PHYTOTHERAPY RESEARCH, VOL. **11**, 198–202 (1997)

Antiviral Activity of Medicinal Plant Extracts

M. J. Abad,[1]* P. Bermejo,[1] A. Villar,[1] S. Sanchez Palomino[2] and L. Carrasco[2]

[1] Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain

[2] Centro de Biologia Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

Dichloromethane and ethanol extracts of 12 plants with a history of use in traditional medicine, were tested for antiviral activity against herpes simplex type I. The most potent inhibition was shown by ethanol extracts of *Eugenia jambos, Cistus populifolius, Lippia alba, Chiranthodendron pentadactylon* and *Tuberaria lignosa*. These extracts, and others that had no effect, were chosen for more extensive studies against poliovirus type 1 and vesicular stomatitis virus. It was found that the ethanol extracts of *Eugenia jambos, Chiranthodendron pentadactylon* and *Santolina oblongifolia* inhibited the replication of VSV, but none of the extracts investigated had any effect on poliovirus replication. © 1997 by John Wiley & Sons, Ltd. Phytother. Res. **11**, 198–202, 1997

(No. of Figures: 2. No. of Tables: 1. No. of Refs: 9.)

Keywords: medicinal plant extracts; antiviral activity; HSV-1; poliovirus type 1; VSV.

INTRODUCTION

Despite the wide availability of clinically useful antibiotics and semisynthetic analogues, a continuing search for new antiinfective agents remains vital. There will be an increasing need for substances with antiviral activity in the near future, since the current treatment of viral infections is often unsatisfactory and limited. Mutant viruses resistant to the existing antiviral agents readily arise upon treatment. Furthermore, new viral pathogens may be discovered.

These compounds could be extracted from sources, such as higher plants, which have for various reasons been explored considerably less than the traditional ones. Many of these plants have been used historically to treat diseases now known to be of viral origin (Vanden Berghe *et al.*, 1986).

Although there have been relatively few studies seeking antiviral agents from plants, those studies have revealed an unexpectedly frequent occurrence of activity in higher plants, and a number of compounds with antiviral effects have been extracted from various medicinal plants (Wachsman *et al.*, 1988; De Rodriguez *et al.*, 1990; Roming *et al.*, 1992). It has been suggested that selection of samples on the basis of ethnomedical considerations, gives a higher hit-rate than screening programmes of general synthetic products (Farnsworth and Kaas, 1981; Vanden Berghe and Vlietinck, 1991). Therefore, the investigations have not been extensive, and thousands of plants still need to be investigated.

In searching for natural products as potential antiviral agents, a series of plant extracts widely studied in our laboratory, and reported in traditional medicine to have antiinfective properties, were first screened for *in vitro* antiviral activity. The results presented here concern several medicinal plants from the following species: *Tanacetum microphyllum* DC., *Santolina oblongifolia* Boiss (Compositae), *Tuberaria lignosa* (Sweet) Sampaio, *Cistus populifolius* L. (Cistaceae), *Teucrium buxifolium* Schreber, *Satureja obovata* Lag., *Sideritis phoetens* Clem. (Lamiaceae), Eugenia jambos L. (Mirtaceae), *Hibiscus sabdarifa* L. (Malvaceae), *Lippia alba* (Mill.) N.E. Browne, ex Britton & Wilson (Verbenaceae), *Chiranthodendron pentadactylon* Larr (Sterculiaceae), and *Tecoma stans* (L.) H.B.K. (Bignoneaceae).

Extracts of these plants were tested for their activity against herpes simplex type I (HSV-1, a DNA virus). The extracts with the greatest antiviral activities were chosen for more extensive studies against poliovirus type 1 and vesicular stomatitis virus (VSV). This is the first report on assays of the antiviral activity of extracts from these plants.

MATERIALS AND METHODS

Plant material. Plant material was provided by the Department of Pharmacology, Faculty of Pharmacy, Universidad Complutense, Madrid, Spain. The different plant species were taxonomically identified by the Department of Botany, and voucher specimens have been deposited at the Herbarium of the Centre.

Preparation of plant extracts. After air drying and grinding, the powdered plant material (100 g) was extracted sequentially by percolation with dichloromethane (DClMe) and ethanol (EtOH). For each solvent, extraction was performed three times at room temperature for 24 h, before evaporation of the solvent and freeze-drying. Solvent was removed from the extract by rotary evaporation at a temperature not exceeding 35°C. For each plant, the percentage yields were (w/w dry weight of plant material): *T. microphyllum* 9.9% DClMe extract and 7.4% EtOH extract, *S. oblongifolia* 6.7% and 6.9%, *T. lignosa* 2.1% and 10.5%, *C. populifolius* 6.9% and 11.9%, *T. buxifolium* 4.4% and 2.8%, *S. obovata* 1.6% and 3.2%, *S. phoetens* 2.6% and 1.4%, *E. jambos* 4.8% and 17.9%, *H. sabdarifa* 0.51% and 23.2%, *L. alba* 2.4% and 7.2%, *C. pentadactylon* 2.6% and 26.3%, and *T. stans* 2.2% and 27%, respectively.

* Correspondence to: M. J. Abad.

CCC 0951–418X/97/030198–05

Accepted (revised) 21 October 1996

In vitro antiviral assay

Cells and virus growth. 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% (v/v) calf serum (CS) (Gibco). 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. The procedures for cell culture and virus titration were as described elsewhere (Gonzalez *et al.*, 1987).

Determination 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. Then, they were infected with the virus indicated in DMEM supplemented with 2% CS, at multiplicities of infection (MOI) of 0.5 for HSV-1 and poliovirus, and MOI of 1 for VSV. Just after addition of the virus inoculum, the different extracts tested were independently added and assayed at concentrations ranging from 1 to 500 μg/mL. Each plate included toxicity, viruses and cell controls. Cultures were incubated at 37°C in a 5% CO_2 atmosphere until the CPE was clearly apparent in non-treated infected cells (48 h for HSV-1 and 24 h for VSV and poliovirus). Then, protection of the cell monolayer and protein synthesis were evaluated. The cytotoxic and protective potential of the extracts tested was quantified by estimation of [^{35}S]methionine incorporation to the proteins of cell monolayers. For that purpose, cell monolayers were washed twice with DMEM without methionine, and incubated for 1 h at 37°C in the same culture medium, but supplemented with [^{35}S]methionine (1.6 μCi/mL). Non-soluble radioactivity was precipitated with 5% trichloroacetic acid, and the cell monolayer was washed twice with EtOH 96°, and solubilized with 0.1N NaOH and 1% dodecyl sulphate sodium. Finally, the radioactivity incorporated in proteins was measured in a liquid scintillation spectrometer. Carrageenan was used as reference for the antiherpetic activity (Gonzalez *et al.*, 1987) and Ro-090179 as an inhibitor of poliovirus replication (Gonzalez *et al.*, 1990).

RESULTS AND 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 (Farnsworth and Kaas, 1981). The plants investigated here are endemic to the Iberian Peninsula and Guatemala, and are employed for the treatment of a variety of ailments, known to be of viral origin (Font-Quer, 1979).

Two extracts were isolated from each plant species, on the basis of relative polarity, ranging from the least polar (DClMe) to most polar (EtOH) solvents. A screening of DClMe and EtOH extracts was carried out for their activity against HSV-1. The toxicity of the different extracts to HeLa cells was also investigated, and the results are summarized in Table 1. In general, all extracts presented more or less pronounced cytotoxicities, which prevented the evaluation of their potential antiviral effects at higher concentrations. DClMe extracts were, as expected, generally more toxic to HeLa cells than EtOH extracts. The DClMe extracts of *C. populifolius*, *S. phoetens*, *E. jambos* and *L. alba*, and both extracts of *T. microphyllum* were especially toxic, suggesting the presence of very active antiproliferative compounds.

At a concentration of 50 μg/mL, carrageenan inhibited HSV-1 replication by 100%, without cytotoxic effects. Of the extracts tested in this survey, five showed antiviral activity against HSV-1. The most active extract was the

Table 1. Medicinal plant extracts used in the present experiment. Toxicity of extracts on HeLa cell cultures

Botanical name (family)	Plant part	NTLC[a] (μg/mL)	Extract
Tanacetum microphyllum	Flower heads	2.5	DClMe
(Compositae)		5	EtOH
Santolina oblongifolia	Flower heads	25	DClMe
(Compositae)		50	EtOH
Tuberaria lignosa	Aerial parts	25	DClMe
(Cistaceae)		12.5	EtOH
Cistus populifolius	Leaf	5	DClMe
(Cistaceae)		25	EtOH
Teucrium buxifolium	Aerial parts	25	DClMe
(Lamiaceae)		50	EtOH
Satureja obovata	Aerial parts	25	DClMe
(Lamiaceae)		25	EtOH
Sideritis phoetens	Aerial parts	5	DClMe
(Lamiaceae)		25	EtOH
Eugenia jambos[b]	Leaf	5	DClMe
(Mirtaceae)		25	EtOH
Hibiscus sabdarifa[b]	Flower heads	25	DClMe
(Malvaceae)		250	EtOH
Chirantodendron pentadactylon[b]	Flower heads	25	DClMe
(Sterculiaceae)		25	EtOH
Tecoma stans[b]	Leaf	50	DClMe
(Bignoneaceae)		50	EtOH

[a] Non-toxic limit concentrations.
[b] Plants from Guatemala; the others are from the Iberian Peninsula.

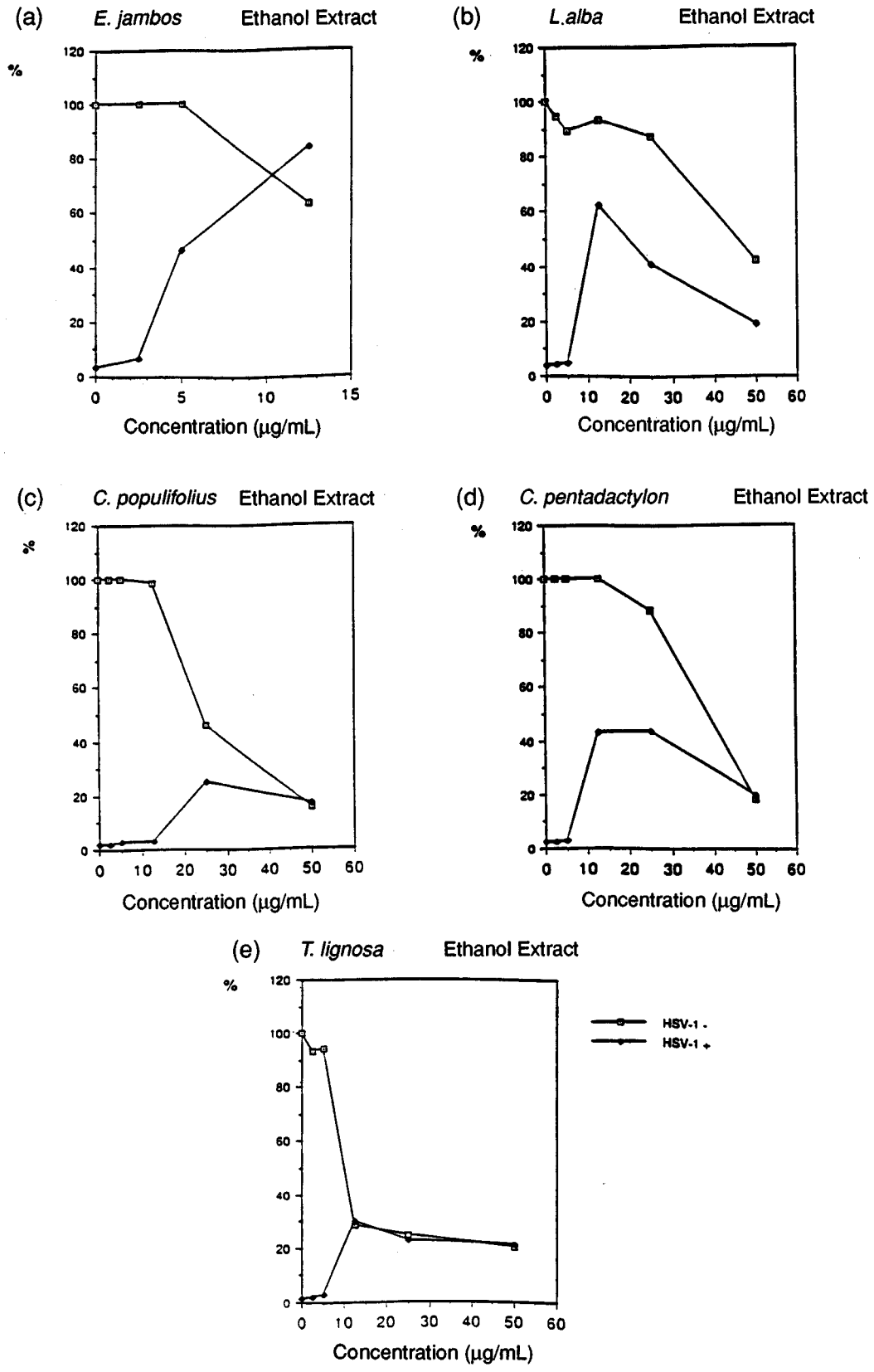

Figure 1. Inhibition by ethanol extracts of herpes simplex type I (HSV-1) replication (◆). Results are expressed as percentages of cellular viability relative to control (100%). The toxicity of the extracts to HeLa cells was also investigated (⊡). (a) *Eugenia jambos;* (b) *Lippia alba;* (c) *Cistus populifolius*; (d) *Chiranthodendron pentadactylon;* (e) *Tuberaria lignosa.*

EtOH extract of *E. jambos*, which exhibited antiviral activity at lower concentrations, ranging from 5 to 25 µg/mL (Fig. 1a). The EtOH extract of *L. alba* was also effective against HSV-1 at concentrations ranging from 12.5 to 50 µg/mL, but toxicity limits, however, closely paralleled the useful concentration range (Fig. 1b). More favourable examples were provided by the EtOH extracts of *C. populifolius* and *C. pentadactylon*, which had antiviral activity over a concentration range from 25 to 50 µg/mL and 12.5 to 50 µg/mL, respectively (Fig. 1c and 1d).

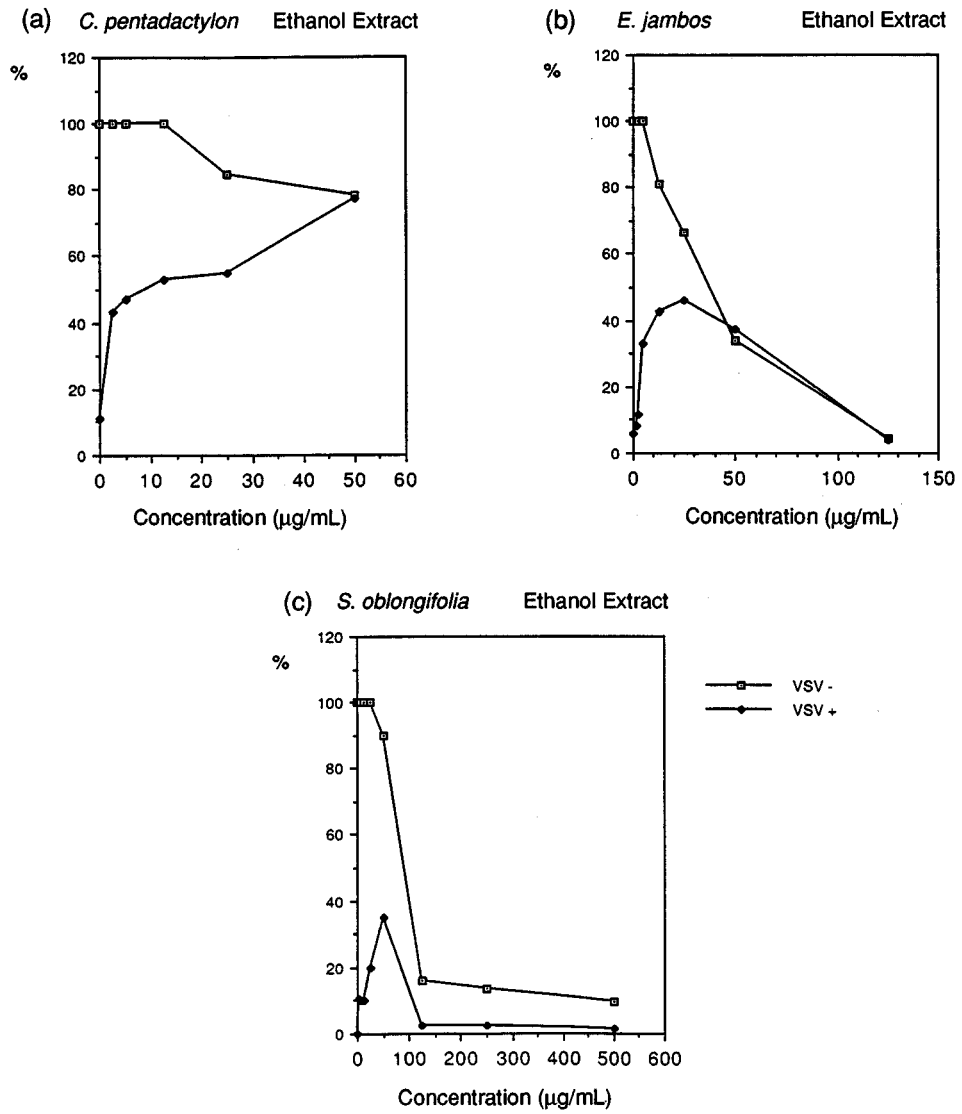

Figure 2. Inhibition by ethanol extracts of vesicular stomatitis virus (VSV) replication (◆). Results are expressed as percentages of cellular viability relative to control (100%). The toxicity of the extracts to HeLa cells was also investigated (⊡). (a) *Chiranthodendron pentadactylon*; (b) *Eugenia jambos*; (c) *Santolina oblongifolia*.

Finally, the EtOH extract of *T. lignosa* was also active at 12.5–50 µg/mL, but markedly more toxic than the above mentioned extracts (Fig. 1e).

These five extracts and some of the extracts that had no effect on HSV-1 replication, were used in subsequent experiments against poliovirus type 1 and VSV. The EtOH extract of *C. pentadactylon* and *E. jambos* showed antiviral activity against VSV, at concentrations ranging from 12.5–50 µg/mL and 2.5–50 µg/mL, respectively (Fig. 2a and 2b). The EtOH extract of *S. oblongifolia* were also slightly active at 50 µg/mL (Fig. 2c).

At a concentration of 1–3 µg/mL, Ro-090179, a flavonoid, inhibited poliovirus replication by 50%. However, none of the extracts had any effect against poliovirus.

In conclusion, of the extracts tested in this survey, five showed significant antiherpetic potency. Two of them were active against two different viruses, i.e. HSV-1 and VSV, which are unrelated from a phylogenetic viewpoint. These data suggest that these extracts contain components which may be effectively utilized as wide-spectrum antiviral agents. Further analysis, including additional purification of the extracts and chemical characterization of isolated compounds, along with further antiviral testing, should permit identification of those compounds possessing specific activities.

Acknowledgements

SSP is a holder of a Brystol Myers fellowship. This work is a collaboration of Department of Pharmacology, Faculty of Pharmacy, University Complutense, Madrid, Spain, with Iberoamerican Programme of Science and Technology for Development (CYTED). The technical assistance of Ms P. Brooke-Turner is gratefully acknowledged.

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