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Article

Antiviral activities of plants occurring in the state of Minas Gerais, Brazil. Part 2. Screening Bignoniaceae species#

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RESUMO: "Atividade antiviral de extratos de plantas coletadas no estado de Minas Gerais. Parte 2. Triagem de Bignoniaceae." Extratos etanólicos de dezoito espécies vegetais pertencentes à família Bignoniaceae, das quais sete são descritas como de uso medicinal, foram avaliados, pelo ensaio colorimétrico do MTT, para atividades citotóxica, em células Vero, e antiviral, frente aos vírus herpes simplex-tipo 1, vaccinia e encefalomiocardite murina. A maior parte dos extratos não apresentou citotoxicidade até a concentração de 500 µg/mL. Dos 28 extratos testados quatorze (50%) apresentaram atividade antiviral com valores de CE50 na faixa de 4,6+03 a 377,2+17,7 µg/mL. Somente duas espécies, *Arrabidaea samydoides* e *Callichlamys latifolia*, foram ativas frente aos três vírus. Os extratos foram caracterizados pelos seus perfis cromatográficos em CCD e CLAE-FR. Análises por CLAE-FR mostraram que a mangiferina é o constituinte majoritário em *A. samydoides* mas a substância isolada foi menos ativa do que o extrato bruto. Esta é a primeira vez que se relata a atividade antiviral de extratos das dezoito espécies avaliadas.

Unitermos: Bignoniaceae, atividade antiviral, citotoxicidade, HHV-1, VACV, EMCV.

ABSTRACT: Ethanol extracts of eighteen Bignoniaceae species have been evaluated by the MTT assay for cytotoxicity in Vero cells and for antiviral activity against Human herpes virus type 1, Vaccinia virus and murine Encephalomyocarditis virus. Among such species, seven are reported to be of traditional medicinal use No cytotoxicity was observed for most of the extracts up to the concentration of 500 µg/mL. Fourteen (50%) of the 28 extracts assayed have disclosed antiviral activity with EC50 values in the range of 4.6+0.3 to 377.2+17.7 µg/mL. Only two species, *Arrabidaea samydoides* and *Callichlamys latifolia*, have shown activity against all the three viruses. The extracts were chemically characterized by their TLC and HPLC-DAD profiles. Mangiferin is the major constituent of *A. samydoides* but the isolated compound has been less active than the crude extract. This is the first report on the antiviral evaluation of the eighteen Bignoniaceae species assayed.

Keywords: Bignoniaceae, antiviral activity, cytotoxicity, HHV-1, VACV, EMCV.

INTRODUCTION

The marked evolution of biodiversity prospecting has been greatly driven by the growing demand for new genes and chemicals coupled to the awareness that an abundant and virtually untapped supply of natural resources exists in wild land biodiversity. Biotechnology has opened a new frontier in the pharmaceutical industry (Reid et al., 1996). The impact of biodiversity as source of new pharmaceuticals can be appreciated when we consider that a species (plants, microorganisms, animals, and insects) may contain hundreds of different chemicals that might result as new pharmaceutical leads. The success in the discovery of promising lead molecules increased by improvements in screening technology

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that allows evaluation of a high number of compounds at a high speed and relatively low cost (High Throughput Screening - HTS). The progress in this area, associated to advances in synthetic chemistry, biotechnology and medical sciences has led to new drugs in the last 25 years, many of which of plant origin or derived from natural products as extensively shown by Newman & Cragg (2007). The data presented confirms that the utility of natural products is not necessarily as the final drug entity but also as sources of novel and peculiar molecules to be used for semi-synthetic drugs or templates for totally synthetic ones. Furthermore, besides the important role of natural products as lead compounds in drug discovery, their significance as components of standardized plant extracts provide unlimited opportunities for new medicines. The phytochemical-biological investigation of traditional medicinal plants affords bioactive compounds that, besides their potential as new drug leads, are needed as chemical markers for standardization of extracts to be evaluated for efficacy and safety in the development of phytomedicines (Gibbons, 2003). Phytotherapy, that should be of great importance in developing countries, is still poorly explored, except in countries such as China and India where it is the basis of traditional medicine (TCM and Ayurveda, respectively) or, more recently, in Germany where pythomedicines (phytopharmaceuticals) are legally marketed once there are scientific evidences on their safety and reasonable confidence in their efficacy. That is the so called scientific or rational phytotherapy. Integration of new biological assays into the screening (bioprospection) of plant extracts and in the bioactivity guided isolation of active constituents are prerequisite for the standardization of extracts when the aim is the scientific validation of a medicinal plant used in traditional medicines aiming to investigate their alleged therapeutic use (Schulz et al., 2001).

Aiming to explore the rich Brazilian biodiversity we have started the bioprospection of plants occurring in the state of Minas Gerais, Brazil, by screening plant extracts for antiviral activity.

A large number of antiviral natural products are described (Chattopadhyay & Naik, 2007). Novel natural anti-HIV compounds, such as the calanolides, michellamines, prostatin and betulinic acid derivatives, have passed through preclinical assays (Butler, 2005). In general, *in vitro* antiviral assays are based on the cytopathic effect (CPE) in cell culture; in these assays, the activity is expressed by the 50% endpoint titration technique (EPTT). Over the last two decades, however, the colorimetric MTT assay, in which the MTT dye is reduced by viable cells, is becoming more and more used. This assay is semi- automated, rapid, requires only a small amount of test sample and directly assesses cell viability. (Betancur-Galvis et al., 2002).

The present paper reports on the evaluation of the susceptibility of one RNA-virus, the murine Encephalomyocarditis virus (EMCV), and two DNA-viruses, Human herpes virus 1 (HHV-1) and Vaccinia virus Western Reserve (VACV-WR) to ethanol extracts of Bignoniaceae species occurring in the state of Minas Gerais, Brazil. Plants for testing have been selected on the basis of ethnopharmacological and taxonomic criteria. HHV-1 and HHV-2 are pathogenic to humans, causing recurrent infections especially in the case of highly susceptible adults. Among HHV-related pathologies, genital herpes is an important sexually transmitted disease. In immuno-compromised patients and neonates, HHV infections can cause serious systemic illnesses. Furthermotre, HHV is involved in several ocular diseases, such as the herpetic stromal keratitis (HSK), an immunopathology which is one of the leading causes of blindness in western world. Acyclovir, a nucleoside analogue, is the most commonly used drug for treatment of HHV infections. However, resistance to acyclovir and related nucleosides occurs frequently (Khan et al. 2005) and, therefore, new antiviral agents are needed.

VACV (Poxvirideae family) is in the group of the Poxviruses genus Orthopoxvirus which includes cowpox, vaccinia, variola and camelpox viruses. Vaccinia virus (VV), but not variola virus, was used in the vaccination against smallpox that was eradicated more than twenty years ago. Extensive use of VV for smallpox vaccination, has rendered most humans vulnerable to smallpox infection (De Clercq, 2001). Re-emergence of human VV infections (Trindade et al., 2007) as well the threat that variola virus, the etiological agent of smallpox, might be used in warfare or terrorism, have motivated the search for measures to control or treat smallpox and poxvirus infections, in general. With the eradication of smallpox and the successful application of the smallpox vaccine, the search for new therapeutic agents against smallpox was not further pursued until the late 1970s when a panel of viruses, including VV strains, was evaluated for their susceptibility to different classes of compounds and has led to a large number of anti-VV leads, most of which are nucleoside analogues (De Clercq, 2001).

EMCV (family Picornaviridae, genus *Cardiovirus*) is a group of closely related virus strains with a wide host range. Infections with EMCV are associated with sporadic cases and outbreaks of myocarditis and encephalitis in domestic pigs, in nonhumans primates and in other mammalian species. The disease is often fatal; frequently sudden death is the first indication of infection and most outbreaks have been associated with captive animals such as those in piggeries, primate research centers and zoos. Virus isolation has been reported from patients with aseptic meningitis, poliomyelitis-like paralysis, encephalomyelitis and fever of unknown origin documented by virus isolation of several specimen types. Few cases of human EMCV infection and disease have been documented; the most recent were in 2004 from two febrile patients in Peru (Oberste et al., 2009).

EMCV was used as a model for RNA virus, especially for viruses from the Picornaviridae family, as it presents a safe animal model to test antiviral drugs (Mujtaba et al., 2006).

Interferons, cell glycoproteins synthesized in response to viral infections and various nonviral inducers, have proved therapeutically effective for viral infections in experimental models and in humans. Their significance as antiviral, antiprotozoal, immunomodulatory and cell growth regulatory agents is well documented. The IFN system can impair various steps of viral replication. However EMC viruses can replicate efficiently in IFN-treated cells what renders these viruses useful in the investigation of compounds whose antiviral effect could be related to stimulation of IFN production. EMCV was also used by our group to study the antiviral activity of interferons (Franco et al., 1999)

A large number of natural products and plant extracts obtained from traditionally used plants has been evaluated against HHV-1 and 2. (Khan et al., 2005). However there are few reports on evaluation of the susceptibility of VACV and EMCV to plant extracts. Many Bignoniaceae species are used in popular (traditional) medicine to the treatment of several diseases. Brazil is the main center of diversity for the Bignoniaceae family with 56 genera and 338 species which are distributed in the tribes Bignonieae, Crescentieae and Tecomeae. (Gentry, 1992).

Chemically, bignoniaceous plants are characterized by the presence of flavonoids, terpenoids, quinones, mainly naphthoquinones, and aromatic compounds such as lignans, cinnamoyl, benzoyl and acetophenone derivatives (Warashina et al. 2006). Naphthoquinones occur in many species of the family and could be responsible for different biological activities, including anticancer, antiinflamatory, antiviral, antimicrobial antifungal, antimalarial and antiparasitic. Flavonoids and terpenoids have been shown to disclose a broad spectrum of biological activities including as inhibitors of the cytopathic effect of several viruses (Chattopadhyay & Naik, 2007). The frequent occurrence of species belonging to the family Bignoniaceae in the state of Minas Gerais and the use of several species in the Brazilian traditional medicine, have motivated the present investigation.

MATERIALS AND METHODS

Plant material, extraction and chromatographic analyses

Plant material was collected in the state of Minas Gerais, Brazil. Voucher species were deposited into the herbarium of UFMG (BHCB), Belo Horizonte, Brazil. The plant material was dried in an air circulating oven at 45 °C for 48 h. The different plant parts were separated, ground and exhaustively extracted by percolation with ethanol 92.8 °GL affording the crude extracts (Table 1). All the extracts were characterized with TLC as well by HPLC-DAD, with online registration of the UV spectra of the constituents. HPLC fingerprints were registered on a Waters 2695 apparatus with a UV-DAD detector (Waters 2996).

Conditions

A LiChrospher 100 RP-18 column (5 µm, 250 x 4 mm *i.d*.) (Merck) was employed at a temperature of 40 °C, flow rate of 1.0 mL/min and detection at wavelengths of 220, 280 and 360 nm.

Sample preparation

To an aliquot (10.0 mg) of each dried extract, HPLC grade MeOH was added, the mixture was dissolved by sonication in a ultrasom bath for 15 min, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was filtered through Millipore membrane (0,2 µm) and injected (10.0 µL) onto the equipment. Elution was carried out with a linear gradient of water (A) and acetonitrile (B) (from 5% to 95% of B in 60 min).

Isolation and characterization of mangiferin

To dried ethanol extracts from *A. samydoides* leaves (10 g) and stems (10 g), MeOH (30 mL) was added and the insoluble part was filtered of, washed with cold MeOH and recrystallized from EtOH-water affording mangiferin (leaves extract 0.98 g, stems extract 0.85 g). M.p. 265 °C (dec.). Mangiferin was identified by usual spectrometric techniques (^{1}H and ^{13}CNMR, IR, UV, HRMS) and comparison with reported data (Gómez-Zaleta et al., 2006).

Cell culture and virus

Kidney cells of the African green monkey *Cercopthecus aeothiops* (Vero cell line ATCC CCL-81) were used in all experiments. Cells were grown in Dulbecco's modified Eagle medium (DMEM), containing 5% fetal bovine serum, 50 µg/mL gentamicin, 100 U/mL penicillin, and 5 µg/mL fungizone. The following strains were used in the assays: a clinical isolate of Human Herpes Virus type 1 (HHV-1) obtained in the Laboratory of Virus, Institute of Biological Sciences, UFMG, Belo Horizonte, Brazil, murine Encephalomyocarditis virus (EMCV), and Vaccinia Virus strain Western Reserve (VACV-WR), which were kindly donated by Dr. I. Kerr (Cancer Research UK, London Research Institute, London, United Kingdom) and Dr. C. Jungwirth (University of Würzburg, Germany), respectively.

Cytotoxicity assay

Vero cell monolayers were trypsinized, washed with culture medium and plated in a 96-well flat-bottomed plate with 6.104 cells per well. After 24 h of incubation, the diluted extracts (800-0.125 µg/mL) were added to the wells and the plates were further incubated for 48 h at 37 ºC in a humidified incubator with 5% CO_2. The supernatants were removed from the wells and 28 µL of MTT (Merck) solution (2 mg/mL in PBS) were added to each well. The plates were incubated for 1.5 h at 37 ºC and DMSO (130 µL) was added to the wells to dissolve the formazan crystals. The plates were placed on a shaker for 15 min and the optical density was determined at 492 nm (OD492) on a multiwell spectrophotometer (Stat Fax 2100) (Twentyman & Luscombe, 1987). The results were obtained from four replicates with at least four concentrations of each sample. Cytotoxicity was calculated using the equation (A-B)/A.100, where A and B are the OD492 values of untreated and treated cells, respectively. The 50% cytotoxic concentration (CC50) of the assayed samples is defined as the concentration that reduces the OD492 value of treated uninfected cells to 50% of that of untreated uninfected cells.

Antiviral assay

The antiviral testing was carried out by the MTT colorimetric assay according to the methodology previously described (Betancur-Galvis et al., 2002). Dilutions of the crude extracts in non-cytotoxic concentrations were added to the wells after viral infection. The plates were incubated at 37 ºC in humidified 5% CO_2 atmosphere for a period of 48 and/or 72 h. Controls consisted of untreated infected, treated non-infected and untreated non-infected cells. Positive controls (acyclovir, Calbiochem, USA; α-2a-interferon, Bergamo, Brazil) were also employed in each assay. Cell viability was evaluated by the MTT colorimetric method as described above for the cytotoxicity assay.

RESULTS AND DISCUSSION

The bignoniaceous species (18) were collected in the state of Minas Gerais, Brazil. The 28 ethanol extracts obtained from different parts have been characterized by their chromatographic profiles on TLC and HPLC (Table 1). All the extracts have been evaluated for cytotoxicity in Vero cell cultures and against HHV-1, VACV and EMCV by the MTT colorimetric assay. (Table 2).

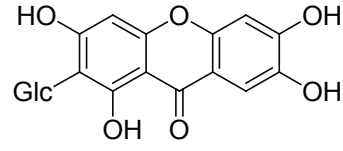
Most of the 28 extracts assayed have not shown cytotoxicity up to the concentration of 500 µg/mL (Table 2). Only two species, *Clytostoma ramentaceum* and *Pleonotoma stichadenium*, have been highly cytotoxic with CC50<20 µg/mL and have been more cytotoxic then *Tabebuia impetiginosa* (synon. *T. avellanedae*) and *T. serratifolia* which are known sources of cytotoxic naphthoquinones (Oliveira et al., 1990).

It is well known that the titer of a virus culture can influence the EC50 in the *in vitro* bioassays. Plant extracts that are able to protect cells from the induced cytopathic effects (CPE) of a virus with a TCID50/ mL>103 are considered relevant and deserve further investigation for the isolation of the active compounds. In the present screening, assays were run in cultures of virus with TCID100/mL of 2.5 x 106 for HHV-1, and 1.0 x 106 TCID100/ mL for both VACV-WR and EMCV.

Fourteen (50%) of the 28 extracts tested have disclosed antiviral activity against one or more of the virus and have shown EC50 values in the range of 4.6+0.3 to 377.2+17.7 µg/mL. Ten of the fourteen active extracts might be considered promising sources of antiviral compounds, as they showed EC50<100 µg/mL (Table 2).

Nine of the 28 extracts have disclosed activity against HHV-1 (Table 2) and two of these extracts, *Arrabidaea samydoides* leaves (EC50 40.6+1.6 µg/mL) and *Callichlamys latifolia* stems (30.4+12.0 µg/mL) have shown EC50 values close to that one of acyclovir, a frequently used anti-herpetic drug, besides presenting good selectivity (SI 12.3 and 16.5, respectively). Mangiferin (1), a *C*-glucosylxanthone, and acylated derivatives, characterized as antioxidants by the DPPH assay, were isolated from a specimem of *A. samydoides* which was collected in the state of São Paulo, Brazil (Pauletti et al., 2003). However no data on the chemistry and biological activity of *C. latifolia* was found.

We have shown by HPLC-DAD analyses that mangiferin is the major constituent of leaves ethanol extract of the *A. samydoides* specimen and, at a first glance, we might expect its presence would explain the activity of this extract. However, mangiferin (**1**) is also the major constituent of the stems extract although it has been about five times less active (EC50 218.1+3.4 µg/ mL). Therefore, besides mangiferin, other more active compounds are supposed to be present in the leaves of *A. samydoides* and a bioactivity guided fractionation of this extract has to be carried out.



1

Glc = glucosyl

VACV which, as HHV, is a double-streamed DNA virus, has exhibited higher susceptibility to the extracts tested with twelve positive results, seven of these with EC50<50 µg/mL. The most potent of the 28 extracts assayed, with EC50<10 µg/mL, are found in

this group: *Adenocalymma ternatum* stems (8.1+0.4 µg/mL) and *Distictella elongata* leaves (4.6+0.3 µg/mL). *A. samydoides* (leaves and stems), *Callichlamys latifolia* (stems), *Distictella elongata* stems and *Xylophragma myrianthum* stems are also promising extracts since they have presented EC50<50 µg/mL.

EMCV was the less susceptible virus species to the extracts assayed. Only one has shown a moderate activity (*Callichlamys latifolia* stems, EC50 103.0+3.2 µg/mL, SI>4.9) and three have been weakly active with EC50>300 µg/mL (*A. samydoides* leaves and stems, *Adenocalymma pedunculatum* stems).

Arrabidaea samydoides and *Callichlamys latifolia* are the only species among the eighteen tested ones to inhibit replication of HHV-1, VACV and EMCV. The leaves extract of *A. samydoides* has been about five-fold more active than the stems extract against HHV-1(EC50 40.6+1.6 µg/mL and 218.1+3.4 µg/mL, respectively). On the other hand, both leaves and stems extracts have had similar effects against VACV (37.13+1.3 and 45.5+2.8 µg/mL, respectively) and EMCV (EC50 323.4+5.6 and 377.2+17.7 µg/mL for leaves and stems, respectively. (Table 2). As shown by HPLC-DAD profiles (Figure 1), mangiferin (**1**) is the major constituent of both leaves and stems of the A. samydoides specimem we have assayed and might be responsible for the observed antiviral effects. Indeed, mangiferin has previously been shown to be active against both HHV-1 and -2. The average plaque reduction rate against HHV-1, by the tissue culture technique, was 56.8% (Zheng & Lu, 1989). Against HHV-2 the EC50 in HeLa cells was 111.7 µg/mL and at the concentration of 80 µg/mL there was 99% reduction of the virus replication (Zhu et al., 1993). However, in the present case, this compound has been less potent (EC50 267,9+6.7 µg/mL) than the leaves extract (EC50 40.6+1.6 µg/mL) and its EC50 is more close to that one of the stems extract (218.1+3.4 µg/mL). These results are a survey for the presumption that the leaves extract might contain compound(s) more active(s) than mangiferin while it might be the major anti-herpetic constituent of the stems extract. We have shown that mangiferin is less active against VACV (EC50 182,7+14,3 µg/mL) than both the leaves and stems extracts (37.13+1.3 and 45.5+2.8 µg/mL, respectively) from *A. samydoides* (Table 2) besides being inactive against EMCV. Bioactivity guided fractionation of these extracts has to be carried out looking for minor constituents more active than mangiferin.

Stems extract of *Callichlamys latifolia* has shown good activity against HHV-1 and VACV (EC50 30.4+12.0 and 56.0+4.0 µg/mL, respectively) and a moderate activity against EMCV (EC50 103.0+3.2 µg/mL). It is interesting to notice that the leaves extract has been much less active against HHV-1 and VACV besides being inactive against EMCV (Table 2). As stated

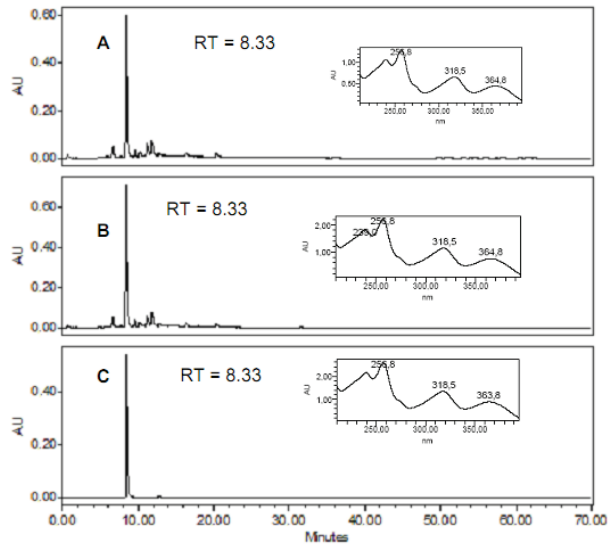


Figure 1. RP-HPLC fingerprints for crude ethanol extracts of *Arrabidaea samydoides* leaves (A), stems (B), mangiferin (C). Detection l 350 nm. UV spectra on line, RT=8.33 min (mangiferin). Chromatographic conditions: see Materials and Methods.

previously, no data were found on the chemistry and biological activity of *C. latifolia.*

Reported medicinal uses of the seven ethnopharmacologically selected species are shown in Table 3. Only three (42.8%) out of these species (*Arrabidaea chica, Callichlamys latifolia* and *Tabebuia impetiginosa*) have shown some activity against one or more virus (Table 2). *A. chica* is a neotropical creeper which occurs, particularly, in the Amazon basin where it is called "pariri", "crajirú", "crajurú" and "carajurú" The leaves of this plant have been widely used by Brazilian indians as dye in body paintings because they produce a dark red pigment that is known as "carajurin". *A. chica* leaves are nowadays widely used in Northern Brazil to treat blood disfunction (anaemia, hemorrhage), uterine inflammation, hemorrhoids and skin affections (Barbosa et al., 2008). Despite the wide use of this species there are few informations on the pharmacological effects of their leaves. Chemically, 3-deoxyanthocynidins and flavones have been isolated and carajunin was shown to be 6,7-dihydroxy-5,4'-dimethoxy-flavylium (Takemura, et al., 1995; Zorn et al., 2001; Devia et al., 2002; Barbosa et al., 2008). The antifungal activity of *A. chica* ethanol extract against *Trychophyton mentagrophytes in vitro* has been demonstrated (Barbosa et al., 2008) as well the *in vitro* and *in vivo* wound healing effects (Jorge et al., 2008).

Tabebuia impetiginosa (synon. *T. avellanedae*) is a neotropical tree that occurs in Mexico, Central and South America and is popularly known as "ipê-roxo", "ipê-preto", "pau-d'arco," "peúva" (Lohmann, 2006), "lapacho" and "taheebo". (Wagner & Seitz

Table 1. Plant species screened for cytotoxicity and antiviral activity and results of the phytochemical screening by TLC and HPLC-DAD.

Extract Number	Family species (Selection method)	Voucher Number	Plantpart	Extractives (%)	TLC	HPLC-DAD
	Bignoniaceae					
1	*Adenocalymma cymbalum* (Cham.) Bureau & K. Schum. (B)	BHCB 21858	S, L	12.8	TS, Fla, Ant	Fla, Ant, Proac, Cin
2	*Adenocalymma pedunculatum* (Vell.) L. G. Lohmann	UEC 69796	L	26.2	TS, Fla	Fla
3	(*Memora pedunculata* (Vell.) Miers)[C] B		S	16.9	TS, Fla, Sa	Fla, Proac, Cin
4	*Adenocalymma ternatum* (Vell.) Mello ex Bureau & K. Schum. (B)	BHCB 24763	L	15.0	Fla	Fla, Proac, Cin
5	(*Adenocalymma pleiadenium* Bureau & K. Schum.)[C]		S	9.0	Ta, TS, Fla	Fla, Proac, Cin
6	*Anemopaegma floridum* Mart. ex DC. (B)	BHCB 21861	F	18.9	Ta, TS, Fla	Fla, Proac
7		BHCB 23859	L	19.8	TS, Fla	Fla
8	*Arrabidaea chica* (Bonpl.) Verl. (A)	BHCB 23859	L	19.8	TS, Fla	Fla
9	(*Fridericia chica* (Bonpl.) L.G. Lohmann)[C]		F	20.3	TS, Fla	Fla, Proac
10	*Arrabidaea rego* (Vell.) DC. (A)	HRCB 27499	L	24.9	TS, Fla, Sa	Fla
11	(*Fridericia rego* (Vell.) L. G. Lohmann)[C]		S	17.5	TS, Ant, Fla	Fla, Ant, Proac
12	*Arrabidaea samydoides* (Cham.) Sandwith (B)	BHCB 23896	L	21.0	TS, Fla	Fla, Xant
13	(*Fridericia samydoides* (Cham.) L.G. Lohmann)[C]		S	15.0	TS, Fla	Fla, Xant
14	*Callichlamys latifolia* (Rich.) K. Schum. (B)	BHCB 80146	L	8.8	Ts, Fla	Fla, Proac, Cin
15			S	20.0	TS, Fla	Proac, Fla
16	*Clytostoma ramentaceum* (Mart. ex DC.) Bureau & K. Schum. (B) (*Cuspidaria ramentacea* Mart. ex DC.)[C]	BHCB 22728	S	17.3	TS	Cin
17	*Cybistax antisyphilitica* (Mart.) Mart. (A)	BHCB 21680	L	23.0	TS, Fla	Fla
18	*Distictella elongata* (Vahl) Urb. (B) (Amphilophium elongatum (Vahl) L. G. Lohmann)[C]	BHCB 21862	L	22.9	TS, Fla	Fla, Cin
19			S	15.0	TS, Fla	Fla, Cin
20			F	17.4	Fla	Fla
21	*Mansoa hirsuta* DC. (A)	BHCB 23862	F	18.6	Ta	Proac
22	*Pleonotoma stichadenium* K. Schum. (B)	BHCB 25328	L	19.0	TS, Fla,	Fla, Proac, Cin
23	*Tabebuia impetiginosa* (Mart. ex DC.) Standl. (A) (*Tabebuia avellanedae* Lorentz ex Griseb.)[C]	BHCB 45512	S	12.0	Ant, TS, Fla	Fla, Ant, Naph, Cin
24	*Tabebuia serratifolia* (Vahl) G. Nichols. (A)	BHCB 32995	S	13.0	Ta, Ant, Fla	Fla, Proac, Ant, Cin
25	*Tabebuia stenocalyx* Sprague & Stapf (B)	BHCB 63698	L	14.0	Ta, TS, Fla	Fla, Ant, Proac
26			S	18.0	Ta, TS, Fla	Naph, Fla, Proac, Cin
27	*Xylophragma myrianthum* (Cham.) (B)	BHCB 24760	L	21.6	Ta, TS, Fla	Fla, Proac, Cin
28			S	6.7	TS, Fla	Fla, Cin

A: Ethnopharmacological selection; B: Taxonomic selection; [C]synonymy; Fla: flavonoids, TS: triterpenes and steroids, Ta: tannins, Sa: saponins, Ant: anthraquinones, Cin: cinnamoyl esters, Proac: proanthocyanidins, Xan: xanthones, Naph: naphthoquinones; L: leaves ; S: stems; F: fruits

Table 2. Cytotoxicity and antiviral activity of ethanol extracts from Brazilian Bignoniaceae species collected in the state of Minas Gerais.

Extract Number	Family species	Plant part	VER0 Cells CC50 (µg/mL)	HHV-1 EC50 (µg/mL)	SI	VACV-WR EC50 (µg/mL)	SI	EMCv EC50 (µg/mL)	SI
	Bignoniaceae								
1	*Adenocalymma cymbalum* (B)	L,S	>500	NA		NA		NA	
2	*A. pedunculatum* (B)	L	>500	272.5+4.7	>1.8	48.3+1.6	>10.4	NA	
3		S	>500	NA		NA		374.2+13.9	
4	*A. ternatum* (B)	L	390.9+11.4	NA		NA		NA	
5		S	>500	NA		8.1+0.4	>61.6	NA	
6	*A. floridum* (B)	F	>500	266.5+2.9	>1.9	303.8+23.8	>1.6	NA	
7	*Arrabidaea chica* (A)	L	>500	245.7+37.1	>2.0	86.3+2.5	>5.8	NA	
8		F	>500	303.4+6.3	>1.6	72.4+2.7	>6.9	NA	
9		S	426.3+12,2	NA		NA		NA	
10	*A. rego* (A)	L	>500	NA		NA		NA	
11		S	>500	NA		NA		NA	
12	*A. samydoides* (B)	L	>500	40.6+1.6	>12.3	37.13+1.3	>13.5	323.4+5.6	>1.5
13		S	>500	218.1+3.4	>2.3	45.5+2.8	>11.0	377.2+17.7	>1.3
14	*Callichlamys latifolia* (A)	L	>500	312.3+10.9	>1.6	150.2+2.3	>3.3	NA	
15		S	>500	30.4+12.0	>16.5	56.0+4.0	>8.9	103.0+3.2	>4.9
16	*Clytostoma ramentaceum* (B)	S	19.7+2.8	NA		NA		NA	
17	*Cybistax antisyphilitica* (A)	L	>200	NA		NA		NA	
18	*Distictella elongata* (B)	L	163.3+3.4	NA		4.6+0.3	36.7	NA	
19		S	>500	NA		24.3+1.8	>20.6	NA	
20		F	>500	NA		NA		NA	
21	*Mansoa hirsuta* (A)	F	>500	NA		NA		NA	
22	*Pleonotoma stichadenium* (B)	L	19.6+0.5	NA		NA		NA	
23	*Tabebuia impetiginosa* (A)	S	>200	166.6+9.8	>1.2	NA		NA	
24	*T. serratifolia* (A)	S	>500	NA		NA		NA	
25	*T. stenocalyx* (B)	L	>500	NA		NA		NA	
26		S	>500	NA		NA		NA	
27	*Xylophragma myrianthum* (B)	L	>500	NA		NA		NA	
28		S	>500	NA		34.1+0.6	>14.7	NA	
	Acyclovir			40					
	α-2a-Interferon					2.5×10^2		1.5×10^2	

CC50: 50% cytotoxic concentration (µg/ mL) for vero cells; EC50: effective concentration (µg/ mL) required to inhibit by 50 % the cytopathic effect (CPE) at a viral title of 2.5×10^6, 1.0×10^6 and 1.0×10^6 TCID100/mL for HHV-1, VACV-WR and EMCV respectively; SI: selectivity index = CC50/EC50; HHV – 1: *Human herpesvirus* 1; EMCV: murine *Encephalomiocarditis virus*; VACV- WR: *Vaccinia virus* Western Reserve; NA: Not Active; NT: Not Tested; L: leaves; S: stems; F: fruits.

1998). Ethnopharmacological data point to the use by Southamerican natives for thousand of years. Spanish and Portuguese conquerors described the use of large quantities by indigenous tribes. However, it was only after spreading news on the antitumoral effect of inner bark decoction in the 1960s that scientific research has greatly increased around the world. Clinical assays of lapachol by the NCI led to its rejection as effective anticancer agent in 1970 and, more recently, the interest has turned to β-lapachone that revealed significant activity against a range of human tumour cell lines. It has been observed that β-lapachone, as camptothecin, inhibits

Table 3. Reported medicinal uses for some of the plant species assayed for cytotoxicity and antiviral activity

Plants	Brazilian names	Traditional uses	References
Bignoniaceae			
Arrabidaea chica	Carajurú, crajurú, crajirú	Anti-inflammatory, astringent, intestinal colic, diarrhea, leucorrhoea, anemia, leukemia, skin affections, wound healing	Correa, 1975; Cruz, 1985
A. rego	Cipó-rego	Skin affections, gonorrhea	Correa, 1975; Cruz, 1985
Callichlamys latifolia	Cipó-guachana amarelo	Intestinal colic, skin affections	Cerón-Martínez and Montalvo-Ayala, 1998; Ríos et al., 2007
Cybistax antisyphilitica	Ipê-verde, cinco-folhas	Diaphoretic, syphilis, anti-edemic, bladder disfuntion	Correa, 1975; Cruz, 1985
Mansoa hirsuta	Alho-bravo	Diabetes, throat pains	Emperaire, 1983; Agra et al., 2007
Tabebuia impetiginosa	Ipê-roxo, pau-d'arco, peúva	Cancer, liver diseases, skin affections, inflammations of the ear and mucosa (gingival, throat, vagina, uterus and anus), ovaries, prostate and muscle	Emperaire, 1983; Cruz, 1985; Agra et al., 2007
T. serratifolia	Ipê-amarelo, pau-d'arco amarelo	The same indications and uses as above	Correa, 1975; Agra et al., 2007

topoisomerase I but by a different metabolic pathway. Synthtetic β-lapachone (ARQ501) is in clinical trials as anticancer at ArQule, USA (Gómez-Castellanos et al., 2009). The bark and heartwood extracts, particularly those in methanol or etanol, are reported to contain lapachol, α- and β-lapachone, furanonaphthoquinones, lignans and iridoids along with simple aromatic compounds (Warashina et al., 2006, Wagner & Seitz, 1998). As far as we are concerned this is first report on the evaluation of an ethanol extract from *T. impetiginosa* against HHV-1, VACV and EMCV. We have detected lapachol, but not β-lapachone, by HPLC-DAD, in the ethanol extract which was presently assayed.

Six of the eleven taxonomically selected Bignoniaceae species (54.5%) have shown some activity in one or more virus species (Table 2). Among these *Distictella elongata* leaves extract has shown the lowest EC50 value (4.6+0.3 μg/mL, against VACV) of the 28 extracts assayed and its chromatographic profile revealed the presence of flavonoids and cinnamoyl esters (Table 1) that might be responsible for the significative antiviral activity observed.

CONCLUSIONS

Fourteen (50%) of the assayed extracts have shown antiviral activity against one or more of the virus and ten of the fourteen active extracts might be considered promising sources of antiviral compounds, as they showed EC50<100 μg/mL. *Arrabidaea samydoides* is one of the two species, among the eighteen tested ones, to inhibit the replication of the three virus assayed and it is a rich source of mangiferin, an anti-herpetic *C*-glucosylxanthone. This is the first report on the antiviral activity of the eighteen screened species and the results demonstrate the high potential of Bignoniaceae species as source of antiviral agents.

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