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General Pharmacology 32 (1999) 499–503

General Pharmacology
The Vascular System

Antiviral activity of Bolivian plant extracts

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Manuscript received April 8, 1998; accepted manuscript July 7, 1998

Abstract

Ethanolic and aqueous extracts of seven plant species used in the traditional medicine of Bolivia have been tested for their antiviral activity against herpes simplex type I (HSV-1), vesicular stomatitis virus (VSV), and poliovirus type 1. The aqueous extracts of most of the species investigated showed antiviral activity. Two of these plants—namely, *Satureja boliviana* and *Baccharis genistelloides*—were active against two different viruses—HSV-1 and VSV.

Keywords: Medicinal plants; Bolivia; Antiviral activity; HSV-1; VSV; Poliovirus type 1

Nucleoside analogues and other synthetic compounds have traditionally been the primary sources for antiviral agents, but recent research proved that higher plants may serve as promising sources of novel viral prototypes as well (Vanden Berghe et al., 1986; Vlietinck and Vanden Berghe, 1991). A number of studies were carried out on the antiviral activity of plants (Abad et al., 1997; Beuscher et al., 1994; Roming et al., 1992), as well as on the identification of the active compounds (Amoros et al., 1992; Hayashi et al., 1997; Marchetti et al., 1996).

Most plants of the Bolivian tropical forests are used empirically by rural and indigenous populations, commonly as infusions or decoctions. In folk medicine, *Baccharis genistelloides* (Lam.) Pers (Asteraceae) ("Quimsba-Kuchu") is used as an anthelmintic and an antiulcer treatment and as a treatment for influenza (Gupta, 1995); *Baccharis rubricaulis* Rusby (Asteraceae) ("Chilca") is used for treating different types of mucous ailments (Killeen et al., 1993); *Ambrosia arborescens* Miller (Asteraceae) ("Markju") is used as an antispasmodic, emmenagogue, and vermifuge drug and as a treatment for amebic dysentery (Correa et al., 1990; Girault, 1987); *Phoradendron crassifolium* (Polh.) Eichier (Loranthaceae) ("Solda Solda") is used to relieve fever, colic, inflammation, and rheumatism (Girault, 1987; Oblitas, 1992); *Rumex obtusifolius* L. (Polygonaceae) ("Kento") is used for skin infections and as a treatment for liver diseases (Oblitas, 1992); *Plantago australis* Lam. (Plantaginaceae) ("Kara Llanten") is used as an antiseptic and antiulcer drug and for treating amebic dysentery (Correa et al., 1990; Girault, 1987); and *Satureja boliviana* (Benth.) Briquet (Lamiaceae) ("Khoa") is empirically used in folk medicine to cure influenza and as an antiseptic, anthelmintic, and insecticide drug (Girault, 1987). Despite wide use of these plants in Bolivian folk medicine, there are no data in the literature on their biological activity, whereas data have been reported on the antibacterial and antiviral activity of several species of these genera (Garcia et al., 1990; Naqui et al., 1991; Panizzi et al., 1993; Rahalison et al., 1995; Verastagui et al., 1996; Wachsman et al., 1988).

In this study, ethanolic and aqueous extracts from seven plants were screened for in vitro antiviral activity against herpes simplex type I (HSV-1), vesicular stomatitis virus (VSV), and poliovirus type 1. This is the first report on assays of the antiviral activity of extracts from these plants.

1. Materials and methods

1.1. Plant material

The plants were collected in the flowering season from different regions of the north and center of La Paz (Bolivia). Voucher specimens are on deposit in the Herbarium of the Faculty of Pharmacy, Mayor University of San Andres, La Paz, Bolivia.

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0306-3623/99/$–see front matter
PII: S0306-3623(98)00214-6

1.2. Extract preparation

Dried plant material (250 g) was ground and extracted by percolation consecutively with hexane (1.5 l), dichloromethane (1.5 l), and ethanol (1.5 l) for 3 days at room temperature. Ethanolic extracts were filtered and evaporated to dryness in a rotary evaporator at a temperature not exceeding 35°C. A separate aqueous extract was prepared with dried plant material (50 g) and hot distilled water (500 ml). After 30 min, the resulting fresh "tea" was lyophilized.

1.3. Cells and viruses

HeLa (human epitheloid cervical carcinoma), Vero (African green monkey kidney), and BHK-21 (baby hamster kidney) cells were routinely cultivated at 37°C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS; GIBCO, Grand Island, NY). HSV-1 was grown in Vero cells, poliovirus type 1 in HeLa cells, and VSV in BHK-21 cells. Virus stocks were maintained at −70°C until use. The procedures for cell culture and virus titration were as described elsewhere (Gonzalez et al., 1987). The infectious titer was 4×10^8 pfu/ml for HSV-1 and 4×10^9 pfu/ml for VSV and poliovirus.

1.4. Assay of antiviral activity

To monitor the antiviral activity, both the cytopathic effect (CPE) and protein synthesis were evaluated by means of [^{35}S]methionine incorporation. HeLa cells were seeded into 24-well plates at a concentration of 5×10^4 cells/well and incubated at 37°C in a 5% CO_2 atmosphere until confluent. Cell monolayers formed after 24 h of cell seeding were infected with the indicated viruses in DMEM supplemented with 2% CS, at multiplicities of infection (MOI) of 0.5 for VSV and poliovirus and an MOI of 1 for HSV-1. Just after addition of the virus inoculum, the different extracts tested were independently added and were assayed at concentrations ranging from 1 to 500 μg/ml. Toxicity controls, cell controls, and virus controls were run simultaneously. The cultures were observed daily for evidence of CPE (partial or complete loss of the monolayer). Viral cytopathogenicity was recorded as soon as it affected 100% of cells in the untreated virus-infected cultures (48 h for HSV-1 and 24 h for VSV and poliovirus). Then, protection of the cell monolayer and protein synthesis were evaluated. First, evaluation of CPE was performed subjectively by semiquantitative criteria. The CPE inhibition was assessed by microscopic observation of cell layers and was graded on a progressive scale of 0 (normal cells) to 4 (complete destruction of the cell monolayer): 0 = 100% inhibition; 1 = 75% inhibition; 2 = 50% inhibition; 3 = 25% inhibition; 4 = 0% inhibition. The cytotoxic and protective potential of the extracts tested was quantified by estimation of [^{35}S]methionine incorporation into the protein of cell monolayers. Microtiter wells were washed twice with DMEM without methionine, pulsed with [^{35}S]methionine (1.6 μCi/ml; The Radiochemical Centre, Amersham, Bucks, UK) in the same culture medium, and incubated for 1 h at 37°C. Nonsoluble radioactivity was precipitated with 5% trichloroacetic acid. The cell monolayer was washed twice with ethanol 96° and solubilized with 0.1 N NaOH and 1% dodecyl sulfate sodium. The radioactivity of samples was determined by means of a liquid scintillation spectrometer. The mean [^{35}S]methionine cpm value of triplicate determinations was calculated. Carrageenan was used as a reference for the antiherpetic activity (Gonzalez et al., 1987), ethanolic extract of *Chirantodendron pentadactylon* (Sterculiaceae) for the anti-VSV ac-

Table 1
Medicinal plant extracts used in the present experiment and toxicity of extracts on HeLa cell culture

Botanical name	NTLC (μg/ml)	Extract
Baccharis genistelloides (Lam.) Pers	25	Ethanolic
Asteraceae	50	Aqueous
Baccharis rubricaulis Rusby	5	Ethanolic
Asteraceae	50	Aqueous
Ambrosia arborescens Miller	25	Ethanolic
Asteraceae	50	Aqueous
Phoradendron crassifolium (Polh). Eichier	125	Ethanolic
Loranthaceae	500	Aqueous
Rumex obtusifolius L.	25	Ethanolic
Polygonaceae	125	Aqueous
Plantago australis Lam.	50	Ethanolic
Plantaginaceae	500	Aqueous
Satureja boliviana (Benth.) Briquet	25	Ethanolic
Lamiaceae	125	Aqueous

Abbreviation: NTLC, nontoxic limit concentrations.

Table 2
Antiviral activity of active plant extracts and inhibition percentages of cytophatic effect

Aqueous extracts	NTLC (μg/ml)	Dose (μg/ml)	HSV-1	VSV
P. crassifolium	500	125	75	0
		250	50	0
		500	50	0
S. boliviana	125	10	50	0
		50	75	50
		125	25	75
B. genistelloides	50	25	75	25
		50	50	25
P. australis	500	150	0	50
		250	0	50
		500	0	75
A. arborescens	50	10	0	25
		25	0	50
		50	0	75
R. obtusifolius	125	50	0	25
		125	0	25

Abbreviation: NTLC, nontoxic limit concentrations.

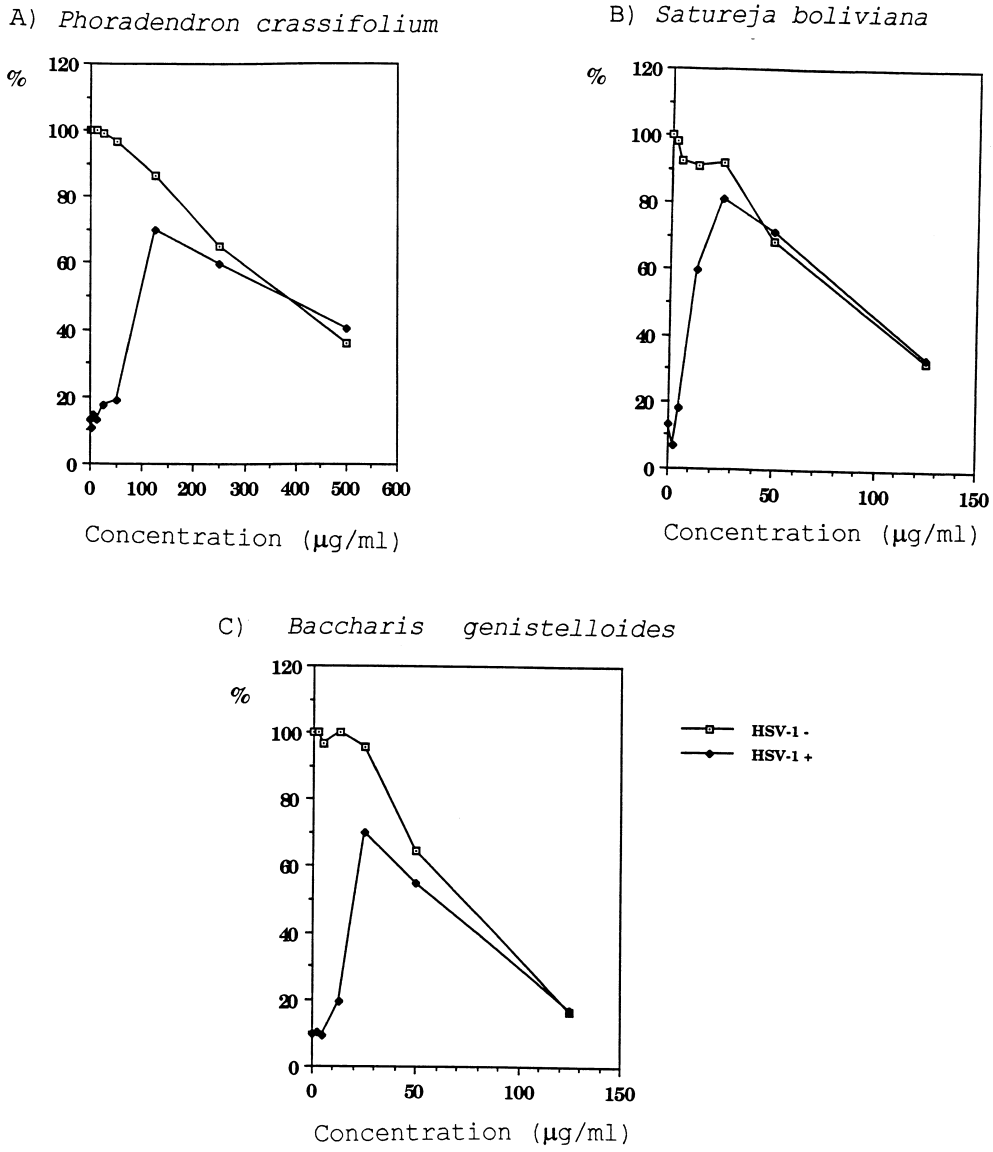

Fig. 1. Inhibition by aqueous extracts of HSV-1 replication. Results are expressed as percentages of cellular viability relative to control (100%). The toxicity of the extracts to HeLa cells was also investigated. (A) *Phoradendron crassifolium*; (B) *Satureja boliviana*; (C) *Baccharis genistelloides.*

tivity (Abad et al., 1997), and Ro-090179 as inhibitor of poliovirus replication (Gonzalez et al., 1990).

2. Results

Extracts from seven Bolivian medicinal plants were investigated. Two extracts, ethanolic and aqueous extract, were tested for their in vitro activity against HSV-1, VSV and poliovirus type 1. The toxicity of extracts to HeLa cells also was evaluated, and the results are summarized in Table 1. Ethanolic extracts were, as expected, generally more toxic to HeLa cells than aqueous extracts, which prevented the evaluation of their potential antiviral effects at higher concentrations. The ethanolic extract of *B. rubricaulis* was especially toxic; even concentrations lower than 5 µg/ml were still cytotoxic.

The antiviral activity resided only in the aqueous rather than in the ethanolic extracts. The inhibition percentages of CPE by the active extracts are shown in Table 2. Of the extracts tested, three showed antiviral activity against HSV-1, a DNA virus. However, the cytotoxicities of the extracts parallel their antiviral activities. The aqueous extract of *P. crassifolium* was effective against HSV-1 at concentrations ranging from 125 to 500 µg/ml, but toxicity limits closely paralleled the useful concentration range (Fig. 1A). More favorable examples were provided by the aqueous extracts of *S. boliviana* and *B. genistelloides*, which had antiviral activity over a concentration range of 10 to 125 µg/ml and 25 to 50 µg/ml, respectively, but were markedly more toxic than the abovementioned extract (Fig. 1B and C).

Two of these extracts, and some of the extracts that

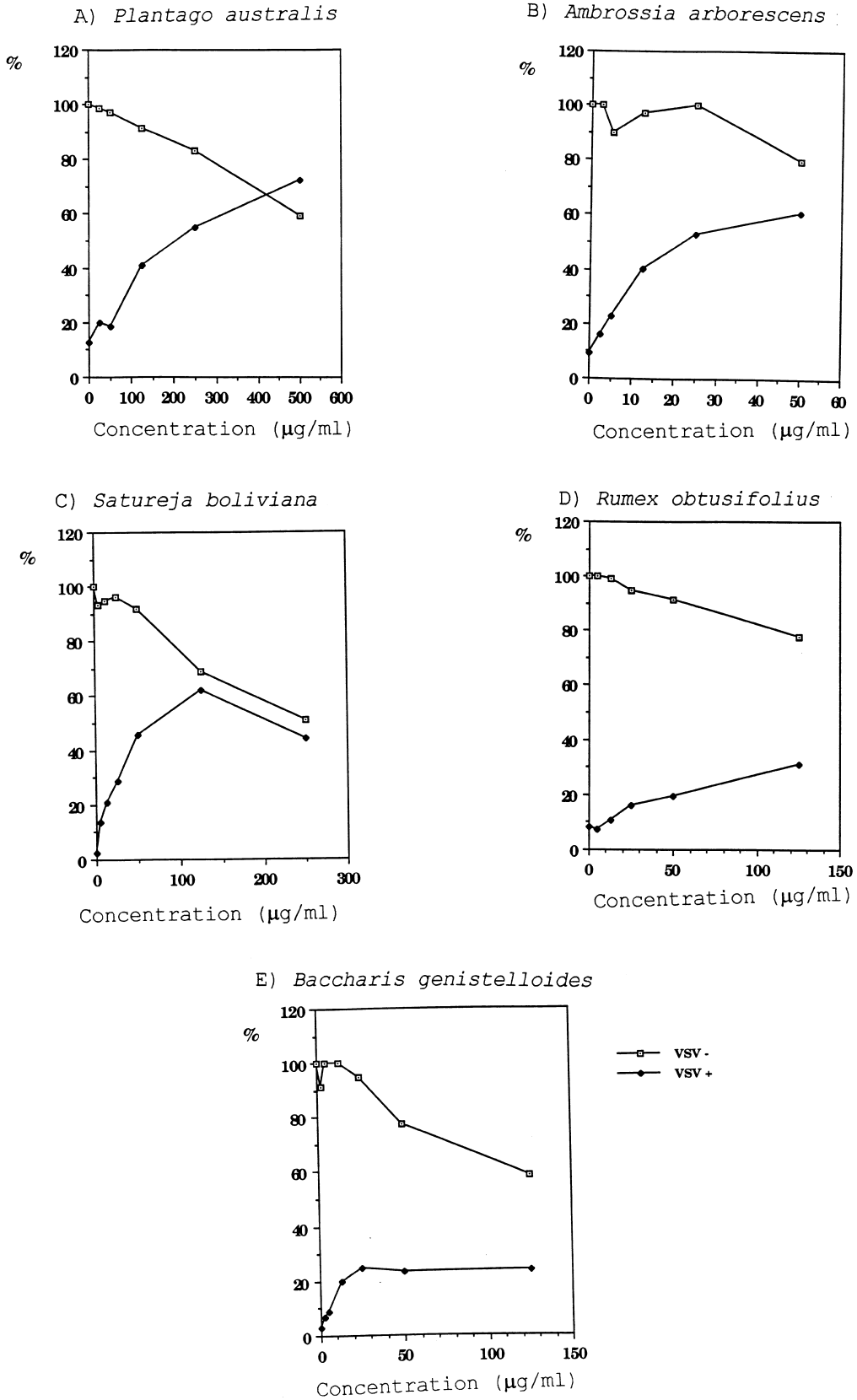

Fig. 2. Inhibition by aqueous extracts of VSV replication. Results are expressed as percentages of cellular viability relative to control (100%). The toxicity of the extracts to HeLa cells was also investigated. (A) *Plantago australis*; (B) *Ambrosia arborescens*; (C) *Satureja boliviana*; (D) *Rumex obtusifolius*; (E) *Baccharis genistelloides.*

had no effect on HSV-1 replication, were also active against a second virus—VSV, an RNA virus. The most active was the aqueous extract of *P. australis*, which exhibited antiviral activity at concentrations ranging from 150 to 500 μg/ml without cytotoxic effects (Fig. 2A). Similar activity was found with the aqueous extracts of *A. arborescens* and *S. boliviana*, which inhibited VSV replication at concentrations ranging from 10 to 50 μg/ml and 50 to 250 μg/ml, respectively. These extracts were only weakly toxic (Fig. 2B and C). The aqueous extracts of *R. obtusifolius* and *B. genistelloides* were also slightly active at 50 to 125 μg/ml and 25 to 125 μg/ml, respectively (Fig. 2D and E).

At a concentration of 1–5 μg/ml, the flavonoid Ro-090179 inhibited poliovirus replication by 100%. However, none of the extracts tested in this survey had any effect against poliovirus.

3. Discussion

The results of the present investigation provide further evidence of the importance of ethnopharmacology as a guide to the screening for biologically active plant material. Most of the aqueous extracts of plants used in folk medicine showed an antiviral activity against several DNA or RNA viruses or both, even if this activity appears rather moderate. It is interesting to point out that two of these plants—namely, *S. boliviana* and *B. genistelloides*, which are unrelated from a phylogenic viewpoint—were active against two different viruses—that is, HSV-1 and VSV. Further investigations are being undertaken to isolate bioactive compounds.

Acknowledgments

This work was supported by DGICYT PB94-0148 and "Proyecto Multidisciplinar" PR182-6738/96 UCM. E.G. is a holder of an Agencia Española de Cooperación Iberoamericana (AECI) fellowship. The technical assistance of Ms. P. Brooke-Turner is gratefully acknowledged.

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