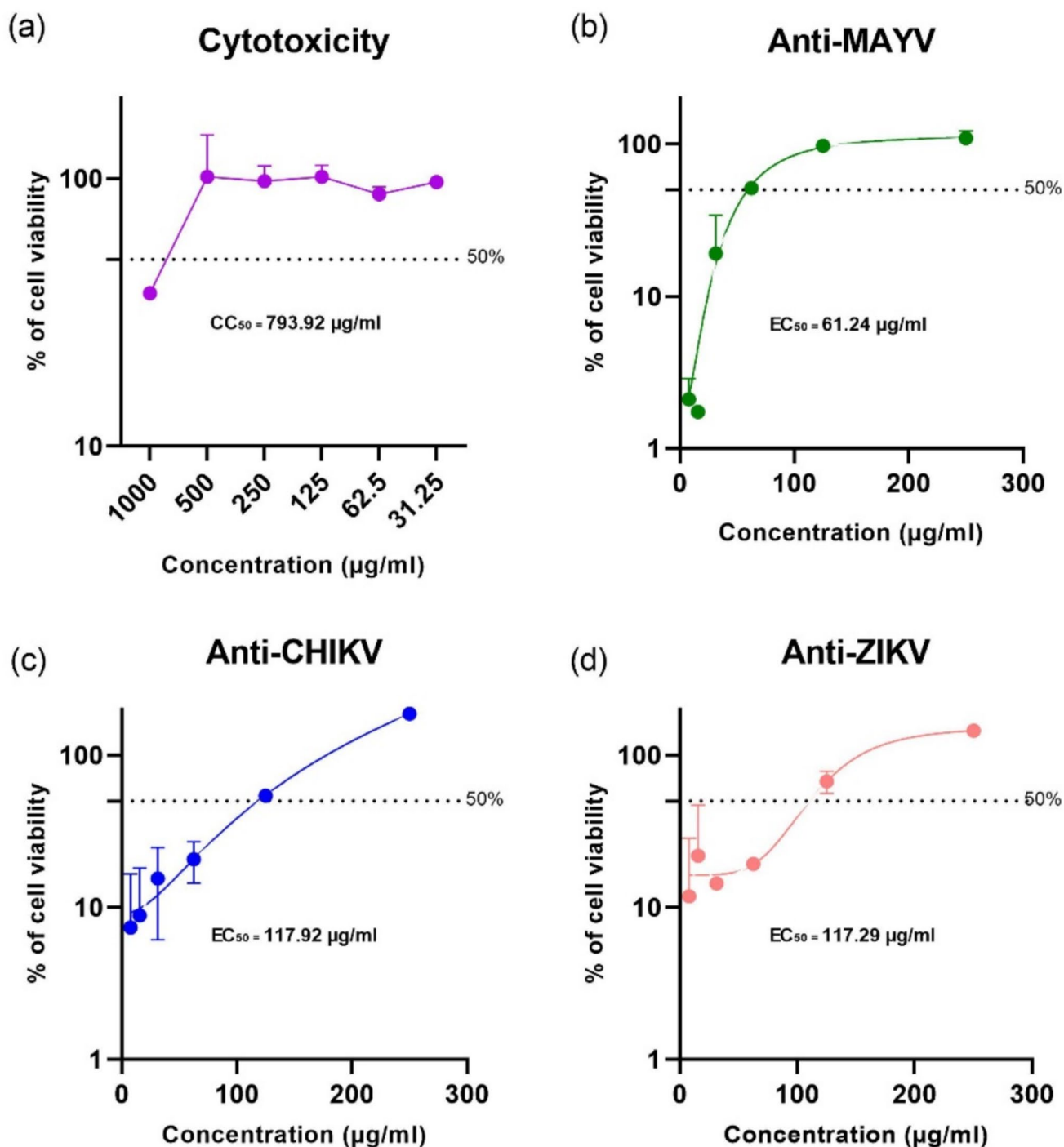


Fig. 1 Cytotoxicity and antiviral activity of ethanolic extract of *Ficus citrifolia* leaves. **a** Cytotoxicity was assessed after 48 h of treatment with different concentrations of the ethanolic extract. Antiviral activity against **b** Mayaro virus, **c** Chikungunya virus, and **d** Zika virus

was evaluated after 48 h of infection with concomitant treatment at different extract concentrations. For both assays, cell viability was measured using the MTT assay. Data are presented as mean $\pm$ standard error of the mean (SEM)

the potential of the extract against Orthoflavivirus and Alphavirus.

High-resolution LC–MS/MS analysis was employed as a dereplication strategy to obtain a chemical profile of the

extract and to perform putative annotation of known compounds that could be associated with the observed antiviral activity. Among the phenolic acids, caffeic acid (1) has been reported to inhibit ILHV and ZIKV by binding to

Table 2 Antiviral activity and cytotoxicity threshold for the ethanolic extract of *Ficus citrifolia* leaves

Cytotoxic concentration (CC ₅₀ , µg/ml)	793.92	
Arbovirus	Effective concentration (EC ₅₀ , µg/ml)	Selectivity index (SI)
MAYV	61.24 ± 5.56	12.96
OROV	nd	nd
CHIKV	117.92 ± 2.52	6.73
ZIKV	117.29 ± 37.16	6.77

Note: MAYV, Mayaro virus; OROV, Oropouche virus; CHIKV, Chikungunya virus; ZIKV, Zika virus; nd, not detected

the viral envelope protein (Saivish et al. 2023). Its derivative, ethyl caffeate (12), also exhibited antiviral effects by inhibiting HIV protease (Wang et al. 2019).

Several flavonoids annotated in the extract have been previously described as antiviral agents. Orientin (2) has been shown to block SARS-CoV-2 entry into host cells (Bhowmik et al. 2021), whereas isoorientin (5) inhibits the spike-ACE2 interaction (Chen et al. 2023). Luteolin (10) displays broad-spectrum antiviral activity against influenza, herpesvirus, and coronaviruses (Lu et al. 2023). Vitexin (3) and isovitexin (8) inhibit H1N1 infection and have demonstrated protective effects against COVID-19 (Sadati et al. 2019; Ferdausi et al. 2022). Apigenin (15) is known to interfere with the replication of DNA and RNA viruses, including HSV, HCV, DENV, and SARS-CoV (Lee et al. 2023).

Flavanones identified in the extract include eriodictyol (9), which modulates inflammatory cytokines relevant in CHIKV and MAYV infections (Deng et al. 2020). Naringenin (14) and its glycoside naringenin-7-O-glucoside (7), along with pinocembrin (16), reduce ZIKV RNA synthesis and affect viral proteins (Lee et al. 2019; Cataneo et al. 2021). Quercetin (11) disrupts MAYV entry and RNA replication (Santos et al. 2014), supporting the strong activity observed against this virus (EC₅₀ 61.24 µg/ml; SI 12.96).

The lignan pinoresinol (13), previously reported in *F. citrifolia*, has demonstrated anti-HBV effects (Wang et al. 2013). Prenylated isoflavones such as isowigtheone (17), alpinumisoflavone (18), and its isomer, known from the genus (Bankeu et al. 2011), may also contribute due to their increased lipophilicity and potential membrane interaction (Ateba et al. 2019).

Monoterpenes like loliolide (6), previously identified in *Ficus* species (Zhang et al. 2024), have been reported to inhibit HCV entry (Chung et al. 2016). Its epimer, (*epi*) loliolide (4), although not associated with antiviral activity, exhibits anti-hepatocellular carcinoma properties (Gangadhar et al. 2020). The triterpene lupenone (19) has shown activity against CHIKV, while stigmasterol (20)

inhibits HSV replication (Petrera et al. 2014; Bhakat and Soliman 2015).

Taken together, these results suggest that the antiviral effects of the extract are likely the result of combined or synergic actions among multiple constituents rather than the activity of a single compound (Caesar and Cech 2019). The chemical diversity revealed by LC–MS/MS-assisted dereplication supports the antiviral activity observed by highlighting *F. citrifolia* as a promising source of bioactive metabolites.

In this context, the present study was designed as an initial antiviral screening combined with chemical profiling to qualitatively associate the observed biological with the known chemistry of the extract using cytotoxicity and global antiviral assays, which are widely applied in early-stage antiviral prospection. Further studies, including bioguided fractionation, direct viral quantification, time-of-addition, virucidal and post-entry assays, as well as molecular docking approaches, will be necessary to elucidate specific mechanisms of action and identify the compounds responsible for the antiviral effects.

Finally, the antiviral activity demonstrated against arboviruses may provide preliminary scientific support for the traditional use of *F. citrifolia* in the treatment of inflammatory and febrile conditions commonly associated with arboviral infections. Although inflammation is often a secondary manifestation of acute viral diseases, the proposed relationship between anti-inflammatory properties and antiviral effects remains hypothetical and was not directly evaluated in this study. Overall, these findings integrate traditional knowledge, chemical profiling, and antiviral screening, highlighting the potential of *F. citrifolia* as a source of bioactive metabolites relevant to arboviral diseases.

Acknowledgements The authors would like to thank the Universidade Federal do Pará and the CAPES for their support in awarding a master's degree scholarship.

Author Contribution HAM and MNS designed the studies. HAM, AMSA, LCRA, and JCCS conducted LC–MS experiments and data processing. CLBG designed antiviral assays. HAM and JCCS performed metabolite characterization. HAM, AMSA, LCRA, and AJBM searched for the pharmacological properties of the annotated metabolites. ACF, MBSM, and CLBM performed and discussed *in vitro* antiviral assays with the extracts. HAM, AMSA, JCCS, AJBM wrote the manuscript. SGSRP, CLBM, AJBM, CYYS, and MNS reviewed the whole manuscript and provided comments. All authors have read and agreed to the published version of the manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Declarations

Ethics Approval Not applicable. This study did not involve human participants or animal experiments.

Competing interests The authors declare no competing interests.

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