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Pharmacognosy
Antiviral activity and chemical characterization of *Cissus erosa* (Vitaceae) ethanol extracts

Adriana Cotta Cardoso Reis[1], *Hélia Maria Marques de Moura*[1], *Breno Mello Silva*[2], *Alaíde Braga de Oliveira*[3] & *Geraldo Célio Brandão*[1,4,5]

Abstract
Cissus erosa (Vitaceae), popularly known in Brazil as Cipó-fogo, is a medicinal plant used in the treatment of warts and external ulcers. The present study aimed to evaluate the activity of stems and leaves ethanol extracts of *C. erosa* against the Dengue and Zika virus by the MTT colorimetric method and to carry on the phytochemical characterization of active extracts by high performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Only the leaves ethanol extract showed anti-Dengue virus activity EC_{50} 18.2 μg/ml (SI > 27.5) and low cytotoxicity for $LLCMK_2$ cells (CC_{50} > 500 mg/ml). Both extracts (stems and leaves) showed anti-Zika virus activity with EC_{50} of 45.8 mg/ml and 82.8 mg/ml, respectively. These extracts presented CC_{50} of 309.2 μg/ml (leaves) and 387.6 μg/ml (stems) to Vero cells. Analysis by CCD and HPLC-DAD detected the presence of triterpenes, steroids, flavonoids and tannins. UPLC-MS analyses of these extracts, allowed the identification of the majority of flavonoids present known as vitexin, isovitexin, orientin, isoorientin and two flavones derivatives, methoxyluteolin-6(8)-*C*-hexosyl and luteolin-7,4'-di-*O*-glycosylflavone. The results of the phytochemical studies here described suggest that flavonoids and terpenoids are the substances that contribute to the antiviral activity of the ethanol extracts within this species.
Key words: antiviral activity, Dengue virus, flavonoids, Zika virus.

Resumo
A espécie *Cissus erosa* (Vitaceae) conhecida popularmente, no Brasil, como Cipó fogo é uma planta medicinal empregada no tratamento de verrugas e úlceras externas. O presente estudo objetivou a avaliação da atividade antiviral frente a Dengue e Zika virus dos extratos etanólicos de caules e de folhas desta espécie pelo método colorimétrico do MTT e determinar a caracterização fitoquímica por cromatografia líquida de ultra eficiência acoplada a espectrometria de massa destes extratos (CLUE-EM). Os extratos apresentaram atividade antiviral e baixa citotoxicidade. O extrato de folhas apresentou CC_{50} > 500 μg/ml para células $LLCMK_2$ e CE_{50} = 18,2 μg/mL, IS > 27,5 contra o Dengue virus 2 (DENV 2). Ambos os extratos (caules e folhas) apresentaram atividade anti-Zika virus com CE_{50} de 45,8 μg/ml e 82,8 μg/ml, respectivamente. Os extratos apresentaram CC_{50} de 309,2 μg/ml (folhas) e 387,6 μg/ml (caules) para células Vero. Nas análises por CCD e CLAE-DAD detectou-se a presença de triterpenos, esteroides, flavonoides e taninos. Análises por CLUE-EM dos extratos permitiram a identificação parcial dos flavonoides majoritárias destes extratos como sendo, vitexina, isovitexina, orientina, isoorientina, luteolina-7,4'-di-O-glicosilflavona, metoxiluteolia-6(8)-C-hexosil. Os resultados dos estudos fitoquímicos até o momento sugerem que flavonoides e terpenoides são os constituintes majoritários dos extratos etanólicos destas espécies e devem contribuírem para a atividade antiviral observada *in vitro*.
Palavras-chave: atividade antiviral, Dengue virus, flavonoides, Zika virus.

[1] Universidade Federal de Ouro Preto, Depto. Farmácia, Escola de Farmácia, Campus Morro do Cruzeiro s/n, Bauxita, 35400-000, Ouro Preto, MG, Brazil.
[2] Universidade Federal de Ouro Preto, Depto. Ciências Biológicas, ICEB, Campus Morro do Cruzeiro s/n, Bauxita, 35400-000, Ouro Preto, MG, Brazil.
[3] Universidade Federal de Minas Gerais, Faculdade de Farmácia, Depto. Produtos Farmacêuticos, Av. Antônio Carlos 6627, 31270-901, Belo Horizonte, MG, Brazil.
[4] ORCID: <https://orcid.org/0000-0002-7330-051X>
[5] Author for correspondence: celiobrandao@ufop.edu.br

Introduction

Dengue and Zika are arbovirus transmitted by Arthropods of the genus *Aedes*. For some years, this disease has been a major public health problem, especially in countries of tropical and subtropical regions of the world. Human infections occur after the bites of contaminated mosquitos (*Aedes aegypti* is the primary vector) (WHO 2019). There are currently four serotypes of the *Dengue virus* causing human infections (DENV 1, DENV 2, DENV 3 and DENV 4). Infections may be asymptomatic or feverish. However, some patients may progress to more severe conditions such as hemorrhagic fever and dengue shock syndrome. In *Zika virus* infections, the patient may also present fever as a symptom of the infection. Recently, however, the *Zika virus* infection has been linked to cases of microcephaly, a rare neurological condition generally identified in the gestational stage, and Guillan-Barré syndrome, which is an autoimmune disease that makes the immune system attack the nervous system causing inflammation on the nerves and muscular weakness (Martinez *et al.* 2019).

Viral infections are still a leading cause of death in the world, therefore, it is necessary to develop effective and safe antiviral agents to control these, through either preventive prophylactic therapeutic measures (vaccines) or healing drugs. In this context, a number of bioactive compounds come from plants, including antiviral substances, becoming an important strategy at the development of promising new antiviral drugs (Chattopadhyay & Naik 2007; Newman & Cragg 2016).

The Vitaceae family is a primarily pantropical family comprised of about 15 genera (Ingrouille *et al.* 2002) with some 700 species (Brizicky 1965). The largest genus is both *Cissus* L. and *Cyphostemma* (Planch.) Alston, and the most well-known is the genus *Vitis* L. (Mabberley 1987).

The *Cissus* L. genus has about 350 species widely distributed in the tropics worldwide with diversity centers spread through southern Asia, Australia, Africa, the Americas and Papuasia (Mabberley 1987). In the Neotropical Centers about 74 species occur in South America (Lombardi 2007; Liu *et al*. 2013). This genus is chemically characterized by the presence of stilbenoids (Wang *et al*. 2007), other compounds are commonly found, such as coumarins, terpenes, flavonoids, steroids (Beltrame *et al*. 2002), quinolizidine alkaloids (Saifah *et al.* 1983), saponins and cardiac glycosides (Nagani *et al.* 2011).

Some species of *Cissus* L. genus are used in folk medicine (Morton 1975; Oliveira *et al.* 2012). They present different biological activities such as analgesic, diuretic, in the treatment of leishmaniosis, kidney and respiratory diseases, topic use against warts and external ulcers (Agra *et al.* 2007), anti-inflammatory activity (García *et al*. 2000; Salazar *et al.* 2018), antioxidant activity and neuroprotective effect (Salazar *et al.* 2018), hypoglycemic and healing activities (Braga 2008) as well as an antifungal. There are also reports about the antiviral activity of this genus species (Mothana *et al*. 2006; Balasubramanian *et al*. 2010), including *Cissus erosa* Rich. (Simoni *et al*. 2007; Brandão *et al*. 2011).

The present study aimed to evaluate antiviral activity against *Dengue* and *Zika viruses* of ethanolic extracts from *Cissus erosa* performed with the MTT colorimetric method and phytochemical characterization by high-performance liquid chromatography coupled to mass spectrometer of this ethanolic extracts.

Materials and Methods

Plant materials

The leaves and stems of *Cissus erosa* Rich. were collected at the Ecological Station - UFMG in Belo Horizonte, Minas Gerais, Brazil (19º55'15"S, 43º56'16"W) and the taxonomic determination was made by the botanist Dr. J.R. Stehman, Botany Department, Institute of Biological Sciences, UFMG, in Belo Horizonte, Brazil. The voucher specie, BHCB48733, was deposited at the BHCB/UFMG herbarium, in Belo Horizonte.

Preparation of extracts

The leaves and stems of *C. erosa* were separated and dried in a forced ventilation oven at 40 ºC. Then the plant material was ground in a knife mill and extracted with 96 % ethanol at room temperature. The solvent was removed in a Buchi Rotary Evaporator under reduced pressure at 50 ºC, leaving dark residues which were kept in a vacuum desiccator up to constant weight.

Phytochemical prospection by TLC

The phytochemical prospection of *C. erosa* extracts were done by thin layer chromatography (TLC) to detect the presence of different classes of natural products. The flavonoids detection of leaves and stem extracts was performed using ethyl acetate: formic acid: acetic acid: water

(100:11:11:27) mobile phase, rutine and orientin as standards sample. Then the plates were observed under UV (254 and 365 nm) and visible light, before and after spraying with natural product reagent (NP-PEG. Tanins detection was done using the same mobile phase to flavonoids, and the plates were observed under UV (254 and 365 nm) and visible light, but as a developer, potassium ferrocyanide was utilized, for this investigation, the standards used were protoanthocyanidine B2 and tannic acid. Terpenes and steroids detection of these extracts were performed using n-hexane: ethyl aacetate (1:1) as mobile phase, and as standards samples the β-sitosterol and betulinic acid were used. The plates were observed under UV (254 and 365 nm) and visible light, before and after with spraying sulfuric anilsaldehyde.

HPLC analyses

The extracts were characterized by HPLC-DAD, with online registration of the UV spectra of the constituents. HPLC fingerprint was registered on a Waters 2695 apparatus with a UV-DAD detector (Waters 2996). Conditions - A LiChrospher 100 RP-18 column (5 µm, 250 × 4 mm i.d.) (Merck) was employed at a temperature of 40 ºC, flow rate of 1.0 ml/min and detection at wavelengths of 220, 280 and 350 nm. Elution was carried out with a linear gradient of water (A) and acetonitrile (B) (from 5% to 95% of B in 60 min) (Brandão *et al*. 2013).

UPLC-DAD-MS and UPLC-ESI-MS/MS analyses

The UPLC-DAD-MS and UPLC-ESI-MS/MS analyses were performed using an UPLC Acquity (Waters) ion trap mass spectrometer in the following conditions: positive and negative ion mode; capillary voltage, 3,500 V; capillary temperature, 320 ºC; source voltage, 5 kV; vaporizer temperature, 320 ºC; corona needle current, 5 mA; and sheath gas, nitrogen, 27 psi. Analyses were run in the full scan mode (100–2000 Da). The UPESI-MS/MS analyses were additionally performed in an UPLC Acquity (Waters) with argon as the collision gas, and the collision energy was set at 30 eV. Chromatographic separation was done on ACQUITY UPLC HSS column (1.7 µm, 50 × 2 mm i.d.) (Waters). The mobile phase consisted of water 0.1% formic acid (solvent A) and acetonitrile 0.1% formic acid (solvent B). The elution protocol was 0–11 min, linear gradient from 5% to 95% B. The flow rate was 0.3 mL min-1, and the sample injection volume was 4.0 µL. The UV spectra were registered from 220 to 400 nm. Mass spectrometry analysis was performed by Waters ACQUITY® TQD equipped with on quadrupole instrument fitted with an electrospray source in the positive and negative ESI mode. Ion spray voltage: -4 kV; orifice voltage: -60 V.

Virus and cell line

Kidney cells of the Rhesus monkey *Macaca mulatta* ($LLCMK_2$ cell line ATCC® CCL-7™) and Kidney cells of the African green monkey *Cercopthecus aeothiops* (Vero cell line ATCC® CCL-81™) were used in the assays. The cells were cultivated in complete cell medium consisting of Dulbecco's modified Eagle medium (DMEM, Cultilab, Campinas, SP, Brazil), supplemented with 5% fetal bovine serum, 50 µg/mL gentamicin, 100 U/mL penicillin and 5 µg/mL amphotericin B (Brandão *et al*. 2013). The cells were grown in 37 ºC in a humidified atmosphere containing 5% CO_2 and harvested in log-phase for experimental use. Dengue virus and Zika virus strains were kindly donated by Dra. E. Kroon (UFMG, Belo Horizonte, Brazil). The virus was titrated by $TCID_{50}$ in Vero cells, as established by Rodriguez *et al*., in 1990 and the titers were 1.0×10^4 $TCID_{50}$/mL and 1.0×10^7 $TCID_{50}$/mL, respectively.

Cytotoxicity assay

The assays were performed using 96-well microtiter plates and $LLCMK_2$ (2.0×10^4 cells per well) and Vero (2.0×10^4 cells per well) cell lines were exposed to different concentrations of extracts for 72 h (Brandão *et al*. 2013). After incubation, cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich) assay at a concentration of 2 mg/mL in PBS (Mosmann 1983; Twentyman & Luscombe 1987). Each sample was assayed in three replicates for concentrations ranging from 400 to 1.5662 µg/mL. The cytotoxicity of each sample was expressed as CC_{50}, i.e., the concentration of sample that inhibited cell growth by 50% (Brandão *et al*. 2013).

Antiviral MTT assay

The antiviral activity measured by the 50% effectivity concentration (EC_{50}) ethanolic extract of *C. erosa* was evaluated by the MTT assay (Betancur-Galvis *et al*. 2002). $LLCMK_2$ cell

monolayer (2.0×10^4 cells per well) was infected with viral suspensions with titers of 1.0×10^4 $TCID_{50}$/mL, (MOI = 1.0), Dengue virus. Vero cell monolayer (2.0×10^4 cells per well) was infected with viral suspensions with titers of 1.0×10^7 $TCID_{50}$/mL, (MOI = 1.0), Zika virus. Dilutions of ethanolic extract in non-cytotoxic concentrations were added to the wells after viral infection, and as positive controls were used interferon α for anti-Dengue virus assay and ribavirin for anti-Zika virus assay. Experiments were carried out with eight different concentrations within the cytotoxic range of the samples. Plates were incubated at 37 ºC in humidified 5% CO_2 atmosphere for a period of 72 h (Brandão *et al*. 2013). The 50% inhibitory concentration of the viral effect (EC_{50}) for compounds and ethanolic extract were calculated from concentration-effect-curves after nonlinear regression analysis (Brandão *et al*. 2013). The selectivity index (SI) is defined as CC_{50} over EC_{50}, the higher the SI, the more promissory an extract is, due to its selectivity in inhibiting the virus multiplication cycle. Statistical calculations were carried out with the GraphPad prism 5.0 software package (Statistica). Results are expressed as the mean ± S.E.M. of 4 independent experiments. The t-test of students was used for statistical analyses; P values > 0.05 were considered to be significant.

Results

Phytochemical investigation by TLC, HPLC and UPLC analysis, as well as the identification of compounds from *C. erosa* extracts.

The preliminary phytochemical prospection of *C. erosa* extracts were performed by thin layer of chromatography (TLC) in order to detect the presence of different classes of natural products and these phytochemical analyses showed the presence of flavonoids, tannins and terpenoids (Fig. 1) in the ethanolic extracts.

The C-glucosylflavones orientin, isoorientin, vitexin and isovitexin were detected in the chromatographic analyzes by HPLC-FR (Fig. 2) of leaves and stems of *C. erosa*. These results were confirmed after co-injection with reference samples.

Identification and further confirmation of many components in the studied extracts were performed by UPLC-DAD-MS, which were used to obtain molecular mass ions and also characteristic fragment ions. Isolated compounds (rutine, orientin, vitexin, isoorientin and isovitexin) were used as

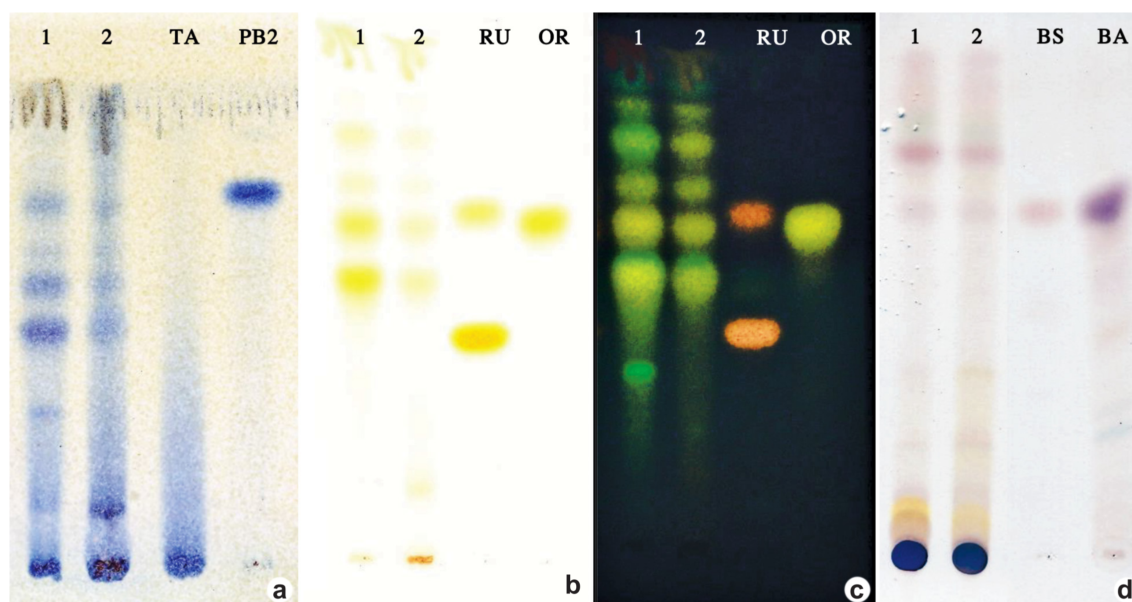

Figure 1 – a-d. CCD profiles of *Cissus erosa* leaf and stem ethanolic extracts – eluents: a-c. ethyl acetate - formic acid - acetic acid - water (100: 11: 11: 27); d. n-hexane - ethyl acetate (1:1). Developer: a. Potassium ferrocyanide; b,c. Natural Product Reagent (NP / PEG) - (B) visible; c. UV365 nm; d. Liebermann-Burchard Reagent. 1 = leaves extract; 2 = stems extract. Standards sample: TA = tannic acid; PB2 = proanthocyanidin B2; RU = rutine; OR = orientin; BS = β-sitosterol; BA = betulinic acid.

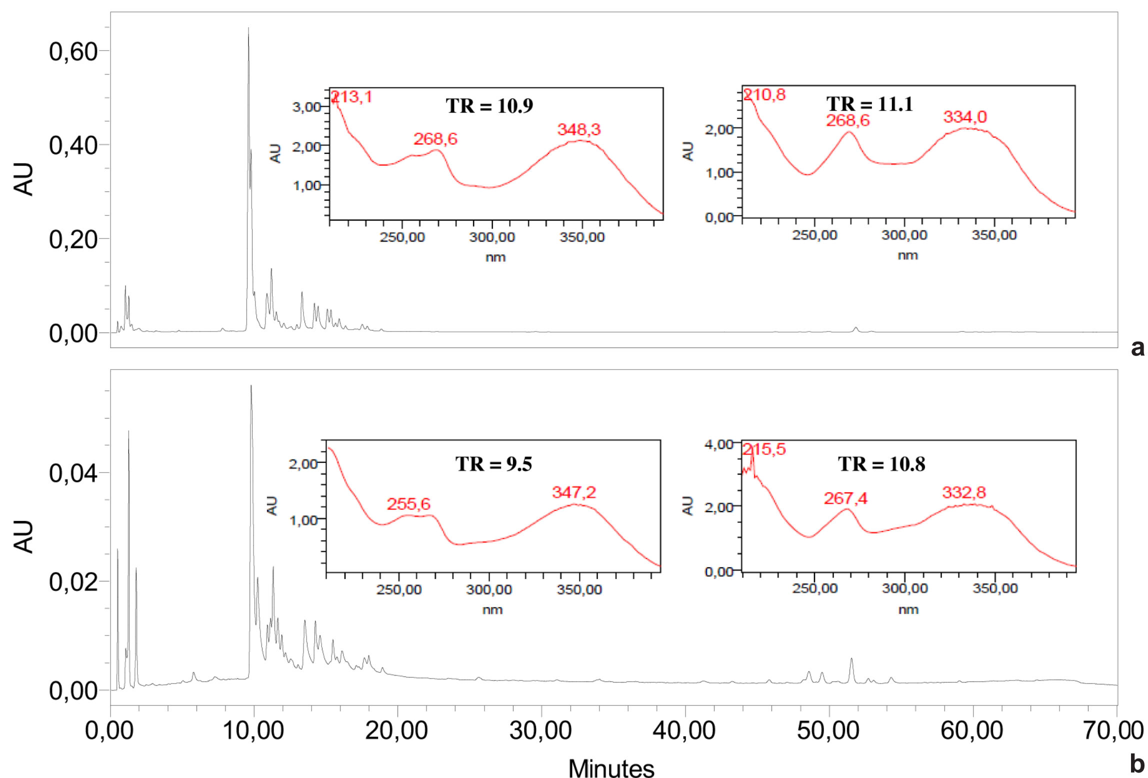

Figure 2 – a-b. HPLC-DAD chromatographic profiles of *Cissus erosa* – a. leaves extracts; stems extracts. Highlighting the UV spectra of orientin peaks (Rt = 9.5 min), vitexin (Rt = 10.8 min), isoorientin (Rt = 10.9 min) and isovitexin (Rt = 11.1 min).

standards for optimization of the separation of phenolic compounds using UPLC, and the ionization and fragmentation using ESI MS^2. The full scan mass spectrum (100–1000 Da) obtained from C. erosa ethanolic extracts by UPLC-DAD-MS in negative and positive ion modes are presented in Figure 3. Sensitivity was greater when chromatograms were acquired in negative-ion mode than in positive-ion mode, in which few peaks were observed. Tentative identification of plant components was performed by detailed fragmentation studies and by comparison with published data from the literature.

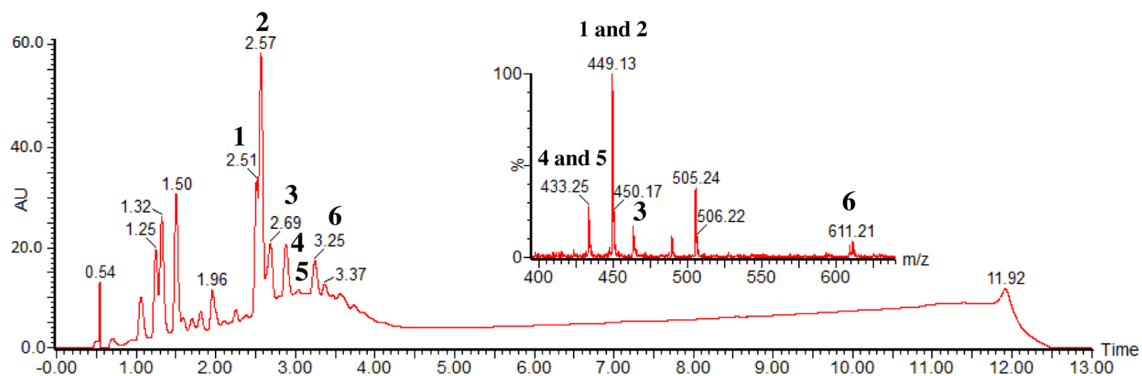

Figure 3 – UPLC-DAD-MS chromatographic profiles of *Cissus erosa* leaves extract. Highlighting the $[M+H]^+$ (*m/z*) spectra of flavonoids.

Furthermore, UPLC-DAD analysis from leaves and stems extracts allowed for the identifying of flavonoids as one of the main constituents of these ethanolic extracts. UPLC-EM analysis allowed the partial identification of the main flavonoids from Cissus erosa extracts as vitexin, isovitexin, orientin, isoorientin, luteolin-7,4'-di-O-glicosilflavon and methoxyluteolin-6(8)-C-hexosil (Tab. 1).

There were two flavones from retention time of 2.51 and 2.57 min and molar mass of 448 Da, which were detected in the *Cissus erosa* extracts. In the full-scan mass spectra in positive mode, protonated molecules of *m/z* 449.19 and 449.13 were observed, as well as the deprotonated molecules of *m/z* 447.17 and 447.10 in negative mode, respectively. The fragments of the ions obtained by MS^2 experiments and the comparison with the literature data allowed the suggestion that the compounds of Rt 2.51/2.57 have as a result a structure similar to orientin/isoorientin.

Other phenolic compound with molar mass of 464 Da exhibiting a retention time of 2.69 min, and in the full-scan mass spectra were observed, the protonated molecule of *m/z* 465.33 in the positive mode and in the negative mode the deprotonated molecule was of *m/z* 463.24. The ions fragments obtained by MS^2 experiment allowed to suggest that this compound has a structure similar to the flavone methoxyluteolin-6(8)-hexosyl.

In the retention time of 2.73 and 2.88, two other C-glycosyl-flavones were identified. In the positive mode of the full-scan mass spectra, the protonated molecules of *m/z* 433.19 and 433.12 were observed, while in the negative mode, the deprotonated molecules were *m/*z 431.23 and 431.36. The ions fragments obtained by MS^2 experiments and the comparison with the literature data allowed to suggest that the compounds of Rt 2.73/2.88 have a structure similar to vitexin/isovitexin,

A luteolin derivate was identified in the *C. erosa* extracts with a retention time of 3.25 and molar mass of 610 Da. The full-scan mass spectra showed in the negative mode the deprotonated molecule of *m/z* 609.35 and the protonated molecule of *m/z* of 611.28 was observed in the positive mode. The ions fragments obtained by MS^2 experiment allowed to suggest that the compound has a structure similar to the flavone luteolin-7,4'-di-O-glycosylflavon.

Antiviral assay of *C. erosa* extract

Leaves and stem ethanolic extracts of the *C. erosa* were evaluated for *in vitro* antiviral activity against the Dengue virus in $LLCMK_2$ cell line at concentrations ranging from 500 to 1.9531 µg/mL. and against the Zika virus in Vero cell line at concentrations ranging from 400 to 1.5625 µg/mL. The leaves extract presented EC_{50} of 18.2 µg/mL against the Dengue virus and EC_{50} of 82.8 µg/mL against the Zika virus. The stems extract was active against the Zika virus presenting EC_{50} of 45.8 µg/mL, however, against the Dengue virus, it was not active. For this assay, two positive controls were used: interferon α and ribavirin that presented EC_{50} of 94.5 µg/mL. The results of mean effectivity concentrations (EC_{50}) for each extract and positive controls (interferon α and ribavirin) are described in Table 2, as well as the results of mean cytotoxicity concentrations (CC_{50}) at the $LLCMK_2$ and Vero cell lines.

In order to predict promising drugs, selectivity index (SI) is the selection parameter used. The SI for the active ethanolic extracts ranged from 27.5 to 3.69, to the Dengue virus

Table 1 – Flavonoids identified in ethanolic extract of leaves and stems of *Cissus erosa*.

	Compounds	Rt (min)	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	MS MS Fragments (*m/z*)
1	Orientin	2.51	265, 347	449.19	447.17	429, 357, 339, 327
2	Isoorientin	2.57	269, 348	449.13	447.10	357, 339, 327
3	Methoxyluteolin-6(8)-C-hexosyl	2.69	268, 342	465.33	463.24	341, 313, 285
4	Vitexin	2.73	269, 334	433.19	431.23	413, 341, 311
5	Isovitexin	2.88	270, 332	433.12	431.36	341, 311
6	Luteolin-7,4'-di-*O*-glycosylflavone	3.25	269, 343	611.28	609.35	447, 285

Table 2 – Antiviral (EC_{50}) and cytotoxic (CC_{50}) activities of *Cissus erosa* extracts.

Extract / Positive control		LLCMK$_2$ CC_{50} µg/mL[a]	DENV-2 EC_{50} µg/mL[b]	SI[c]	Vero CC_{50} µg/mL[a]	ZIKV EC_{50} µg/mL[b]	SI[c]
Cissus erosa	Leaves	> 500.0	18.2 ± 0.7	> 27.5	309.2 ± 14.1	82.8 ± 2.4	3.7
	Stems	83.5 ± 16.9	NA[d]		387.6 ± 9.0	45.8 ± 3.1	8.5
Interferon α				2.5×10^{3} [e]			
Ribavirin					370.4 ± 1.2	94.5 ± 2.7	3.9

[a] = 50 % cytotoxic concentration; [b] = 50 % effective concentration of viral replication; [c] = SI (Selectivity index): ratio between extract CC_{50} and EC_{50} ;
[d] = NA: No active; [e] = 80 a 100 % cytopathic effect inhibition and UI/mL concentration.

type 2, the leaves extract presented a higher SI, on the other hand, the stem extract presented SI of 8.40 that is a better SI to the Zika virus. The positive control ribavirin was 3.92, thus showing an interesting antiviral activity of the *C. erosa* extracts against the Zika virus.

Discussion

The phytochemical investigation of *C. erosa* ethanolic extracts showed the presence of phenolic compounds such as tannins and flavonoids, besides triterpenes and steroids. The prospection phytochemical was first performed by TLC as a preliminary test, and then the UPLC-DAD-MS and UPLC-ESI-MS/MS analyses were performed, which confirmed the presence of flavonoids. The main flavonoids of leaves and stem extracts were partially identified by the UPLC-DAD-MS analyzes as the following flavones: vitexin, isovitexin, orientin, isoorientin, luteolin-7,4'-di-O-glycosylflavon and methoxyluteolin-6(8)-C-hexosyl. The stems ethanolic extract were more cytotoxic when compared to the leaves extract, however, this cytotoxicity was considered low, which is desired for the screening of antiviral activity.

Flavonoids identified in all *C. erosa* extracts belong to the C-glycosyl-flavones class. Literature data show that in the Vitaceae family, the occurrence of this class of natural products was previously reported (Moore 1987), but in the *C. erosa* they were first identified.

Potential antiviral of the flavonoids is widely reported in the literature, and in the review of Perez (2003), a large number of flavonoids was described as antiviral compounds such as quercetin, rutine, luteolin, apigenin, kaempherol, naringenin and their glycoside derivates.

Evaluation of *in vitro* antiviral activity of the ethanolic extracts of *C. erosa* against Dengue and Zika viruses showed that leaves extract was active against the two-virus tested, inhibiting the virus multiplication cycle with EC_{50} of 18.2 µg/mL against DENV-2 and ZIKV an EC_{50} of 82.8. Thus, it presented better activity against DENV-2 with a selectivity index of 27.5 while with ZIKV it was 3.7. Regarding the stem extract, it was only active against the Zika virus, which inhibits the virus multiplication cycle with EC_{50} of 18.2 µg/mL and the selectivity index of 8.4. Comparing the two active *C. erosa* extracts against ZIKV, the SI of stems extract were higher than the leaves extract, because it was less cytotoxic and protective to the cell monolayer in a lower concentration.

In the literature there are some reports about the antiviral activity of *C. erosa* that evaluated the *in vitro* antiviral activity against Human herpes simplex virus type 1 (HSV-1), Vaccinia virus Western Reserve (VACV-WR) and Murine encephalomyocarditis virus (EMCV). As a result, it was observed that leaves extract showed low activity against the HSV-1 (EC_{50} = 97.2 µg/mL and SI = 3.1) and it was not active against VACV-WR and EMCV. While the stems extract presented itself as moderately active for HSV-1 (EC_{50} = 304.1 µg/mL and SI = 3.2) and a favorable activity against VACV-WR (EC_{50} = 27.9 and SI = 13.8) (Brandão *et al*. 2011). Furthermore, Simoni *et al*. (2007) investigated the antiviral activity of *C. erosa* against Bovine herpesviruses type 1 (BoHV-1), Infectious bursal disease virus (IBDV) and Avian reovirus, consequently, an antiviral activity against BoHV-1 was observed with 96 % inhibition percentage and an inhibition viral index of 1.41.

There are other reports in the literature about antiviral activity of the genus *Cissus* species, such

as antiviral activity of *C. quadrangulares* against HSV-1 and HSV-2 (Balasubramanian *et al*. 2010), anti-Influenza A virus and anti-HSV-1 activities of *C. hamaderohensis* and *C. subaphylla* (Mothana *et al*. 2006). Despite this, in the literature there are no reports about *in vitro* evaluation of anti-Zika virus and anti-Dengue virus type 2 activities of *C. erosa*.

The phytochemical characterization by TLC and UPLC-EM and the antiviral activity of *Cissus erosa* observed in this study are in agreement with the literature data, since poliphenols, like flavonoids are one of the major class of secondary metabolites with antiviral activity against several viruses (Kamboj *et al*. 2012; Johari *et al.* 2012; Brandão *et al.* 2017).

In addition, the antiviral activity can be associated with the synergistic effect between flavonoids and other natural constituents, and this increased potency of the biological activity due to the combined effect of various compounds has been previously reported in the literature (Amoros *et al*. 1992; Mucsi *et al*. 1992). Thus, this data suggested that *C. erosa* can be a promissory source to continue investigating antiviral activity against the Dengue and Zika viruses.

Conclusions

The antiviral activity observed for the *C. erosa* ethanolic extracts joined with the phytochemical screening results until this moment, hence, allowing the suggestion that this activity may be related with the flavonoids identified in the extracts. Therefore, *Cissus erosa* is an interesting promisor specie with *in vitro* anti-Zika virus and anti-Dengue virus activity and it was necessary to continue studies in order to elucidate the compounds responsible for the observed activity.

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