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

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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).

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\textsubscript{50}) of AEs on Vero cells and the 50% inhibitory concentration (IC\textsubscript{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\textsubscript{50} over IC\textsubscript{50}.

RESULTS AND DISCUSSION

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Antiviral activity (µg/mL)</th>
<th>Cytotoxicity (µg/mL)</th>
<th>Selectivity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEs</td>
<td>IC\textsubscript{50}</td>
<td>CC\textsubscript{50}</td>
<td>SI (CC\textsubscript{50}/IC\textsubscript{50})</td>
</tr>
<tr>
<td></td>
<td>23.59±0.11</td>
<td>400.60±0.11</td>
<td>17.00</td>
</tr>
</tbody>
</table>

*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\textsubscript{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\textsuperscript{-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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