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Search for Antiviral Activity of Certain Medicinal Plants from Córdoba, Argentina

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ABSTRACT. The antiviral activity of alcoholic extracts of several species belonging to the Asteraceae, Labiatae, Plantaginaceae, Schizaceae, Umbelliferae, Usneaceae and Verbenaceae families has been studied. The tests were carried out in Vero celís-pseudorabies virus strain RC/79 (herpes suis virus) system. Eight plant extracts (*Achyrocline satureioides, Ambrossia tenuifolia, Baccharis articulata, Eupatorium buniifolium, Mynthostachys verticillata, Plantago brasiliensis, Plantago mayor* L and *Verbascum thapsus*) were able to inhibit at least 2 log, the viral infectivity.
Key words: Antiviral, Medicinal Plants

RESUMEN. Se estudió la actividad antiviral de extractos alcohólicos de vegetales pertenecientes a las familias Asteraceae, Labiatae, Plantaginaceae, Schizaceae, Umbelliferae, Usneaceae y Verbenaceae. Los ensayos fueron realizados en el sistema células Vero-virus de la pseudorrabia (virus herpes suis) cepa RC/79. Ocho extractos de plantas (*Achyrocline satureioides, Ambrossia tenuifolia, Baccharis articulata, Eupatorium buniifolium, Mynthostachys verticillata, Plantago brasiliensis, Plantago mayor* L *y Verbascum thapsus*) presentaron capacidad de inhibir al menos en 2 log, la infectividad viral.
Palabras clave: Antivirales, Plantas Medicinales.

INTRODUCCION

Infectious virus diseases remain an important worldwide problem, because of the nature of these infectious agents which totally depend upon the cell that they infect for their multiplication and survival. This characteristic has made development of effective chemotherapeutic agents for treatment of viral infections very difficult. And as a consequence, there are only a few antiviral drugs available for the cure of virus diseases.[8,9,22]

During the last years, the search for natural compounds with antiviral activity have been intensified.[2,16,19,21]

At the moment there are several higher plant extracts able to inhibit animal and vegetal virus replication.[7,15,24]

In most cases, the chemical nature of the active principle has not been well established, but for some plants it has been demonstrated that the reported antiviral activity is associated with proteins, phenolics, flavonoids, tannins, carbohydrates or alkaloids.[5,14,23]

This antiviral screening of Argentine medicinal plants is very important not only for the contribution made for the knowledge of our flora, but also as a contribution to solve the problems related to the virus diseases treatment.[23]

In this work, certain medicinal plants from south Córdoba province with medicinal popular use, were assayed.[17] Extracts from mostly leaves and/or branches were tested for antiviral activity using cell culture system.

MATERIAL AND METHODS

Plant material. Thirteen medicinal plants were collected during spring and summer from south Córdoba province (Argentina). Species taxonomy was confirmed by Dr. Norma Vischi from the area of Botany, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional Río Cuarto; see Table 1

The voucher specimens have been deposited at the Herbarium of this University.

The material was washed with distilled water and air-dried. It was kept at -20°C till use.[2]

Extract preparation. Mostly leaves and/or branches from vegetal material were cut into small pieces in a Waring Blender and macerated with 80% ethanol (5 g material x 30 ml) to obtain the alcoholic extracts.[5,11] It was maintained at 37°C during 24 h[10]. After percolation it was dried at 37°C. One gram of each residue was stirred in 5 ml of phosphate buffered saline. The soluble fraction was filtered with Wattman N° 2 paper and sterilized by membrane-filtration (0.22 μm), and was kept at -20°C.

Cell culture and cytotoxicity assay. Bioassays were per-

Table 1. Citotoxicity and antiviral activity of undergone plants from Cordoba province (Argentine).

Species listed by plant family	Part used[a]	MCNC[b]	Antiviral activity[c] (log_{10})	Popular use
Asteraceae				
Achyrocline satureioides	LF, BR	0.47	4	Digestive, antidiabetic[3,13,17]
Ambrosia tenuifolia	LF, BR	1.40	3	Febrifuge, stimulant[17]
Baccharis articulata	LF, BR	2.20	3	Stimulant bilis secretion, diuretic[17]
Eupatorium buniifolium	LF, BR	4.09	4	Tinctorial, medicinal
Labiatae				
Mynthostachys verticillata	LB, BR	0.80	4	Digestive, carminative, stimulant[17]
Plantaginaceae				
Plantago brasiliensis	LF, BR	8.40	4	Medicinal
Plantado mayor L	LF, BR	2.10	2	Astringent, antiinflamatory, pectoral[3,17,20]
Schizaceae				
Anemia tomentosa	LF, BR	0.40	-	Pectoral, stimulant, cicatrizant[17]
Umbelliferae				
Hydrocotyle bonariensis	LF, BR	5.20	1	Hepatic, emetic[17]
Usneaceae				
Usnea barbata	WP	1.93	-	Medicinal[13]
Verbenaceae				
Aloysia gratissima	FL	0.25	-	Stimulant, tonic, digestive[13]
Lippia turbinata	LF, BR	0.85	-	Digestive, tonic[17]
Verbascum thapsus	LF, BR	1.40	2	Antidiarrheic, antirheumatic, expectorant, against respiratory diseases[13,20]

[a]Part used: LF, leaf; BR, branch; WP, whole plant; FL, flower
[b]MCNC: Maximum non.cytotoxic concentration (mg/ml)
[c]Antiviral activity: viral yield reduction

formed in Vero cells (kidney fibroblasts of *Cercopithecus aethiops*) grown on 96 well-microtiter plates in Minimun Essential Medium with 8% inactivated calf serum, 2 mM of glutamine and, 200 UI/ml of penicillin G and 100 ug/ml of streptomycin. These monolayers were incubated at 37°C with Maintenance Medium (MM) with 2% inactivated calf serum containing two-fold serial dilutions of extract.

The cultures were examined microscopically for evidence of toxicity. Maximum non-cytotoxic concentration (MCNC) was defined as the highest concentration extract that did not alter the monolayer cells after 96 h inoculation.[2,24]

Viral stock and antiviral assays. The virus used was pseudorabies virus (herpes suis virus) strain RC/79 isolated in our laboratory.[1]

Virus stock was prepared in Vero cells and the titer of virus was obtained by 50% end-point titration technique. The titer was 10^{10} $TCID_{50}$/ml (Tissue Culture Infectious $Dose_{50}$/ml).[8]

Antiviral activity was tested by infecting monolayers grown on 96 wells microtiter plates with ten-fold serial dilutions of virus. After 1.5 h adsorption at 37°C, MM containing the MCNC of each extract was added. The cultures were incubated at 37°C and examined daily for cytopathic effect by inverted microscopic, at least 72 h after infection. Each extract was assayed at least twice.

Inhibitory effect was determined as the viral yield reduction expressed as the ratio between virus production in cultures treated with extracts and virus yield in non-treated infected cultures.[10,24]

RESULTS

The power of an antiviral agent is related to its capacity in being non toxic for the host cell, since it is necessary to be sure that the inhibition observed is due only to a direct action in the viral replication.[12] Thus, the toxic effect from the extracts was determined in monolayers of Vero cells.

Positive toxicity was considered when the culture cells presented partial or complete loss of monolayer, rounding and shrinkage of the cells, or granular appearance in the

cytoplasm

Extract plants at the maximum non-cytotoxic concentrations of each one are shown in Table 1. These values were at the range of 0.25-8.40 mg plant material per ml.

Extracts which showed decrease in the viral production at least 2 log were considered as active antiviral extracts.[2]

From the 13 extracts studied, 8 showed at least 2 log in the reduction of the viral yield at the maximum non-cytotoxic concentration (range 0.47-8.40 mg/ml). Four plant extracts did not present an antiviral effect. And Hydrocotyle bonariensis species was not considered as an antiviral agent due to the decrease was 1 log in the viral infectivity.[10]

DISCUSSION

In this paper we report the results of the screening of in vitro antipseudorabies virus activity of 13 plant extracts from 7 pl ant families. It is important to emphasize that four of the plants showing a clear antiviral activity are members of the Asteraceae family. Similar results were obtained by García et al.,[10] who have reported that plant extracts of this family showed an antiviral effect.

The fact that 8 of 13 plants tested demonstrated antiviral activity, emphasize the important role of traditional medicine in the search for antiviral compounds from natural sources.

The antiviral activity can affect the viral particle, interfere the adsorption and penetration or inhibit some step of intracellular viral multiplication.[4,6,11,25]

The results presented here suggest that the activity of the extracts was due to intracellular viral reproduction because of the extracts were added only to monolayer cells post-adsorption viral.

Work is in progress to determine which step of the viral intracellular cycle is interfered by plant extracts.

Our aim is to study also the extracts possible effect against other members of the Herpesviridae family for its application in antiviral therapies

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