academicJournals

Vol. 12(10), pp. 106-115, 24 March, 2018
DOI: 10.5897/JMPR2018.6567
Article Number: EF66B9556559
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antiviral activity of Brazilian Cerrado plant extracts against animal and human herpesviruses

Marina A. Padilla[1,2], Isabela C. Simoni[1]*, Verônica Moreira H. Hoe[1], Maria Judite B. Fernandes[1], Clarice W. Arns[2], Juliana R. Brito[3] and João Henrique G. Lago[4]

[1]Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico, Av. Cons. Rodrigues Alves, 1252, CEP 04014-900, São Paulo, SP, Brasil.
[2]Laboratório de Virologia Animal, Universidade Estadual de Campinas, Campinas, SP, Brasil.
[3]Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, São Paulo, Brazil.
[4]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, São Paulo, Brazil.

Received 9 February, 2018; Accepted 16 March, 2018

The Brazilian savanna known as "Cerrado" is very rich in medicinal plants that are used by the local population for treatment of several illnesses. The herpesvirus is a serious problem worldwide, and affects both animal and human health. This work aimed to study the antiviral activity of eight extracts from plants natives of "Cerrado" region against human (HSV-1), equine (EqHV-1) and swine herpesviruses (SuHV-1). The results showed that all plant extracts: *Banisteriopsis variabilis, Byrsonima intermedia, Campomanesia xanthocarpa, Erythroxilum deciduum, Lacistema hasslerianum, Ocotea pulchella, Stryphodendron adstringens and Xylopia aromatica* presented antiviral activity against at least one herpesvirus. Furthermore, it was observed a direct anti-herpes effect of extracts from *B. variabilis* and *B. intermedia* in non-toxic concentrations against all herpesviruses. *B. intermedia* crude aqueous extract showed the most promising results with selective index values of the 41.76 ± 0.04; 4.12 ± 0.1 and 193.97 ± 0.09 respectively against HSV-1, EqHV-1 and SuHV-1. Due to this, *B. intermedia* extract was also analyzed by HPLC/MS allowing for the identification of gallic acid and quercetin as main compounds.

Key words: Brazilian savanna**,** Cerrado, *Byrsonima intermedia*, antiviral, herpesvirus, gallic acid, quercetin.

INTRODUCTION

The herpes viruses are a serious worldwide problem that can affect the health of both animals and human beings. The herpes simplex virus (HSV-1) is pathogenic to humans (Wyler et al., 2017), whereas equine (EqHV-1) and swine herpesviruses (SuHV-1), are responsible for causing serious diseases in horses and pigs respectively, resulting in large economic losses (Wernike et al., 2013; Gulati et al., 2016). These viruses belong to the subfamily Alphaherpesvirinae, characterized by a rapid lytic viral cycle and the establishment of neuronal latency, which can be reactivated (Riaz et al., 2017). The emerging of resistant virus strains to available drugs against human

*Corresponding author. E-mail: isabelasimoni@gmail.com, simoni@biologico.sp.gov.br.

herpesvirus is a growing problem, particularly in immunocompromised patients (Vadlapudi et al., 2013). More recently, outbreaks of equine herpesvirus have had an impact on the equine industry, and stimulated interest in antiherpetic interventions (Vissani et al., 2016). The SuHV-1, also known as pseudorabies virus, is the causative agent of Aujeszky's disease affecting other mammals besides pigs, is still present in wild boar and there are no antiviral drugs approved for veterinary use (Zouharova et al., 2016). Therefore, it is necessary to find new alternative drugs against these diseases that affect both humans and animals (Martinez et al., 2015; Nocchi et al., 2016). Medicinal plants are natural resources, leading to valuable herbal products often used to treat a large number of various diseases in traditional medicine (Katiyar et al., 2012; Wachtel-Galor and Benzie, 2011).

The South American flora represents one of the world's richest sources of material with pharmacological activity, and Brazil has the biggest vegetal diversity (Dutra et al., 2016). One of them is the "Cerrado" (Brazilian Savannah) covering more than 2 million km^2 and land representing 25% of the area of the country (Sawyer, 2016). This vegetation consists of more than 6,000 vegetal species, having many compounds such as saponins, tannis, steroids, and others (Bessa et al., 2013), with biological activities including antifungal, antibacterial, antiprotozoal and antiviral ones (Alves et al., 2000; Mesquita et al., 2005, 2007; Silva Junior et al., 2009; Brandão et al., 2010).

Many plant extracts have been described for antiviral activity against herpesviruses *in vitro* such as *Conocephalum conicum*, *Polypodium glycyrrhiza* and the water-soluble extract from the narcissus bulb *Flos verbasci*, showed anti-HSV activity. The aqueous extracts of *Helichrysum aureonitens* (Asteraceae) shoots inhibited HSV-1 (Chattopadhyay and Khan, 2008). Compounds in green tea extract could also inhibit HSV-1 and black tea extract enriched with theaflavins has the potential to prevent the spread of HSV-1 (Cantatore et al., 2013). Moreover, *Lithraea molleiodes*, *Sebastiana brasiliensis* and *Sebastiania klotzschiana* used against infectious diseases showed antiherpetic activity with no cytotoxicity, as did the aqueous extract of *Beta vulgaris* (Betancour-Galvis et al., 1999). The aqueous extracts from *B. vulgaris* and *S. brasiliensis* also exhibited antiviral activity against swine herpesvirus (Koseki et al., 1990; Simoni et al., 2014) and *Cecropia pachystachya*, *Melochia villosa* and *P. acuminatum* presented the most relevant results against bovine and swine herpesviruses (Simoni et al., 2014).

Simoni et al. (2007) reported on the *in vitro* research for potential antiviral properties of the 16 species collected from the Brazilian Cerrado against bovine herpesvirus (BoHV-1), infectious bursal disease virus (IBDV) and avian reovirus. They found 8 plant extracts active against BoHV-1 including *Byrsonima intermedia*, *Banisteriopsis variabilis*, *Stryphodendron adstringens* and *Xylopia aromatica*.

Species of *Banisteriopsis* are described as having antioxidant and antibacterial properties, while also inhibiting the activity of monoamine oxidases. *Banisteriopsis caapi* is the most studied species of this genus because it is used by some religious groups in Brazil as an ingredient of the drink locally known as *ayahuasca*. Chemical compounds identified in this plant species include alkaloids, harmine and harmaline (Wang et al., 2010; Santos et al., 2017). *B. intermedia* belonging to the Malpighiaceae family popularly known as "murici" has been used in folk medicine to treat fever, as diuretic, for skin infections and ulcers. *B. intermedia* is described mainly as being antimicrobial and as anti-inflammatory (Moreira et al., 2011; Sannomiya et. al., 2007). *S. adstringens* is one of the most widely used medicinal species by the Brazilian population. The stem bark is usually used to treat leucorrhea, gonorrhea, vulvo-vaginal candidiasis, gastritis, sore throat, diarrhea and bleeding (Costa et al., 2010). Extracts from the leaves present trypanocidal activity associated with the chemical compound tannin (Ishida et al., 2009). Extracts from *X. aromatica* have been described for antibacterial, antifungal, insecticidal, antimicrobial, and antiparasitic activities. Many compounds in this genus have been identified, but the triterpenoids are present in higher concentrations (Stashenko et al., 2004; Costa et al., 2013).

The aim of this work was continue the search with these same eight positive plant extracts to evaluate the antiviral activity against equine, swine and human herpesviruses together with additional studies of antiviral and virucidal potential. Additionally, the phytochemical profile of the extract from *B. intermedia*, which displayed higher antiviral activity, was performed by HPLC/MS analysis.

MATERIALS AND METHODS

Plant materials

The "Cerrado" plant species *B. variabilis* B. Gates, *B. intermedia* A. Juss., *Campomanesia xanthocarpa* O. Berg, *Erythroxylum deciduum* A. St.-Hil., *Lacistema hasslerianum* Chodat, *Ocotea pulchella* (Nees) Mez, *S. adstringens* (Mart.) Coville, and *X. aromatica* (Lam.) Mart were collected in Mogi-Guaçu (22º18'S and 47º20'W), São Paulo State, Brazil. The specimens were authenticated by a taxonomist in the Herbarium of the Instituto de Botânica, São Paulo, Dr. Eduardo Luis Martins Catharino.

Preparation of crude extracts

Crude aqueous extracts were prepared by grinding dried leaves with de-ionized distilled water (10%, w/v) in a mixer and maintenance at 4ºC overnight. The aqueous extracts were filtered on Whatman-1 filter paper and freeze-dried in Flex-dry MP FTS systems. Lyophilized extracts were dissolved in equal parts of sterile de-ionized distilled water and Eagle minimum essential medium (MEM 2x) at a final concentration of 10,000, 4,000 or 2,000

µg/mL. The extracts were centrifuged at 2,500 g/10 min in a Hettich Zentrifugen model Rotina R and sterilized by filtration (0.22 µm filter).

HPLC/MS analysis

Crude aqueous extract from *B. intermedia* was filtered on a Sep-Pak column using MeOH as eluent. Samples containing 1 ml of the crude extract was analyzed by HPLC using a Luna C-18 (Phenomenex) column (5 mm, 250 X 4 mm), with a gradient from MeOH:H_2O:HCCOH 1% 75:25 (0 min) to MeOH 100% (30 min), flow rate 1.0 ml/min and detection at 254 nm. HPLC/MS (negative form) were acquired on a Bruker micrOTOF-QII coupled to an Apollo ion source set as follows: dry temperature at 180°C and voltage at 4.5 kV. The mass/charge ratios were detected in scan (*m/z* 100–1200 Da) and product ion scan (*m/z* 50–1200 Da) modes, using the same chromatographic method described above.

Cell cultures and viruses

The equine herpesvirus 1 (EHV-1), strain A4/72 (Moreira et al., 1998) and the herpes simplex virus 1 (HSV-1), strain KOS (Silva et al., 2010) were propagated in Vero (African Green monkey – ATCC CCL 81). The swine herpesvirus 1 (SuHV-1) strain NP (Nova Prata) (Fonseca et al., 2010) was propagated in MDBK (Mardin and Darby Bovine Kidney – ATCC CCL 22). All cells were grown in MEM with 10% fetal bovine serum (FBS) (Simoni et al, 2007).

Cytotoxicity assay

The assays were performed using 96- well microtiter plates with 30,000 cell/well. After 24 h of incubation at 37ºC in a humidified 5% CO_2 atmosphere, each cell type was exposed to decreasing concentrations of plant extract in triplicate. Any cell morphology alteration was observed at light microscopy during the next 3 days to determine the maximum non-cytotoxic concentration (MNCC). Monolayers of cells incubated only with MEM were used as a control.

Antiviral assay

For the assays, MDBK or Vero cells were prepared in 96-well microplates at density of 3×10^4 cells per well and incubated for 24 h at 37ºC with 5% CO_2. After 24 h of incubation, the medium was poured off and 100 µL of extracts at dilutions corresponding at MNCC were added. The cells were incubated for 1 h and after this period, 50 µL of logarithmic dilutions of viruses were inoculated for 96 h. MDBK cells were infected with SuHV-1 and Vero cells were infected with EHV-1 or HSV-1. Controls consisted of untreated infected (virus titer), treated non-infected (extract control), untreated non-infected (cell control) cells. In addition, to verify if the antiviral activity of the extract remained below its MNCC, a dose-response experiment was carried out using it at range of 250 until 1.9 µg/mL.

Direct anti-herpes assay

The assay was described previously by Simoni et al. (1996). Briefly, 100 µL of each extract at MNCC was mixed with 100 µL of logarithmic dilutions of each virus, incubated at 37°C in 5% CO_2 for 1 h. Then, 50 µL of mixture was inoculated at the monolayers in each well of a 96-well plate. In addition, to verify if the direct anti-herpes effect of the extract remained below its MNCC, a dose-response experiment was carried out using it at range of 250 until 1.9 µg/mL.

MTT assay

The MTT assay was performed to confirm qualitatively the MNCC and the antiviral activity of the extracts that presented PI% more than 97%. Cells in a 96-well microplate were incubated with 100 µL/well of increasing concentrations of crude extracts in quadruplicate with at least 5 different concentrations. Thereafter, 100 TCID of virus was added. After incubation for 72 h, the medium was removed and added on cell monolayer 50 µL of MTT (1 mg/mL) to each well. The microplate was then incubated at 37ºC for 4 h. The supernatant was then removed from each well without disturbing the cell clusters containing formazan crystals. To solubilize the formazan crystal, 100 µL of SDS was added and the microplate was incubated again overnight. The absorbance of the wells was read in a computer-controlled microplate reader (Spectra Max Plus 384) at 540 nm wavelengths. The 50% cytotoxic concentration (CC_{50}) of the test compound was defined as the concentration that reduced the absorbance of mock-infected cells by 50% of that of the control. The 50% antiviral effective concentration (IC_{50}) was expressed as the concentration that achieved 50% protection of virus- infected cells from the HSV-1, EHV-1 and SuHV-1 induced destruction (Takeuchi et al., 1991).

Data and statistical analysis

The antiviral and virucidal activities were based on reduction of viral titers using CPE criteria. Values were expressed as titer (TCID 50 µL) (Reed and Muench, 1938) and viral inhibition index (VII) calculated as the difference of virus titer between treated and untreated infected control cultures. The VII was considered positive when ≥ 1.5 (Barros et al., 2012).

The 50% cytotoxic (CC_{50}) and 50% inhibition (IC_{50}) concentrations were calculated from concentration-effect curves obtained from nonlinear regression analysis of concentration-effect curves by the GraphPad Prism 5 Demo program. The results were obtained from triplicate independent assays. The percentage of cytotoxicity was calculated as [(A – B)/A] × 100, where A and B are the OD540 of untreated and of treated cells, respectively. The percentages of protection were calculated as [(A − B) × 100/(C − B)], where A, B and C indicate the absorbance of the extracts/fractions, virus and cell controls, respectively. Each obtained EC_{50} value was defined as the effective concentration that reduced the absorbance of infected cells to 50% when compared with cell and virus controls. The selectivity index (SI) was determined by the ratio of CC_{50} to EC_{50} and expressed as mean ± s.e.m. The statistically different effects of tested extracts on the inhibition of virus replication were compared with the control group using the Student's t-test with $p \leq 0.05$ for significant result.

RESULTS

Cytotoxicity

The MNCCs of the extracts were determined in two different cell lines, MDBK and Vero and the concentrations used in bioassays are presented in Table 1. In Vero cells, the CC_{50} was lower than that one observed in MDBK cells. *X. aromatica* presented the lowest cytotoxic to Vero and MDBK cells with MNCC of 1250 and 625 µg/mL, respectively. The extract from *O. pulchella* was the most cytotoxic for both cell lines with

Table 1. Antiviral activity of extracts at MNCC and CC_{50} against HSV-1, EHV-1 and SuHV-1.

Plant extract	MNCC[a] Vero	CC_{50}[b]	HSV-1[c]	EHV-1	MNCC MDBK	CC_{50}	SuHV-1
Banisteriopsis variabilis	625	435.8	2.25	3.75	312	>2,000	3.24
Byrsonima intermedia	31.2	141.1	3.25	4.87	250	782.1	5.45
Campomanesia xanthocarpa	62.5	n.t	0	0.62	250	1,299	2.0
Erythroxilum deciduum	125	n.t.	1.0	0.26	31.2	n.t.	1.16
Lacistema hasslerianum	62.5	n.t.	0.5	0.26	62.5	821	2.0
Ocotea pulchella	31.2	n.t.	0	0.26	31.2	290.9	1.76
Stryphodendron adstringens	625	n.t.	2.75	1.0	62.5	>2,000	2.0
Xylopia aromatica	1250	203.1	1.5	1.76	625	>2000	3.24

a: MNCC: Maximum non-cytotoxic concentration; HSV-1: Human Herpesvirus type 1 SuHV-1: Swine Herpesvirus type 1; EHV-1: Equid Herpesvirus type 1; b: 50% cytotoxic concentration (µg/mL); c: VII: Viral inhibition index; n.t.: CC_{50} not tested.

Table 2. Direct anti-herpes effect of extracts at MNCC against herpes simplex virus, equine herpesvirus and swine herpesvirus in cell cultures.

Plant extract	VII[a]	VII	VII
	HSV-1[b]	EHV-1[c]	SuHV-1[d]
Banisteriopsis variabilis	3.0	3.67	3.34
Byrsonima intermedia	4.5	4.0	5.22
Stryphodendron adstringens	n.t.	n.t.	0.75
Xylopia aromatica	1.5	0.84	1.67

a: VII: Viral inhibition index; b: Human Herpesvirus type 1; c: Equid Herpesvirus type 1; d: Swine Herpesvirus type 1; n.t.: Not tested. MNCC: Maximum non-cytotoxic concentration.

MNCC of 31.2 µg/mL. The other extracts showed MNCC ranging between these values for both cells.

Antiviral activity

Results are presented in Table 1. The extracts from *B. variabilis*, *B. intermedia, S. adstringens* and *X. aromatica* presented VII greater than or equal to 1.5 for HSV-1. The extracts of *B. variabilis*, *B. intermedia* and *X. aromatica* were positive for EHV-1 while the extracts *B. variabilis*, *B. intermedia, C. xanthocarpa, L. hasslerianum, O. pulchella, S. adstringens* and *X. aromatica* were effective for SuHV-1.

Direct anti-herpes effect

Assays to study the direct anti-herpes effect were done only with the extracts that were effective for at least two of the viruses tested in the antiviral assays. These results are presented in Table 2.

The extracts of *B. variabilis* and *B. intermedia,* presented an inhibition against the three viruses with VII greater or equal to 3.0. *X. aromatica* was only effective for SuHV-1and HSV-1, while the extracts from *S. adstringens* had action only on HSV-1.

Additional studies with *Byrsonima intermedia*

B. intermedia was selected for additional studies and to characterize its antiviral properties because it showed the highest values of VII against all herpesviruses.

Table 3 shows the results of the cytotoxic, antiviral activities and selectivity index (SI) obtained from *B. intermedia* extracts. The MNCC of *B. intermedia* extracts that did not cause alterations in the morphology of the cells visible under the optical microscope (Table 2) were lower than that obtained with CC_{50} of 141.1 for Vero cells and 782.1 for MDBK cells. *B. intermedia* presented SI values of 41.76 ± 0.04; 4.12 ± 0.1 and 193.97 ± 0.09 respectively against HSV-1, EqHV-1 and SuHV-1.

Figure 1 shows the antiviral activity of extract from *B. intermedia* at 8 set concentrations against the three viruses showing that treatment with *B. intermedia* extract resulted in reduced viral titers in dose-response curves. At 15.6 µg/mL, the initial reduction occurred firstly with SuHV-1 and then with EHV-1 and HSV-1 at 62.5 µg/mL. At 250 µg/mL, the greater VII was also obtained with

Table 3. Selectivity index, cytotoxic and antiviral activities from *B. intermedia* aqueous extract against swine herpesvirus, equine herpesvirus and herpes simplex virus by the MTT assay.

Plant extract	Vero	HSV-1	SI[c]	EqHV-1	SI	MDBK		SuHV-1
	CC_{50}[a]	IC_{50}[b]		IC_{50}		CC_{50}	IC_{50}	SI
Byrsonima intermedia	141.1	3.37	41.76 ± 0.04	34.24	4.12 ± 0.1	782.1	4.03	193.97 ± 0.09

a: 50% cytotoxic concentration (µg/mL); b: 50% inhibitory concentration (µg/mL); c: SI - Seletivity Index - ratio of CC_{50} to IC_{50}. SuHV-1: Swine Herpesvirus type 1; EHV-1: Equid Herpesvirus type 1; HSV-1: Human Herpesvirus type 1.

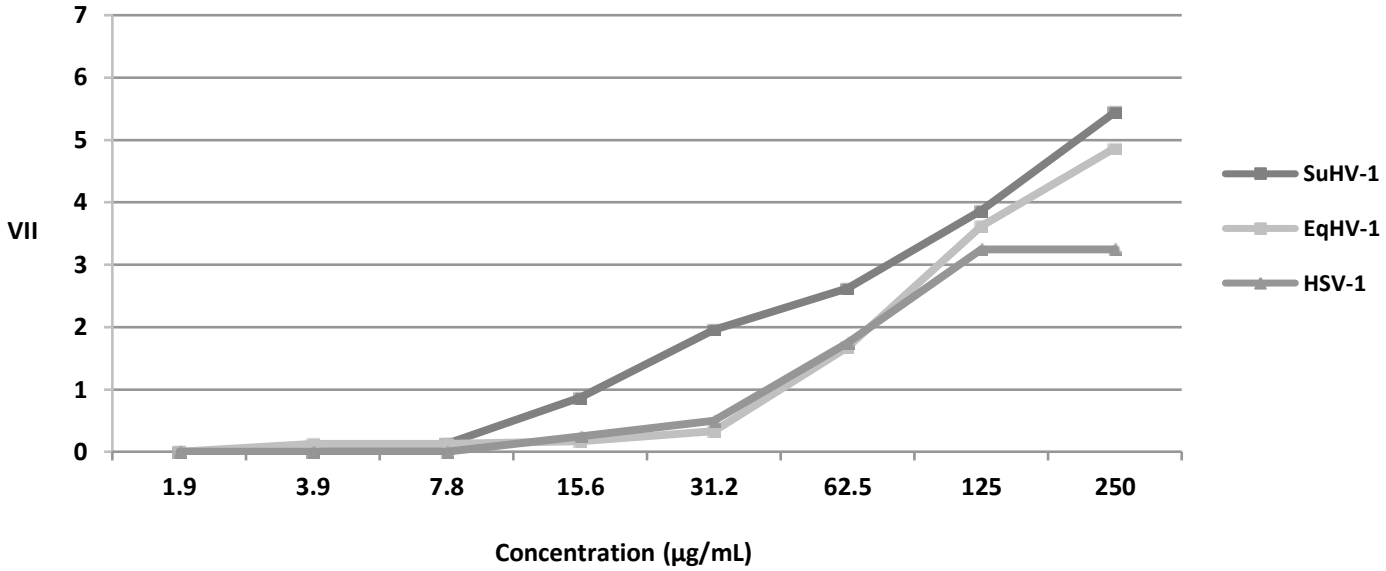

Figure 1. Antiviral activity of different concentration of extract from *B. intermedia* against Swine Herpesvirus type 1 (SuHV-1); Equine Herpesvirus type 1 (EHV-1); Human Herpesvirus type 1 (HSV-1); VII: Viral Inhibition Index.

SuHV-1 followed by EHV-1 and HSV.

Likewise, Figure 2 shows the direct anti-herpes effect against SuHV-1, EHV-1 and HSV-1 showing that treatment of virions with this various concentrations of extracts for one hour resulted in reduced viral titers. The extracts were active at the concentrations range 250 to 31.2 µg/mL. At 3.9 µg/mL, the reduction occurred firstly with SuHV-1 and then with EHV-1 and HSV-1 at 7.8 µg/mL. At 250 µg/mL, the greater VII was obtained for SuHV-1 and EHV-1 followed by HSV.

Phytochemical profile of *Byrsonima intermedia*

As follow-up, the *B. intermedia* extract was subjected in the current study to HPLC/MS analysis (Figure 3). The peak at R_t = 10.2 min corresponds to a $[M - H]^-$ *quasi*-molecular ion peak at *m/z* 169, suggesting the occurrence of gallic acid (A). The second peak, detected at Rt = 13.6 min corresponds to a $[M - H]^-$ *quasi*-molecular ion peak at *m/z* 301, suggesting the presence of flavonoid quercetin (B).

DISCUSSION

Cytotoxicity

The cytotoxic assay at the first stage of screening is very important to determine the maximum concentration of the aqueous extract that did not induce changes in cell morphology. This step is critical because the virus is an intracellular parasite and uses the cell machinery to replicate, so it must be ensured that the virus have the ideal conditions for their growth (Cos et al., 2006).

The study of cytotoxicity in different cell lines is important because one of the inherent drawbacks of *in vitro* antiviral testing is the environmental sensitivity of animal cells in culture, although the *in vitro* methodology is faster and less costly (McCutcheon et al., 1995).

Antiviral activity

Brazilian Cerrado plants may lead to the study of a broad source of new natural compounds; however, most are

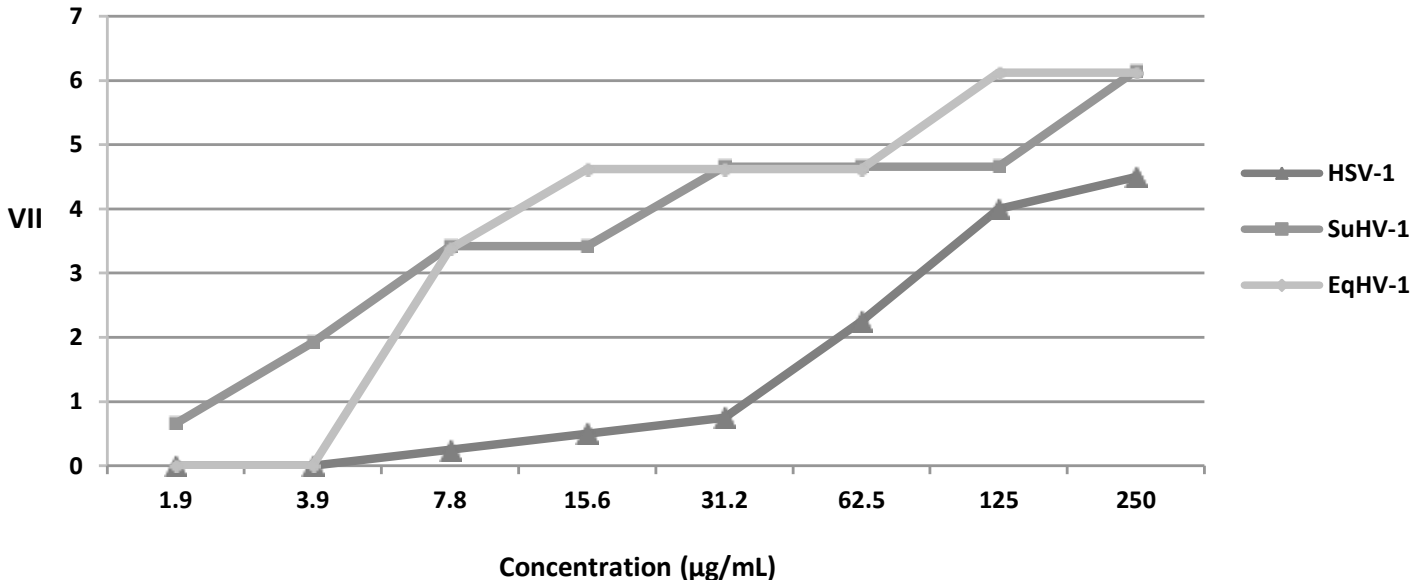

Figure 2. Direct anti-herpes effect of different concentrations of extract from *B. intermedia* against Swine Herpesvirus type 1 (SuHV-1); Equine Herpesvirus type 1 (EHV-1); Human Herpesvirus type 1 (HSV-1); VII: Viral Inhibition Index.

being developed for use as antibiotics more than antiviral drugs. This is because the viruses present certain characteristics among others of replicating within the cell and persisting in the host. Their combat must be more rational by the choice of species of plants that have already been used by the population to treat the diseases.

Many Brazilian Cerrado species have already been described as having antiviral activity. Chattopadhyay et al. (2015) showed that *Byrsonima verbascifolia* had strong anti-HSV activities, whereas Maldini et al. (2011) found that *B. crassifolia* used as anti-inflammatory has been described as having compounds that also display antiviral activity against HSV-1 and HIV.

In the present study, eight extracts reduced at least one of the viral titers, as compared to untreated control cells. *B. variabilis*, *B. intermedia* and *X. aromatica* species were active against all these herpesviruses. Furthermore, a strong inhibition was obtained from *B. variabilis* and *B. intermedia*. *S. adstringens* extract also showed an effective inhibition against SuHV-1 and HSV-1. Extract from *X. aromatica* presented the highest values of VII against SuHV-1. Antiviral activity was also observed for extracts from *C. xanthocarpa* and *O. pulchella* only against SuHV-1.

Frias et al. (2012) isolated from metanolic extract of *B. variabilis*, the flavonoids quercetin, rutin, and apigenin. Quercetin is found in a wide variety of plants and studies showed that it could reduce infectivity of target cells and replication against herpesviruses. The results of our work indicated that the extracts of *B. variablis* showed inhibitory effect against the three herpesviruses and probably this inhibitory effect is due to presence of these flavonoids.

Felipe et al. (2006) described the inhibition BoHV-1 replication of aqueous extracts from *S. adstringens* stem bark. Simoni et al. (2007) showed that the leaves presented moderate activity against BoHV-1. In the present work, leaves extracts from *S. adstringens* also presented moderate activity against EHV-1 but strong inhibition against HSV-1 and SuHV-1.

Antiviral drugs have been reported for herpetic infections for both human and animal herpesviruses. Although the regular use of vaccines helps in improvement of animal herds and avoids viral shedding, treatment of herpesvirus infections is not completely prevented by immunization (Gulati et al., 2016; Maxwell, 2017). Thus, antiviral agents can be useful not only as preventives but also as therapeutic in herpesvirus infections which leads to a great interest in discovering new effective and safe drugs. The results of this present work suggest the importance of searching for new compounds for veterinary and human medicine in Brazilian Cerrado plants because they have shown potential for developing new antivirals.

Several plant extracts with promising results have been described for their antiviral potential against diverse herpes viruses. For example, *Cardamine angulata*, *Conocephalum conicum* and *Polypodium glycyrrhiza* showed activity against bovine herpesvirus and HSV-1 (McCutcheon et al., 1995). Aqueous extract from *Guettarda angelica* seeds exhibited strong inhibition for three animal herpesviruses including bovine, swine and equine herpesviruses 1 (Barros et al., 2012).

The present results were promising especially for *B. intermedia* and indicating that materials derived from their

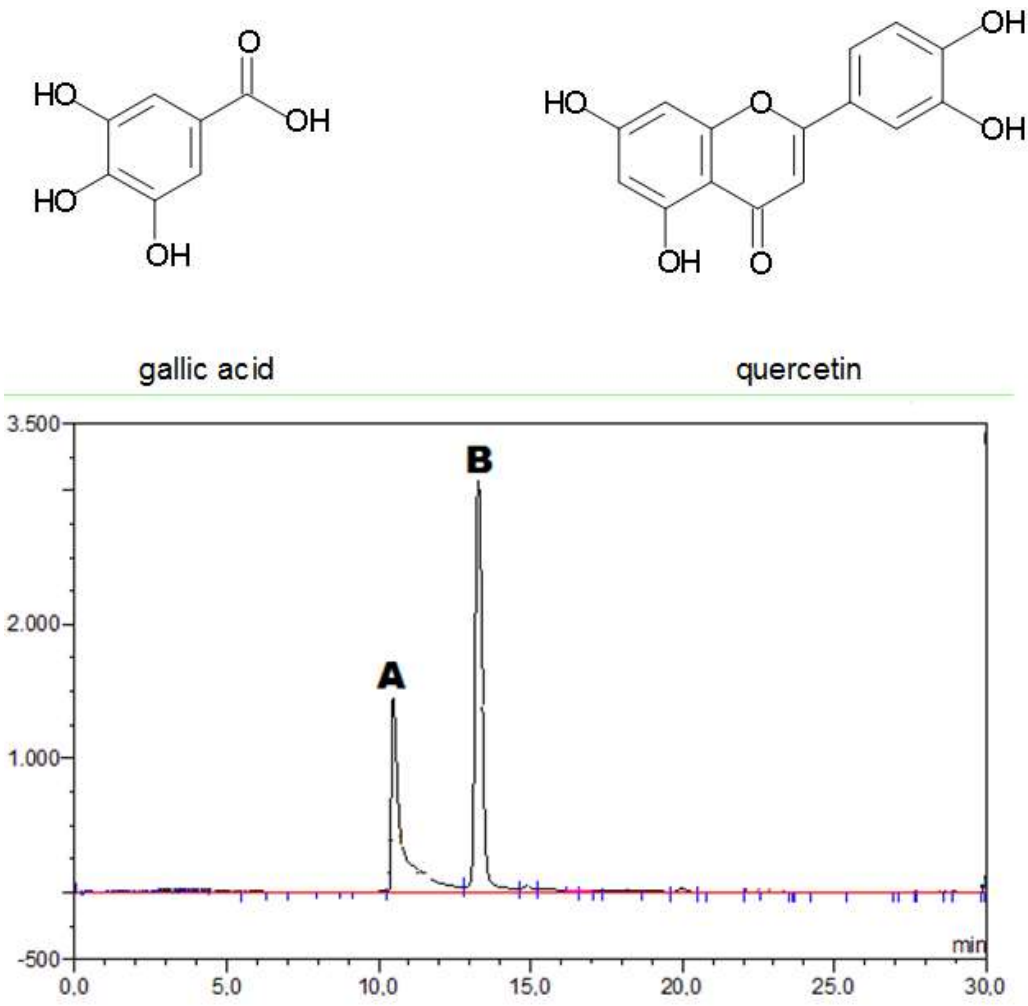

Figure 3. HPLC analysis of crude aqueous extract from *B. intermedia* leaves and identified compounds gallic acid (A) and quercetin (B).

leaves exhibited antiviral activity and selectivity toward three herpesviruses HSV-1, EqHV-1 and SuHV-1. In 2007, Simoni et al. (2007) described the antiviral activity of the *B. intermedia* against bovine herpesvirus (BoHV-1) and against avian reovirus indicating a broad spectrum of action of this plant species.

Direct anti-herpes effect

To verify if the mechanism of action of extracts is also due to inhibition on the viruses in extracellular conditions, the selected extracts showing antiviral activity were studied.

Many plants may exhibit different mechanisms of viral inhibition acting on more than one target during viral replication. The *Opuntia streptacantha* inhibited virus replication and inactivated extracellular virus, such as HSV, equine herpes virus, pseudorabies virus (Jassim and Naji, 2003). *Bidens pilosa* extract was effective against HSV-1 and HSV-2 with potent virucidal activity (Vadlapudi et al., 2013). Virucidal activity has also been described in crude hydroethanolic extract from the stem bark of *S. terebinthifolia* and was effective against HVS-1 in the attachment and penetration stages (Nocchi et al., 2016). Moreover, hot water extract of *B. pilosa* showed potent inhibitory activity against HSV-1 and HSV-2 in Vero cells. The authors suggest multiple targets of activity because this extract showed virucidal activity to block the binding of virus to host cells and viral cell penetration. The *B. pilosa* extract also inhibits virus adsorption to cells and affects some intracellular steps of viral replication (Nakama et al., 2012). These findings are in agreement with those observed in this study.

Additional studies with *Byrsonima intermedia*

The extracts from B. *intermedia* were tested in their

maximum non-toxic concentrations in which no alteration of normal cell morphology was observed, and the CC_{50} for both MDBK and Vero cells was obtained. Therefore, the extract showed no toxicity even when used at high concentrations in the reduction of viral titers assay and direct anti-herpes assay. Further, B. *intermedia* extracts showed anti-herpes activity in a concentration-dependent manner in both assays, suggesting that this effect may have occurred either acting directly on the viral particle or in other stages of the replicative cycle.

Additional studies should be done to study the mechanism of action of extracts of *B. intermedia* against those herpesviruses indicating at which stage the extract has activity against viruses.

Phytochemical profile

Using dereplication procedures by HPLC/MS, the occurrence of gallic acid and quercetin in the active extracts from *B. intermedia* was identified. As previously reported, these compounds were detected in the MeOH leaves extract from *B. intermedia* which displayed mutagenic activity (Sannomiya et al., 2007).

Gallic acid is a phenolic compound present in many plants, fruits and vegetables. Studies have demonstrated that gallic acid exhibits a strong inhibitory effect against several viruses such as the herpes simplex virus, hepatite C virus, HIV and enterovirus 71. Therefore, the potential activity observed by GA suggests that this activity is due to the hydrophobic interaction between the functional group, (hydroxyl) and virion components of enveloped herpesvirus. In addition, gallic acid showed an inhibitory effect in a time-dependent manner, notably similar to the current study (Govea‐Salas et al., 2016). Quercetin, a flavonoid found in several plant species, including fruits and edible vegetables, displayed several biological potentials (Sharma et al., 2018), including antiviral against HSV-1 (Garrett et al., 2012). Additionally, Choi et al. (2009) described that quercetin-3-rhamnoside from *Houttynia cordata* extract inhibit influenza A replication in the initial stage of virus infection by indirect interaction with virus particles.

New studies are needed to bring the active substances present in *B. intermedia* extract and to identify in detail the ways in which they act. The other extracts that can be considered promising must also pass through the processes of fractionation and isolation of substances in order to verify the potential of this activity. All of them here have shown their potential to lead to the development of an alternative therapy for the treatment of such important infections worldwide.

Conclusion

This study has demonstrated a broad antiviral activity of Brazilian Cerrado plants and indicated that they can be effective potential candidates for the development of new strategies to treat viral infections. *B. intermedia* was chosen for further research as well as gallic acid and quercetin all necessary to elucidate the mechanism of action of the extract or compounds both on herpesviruses replication and on direct antiviral effect.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are thankful to the Enrica G. D'Alessandro for reviewing English.

REFERENCES

Alves TMA, Silva AF, Brandão M, Grandi TSM, Smânia EFA, Smânia Jr A, Zani CL (2000). Biological screening of Brazilian medicinal plants. Mem. Inst. Oswaldo Cruz 95(3):367-373.

Betancour-Galvis L, Saez J, Granados H, Salazar A, Ossa J (1999). Antitumor and antiviral activity of Colombian medicinal plants extracts. Mem. Inst. Oswaldo Cruz 94(4):531-535.

Brandão GC, Kroon EG, dos Santos JR, Stehmann JR, Lombardi JA, Braga de Oliveira A (2010). Antiviral activity of Bignoniaceae species occurring in the State of Minas Gerais (Brazil): part 1. Lett. Appl. Microbiol. 51(4):469-476.

Barros AV, Araújo LM, Oliveira FF, Conceicao AO, Simoni IC, Fernandes MJB, Arns CW (2012). In vitro evaluation of the antiviral potential of *Guettarda angelica* against animal herpesviruses. Acta Sci. Vet. 4:1068.

Bessa NGFdel, Borges JCM, Beserra FP, Carvalho RHA, Pereira MAB, Fagundes R, Campos SL, Ribeiro LU, Quirino MS, Chagas Jr AF, Alves A (2013). Prospecção fitoquímica preliminar de plantas nativas do cerrado de uso popular medicinal pela comunidade rural do assentamento vale verde-Tocantins. Braz. J. Med. Plants 15(4):692-707.

Cantatore A, Randall SD, Traum D, Adams SD (2013). Effect of black tea extract on herpes simplex virus-1 infection of cultured cells. BMC Complement. Altern. Med. 13:139.

Chattopadhyay D, Khan MTH (2008). Ethnomedicines and ethnomedicinal phytophores against herpesviruses. Biotechnol. Annu. Rev. 14:297-348.

Chattopadhyay D, Ojha D, Mondal S, Goswami D. (2015) Chapter 8 – Validation of Antiviral Potential of Herbal Ethnomedicine. Evid. Based Valid. Herbal Med. 175-200.

Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH Baek SH (2009). Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Res. 81(1):77-81.

Cos P, Vlietinck AJ, Vanden Berghe D, Maesa L (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol. 106(3):290-302.

Costa MA, Ishida K, Kaplum V, Koslyk EDA, Mello JCP, Ueda-Nakamura T, Dias Filho BP, Nakamura CV (2010). Safety evaluation of proanthocyanidin polymer-rich fraction obtained from stem bark of *Stryphnodendron adstringens* (BARBATIMAO) for use as a pharmacological agent. Regul. Toxicol. Pharmacol. 58(2):330-335.

Costa EV, Silva TB, Menezes LRA, Ribeiro LHG, Gadelha FR, Carvalho JE, Souza LMB, Silva MAN, Siqueira CAT, Salvador MJ (2013): Biological activities of the essential oil from the leaves of *Xylopia laevigata* (Annonaceae), J. Essential Oil Res. 25(3):179-185.

Dutra RC, Campos MM, Santos ARS, Calixto JB. (2016). Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges

and perspectives. Pharm. Res. 112:4-29.
Felipe AMM, Rincão VP, Benati FJ, Linhares REC, Galina KJ, De Toledo CEM, Lopes GC, De Mello JC, Nozawa C (2006). Antiviral effect of *Guazuma ulmifolia* and *Stryphnodendron adstringens* on poliovirus and bovine herpesvirus. Biol. Pharm. Bull. 29(6):1092-1095.
Fonseca AAJr, Carmagos MF, D´Ambros RMF, Braga AC, Ciacci-Zanella J, Heinemann MB, Leite RC, Reis JKP (2010). Diagnóstico e genotipagem do vírus da pseudoraiva por nested-PCR e análise de restrição enzimática. Ciênc. Rural 40(4):921-927.
Frias UA, Costa MCM, Takahashi JA, Oki Y (2012). *Banisteriopsis* Species: A Source of Bioactive of Potential Medical Application. Int. J. Biotechnol. Wellness Ind. 1(3):163-171.
Garrett R, Romanos MTV, Borges RM, Santos MG, Rocha L, Silva AJRD (2012). Antiherpetic activity of a flavonoid fraction from *Ocotea notata* leaves. Rev. Bras. Farmacogn. 22(2):306-313.
Govea-Salas M, Rivas-Estilla AM, Rodríguez-Herrera R, Lozano-Sepúlveda SA, Aguilar-Gonzalez CN, Zugasti-Cruz A, Salas-Villalobos TB, Morlett-Chávez JA (2016). Gallic acid decreases hepatitis C virus expression through its antioxidant capacity. Exp. Therap. Med. 11:619-624.
Gulati BR, Anagha G, Riyesh T, Khurana SK (2016). Emergence of equine herpes virus 1 myeloencephalopathy: a brief review. J. Exp. Biol. Agric. Sci. 4:S132-138.
Ishida K, Rozental S, Mello JCP, Nakamura CV (2009). Activity of tannins from *Stryphnodendron adstringens* on *Cryptococcus neoformans*: effects on growth, capsule size and pigmentation. Ann. Clin. Microbiol. Antimicrob. 8:29.
Jassim SAA, Naji MA (2003). Novel antiviral agents: a medicinal plant perspective. J. Appl. Microbiol. 95:412-427.
Katiyar C, Gupta A, Kanjilal S, Katiyar S (2012). Drug discovery from plant sources: An integrated approach. AYU 33:10-9.
Koseki I, Simoni IC, Nakamura IT, Noronha AB, Costa SS (1990). Antiviral activity of plant extracts against aphovirus, pseudorabies virus and pestivirus in cell cutures. Microb. Lett. 44:19-30.
McCutcheon AR, Roberts TE, Gibbons E, Ellis SM, Babiuk LA, Hancock REW, Towers GHN (1995). Antiviral screening of British Columbian medicinal plants. J. Ethnopharmacol. 49(2):101-110.
Maldini M, Montoro P, Pizza C (2011). Phenolic compounds from *Byrsonima crassifolia* L. bark: Phytochemical investigation and quantitative analysis by LC-ESI MS/MS. J. Pharm. Biomed. Anal. 56:1-6.
Martinez JP, Sasse F, Brönstrup M, Diez J, Meyerhans A (2015). Antiviral drug discovery: broad-spectrum drugs from nature. Nat. Prod. Rep. 32(1):29-48.
Maxwell LK (2017). Antiherpetic Drugs in Equine Medicine. Vet. Clin. Equine 33(1):99-125.
Mesquita ML, Desrivot J, Bories FC, Fournet A, Paula JE, Grellier P, Espindola LS (2005). Antileishmanial and trypanocidal activity of Brazilian Cerrado plants. Mem. Inst. Oswaldo Cruz 100(7):783-787.
Mesquita ML, Grellier P, Mambu L, De Paula JE, Espindola LS (2007). In vitro antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. J. Ethnopharmacol. 110(1):165-170.
Moreira N, Kruger ER, Warth JFG, Biesdorf SM, Goularte MMM, Weiss RR (1998). Aspectos etiológicos e epidemiológicos do aborto equino. Arch. Vet. Sci. 3(1):25-30.
Moreira LQ, Vilela FC, Orlandi L, Dias DF, Santos ALA, Silva MA, Paiva R, Alves-da-Silva G, Giusti-Paiva A (2011). Anti-inflammatory effect of extract and fractions from the leaves of *Byrsonima intermedia* A. Juss. in rats. J. Ethnopharmacol. 138(2):610-615.
Nakama S, Tamaki K, Ishikawa C, Tadano M, Mori N (2012). Efficacy of *Bidens pilosa* Extract against Herpes Simplex Virus infection *in vitro* and *in vivo*. Evidence-Based Complement. Altern. Med. 2012:413453.
Nocchi SR, de Moura-Costa GF, Novello CR, Rodrigues J, Longhini R, de Mello JCP, Ueda-Nakamura T (2016). In vitro cytotoxicity and anti-herpes simplex virus type 1 activity of hydroethanolic extract, fractions, and isolated compounds from stem bark of *Schinus terebinthifolius* Raddi. Pharmacogn. Mag. 12(46):160-164.
Reed LJ, Muench H (1938). Simple method of estimating fifty percent and point. Am. J. Hyg. 27(3):493-497.
Riaz A, Murtaz-ul-Hasan K, Akhtar N (2017). Recent understanding of the classification and life cycle of herpesvirus: a review. Sci. Lett. 5(2):195-207.
Sannomiya M, Cardoso CR, Figueiredo ME, Rodrigues CM, Santos LC, Santos FV, Serpeloni JM, Cólus IMS, Vilegas W, Varanda EA (2007). Mutagenic evaluation and chemical investigation of *Byrsonima intermedia* A. Juss. leaf extracts. J. Ethnopharmacol. 112(2):319-326.
Santos AFA, Vieira ALS, Pic-Taylor A, Caldas ED (2017). Reproductive effects of the psychoactive beverage ayahuasca in male Wistar rats after chronic exposure. Rev. Bras. Farmacog. 27(3):353-360.
Sawyer DR (2016). Ecosystem Profile: Cerrado Biodiversity Hotspot. Available at: http://legacy.cepf.net/SiteCollectionDocuments/cerrado/CerradoEcosystemProfile-EN.pdf
Sharma A, Kashyap D, Sak K, Tuli HS, Sharma AK (2018). Therapeutic charm of quercetin and its derivatives: a review of research and patents. Pharm. Pat Anal. 7(1):15-32.
Silva IT, Costa GM, Stoco PH, Schenkel EP, Reginatto FH, Simões CM (2010). In vitro antiherpes effects of a C-glycosylflavonoid-enriched fraction of *Cecropia glaziovii* Sneth. Lett. Appl. Microbiol. 51(2):143-148.
Silva Junior IE, Cechinel Filho V, Zacchino SA, Lima JC, Martins DT (2009). Antimicrobial screening of some medicinal plants from Mato Grosso Cerrado. Rev. Bras. Farmacog. 19(1):242-248.
Simoni IC, Fernandes MJB, Gonçalves CR, Almeida AP, Costa SS, Lima AP (1996). A study on the antiviral caracteristics of *Persea americana* extracts against Aujesky's disease vírus. Biomed. Let. 54:173-181.
Simoni IC, Manha APS, Sciessere L, Hoe VMH, Takinami VH, Fernandes MJB (2007). Evaluation of the antiviral activity of Brazilian Cerrado plants against animal viruses. Virus Rev. Res. 12(1/2):25-31.
Simoni IC, Fernandes MJB, Camargo LMM, Biltoveni LR, Manha APS, Tomitão MTP, de Oliveira DB, Negrelle R, Costa SS (2014). Plants from deer diet in the Brazilian Pantanal wetland as potential source of antiviral and antioxidant compounds. Virus Rev. Res. 19:1-12.
Stashenko EE, Jaramillo BE, Martínez JR (2004). Analysis of volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) by different extraction and headspace methods and gas chromatography. J. Chromatogr. A 1025(1):105-113.
Takeuchi H, Baba M, Shigeta S (1991). An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. J. Virol. Methods 33(1-2):61-71.
Vadlapudi AD, Vadlapatla RK, Mitra AK (2013). Update On Emerging Antivirals For The Management Of Herpes Simplex Virus Infections: A Patenting Perspective. Recent Pat Antiinfect. Drug. Discov. 8(1):55-67.
Wyler E, Menegatti J, Franke V, Kocks C, Boltengagen A, Hennig T, Theil K, Rutkowski A, Ferrai C, Baer L, Kermas L, Friedel C, Rajewsky N, Akalin A, Dölken L, Grässer F, Landthaler M (2017). Widespread activation of antisense transcription of the host genome during herpes simplex virus 1 infection. Genome Biol. 18:209.
Wernike K, Hoffmann B, Beer M (2013). Single-tube multiplexed molecular detection of endemic porcine viruses in combination with background screening for transboundary diseases. J. Clin. Microbiol. 51(3):938-944.
Wachtel-Galor S, Benzie IFF (2011). Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie IFF, Wachtel-Galor S (eds,). Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 1. Available at: https://www.ncbi.nlm.nih.gov/books/NBK92773/
Wang YH, Samoylenko V, Tekwani BL, Khana IA, MillerLS, Chaurasiya ND, Md. Rahman M, Tripathi LM, Khan SI, Joshi VC, Wigger FT, Muhammada I (2010). Composition, standardization and chemical profiling of *Banisteriopsis caapi*, a plant for the treatment of neurodegenerative disorders relevant to Parkinson's disease. J. Ethnopharmacol. 128(3):662-671.
Vissani MA, Thiry E, Dal Pozzo F, Barrandeguy M (2016). Antiviral agents against equid alpha herpesviruses: Current status and perspectives. Vet. J. 207:38-44.
Zouharova D, Lipenska I, Fojtikova M, Kulicha P, Neca J, Slanya M, Kovarcik K, Turanek-Knotigova P, Hubatka F, Celechovska H, Maseka J, Koudelka S, Prochazka L, Eyer L, Plockova J,

Bartheldyova E, Miller AD, Ruzek D, Turaneka J (2016). Antiviral activities of 2,6-diaminopurine-based acyclic nucleoside phosphonates against herpesviruses: In vitro study results with pseudorabies virus (PrV, SuHV-1) Vet. Microbiol. 184(29):84-93.