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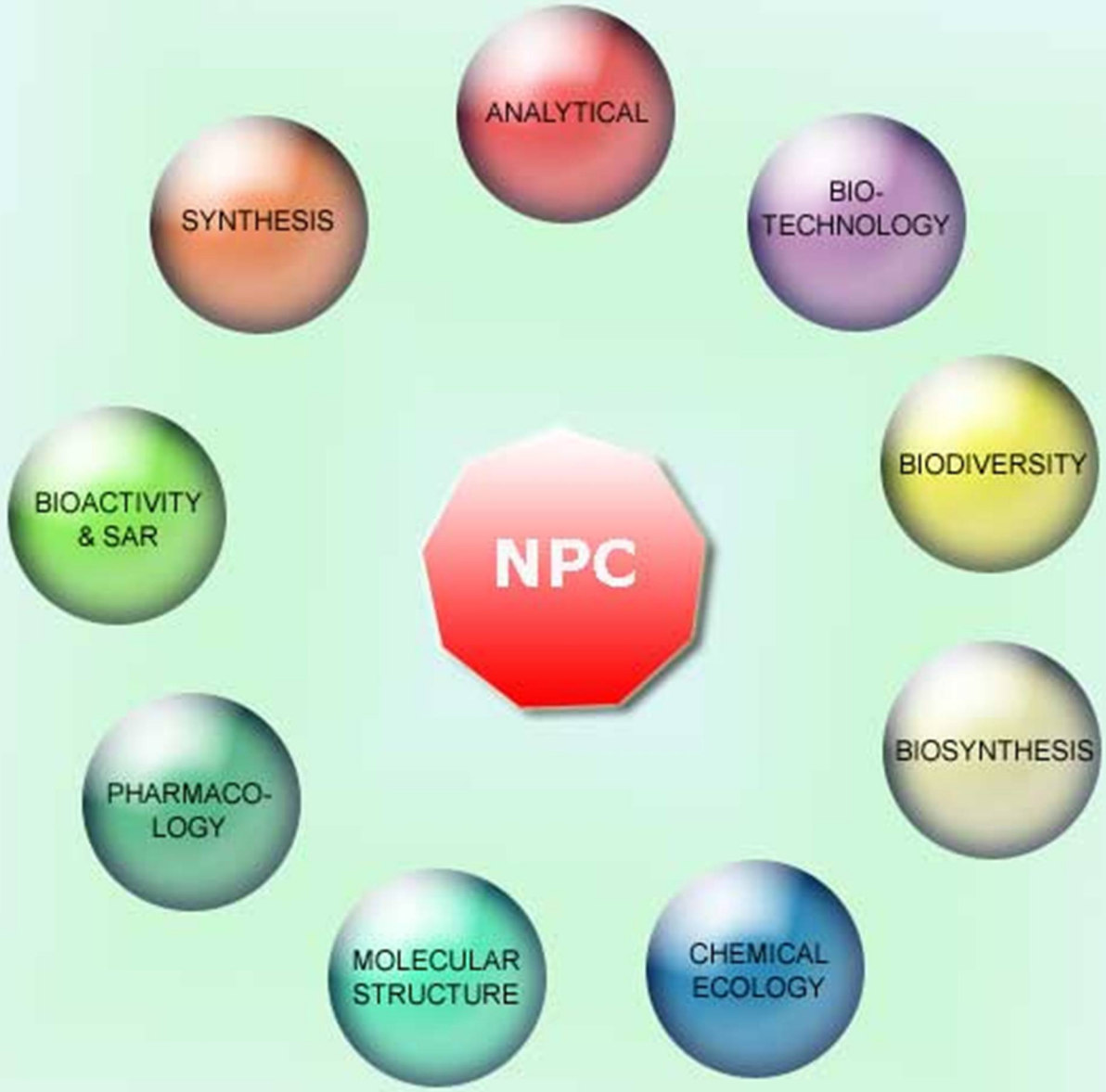

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Study of Antiviral and Virucidal Activities of Aqueous Extract of *Baccharis articulata* against *Herpes suis* virus

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Baccharis articulata is native of América and traditionally used for the treatment of digestive disorders and urinary infections. Cytotoxicity of aqueous extracts of *B. articulata* was investigated in Vero cells. As the maximal non cytotoxic concentration has been established, this concentration has been used to evaluate antiviral and virucidal activities against *Herpes suis virus type 1*, member of the same subfamily of *Herpes simplex virus.* Aqueous extracts of *B. articulata* exhibited more than 95% of virucidal activity. These findings support their potential application as a disinfectant or antiseptic with low toxicity and provide a valuable knowledge to ethnopharmacology properties of *Baccharis articulata*.

Keywords: cytotoxicity, virucidal, antiviral activity, aqueous extract, *Baccharis articulata, Herpes suis* virus.

Baccharis articulata, commonly known as carqueja, frequently found in the hills region of Cordoba province of Argentina, exhibit antioxidant, antibacterial, anti-HIV and antifungal abilities, [1a-1c]. In treatment of herpetic infections one or more drugs currently available were used, but both continuous use and self-medication promote the development of resistance and tolerance of these viruses [2]. This background encouraged the study of cytotoxic, antiviral and virucidal activities of aqueous extracts of *B. articulata* against *Herpes suis* type 1, virus closely related to *Herpes simplex types 1* and *2*.

The aim of the present study was to determine concentration of aqueous extracts that do not affect monolayer cell and to be used in later assays. Therefore cytotoxic effect on Vero cells of aqueous extracts was evaluated by daily microscopic observation of treated cells to determine the MNCC (Maximal Non Cytotoxic Concentration). The MNCC values were 1000 µg/mL and 600 µg/mL for cold aqueous extract (CAE) and hot aqueous extract (HAE), respectively. The cultures exposed to *extract* concentrations lower than MNCC exhibited morphology similar to control cultures. The cytotoxic effect was characterized by retraction cell and disruption of cell monolayer. Virucidal and antiviral assays performed at different stages of virus replication revealed percentages of inhibition shown in Table 1. The CAE inhibited 25 and 33% viral replication when the extract was added at 1000 µg/mL (MNCC) during the viral adsorption and later that step, respectively. The HAE, at 600 µg/mL, demonstrated to exert the inhibitory activity

Table 1: Cytotoxic effect and inhibition of viral replication of aqueous extracts of *Baccharis articulata*.

	Cytotoxicity	Antiviral activity (%)			Virucidal (%)	
	MNCC*	A	B	C	MNCC	MNCC 2X
CAE	1000 µg/mL	25.6	33	0	31.5	97.8
HAE	600 µg/mL	54	6.8	11	80	96

* Maximal non-cytotoxic concentration, A: viral adsorption, B: post-viral adsorption, C: pre-treatment cell.

(54%) during the stage of viral adsorption and penetration. This value showed that *Herpes suis* virus were more sensitive at HAE than CAE of *B. articulata.*

Only the pre-treatment of Vero cells with HAE slightly interfered *Herpes suis type 1* adsorption to cellular receptor. Therefore, both extracts would not induce antiviral state in the cells and neither would interfere to the mechanism of endocytosis used in the entry of virus into the cell. The CAE and HAE demonstrated to exert strong extracellular virus inactivation mostly when the assays were carried out with extracts at double concentration of MNCC (97.8 and 96% respectively). Results obtained in this work allow concluding that the aqueous extracts of *B. articulata* exert slight antiviral activities against Herpes suis virus type 1.

It is known that plant aqueous extracts, among other components, contain anthocyanins, saponins, polypeptides and terpenes [3a]. Studies on the chemical composition of *B. articulata* have reported presence of several terpenes such as articulina, germacrene and α-pinene [3b]. These compounds in other plant species (*Glyptopetalum sclerocarpum, Thymus vulgaris*) have exhibited antiviral

activity [3a,3c]. As a consequence they could be responsible of the antiviral activity demonstrate in this work. Furthermore, additional studies are needed in order to identify which compounds could be responsible for this effect and how they exert antiviral action.

Experimental

Plant material: *Baccharis articulata* was collected in the hills of Cordoba, Argentina. Taxonomic identification was performed by Prof. Margarita Grosso of the Universidad Nacional de Río Cuarto. A specimen of the plant was deposited (Nº RCV 1810) in the Herbarium. Dried aerial parts (branch and leaf) (15 g) were submitted to extraction with 700 mL of cold water at 4°C for 2 days (cold aqueous extract, CAE) and at 70°C for 2 days (hot aqueous extract, HAE). The extracts were filtered and lyophilized.

Viruses and cells: Vero cells (African green monkey kidney) were grown in Eagle´s minimum essential medium (MEM) supplemented with 8% FCS, 1% gentamicin and 1% L-glutamine and maintained at 37ºC in 5% CO_2 atmosphere. *Herpes suis* virus *type 1* strain *RC/79* was isolated in Río Cuarto in 1979 [4a].

Cytotoxicity assays: Confluent cell monolayers cultivated in 96-well culture plates were treated with different concentrations of extracts and incubated at 37ºC for 72 h. At this time, maximal non cytotoxic concentration (MNCC) was determined by microscopic observation.

Antiviral assays: The antiviral activity of tested extracts was evaluated at different stages of viral replication by plaque reduction method. Virus titres were calculated by plaque forming units per mL (PFU/mL) [4b]. The percentage of inhibition was calculated as the ratio between virus titres in treated cells and in untreated cells.

During adsorption and viral penetration: Monolayer cells grown in 24-well culture plates were incubated for 90 min with *Herpes suis* virus *type 1* (10^5 PFU/mL) in combination or not with extract at MNCC. Then, residual virus was discarded. The cells were overlaid with an overlay medium containing 1% of methylcellulose. The plates were further incubated at 37ºC for 72 h. Later cell monolayer was fixed with 10% formalin. The virus plaques formed on Vero cells were stained with 1% crystal violet. Percentage of viral inhibition was determined.

Post-adsorption and penetration of virus: Confluent monolayer of Vero cells grown in 24-well culture plates were infected with *Herpes suis type 1* (10^5 PFU/mL) and incubated at 37ºC for 90 min. After residual virus was removed, the cells were covered with the overlay medium containing 1% of methylcellulose and extract at MNCC, and incubated at 37ºC for 72 h. Percentage of viral inhibition was determined.

Pretreatment: Monolayer cells grown in 24-well culture plates were incubated for 2 h with extract at MNCC. After the extract was discarded, culture cells were inoculated with *Herpes suis type 1* (10^5 PFU/mL) and incubated at 37ºC for 90 min. The remainder virus was discarded and the cells were incubated with the overlay medium containing 1% of methylcellulose at 37°C for 72 h. Percentage of viral inhibition was determined.

Virucidal activity assay: Viral suspensions (10^5 PFU/mL) were incubated with extract at MNCC and at double concentration. After incubation of virus at 37°C for 2 h, monolayer cells grown in 24-well culture plates were infected with treated virus. The infected cells were incubated at 37°C for 90 min. The remainder virus was discarded and the cells were incubated with overlay medium containing 1% of methylcellulose at 37°C for 72 h. Percentage of viral inactivation was determined.

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