Cytostatic Activity of Some Phenolic Acids of *Scrophularia frutescens* L. var. *frutescens*

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The cytostatic activity of seven phenolic acids: coumaric, caffeic, ferulic, gentisic, protocatechuic, syringic and isovanillic acid previously isolated from *Scrophularia frutescens* has been evaluated against Hep–2 cells (derived from a human epidermoid carcinoma of the larynx) and McCoy cells (derived from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis). The compounds belonging to the cinnamic group present the highest activity.

Introduction

An extensive bibliography is now avaible concerning natural products from higher plants which have been tested for their cystostatic activity in animal and plant cells. A great variety of active compounds of natural origin and novel agents isolated from plants have shown antitumoral properties (Gomez *et al.,* 1996).

Different species of the genus *Scrophularia* (Scrophulariaceae) have been used in traditional medicine to treat a wide diversity of diseases, of which dermatosis: scabies, tumors, slong, etc. (Viola, 1966; Font Quer, 1990) and inflammatory affections (Swiatek, 1970) stand out.

Scrophularia frutescens grow in south west Spain and northwest Africa, but in different habitats. We previously carried out a phytochemical and pharmacological study of *Scrophularia frutescens* and the isolation and identification of the several phenolic acids and their antibacterial and anti-inflammatory activity was reported (Fernandez *et al.,* 1996; Garcia *et al.,* 1996).

The aim of this study is to determine the cytostatic effects of seven phenolic acids: *p*–coumaric, caffeic, ferulic (derivates of cinnamic acid) and gentisic, protocatechuic, syringic and isovanillic (derivates of benzoic acid), isolated from *Scrophularia frutescens* on two cell lines: Hep–2 (derived from a human epidermoid carcinoma of the larynx) and McCoy (derived from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis). With the obtained results we had attempted to establish some structure–activity relationship.

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Material and Methods

Plant material

The aerial parts of *Scrophularia frutescens* L. var. *frutescens* were collected in southwest Spain, in Matalascañas (province of Huelva), in March 1997 and were identified by the Botany Department, Faculty of Pharmacy, Sevilla. A voucher specimen was deposited in the herbarium of this Faculty (SEV–F).

Compounds tested

Phenolic acids: *p*–coumaric, caffeic, ferulic, gentisic, protocatechuic, isovanillic and syringic were isolated of a aqueous acid extract from *Scrophularia frutescens* (Fernandez *et al.,* 1996). The selected method was the Lescao technique (Lescao *et al.,* 1972) slightly modified by Marhuenda and Garcia (Marhuenda *et al.* 1985). Dried aerial parts of plant were reduced to powder, boiled in acid water with HCl (pH = 2) for 20 min and filtered. The aqueous solution was treated with diethyl ether and purified through successive changes of pH to give ethereal extract which was evaporated to dryness under vacuum. The dry residue obtained was fractionated by column chromatography on silicagel 60 (Merck), using different proportions of *n*-hexane/ethyl acetate and ethyl acetate/methanol as solvent systems.

Cytostatic test procedure

The cytostatic activity was determined by measuring the inhibition of the development of a single-layer culture of Hep-2 and McCoy cells (collection of Departement of Microbiology and Parasitology, Faculty of Medicine, University of

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Sevilla), cultivated in Eagle's minimum essential medium (MEM), according to the method described by Geran *et al.* (1972).

Cells were grown in MEM supplemented with 5% of bovine fetal serum and a 2% solution of penicillin (5000 IU/ml) and streptomycin (5000 μg/ml) to pH 7.2 and 36 °C. After distribution in the nutrient medium and when a continuous monolayer culture had been obtained, the samples in the study were sterilized through a filter Millipore 0.22 μm and then inoculated.

Samples were dissolved in a 1% hydroalcoholic solution as vehicle and diluted to give different concentrations. A solution of 6-mercaptopurine (0.5 μg/ml) as a positive control and a blank control were used under identical conditions.

72 h after inoculation of the samples, incubated at 36 °C, the cellular protein concentration was determined to evaluate the inhibitory effect on growth. The colorimetric method of Bradford (1976) was followed, using a calibration gauge with different concentrations of a standard solution of human albumin. Each assay was carried out in triplicate and the average of the reading is documented.

Statistical analysis

Student's t-test was used to compare results against the control group. The values are expressed as mean ± SE.

Results and Discussion

The results (expressed as ID_{50} values) obtained on cytostatic activity are summarized in Table I.

Table I. Cytostatic activity (ID_{50}) on Hep-2 and McCoy cells.

	ID_{50} [μM/ml]	
COMPOUND	**Hep-2 cells**	**McCoy cells**
Caffeic acid	$28.55 .10^{-3} \pm 0.028$	$13.61 .10^{-3} \pm 0.026$
Ferulic acid	$17.21 .10^{-3} \pm 0.022$	$7.47 .10^{-3} \pm 0.016$
p-Coumaric acid	$23.17 .10^{-3} \pm 0.018$	$9.88 .10^{-3} \pm 0.010$
Syringic acid	$49.14 .10^{-3} \pm 0.041$	$19.54 .10^{-3} \pm 0.029$
Gentisic acid	$203.37 .10^{-3} \pm 0.019$	$126.62 .10^{-3} \pm 0.025$
Protocatechuic acid	$234.67 .10^{-3} \pm 0.042$	$153.90 .10^{-3} \pm 0.038$
p-OH benzoid acid	$212.10 .10^{-3} \pm 0.088$	$97.54 .10^{-3} \pm 0.043$
Isovanillic acid	$161.54 .10^{-3} \pm 0.024$	$31.25 .10^{-3} \pm 0.067$

All the compounds tested show a higher activity against Hep-2 and McCoy cells. The phenolic acids of the cinnamic group present a ID_{50} inferior than those recommended by protocols of the National Cancer Institute (N. C. I.) of USA for natural products, 6μg/ml for first stage and 4 μg/ml for second stage, as interesting for further investigations.

However the data obtained with the phenolic acid of the benzoic group show that the ID_{50} for these samples are highest than those recommed by N. C. I. except the syringic acid ($ID_{50} = 3.87 \pm 0.34$) and isovanillic acid ($ID_{50} = 5.25 \pm 0.70$) against McCoy cells.

The results are in accord with the popular uses of different species of *Scrophularia* genus, traditionally used in scrofulas, and indicate that some of the phenolic acids assayed may be promising for the therapy malignant skin inflammatory affections; related to this application, we demonstrated previously the antiseptic and antiinflammatory effects of the phenolic acids isolated from this plant.

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