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Avaliação *in vitro* do potencial antiviral de *Guettarda angelica* contra herpesvírus animais

In Vitro Evaluation of the Antiviral Potential of *Guettarda angelica* Against Animal Herpesviruses

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ABSTRACT

Background: The number of antiviral studies using plant extracts has increased in the last decades, and the results have shown that plants are potential sources of compounds that are able to inhibit and/or decrease viral infections. The selection of these plants by ethnopharmacological criteria increases the probability of finding new substances with significant pharmacological and biological activities. Hence, Brazil has an advantage in this area due to its extensive biodiversity and ethnological diversity. *Guettarda angelica* is a plant from the Brazilian Caatinga region the roots of which are popularly used for various therapeutic purposes, including veterinary use. The aim of this work was to investigate the *in vitro* antiviral activity of extracts of plant parts from *G. angelica* against three animal herpesviruses: bovine (BoHV-1), suid (SuHV-1) and equine (EHV-1) herpesviruses type 1.

Materials, Methods & Results: The extracts of roots, leaves and seeds of *G. angelica* were initially screened for *in vitro* antiviral activity against these herpesviruses using the virus yield reduction assay. The MDBK cells were used in assays with BoHV-1 and SuHV-1, and the Vero cells with EHV-1. For these assays, the cells previously treated with the extracts in non-cytotoxic concentrations were inoculated with logarithmic dilutions of each virus. The viral inhibitory activity of extracts was calculated by difference of virus titer between treated infected cells and non-treated infected cells. Only the aqueous extract from seeds (AEs) showed a significant antiviral activity ($P < 0.01$, ANOVA followed by Tukey test) against all herpesviruses leading continuous studies. Thus, the selectivity index (SI) of this extract was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay by calculating the ratio CC_{50} over IC_{50} . The 50% cytotoxic concentration (CC_{50}) was defined as the extract concentration that reduced the cell viability by 50% when compared to untreated controls; the 50% inhibitory concentration (IC_{50}) was defined as the concentration of the extract that inhibited 50% of viral replication when compared to the virus control. The CC_{50} and IC_{50} were obtained from nonlinear regression analysis of concentration-effect curves by the GraphPad Prism 5 Demo program and represented the means \pm standard deviation of three independent experiments. The CC_{50} for Vero cells was $400.60 \pm 0.20 \mu\text{g/mL}$, while the CC_{50} for MDBK cells was $920.50 \pm 0.19 \mu\text{g/mL}$. The IC_{50} values of the AEs on the BoHV-1, SuHV-1 and EHV-1 were $22.79 \mu\text{g/mL}$, $91.30 \mu\text{g/mL}$ and $19.95 \mu\text{g/mL}$, respectively. The SI values of this extract for each virus obtained from these data were 40.39, 10.08 and 20.08 for BoHV-1, SuHV-1, and EHV-1, respectively.

Discussion: To ensure the antiviral activity of a plant extract and consequently its future use as antiviral agent is crucial the obtainment of its selectivity index or safety index. It is guarantee of a true antiviral effect and not the result of cytotoxicity of the extract on cells, and that could be confused with an antiviral activity. Other important point are the extract IC_{50} values less than $100 \mu\text{g/mL}$. The results of the AEs of *G. angelica* are in accordance with these considerations indicating that the *G. angelica* seeds may be a potential source of antiviral compounds insurance and encouraging further investigation of them.

Keywords: *Guettarda angelica*, bovine herpesvirus type 1, suid herpesvirus type 1, equine herpesvirus type 1, antiviral activity.

Descritores: *Guettarda angelica*, herpesvírus bovino tipo 1, herpesvírus suíno tipo 1, herpesvírus equino tipo 1, atividade antiviral.

INTRODUCTION

Herpesviruses are responsible for important diseases in almost all domestic animal species. Bovine herpesvirus 1 (BoHV-1) is the major pathogen of cattle, responsible for various clinical manifestations including the infectious bovine rhinotracheitis (IBR) and reproductive disorders. The suid herpesvirus 1 (SuHV-1) causes a disease in pigs known as Aujeszky's disease where the economic impact on the pig industry worldwide is due to the high rates of morbidity and mortality leading to restrictions on the international trade of swine products. Equine herpesvirus 1 (EHV-1) is an important ubiquitous enzootic equine pathogen [22].

Despite advances in medical strategies to control viral infections have taken place, the viruses are still a cause of concern and represent a risk for public health. The search for antiviral drugs more effective and affordable is one of strategies to control the viral infections [8,9,20]. Plants and their derivatives may serve as promising sources of novel antiviral compounds [6,10,18,20,25].

Guettarda angelica, is a plant found in the "Caatinga" region of northeastern Brazil with a broad medicinal use of its roots including in veterinary area. In our previous studies, extracts from *G. angelica* root showed *in vitro* antibacterial activity [2].

The number of antiviral studies with plant extracts has increased in the last decades, and the results have shown that plants are potential sources of compounds that are able to inhibit and/or decrease viral infections [6,10,18,20]. Therefore, the aim of this study was to evaluate the *in vitro* antiviral activity of *G. angelica* extracts against these three animal herpesviruses.

MATERIALS AND METHODS

Plant material

The root barks, leaves and seeds of *Guettarda angelica* Mart. ex Müll. Arg. (Rubiaceae) were collected at a small beach in Lomanto, in the region of Jequié (13° 51' S, 40° 14' W), Bahia State, Brazil, in December, 2003. A voucher specimen was deposited in Herbarium of the Centro de Pesquisa do Cacau (BA) and identified as NY Specimen ID 1015301.

Preparation of extracts

The crude aqueous extracts from seeds (AEs) and leaves (AEI) were prepared by grinding the dried

materials in a mixer, followed by the addition of distilled water (10%, w/v) and incubation at 4°C for 8 h (AEs) or 24 h (AEI). To prepare the aqueous extract from the root barks (AEr), the material was boiled for 15 min. All aqueous extracts were filtered through filter paper and freeze-dried. The methanolic extract from the root barks (MeOHer) was also prepared and subsequently partitioned with organic solvents to obtain the hexane (HexF), ethyl acetate (AcOEtF) and aqueous (AqF) fractions. Briefly, 5 g of powdered root bark was initially soaked in 50 mL of methanol for 24 h and then soaked again for another 24 h. The resultant extract was filtered and concentrated in a rotary evaporator under reduced pressure at a temperature of 30°C. Organic extracts were solubilized in a 45% solution of dimethyl sulfoxide (DMSO)¹ and freeze-dried.

All freeze-dried fractions and extracts were solubilized in equal parts of distilled water and Earle minimum essential medium (MEM)² twice concentrated to reach a final concentration of 5,000 µg/mL, except for the EA_r (4,000 µg/mL). The samples were then sterilized by filtration (0.22 µm filter) and stored at -20°C.

Viruses and cell lines

The Los Angeles strain of BoHV-1 and Nova Prata strain of SuHV-1 were propagated in Madin Darby bovine kidney cells - MDBK (ATCC-CCL 22). The A4/72 strain of EHV-1 was propagated in African green monkey kidney cells - Vero (ATCC-CL 81). The MDBK and Vero cells were maintained in MEM with 10% fetal calf serum. To perform the tests, the cells were seeded in 96-well sterile microplates at 3x10⁴ cells/well and incubated at 37°C in a humidified 5% CO₂ atmosphere.

Determination of cytotoxicity by cell morphology analysis

After 24 h of cell seeding, the medium was removed, and the cells were exposed to 100 µL of increasing concentrations of plant fractions and extracts, ranging from 5.0 to 2,500 µg/mL, except for EA_r (4.0 to 2,000 µg/mL), in triplicate. The controls consisted of cells incubated with MEM only. To determine the maximum non-cytotoxic concentration (MNCC), the cell morphology alterations were observed until 96 h after the treatment by inverted light microscopy.

Determination of antiviral effect

Antiviral screening was based on the viral yield reduction technique [9]. Briefly, 24 h after cell seeding and withdrawal of the medium, the cells were treated with 100 μ L of extracts or fractions at dilutions corresponding to each MNCC. After 1 h of incubation, 50 μ L of logarithmic dilutions of each virus were added, in triplicate, and the microplates were incubated again. The controls consisted of untreated infected cells (virus titer), untreated non-infected cells (cell control) and the positive antiviral control (Acyclovir - SQRFB)³. The cells were observed for the presence of the cytopathic effect (CPE) every day for up to 4-6 days after treatment. The viral titers were determined by the 50% infective dose in tissue culture (TCID₅₀), and the antiviral activity was determined by the difference of the viral titers between treated-infected cells and untreated-infected cells and expressed as viral inhibition index (VII). A plant extract was considered an inhibitor when the VII was ≥ 1.5 [26]. ANOVA followed by Tukey test was used to evaluate the difference between the VII values. A *P* value of less 0.01 was considered statistically significant. The inhibitor extracts were further assessed by the MTT⁴ (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-Sigma) colorimetric method.

MTT colorimetric assay

Both cell viability and antiviral activity were evaluated by the MTT technique as described elsewhere [30] with minor modifications. For the cell viability assay, after 24 h of cell seeding and withdrawal of the medium, the cells were incubated with 100 μ L/well of increasing concentrations of plant extracts in quadruplicate. After 48-72 h, the medium was removed, and 50 μ L of MTT solution (1 mg/mL, Sigma) prepared in MEM was added to each well. After 4 h of incubation, the MTT solution was removed, and 100 μ L of sodium dodecyl sulfate¹ in 10% 0.001 N HCl was added to dissolve formazan crystals. The microplates were incubated overnight, and the intensity of the

color (absorbance) was automatically measured using the spectrophotometer (Spectra Max Plus 384) and a wavelength of 540 nm. The controls consisted of cells incubated with MEM only. The 50% cytotoxic concentration (CC₅₀) was defined as the material concentration that reduced the cell viability by 50% when compared to untreated controls.

This same procedure was used to obtain the minimal concentration of extracts required to inhibit 50% of viral growth, except for the addition of viral suspension containing 200 TCID₅₀/100 μ L after adding the extract dilutions. The viral controls consisted of the cells incubated with MEM and virus. The 50% inhibitory concentration (IC₅₀) was defined as the concentration of the material that inhibited 50% of viral replication when compared to the virus control.

The CC₅₀ and IC₅₀ were obtained from nonlinear regression analysis of concentration-effect curves by the GraphPad Prism 5 Demo program and represent the means \pm standard deviation of three independent experiments. The selectivity index (SI) was calculated from the ratio CC₅₀/IC₅₀.

RESULTS

The maximum non-cytotoxic concentrations (MNCCs) of the each plant extract and fraction from *G. angelica* are shown in Table 1. In MDBK cells, the MNCCs for aqueous extracts from the leaves (AE1), seeds (AEs) and roots (AEr) were 800 μ g/mL, 400 μ g/mL and 250 μ g/mL, respectively; in addition, the MNCC for the methanolic extract from roots (MeOHEr) was 200 μ g/mL. In Vero cells, these values were 1,250 μ g/mL (AE1), 625 μ g/mL (AEs), 500 μ g/mL (AEr) and 312 μ g/mL (MeOHEr). The MeOHEr fractions AqF and AcOEtF showed the highest values of MNCC and were equal for both cells, corresponding to a low cytotoxicity (1,600 μ g/mL for MDBK cells and 1,250 μ g/mL for Vero cells). On the other hand, the HexF fraction demonstrated higher cytotoxicity, with a MNCC of 80 μ g/mL (Vero cells) and 50 μ g/mL (MDBK cells).

Table 1. Cytotoxicity of *Guettarda angelica* extracts and fractions and their antiviral activities against bovine (BoHV-1), suid (SuHV-1) and equine (EHV-1) herpesviruses 1.

Extracts	MNCC (µg/mL)		VII*		
	Vero Cells	MDBK Cells	BoHV-1	SuHV-1	EHV-1
AEr	500	250	0.57	0.11	0.69
MeOHEr	312	200	0	0.62	0.60
AqF	1,250	1,600	0.50	0.50	0.50
AcOEtF	1,250	1,600	1.13	0	0.56
HexF	80	50	0	0	0.13
AEI	1,250	800	0.63	0.24	1.13
AEs	625	400	2.30	2.72	1.57

AEr: aqueous extract from root barks; MeOHEr: ethanolic extract from root barks; AqF (aqueous), AcOEtF (ethyl acetate), and HexF (hexane) fractions from MeOHEr fractionation; AEI: aqueous extract from leaves; AEs: aqueous extract from seeds. MNCC: maximum non-cytotoxic concentration; VII: viral inhibition index; **P* < 0.01 by Anova followed by Tukey test.

The antiviral screen of plant extracts and fractions was carried out in MDBK cells infected with BoHV-1 and SuHV-1 and in Vero cells infected with EHV-1. As seen in Table 1, AEs showed antiviral activity against the three viruses, with VII of 2.30 (BoHV-1), 2.72 (SuHV-1) and 1.57 (EHV-1). Unlike, the remaining extracts and fractions reached a VII lower than 1.5 and did not demonstrate an antiviral effect against any of the viruses. The three virus strains were resistant to acyclovir (data not shown).

As the AEs was the only extract to present significant antiviral activity in the screening assays, it was evaluated by the MTT assay (Table 2). The CC₅₀ for Vero cells was 400.60±0.20 µg/mL, while the CC₅₀ for MDBK cells was 920.50 ± 0.19 µg/mL. The IC₅₀ values of the AEs on the BoHV-1, SuHV-1 and EHV-1 were 22.79 µg/mL, 91.30 µg/mL and 19.95 µg/mL, respectively. The SI values of this extract for each virus obtained from these data were 40.39, 10.08 and 20.08 for BoHV-1, SuHV-1, and EHV-1, respectively.

Table 2. Selectivity index (SI) and cytotoxic and antiviral activities of the aqueous extract from *Guettarda angelica* seeds (AEs) against bovine (BoHV-1), suid (SuHV-1) and equine (EHV-1) herpesviruses 1 by the MTT assay.

Extract	Vero cells MDBK cells		BoHV-1		SuHV-1		EHV-1	
	CC ₅₀ (µg/mL) ^a	IC ₅₀ (µg/mL) ^b	SI ^c	IC ₅₀ (µg/mL)	SI	IC ₅₀ (µg/mL)	SI	
AEs	400.60 ± 0.20	920.50 ± 0.19	22.79 ± 0.04	40.39	91.30 ± 0.03	10.08	19.95 ± 0.01	20.08

^a 50% cytotoxic concentration; ^b 50% inhibitory concentration; ^c ratio of CC₅₀ to IC₅₀.

DISCUSSION

The initial strategy of the present work was based on an ethnopharmacology approach to *G. angelica*. The medicinal use of its roots includes the treatment of anemia, dyspepsia, pains and fever, and in veterinary medicine, it is used against fever and to “cure” bovine and equine diarrhoea [1,5]. In our previous studies, methanolic and aqueous extracts from *G. angelica* root bark showed in vitro antibacterial activity against Gram-positive bacteria [5]. This work focused on the antiviral activity of different parts of this plant against the animal herpesviruses.

In a study using the roots from another species of *Guettarda*, *G. platypoda* demonstrated an

antiviral activity against the vesicular stomatitis virus and rhinovirus [2]. Other studies reported the antiviral activity of roots or stem barks of other Brazilian plants against the BoHV-1 [13] and SuHV-1 [16,21]. Despite these reports, in our initial studies using the viral yield reduction assay, neither root nor leaf extracts of *G. angelica* presented antiviral activity against the three animal herpesviruses. Although, several leaf extracts from other plant families showed promissory antiviral results against the bovine and suid herpesviruses [12,16,17,21,26].

Otherwise, the seed extracts demonstrated antiviral activity against these viruses. This assay as well as other techniques, such as plaque reduction/

inhibition assay [9,13], inhibition of virus-induced cytopathic effect [9,24] and endpoint titration technique [4,9] have been used for the *in vitro* determination of antiviral activity of plant extracts or compounds. The ACV was used as a positive control in this assay. However, all herpesvirus strains showed resistance to this drug. Previous reports have described both resistance and susceptibility of BoHV-1 and SuHV-1 to this antiviral [11,28]. Another study [14] reported a moderate effect of ACV against EHV-1 *in vitro* and the ACV has been used on a limited basis in veterinary medicine. Mutant viruses resistant to the existing antiviral agents always arise upon treatment (9,25). Furthermore, the synthetic antiviral drugs that are available in the market are often expensive and unsatisfactory, with restricted targets and potential adverse effects [8,9,20]. In this context, research on antiviral compounds of plant origin that are effective against ACV-resistant viral strains is worth pursuing.

The preliminary results obtained with the seeds of *G. angelica* were promising and motivated us to continue this study. Herein, AEs was also evaluated by the MTT method to obtain its SI. The SI is fundamental to determine any possible toxic effect of the extract on the cells that could be confused with an antiviral activity. According with the SI value, it can be concluded that the antiviral activity of a plant extract is real and not a result of its cytotoxic effect on cells. Therefore, the SI verifies the safety index of the material tested. Moreover, an antiviral activity is relevant when the extract tested has IC_{50} values below 100 $\mu\text{g/mL}$ [9]. Taken together, the IC_{50} and SI values of the AEs extract are in accordance with this conclusion. In another studied carried out by our research group [3] the mechanism of antiviral action of AEs extract against the BoHV-1 and SuHV-1 was also investigated. In this study, this plant extract inhibited the viral replication of the two viruses during and after the viral penetration, being only BoHV-1 inhibited by the AEs in the pretreatment.

Moreover, AEs had a virucidal effect on both viruses. Thus, these results revealed an intra and extracellular effect of AEs.

The antiviral activity of the seeds from *G. angelica* could be attributed to the presence in the extract of compounds, such as proteins, peptides or flavonoids, known for their antiviral properties [10,18]. Indeed, many studies have been published on the antiviral activity from seed extracts against both human and other animal viruses [15,24,27,29]. Specifically about the viruses examined here, researches demonstrated an antiviral activity of proteins extracted from seeds of *Senna occidentalis* against BoHV-1 [19] and a virucidal activity of a peptide isolated from *Sorghum bicolor* against diverse viruses, including BoHV-1 [7].

The inhibition of the three animal herpesviruses by an extract of the seeds of *G. angelica* encourages further bioassay-guided fractionation to identify the compounds responsible for this antiviral effect. This is the first description of an antiviral activity of *G. angelica* and its broad antiherpesviral spectrum, which makes it a promising source of antivirals.

SOURCES AND MANUFACTURERS

¹Merck KGaA, Darmstadt, Germany.

²Vitrocell Embriolife, Campinas, Brasil.

³FIOCRUZ, Manguinhos - RJ, Brasil.

⁴Sigma®, St. Louis, Estados Unidos.

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REFERENCES

- 1 **Agra M.F., Silva K.N., Basilio I.J.L.D., Freitas P.F.F. & Barbosa-Filho J.M. 2008.** Survey of medicinal plants used in the region Northeast of Brazil. *Revista Brasileira de Farmacognosia*. 18(3): 472-508.
- 2 **Aquino R., De Simone F., Pizza C., Cobti C. & Stein M.L. 1989.** Plant metabolites. Structure and *in vitro* antiviral activity of quinovic acid glycosides from *Uncaria tomentosa* and *Guettarda platypoda*. *Journal of Natural Products*. 52(4): 679-685.
- 3 **Barros A.V., Conceição A.O., Simoni I.C., Arns C.W. & Fernandes M.J.B. 2010.** Mechanisms of antiviral action of seeds from *Guettarda angelica* Mart. against bovine and swine herpesviruses *in vitro*. *Virus Review & Research*. 15(2): 47-52.

- 4 **Betancour-Galvis L.A., Morales G.E., Forero J.E. & Roldan J. 2002.** Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Memórias Instituto Oswaldo Cruz*. 97(4): 541-546.
- 5 **Bispo N.J., Francisco N.M.C. & Schmitt A.C. 2007.** Atividade antimicrobiana in vitro do extrato metanólico da planta *Guettarda angelica* sobre bactérias gram-positivas e gram-negativas. *Laes and Haes*. 4: 164-169.
- 6 **Brandão G.C., Kroon E.G., dos Santos J.R., Stehmann J.R., Lombardi J.A. & de Oliveira A.B. 2010.** Antiviral activities of plants occurring in the state of Minas Gerais, Brazil. Part 2. Screening Bignoniaceae species. *Revista Brasileira de Farmacognosia*. 20(5): 742-750.
- 7 **Camargo Filho I., Cortez D.A.G., Ueda-Nakamura T., Nakamura C.V. & Dias Filho B.P. 2008.** Antiviral activity and mode of action of a peptide isolated from *Sorghum bicolor*. *Phytomedicine*. 15(3): 202-208.
- 8 **Chattopadhyay D. & Naik T.N. 2008.** Antivirals of ethnomedicinal origin: structure-activity relationship and scope. *Mini Reviews in Medicinal Chemistry*. 7: 275-301.
- 9 **Cos P., Vlietinck A.J., Vanden Berghe D. & Maesa L. 2006.** Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology*. 106(3): 290-302.
- 10 **Cowan M.M. 1999.** Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 12(4): 564-582.
- 11 **De Clercq E. 1982.** Specific targets for antiviral drugs. *Biochemistry Journal*. 205: 1-13.
- 12 **del Barrio G. & Parra F. 2000.** Evaluation of the antiviral activity of an extract from *Phyllanthus orbicularis*. *Journal of Ethnopharmacology*. 7(1-2): 317-322.
- 13 **Felipe A.M.M., Rincão V.P., Benati F.J., Linhares R.E.C., Galina K.J., Toledo C.E.M., Lopes G.C., Mello J.C.P. & Nozawa C. 2006.** Antiviral effect of *Guazuma ulmifolia* and *Stryphnodendron adstringens* on Poliovirus and Bovine Herpesvirus. *Biological and Pharmaceutical Bulletin*. 29(6): 1092-1095.
- 14 **Garré B., Van Der Meulen K., Nugent J., Neyts J., Croubels S., De Backer P. & Nauwynch H. 2007.** *In vitro* susceptibility of six isolates of equine herpesvirus 1 to acyclovir, ganciclovir, cidofovir, adefovir, PMEDAP and foscarnet. *Veterinary Microbiology*. 122: 43-51.
- 15 **Gonçalves J.L., Lopes R.C., Oliveira D.B., Costa S.S., Miranda M.M., Romanos M.T., Santos N.S. & Wigg M.D. 2005.** *In vitro* anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. *Journal of Ethnopharmacology*. 99(3): 403-407.
- 16 **Hsuan S.L., Chang S.C., Wang S.Y., Liao T.L., Jong T.T., Chien M.S., Lee W.C., Chen S.S. & Liao J.W. 2009.** The cytotoxicity to leukemia cells and antiviral effects of *Isatis indigotica* extracts on pseudorabies virus. *Journal of Ethnopharmacology*. 123: 61-67.
- 17 **Ikuno A.A., Braggio M.M. & Haraguchi M. 2003.** Antiherpes activities of fractions from *Sesbania virgata* leaves. *Arquivo do Instituto Biológico*. 70(2): 183-185.
- 18 **Jassim S.A.A. & Naji M.A. 2003.** Novel antiviral agents: a medicinal plant perspective. *Journal of Applied Microbiology*. 95(3): 412-427.
- 19 **Lombardo M., Ikuno A.A., Ferreira V.C.A. & Kiyota S. 2004.** Cytotoxicity and antiviral activities of protein or peptide toxins present in the purification products of active protein fraction from *Senna occidentalis* seeds extracts. *Arquivo do Instituto Biológico*. 71(supl): 466-469.
- 20 **Martim K.W. & Ernest E. 2003.** Antiviral agents from plants and herbs: a systematic review. *Antiviral Therapy*. 8(2): 77-90.
- 21 **Melo F.L., Benati F.J., Roman Jr. W.A.R., Mello J.C.P., Nozawa C. & Linhares R.E.C. 2008.** The antiviral activity of an aliphatic nitro compound from *Heteropteris aphrodisiaca*. *Microbiological Research*. 163: 136-139.
- 22 **Murphy F.A., Gibbs E.P.J., Horzinek M.C. & Studdert M.J. 1999.** Herpesviridae. In: *Veterinary virology*. 3rd edn. London: Academic Press, pp.301-325.
- 23 **New York Botanical Garden (NYBG). 2003.** *The C.V. Starr Virtual Herbarium*. Disponível em: <<http://sweetgum.nybg.org/vh/specimen.php?irn=1021504>>. Acessado em 03/2006.
- 24 **Özcelik B., Aslan M., Orhan I. & Karaoglu T. 2005.** Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera*. *Microbiological Research*. 160(2): 159-164.
- 25 **Rates S.M.K. 2001.** Plants as source of drugs. *Toxicon*. 39: 603-613.
- 26 **Simoni I.C., Manha A.P.S., Sciessere L., Hoe V.M.H., Takinami V.H. & Fernandes M.J.B. 2007.** Evaluation of the antiviral activity of Brazilian cerrado plants against animal viruses. *Virus Review and Research*. 12(1-2): 25-31.

- 27 Soltan M.M. & Zaki A.K. 2009.** Antiviral screening of forty-two Egyptian medicinal plants. *Journal of Ethnopharmacology*. 126(1): 102-107.
- 28 Thiry E., Vindevogel H., Leroy P., Pastoret P.P., Schwers A., Brochier B., Anciaux Y. & Hoyois P. 1983.** *In vivo* and *in vitro* effect of acyclovir on pseudorabies virus, infectious bovine rhinotracheitis virus and pigeon herpesvirus. *Annals of Veterinary Research*. 14(3): 239-245.
- 29 Wei F., Ma S.C., Ma L.Y., But P.P., Lin R.C. & Khan I.A. 2004.** Antiviral flavonoides from seeds of *Aesculus chinensis*. *Journal of Natural Products*. 67(4): 650-653.
- 30 Zandonai R.H., Coelho F., Ferreira J., Mendes A.K.B., Biavatti M.W., Niero R., Filho V.C. & Bueno E.C. 2010.** Evaluation of the proliferative activity of methanol extracts from six medicinal plants in murine spleen cells. *Brazilian Journal of Pharmaceutical Sciences*. 46(2): 323-333.