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In vitro antiviral activity of seeds from *Guettarda angelica* against avian viruses

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ABSTRACT

The aim of this study was to evaluate the inhibition of the aqueous extract of seeds (AEs) from *Guettarda angelica* on cell infection by two avian RNA viruses: avian reovirus (ARV) and metapneumovirus (AMPV). The cytotoxic and antiviral activities were evaluated by MTT assay to determine the 50% cytotoxic (CC_{50}) and inhibitory concentrations (IC_{50}). The selectivity index ($SI=CC_{50}/IC_{50}$) also carried out. AEs exhibited antiviral activity only against ARV presenting IC_{50} of 23.59µg/mL. This inhibition was not due to any cytotoxic effect of AEs since the CC_{50} on Vero cells was of 400.60µg/mL and its SI was of 17.00; this extract also showed a virucidal effect on ARV. Previous studies also demonstrated antiviral activity of AEs extract against three animal herpesviruses. Thus, the seeds from *G. angelica* showing antiviral activity against DNA and RNA viruses, enveloped and non-enveloped, could be promising source of new antiviral agents encouraging its fractionation to isolate the active compound.

INTRODUCTION

Medicinal plants are used alternatively in medicine or as base to new synthetic formulations (Rates, 2001; Newman *et al.*, 2003); and still are source of compounds with the most diverse biological activities including antivirals (Jassim & Naji, 2003; Chattopadhyay & Naik, 2007). The most effective strategy to prevent the viral diseases is the vaccination, which provides resistance to infections, and the use of synthetic antiviral drugs is applied in treatment (Jassim & Naji, 2003; Chattopadhyay & Naik, 2007).

However, there is still great need to develop new antiviral drugs that can substitute for or complement the therapy with the currently available drugs. Thus, new antiviral agents from natural sources, especially those that have high efficacy on resistant mutant viral strains and low toxicity to host, are considered to be most promising. *Guettarda angelica* Mart. ex Müll. Arg. (Rubiaceae) also known as Angélica-do-mato is found in the Northeast region of Brazil (Agra *et al.*, 2008). Its root barks have various therapeutic uses and also presented an in vitro antibacterial activity (Bispo *et al.*, 2007). However in our previous studies this same root extract did not show antiviral activity against bovine (BoHV-1) and swine herpesviruses (SuHV-1) type 1, although its seed extract has presented an antiviral activity against these same DNA viruses (Barros *et al.*, 2012). The inhibition of seeds of this plant on DNA viruses motivated us to expand the study for RNA viruses specifically, the avian reovirus (ARV), member of the *Reoviridae* family and ubiquitous in commercial poultry (Rosenberger, 2003), and avian metapneumovirus (AMPV), *Paramyxoviridae* family, that causes an upper respiratory tract infection in turkeys and is involved in the swollen head syndrome of chickens (Gough & Jones, 2008).

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MATERIAL AND METHODS

Plant material

The aqueous extract of seeds (AEs) from *Guettarda angelica* Mart. ex Müll. Arg. was previously prepared at 5 mg/mL (Barros *et al.*, 2012). The plant was collected in Bahia State, Brazil, in December, 2003 and a voucher specimen was deposited in the Herbarium of the "Centro de Pesquisa do Cacau" (Bahia, Brazil) with ID 1015301.

Cell line and viruses

The strain S1133 of avian reovirus (ARV) was kindly provided by Biovet Laboratory, São Paulo, Brazil. The strain SHS-BR-121 of avian metapneumovirus (AMPV) was provided by Laboratory of Virology of University of Campinas (UNICAMP), São Paulo, Brazil. Both strains were propagated in Vero cell line (African green monkey kidney cells).

MTT colorimetric assay

The cytotoxicity and antiviral assays were performed on Vero cells seeded on 96-well microplates using the MTT colorimetric method described in Barros *et al.* (2012). From these assays it was calculated the 50% cytotoxic concentration (CC_{50}) of AEs on Vero cells and the 50% inhibitory concentration (IC_{50}) on viruses. These values were obtained from nonlinear regression analysis of concentration-effect curves using the GraphPad Prism 5 Demo program. The selectivity index (SI) was calculated from the ratio CC_{50} over IC_{50}.

RESULTS AND DISCUSSION

In this study, AEs extract did not show antiviral activity against AMPV but acted on replication of ARV with IC_{50} of 23.59µg/mL (Table 1). This antiviral activity was at concentrations almost more than 20-fold lower than concentrations exhibiting cytotoxicity (CC_{50} of 400.60 µg/mL).

Table 1. Cytotoxic and antiviral activities and selectivity index of aqueous extract from *Guettarda angelica* seeds (AEs) against avian reovirus by MTT method.

	Antiviral activity	Cytotoxicity	Selectivity index
Extract	**IC_{50}(µg/mL)**	**CC_{50}(µg/mL)**	**SI (CC_{50}/IC_{50})**
AEs	23.59±0.11	400.60±0.11	17.00

*Values obtained from nonlinear regression analysis of concentration-effect curves using the GraphPad Prism 5 Demo program.

From these values, the SI of the extract was of 17.00. In research of biological activity of plant extracts is fundamental a preliminary study of toxicity to ensure its therapeutic uses. The factors of quality, safety and efficacy of the extracts are essential requirements and of extreme importance in any study of plants. Furthermore, a significant and selective antiviral activity of plant extracts is related to IC_{50} values below 100µg/mL (Cos *et al.,* 2006) and a SI higher than 1 as criterion for a true antiviral agent (Tsuchiya *et al.,* 1985, Dezegrini *et al.,* 2010). Taken together the AEs results are in accordance with these values. The virucidal effect of AEs on ARV was also assessed using the viral yield reduction technique according to Barros *et al.* (2010a). There was a significant reduction in viral titer of ARV in 1 x $10^{2.26}$ ($p<0.01$, variance analysis-ANOVA). Although ARV is a non-enveloped virus using endocytosis process to entry the cell cytoplasm, it was inactivated directly and extracellular by AEs. Several plant extracts have been studied showing anti reovirus action. Lupini *et al.* (2009) showed the *Castanea* spp. and *Schinopsis* spp. wood extracts had extracellular antiviral effect against this virus and AMPV; these authors suggested that tannins presented in those extracts maybe the effective substances against poultry enteric viral infections. Also, the aqueous extracts of *Quillaja saponaria* presented antiviral activity against a reovirus serotype 3 but not act directly to inactivate it (Roner *et al.*, 2007). Leaf extracts from some Brazilian Cerrado plant species also presented antiviral activity against ARV (Simoni, 2007) and AMPV (Barros *et al.*, 2010b): *Lithraea molleoides* and *Gochnatia polymorpha* were active for both viruses while those of *Banisteriopsis variabilis*, *Bumelia sartorum* and *Byrsonima intermedia* were active only against AMPV. In other studies, the AEs extract showed antiviral activity against BoHV-1, SuHV-1 and equine herpesviruses type 1 (EHV-1) besides virucidal effect against the two first viruses (Barros *et al.,* 2010a, 2012). Since the seed from *G. angelica* showed antiviral activity against DNA and RNA viruses, enveloped and non-enveloped, it could be promising source of new antiviral agents encouraging its fractionation to isolate the active compound (s).

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