

Full Length Research Paper

***In vitro* inhibitory effect of *Urera baccifera* (L.) Gaudich. extracts against herpes simplex**

Fernanda Otaviano Martins¹, Michelle Mendes da Rocha Gomes¹, Fábio Luis Pereira Nogueira², Gabriel Rocha Martins³, Maria Teresa Villela Romanos¹, Maria Auxiliadora Coelho Kaplan⁴ and Fábio de Sousa Menezes^{5*}.

¹Experimental Laboratory of Cytotoxic and Antiviral Drugs, Virology Department, Microbiology Institute Professor Paulo de Góes, Centre of Health Sciences, Block I, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

²Programme on Plant Biotechnology, Centre of Health Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

³Department of Food and Natural Products, Faculty of Pharmacy, Centre of Health Sciences, Block A, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

⁴Natural Products Research Centre, Centre of Health Sciences, Block H, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

⁵School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, University of Dublin - Dublin 2 – Ireland.

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The inhibitory activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) *in vitro*, at non-cytotoxic concentrations of *Urera baccifera* (L.) Gaudich. extracts have been evaluated. As for the antiviral activity, the maximum non toxic concentration has been established and this concentration has been used in the anti herpes assay, *in vitro*. Antiviral activity was determined by reduction of the virus titres. *U. baccifera* extracts showed the maximum percentage of inhibition against HSV-1-ACVr and the order of activity was found to be butanol fraction > ethyl acetate fraction > ethanol extract. However, the same extracts have not shown expressive percentage of viral inhibition against HSV-2-ACVr.. This is the first report showing antiviral activities for *U. baccifera* extracts.

Key words: *Urera baccifera*, antiviral activity, HSV-1, HSV-2

INTRODUCTION

Herpes Simplex Virus (HSV) is widely spread around the world and represents an important cause of orolabial and genital ulceration. It is also responsible for neonatal morbidity, a co-factor for HIV transmission, particularly in developing countries (Patel and Pompalo, 2005) and it is also involved in several ocular diseases (Pepose et al., 2006; Liesegang, 2001). The two forms of Herpes simplex virus (HSV-1 and HSV-2) are morphologically similar and produce indistinguishable characteristics during the initial infection. However, HSV-1 and HSV-2 have an anatomic tropism and site-dependent incidence of reactivation, thus, HSV-1 is more likely to reactivate produc-

ing orofacial infection while genital infection is frequently produced by HSV-2 (Pepose et al., 2006). Several antiviral agents have been used for the treatment of Herpes simplex virus infections such as acyclovir, ganciclovir and penciclovir. Furthermore, plant extracts and pure compounds isolated from natural sources have already been tested and can be considered an important tool on herpes infection treatment (Pandey et al., 2004; Khan et al., 2005; Da-Silva-Nunes and Pereira 2008; Chattopadhyay and Khan, 2008; Ghaemi et al., 2009). *Urera baccifera* (L.) Gaudich. (Urticaceae) is a woody shrub which occurs from Central America to Argentina including Andean region. In Brazil, this species is distributed throughout Atlantic Tropical Forest in altitude over 800 m. *U. baccifera* is employed by the Costa Rica Amerindians, when crossing high mountains, to ward off chills. They chastise themselves with the spiny stems and

*Corresponding author. E-mail: desouzaf@tcd.ie. Tel: +35318964154. Fax: +35318962810.

the same rubefacient effect is also employed on rheumatic pains. Tests have shown anti-inflammatory and analgesic activities of the aqueous extracts of *U. baccifera* in rats. Other species belonging to the Urticaceae family have shown antiviral (Uncini et al., 2005), hypoglycemic (Kavalali et al., 2003) and cardiovascular (Testai et al., 2002) activities. There are no data in the literature on their chemical constituents.

This work was undertaken to evaluate the *in vitro* antiviral activity of *U. baccifera* extracts against Herpes simplex types 1 and 2.

MATERIALS AND METHODS

Plant material

U. baccifera aerial parts collected in Rio de Janeiro city, Brazil, were identified by Dr. Jorge P. P. Carauta (National Museum, Federal University of Rio de Janeiro). Voucher specimens are deposited in the Herbarium Henrique Castellanos – GUA (FEEMA) under the number 32456.

Extract preparations

Dried plant material was powdered and subjected to exhaustive extraction with ethanol at room temperature. The obtained extract was concentrated under reduced pressure. The residue was suspended in water followed by a sequential liquid-liquid extraction with the different solvents such as: hexane, ethyl acetate and n-butanol, according to Menezes et al. (2005).

Sample preparation

Extracts were lyophilized and dissolved in water to a final concentration of 400 µg/ml. Each solution was sterilized by filtration using a 0.22 µm Milipore membrane, aliquoted and stored at –20°C.

Antiviral assay

In order to evaluate the antiviral activity, the extracts were assayed at the Maximum Non-Toxic Concentrations (MNTC), which is unable to produce any morphological alterations on the cells tested. Vero cells (African green monkey's kidney) were grown in Eagle's Minimum Essential Medium (MEM) supplemented with 10% of Fetal Bovine Serum (FBS) and maintained at 37°C in atmosphere of 5% of CO₂. Antiviral activity was determined by reduction of the virus titres. Logarithmical dilutions of virus suspension were added to the treated and untreated cell cultures and incubated at 37°C in atmosphere of 5% of CO₂. After that, the virus titres determination was performed using Reed and Muench (1938) statistical method and expressed as TCID₅₀ values (Reed and Muench, 1938). The results were expressed as Percentage of Inhibition (PI) and Viral Inhibition Index (VII) according to Gonçalves et al. (2001). In order to determine the mechanism of action involved in the antiviral activity, experiments were performed to evaluate the inhibitory effect at different stages of virus infection. The virucidal assay was carried out as described by Chen (1988) with slight modifications (Wigg, 1996; Chen et al., 1988; Wigg et al., 1996). Essentially, 100 µL of the virus suspension was added at 900 µL of the extracts at the MNTC and incubated at 37°C for 2 h. Then, the treated and

untreated virus suspensions were added to the Vero cell monolayer culture and kept at 37°C for 48 h. After incubation, the residual titres of the treated and untreated virus were determined and expressed as the percentage inhibition as previously described.

The action of extracts on cell receptors was evaluated by adding them on their MNTC to the Vero cell cultures. After that, the cells were washed and inoculated with ten-fold serial dilutions of viral suspension and incubated at 37°C for 48 h. The virus titres in treated and untreated cells were determined and the activity was expressed as the percentage inhibition.

In the penetration assay, Vero cell monolayers were inoculated with logarithmical dilutions of viral suspension and incubated for 1 h at 4°C. After adsorption, the extracts were added at MNTC and incubated for 1 h at 37°C. The cells were then washed to the total removal of the extracts and re-incubated at 37°C for 48 h.

In the intracellular assay, Vero cell monolayers were inoculated with viral suspension and incubated at 37°C for 2 h. The cells were washed and extracts at the MNTC were added and the cultures incubated at 37°C for 16 h. After incubation, the cells were washed to remove the extracts before the release of viral particles. MEM-Eagle was added and the cultures incubated for 32 h at 37°C. The virus titres determination was performed as previously described.

RESULTS

The maximum non-toxic concentrations of the tested samples are shown in Table 1. The sample with the highest cytotoxicity potential was the hexane extract of *U. baccifera* (50 µg/ml).

U. baccifera extracts have shown the maximum percentage of inhibition against HSV-1-ACVr. The ranking of extract activities from higher to lower was butanol > ethyl acetate > ethanol extract. However, the extracts did not show expressive inhibitory percentage against HSV-2-ACVr (Table 1).

The mechanism of action involved in the HSV-1-ACVr inhibition by *U. baccifera* against HSV-1-ACVr is showed in Table 2. The butanol fraction showed antiviral activity with high virucidal activity (85.9%). This extract had also presented a slight action on cellular receptors. For the ethyl acetate extract, the main mechanism involved was the inhibition of cellular receptors (90%). A great inhibition of viral penetration (85.9%) and no virucidal activity have also been seen. The ethanol extract showed antiviral activity by inhibition of viral penetration (94.4%).

DISCUSSION

The results of the present investigation provide further evidence of the importance of this species on Herpes simplex treatment.

The *U. baccifera* extracts tested appeared to be effective against Herpes simplex virus infection. Uncini Manganelli et al. (2005) have demonstrated an antiviral property of *Urtica dioica*. In addition, fractions with different polarities showed antiviral activities by different mechanisms. *U. baccifera* showed to produce very versatile and potent extracts against HSV-1, being capable to

Table 1. Maximum Non-Toxic Concentration (MNTC), the Viral Inhibitory Index (VII) and Percentage of Inhibition (PI) for *U. baccifera* extracts against the herpes simplex virus types 1 and 2.

Samples*	MNTC	HSV – 1-ACVr		HSV – 2-ACVr	
	µg/mL	VII	PI	VII	PI
UBGE	200	1.25	94.4	0.1	20.6
UBGH	50	0	0	0.1	20.6
UBGA	200	1.5	96.8	0	0
UBGB	200	2.25	99.4	0.1	20.6

UBGE - Ethanol extract from *U. baccifera*; UBGH - Hexane fraction from *U. baccifera*; UBGA - Ethyl acetate fraction from *U. baccifera*; UBGB - Butanol fraction from *U. baccifera*.

Table 2. Mechanism of action involved in the inhibition of viral infection by *U. baccifera* against HSV-1-ACVr. Maximum Non-Toxic Concentration (MNTC), the Viral Inhibitory Index (VII) and Percentage of Inhibition (PI).

Samples	MNTC µg/mL	Virucidal activity		Intracellular activity		Receptor activity		Penetration activity	
		VII	PI	VII	PI	VII	PI	VII	PI
UBGE	200	0.5	68.4	0	42.5	1.25	0	0.24	94.4
UBGA	200	0	0	1.0	55.3	0.85	90.0	0.35	85.9
UBGB	200	0.85	85.9	0.35	20.6	0	55.3	0.1	0

inhibit different steps of viral replication differently from acyclovir. This fact reinforces the possible contribution of different compounds in an antiviral activity showed by different *U. baccifera* extracts. Further studies are needed to isolate and identify the bioactive compounds. Recent paper from our research group has investigated the anti-herpes activity of two Asteraceae species, *Pterocaulon alopecuroides* and *Bidens pilosa*.

In that study, we were able to show the potential of the extracts obtained with these plant species as well as to isolate one molecule from *P. alopecuroides*, 7-(2',3'-epoxy-3'-methylbutyloxy)-6-methoxycoumarin, which was also tested and showed a great activity, suggesting that it could be one of the responsible for the antiviral activity showed by *P. alopecuroides* extracts, especially those containing the coumarin (Silveira et al, 2009). It is our intention to follow the same route for *Urera baccifera* and try and isolate the compounds responsible for the anti HSV-1-ACVr shown for their extracts. This is the first report that shows antiviral activities of *U. baccifera* extracts.

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