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SCIENTIFIC COMMUNICATION

ANTIHERPES ACTIVITIES OF FRACTIONS FROM *SESBANIA VIRGATA* LEAVES

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ABSTRACT

The fractions obtained from ethanolic extract from *Sesbania virgata* leaves showed cytotoxic activities on Vero and MDBK cells and promising antiviral activities against bovine and swine herpesvirus.

KEY WORDS: *Sesbania virgata*, antiherpes activity, bovine herpesvirus, swine herpesvirus, cytotoxicity.

RESUMO

ATIVIDADES ANTIHERPES DAS FRAÇÕES DAS FOLHAS DE *SESBANIA VIRGATA*. As frações obtidas do extrato etanólico das folhas da *Sesbania virgata* mostraram atividade citotóxica em células Vero e MDBK e um importante efeito antiherpético contra os herpesvírus bovino e suíno, indicando a necessidade de identificação das substâncias ativas da planta.

PALAVRAS-CHAVE: *Sesbania virgata*, atividade antiherpes, herpesvírus bovino, herpesvírus suíno, citotoxicidade.

The research of chemical substances with therapeutic potential from natural sources has been increasing in the last decades. Some compounds isolated from plants have shown potent antiviral activities such as castanospermine of *Castanospermum australi*, hypericin of *Hypericum* sp., glycyrrhizin of *Glycyrrhiza glabra* and 3-methoxiflavone derivative from *Euphorbia grantii* (EVANS, 1998). The great totality of these researches has been conducted to attack HIV (human immunodeficiency virus), the agent of AIDS. However, they have not been directed to the bovine herpesvirus type 1 (BHV-1) and swine herpesvirus type 1 (SHV-1), diseases of great economic importance for livestock production. The pathogenic agent of Infectious Bovine Rhinotracheitis / Infectious Postular Vulvovaginitis (IBR / IPV), is BHV-1, being responsible for a wide variety of clinical symptoms including respiratory disease, reproductive tract lesions and abortion. SHV-1 is the agent of the disease of Aujeszky which provokes an infection characterized by high lethality and nervous signs. In the search of natural alternative source with antiviral activities, the propose of the present study was to evaluate the cytotoxic activities as well as antiviral activities of BHV-1 and SHV-1 using the extract and its fractions of *Sesbania virgata* forage (Leguminosae-Papilionoideae). This plant, common in the fields of Brazil, presents a high protein level of 16 to 28% (VEASEY et al., 1998) being an alternative source of proteic supplement. Previously, we have reported on the isolation and characterization of flavonoid glycosides from *S. virgata* seed (TSUHAKO et al., 1989) but there is no study in the literature relating the genus *Sesbania* to antiviral principles.

The specimen *Sesbania virgata* (Cav.) Pers. was cultivated and collected at the Zootechny Institute, Nova Odessa city, São Paulo State, Brazil and identified by taxonomist Dr. Reinaldo Monteiro from Universidade Estadual de São Paulo from Rio Claro city, São Paulo State, Brazil. A voucher specimen HRCB 17378 was deposited in the Herbarium Rio Clarense of the Instituto de Biociências for UNESP.

The fresh leaves were crushed in a mixer and exhaustively macerated with 96% ethanol at room temperature. The combined extracts were evaporated to dryness under reduced pressure to yield ethanolic extract (EE - 7.6%). This extract was treated with water and the insoluble green material obtained in the process was removed by filtration and next lyophilized to yield a water insoluble fraction (WIF-1.6%). The aqueous filtrate was extracted sucessively with ethyl acetate and butanol saturated with water to yield, after evaporation, the following fractions: ethyl acetate fraction (EAF-0.2%) and butanol fraction (BF-0.2%).

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Table 1- Cytotoxicity and antiviral activity of fractions from *S. virgata* leaves against BHV-1 and SHV-1 on MDBK and Vero cells

Tested materials	MDBK cells			Vero cells		
	NTMC (µg / mL)	Antiviral activity (SI)		NTMC (µg / mL)	Antiviral activity (SI)	
		BHV-1	SHV-1		BHV-1	SHV-1
EE	125	4,0	1,0	125	1,0	1,0
WIF	125	4,0	1,0	125	1,0	1,0
EAF	125	8,0	1,0	62,50	1,0	1,0
BF	250	2,0	1,0	62,50	1,0	1,0
F-1	3,91	32,58	1,0	15,62	2,0	2,0
F-2	3,91	16,29	1,0	15,62	2,0	2,0
F-3	15,62	3,99	1,0	31,25	1,0	1,0
F-4	15,62	3,99	1,0	31,25	1,0	1,0
F-5	15,62	3,99	1,0	31,25	1,0	1,0

$TCID_{50}$ = 50% tissue culture infectious dose (mg / mL); virus titre of BHV-1 = $10^{7,3}TCID_{50}$; virus titre of SHV-1 = $10^{6,5}TCID_{50}$
NTMC = non-toxic maximum concentration which causes destruction in 50% of monolayer cells
SI = selectivity index

The WIF, after extraction with the following solvents: hexane, methylene carbon and sulphuric ether, yielded an insoluble fraction. Next, this was subjected to chromatographic column on DIAION-HP-20 eluted with mixtures of water and methanol (0%, 20%, 40%, 60%, 80%) in decreasing polarity to give the following residues: F-1 (0.03%), F-2 (0.015%), F-3 (0.008%), F-4 (0.002%), F-5 (0.004%).

For cytotoxic assay, African green monkey kidney (Vero cell line nº ATCC CCL28) and Madin Darby bovine kidney (MDBK cell line nº ATCC CCL22) cultures were grown in Eagle's minimum essencial medium (Eagle-MEM) supplemented with 10% fetal bovine serum (FBS) (Schmid et al.,1989). The samples EE, WIF, EAF, BF, F-1, F-2, F-3, F-4 and F-5 were prepared in DMSO (5%) and Eagle MEM. Each dilution (ratio ½) was added in triplicate to confluent one-day-old monolayers of Vero and MDBK cells grown in 96-well microtitre plates (Flacon NJ) (3.5 x 10^4 cells / mL) and incubated (treated and untreated cells with the samples at non-toxic maximum concentration - NTMC) at 37ºC in a 5% CO_2 atmosphere during a period of 3 days. (Betancur-Galvis et al., 1999) The antiviral activity was assayed against BHV-1 and SHV-1 obtained from the Pan-American Center of Foot and Mouth Disease, Rio de Janeiro, Brazil. Virus titres ($10^{7,3}$ and $10^{6,5}$ $TCDI_{50}$ / mL, respectively) were determined by a statistical method according to Reed & Muench (1938).

The effect of the plant extracts on the proliferation of cell culture was based on cellular morphological alterations for microscopic examination determined by a method similar to that described by Miranda et al. (1997) expressed as the NTMC which is the concentration of substances to inhibit the growth of cells up to 50%. The antiviral activity was evaluated by inhibition of the viral cytopathic effect (VCE) of herpesvirus and the effective concentration which inhibited 50% of VCE (EC), while the selectivity index (SI=NTMC / EC) of EE, WIF, EAF, BF, F-1, F-2, F-3, F-4 and F-5 fractions were estimated as described by Piñeros et al.(1992) and Simões et al. (1999).

The fractions obtained from ethanolic extract from *S. virgata* leaves presented high cytotoxicity in MDBK and Vero cells. All of the fractions evaluated showed in MDBK cells promising activity against BHV-1 replication and only F-1 and F-2 subfractions presented in Vero cells moderated activity against SHV-1 (Table 1). Thus, it could be suggested chemically that this (these) antiviral active principle(s) of this plant might be of polar nature.

In conclusion, despite a moderate antiviral activity of the fractions of *S. virgata* leaves, further studies are required to use this forage as a possible alternative for the treatment of bovine and swine herpesvirus.

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