



## Antiviral activity of *Larrea divaricata* extracts on Junin virus

Brenda Konigheim<sup>1</sup>, Javier Aguilar<sup>1</sup>, Marta Goleniowski<sup>2</sup> and Marta Contigiani<sup>1</sup>

<sup>1</sup> Instituto de Virología Dr. J. M. Vanella, Córdoba, Argentina; <sup>2</sup> Centro de Excelencia en Productos y Procesos Córdoba (CEPROCOR), Argentina. e-mail: bskonigheim@fullzero.com.ar; contigia@cmefcm.uncor.edu

### INTRODUCTION

Viral infections constitute worldwide an important problem and plants offer an excellent source of products with activity for the treatment and control of such infections. The use of medicinal plants has been reported since old times, existing a wide range of active principles with a variety of biological activities (Andrei *et al.*, 1985).

Ethnobotanical studies demonstrate that several plant species of our country, mainly those belonging to the central region, can be selected according to their use in the traditional medicine. Among the species that grow in this area, those belonging to the genus *Larrea* (common name: 'jarillas') are outstanding, e.g., *L. divaricata* (Zygophyllaceae family), which is a species native to Argentina representative of the phytogeographic province of The Mount (Monte). An interesting characteristic of the genus is the great number of chemical constituents of the leaf resin, especially the high lignan content and flavonoids with a wide bioactivity spectrum. One of the most studied active principles is nordihydroguaiaretic acid (NDGA). NDGA exhibits inhibitory property of HIV transcription process, inhibiting the TAT- transactivation, activity that share a wide range of medications against AIDS (Gnabre *et al.*, 1995).

We have previously reported that it is also able to inhibit Junin virus replication (Konigheim *et al.*, 2005). Furthermore, activity against virus of the Herpesviridae family (herpes simple I and II) has also been reported in extracts of another species belonging to this genus, e.g., *L. tridentata* (Larrestat., 2000).

The aim of our work was to evaluate the effect of aqueous (Aq), ethanolic (EtOH) and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) extracts obtained from *L. divaricata* on Junin virus (Arenaviridae family). This virus, of great sanitary importance for our country, is the etiologic agent of the Argentinean Hemorrhagic Fever (AHF), which is an endemo-epidemic zoonotic illness, transmitted to man by rodents, e.g., *Calomys musculinus* (Enria *et al.*, 1998). The specific treatment of this illness is the administration of immune plasm, with the risk implicances of using sanguine derivatives.

### MATERIALS AND METHODS

Different concentrations of extracts were added to monolayers of Vero cells (kidney of African green monkey). During 3 days the appearance of cytopathic effect was registered and the cellular viability was measured using the neutral red captation, establishing the maximum non cytotoxic concentration (MNCC) for each extract.

Antiviral tests were carried out in quadruplicate with the attenuated XJCL3 strain of Junin virus using the reduction of plate forming units (pfu) assay under agarose in monolayers of Vero cells.

The activity of extracts was determined by three different treatments carried out simultaneously under the same conditions:

\* Protocol 1: the virus is previously incubated with the extracts at room temperature for 1 hour. Later on the mixture is inoculated into cellular monolayers and are kept at 36 °C for 1 hour.



\* Protocol 2: the virus is previously incubated with the extracts at 36 °C for 1 hour. Later on the mixture is inoculated into cellular monolayers, and are kept at 36 °C for 1 hour.

\* Protocol 3: the virus, the extracts and the cells are simultaneously incubated at 36 °C for 1 hour.

In all the cases, after 7 days post-infection, cells were fixed, pfu were counted and compared with the respective cellular controls without extracts.

## RESULTS AND DISCUSSION

The MCNC was 650 microg/ml for Aq extract, 63 microg/ml for EtOH extract and 50 microg/ml for CH<sub>2</sub>Cl<sub>2</sub> extract.

The inhibition percents of the activity of Junin virus against different concentrations of Aq, EtOH and CH<sub>2</sub>Cl<sub>2</sub> extracts are shown in the Table 1.

**Table 1.** Inhibition\* percent of Junin virus

Extract	Inhibition percent as protocol (%)			
	µg/ml	Prot. 1	Prot. 2	Prot. 3
AC	650	100	100	96
	325	66	100	71
	130	25	65	48
Cl <sub>2</sub> CH <sub>2</sub>	50	98	100	98
	25	75	100	88
	5	31	59	12
EtOH	63	100	100	98
	31.5	93	100	93
	6.3	45	48	35

\* Measured as: (extracts pfu / virus control pfu) x 100

Comparing the results of protocol 1 and 2, for Aq and CH<sub>2</sub>Cl<sub>2</sub> extracts viral inhibition was more remarkable when both virus and extracts were previously incubated at 36 °C. For EtOH we could not determine high differences.

## CONCLUSIONS

The obtained results show that the extracts have capacity to inhibit the replication of Junin virus under the studied experimental conditions.

Likewise, it can be stand out that protocol 2 was more effective than protocol 1 for Aq and CH<sub>2</sub>Cl<sub>2</sub> extracts. Accordingly, these extracts have

a virucide action without discarding an antiviral activity (Konigheim *et al.*, 2005). Concerning EtOH extract, there were no differences observed between the three protocols. These results justify the planning of future works, including studies on the identification of the metabolites present in each extract, as well as the mechanism(s) by which produce their inhibitory activity on Junin virus and other viral models.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

## REFERENCES

- Andrei G. M., Coto C. M. and Torres R. A. de (1985) Ensayos de citotoxicidad y actividad antiviral de extractos crudos y semipurificados de hojas verdes de *Melia azedarach* L. *Revista Argentina de Microbiología* **4**: 187-194.
- Enria D., Briggiler A. and Feuillade M. (1998) An overview of the epidemiological, ecological and preventive hallmarks of Argentinean hemorrhagic fever. *Bull. Inst. Pasteur* **96**: 103-114.
- Gnabre J. N., Bates R. B., Caldera S. and Huang R. C. (1995) Chemical structure of HIV-inhibitory lignans from *Larrea tridentata*. *Tetrahedron* **51**: 12203-12210.
- Larreestat™ (2000) Herpes Viruses: Method of treating herpes lesions and herpes viruses in humans using an effective amount of an extract of *Larrea tridentata*. *U.S Patent* 5,837,252 issued 17 November 1998 to Larreacorp, Ltd.
- Konigheim B. S., Golenioswki M. E. and Contigiani M. S. (2005) Cytotoxicity and antiviral activity of a lignan extracted from *Larrea divaricata*. *Drug Design Reviews* **2**: 81-83.
- Konigheim B., Aguilar J., Golenioswki M. and Contigiani M. (2005) Determinación de la actividad antiviral de extractos de *Larrea divaricata*. *Revista Argentina de Microbiología* **37** (1).