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In vitro antitumoral activity determination of native plant extracts of the central region of Argentina

Guillermina Bongiovanni[1,3], Noelia Luchino[2], Lorena Palacio[2], Aldo R. Eynard[1,3], Juan José Cantero[2,3] and Marta E. Goleniowski[2,3].

[1]Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina. [2]CEPROCOR Agencia Córdoba Ciencia, Argentina. [3]CONICET, Argentina.
gbongiovanni @ ceprocor.uncor.edu

INTRODUCTION

Many cancer patients have received chemotherapeutic agents during the course of illness, treatment based on cell destruction potential by growth interruption. Within this group, plants have been thoroughly used in the treatment of these maligned diseases. Many drugs have been extracted from plants. It is worth to mention as example the use of vinblastine, vincristine, podophyllotoxin, taxol. The latter is a potent anticancer agent, which was extracted from species of the genus *Taxus*, constituting the most used antineoplastic agent at the present time. These drugs that at the moment are part of international pharmacopeia products, arose out of screenings of the National Institute of Cancer (USA) and diverse pharmaceutical laboratories, *e.g.*, Lilly and Bristol-Myers Squibb.

The antecedents of ethnobotanical studies demonstrate that several plant genera, belonging to the central region of Argentina, can be selected according to their use in traditional medicine as source of new oncological drugs. The hypothesis of work is that extracts with anticancer capacity can be found, and after chemical studies, new drugs can be obtained.

Any plant species can be essentially subjected to a screening, *e.g.*, an anticancer *in vitro* screening, and furthermore the species of the central region of Argentina, few of which have been subjected to this type of analysis.

Aims: Medicinal plants of the central region of Argentina used in domestic medicine were studied by an *in vitro* test, which allowed to select quickly those extracts that inhibited tumor cell proliferation.

MATERIALS AND METHODS

Plant material.

Plants were collected in spring in different areas of the Mountains of Córdoba, dried at room temperature, and ground for a later extraction with different solvents. The following species were used: *Aspidosperma quebracho-blanco, Mandevilla laxa, Mandevilla pentladiana* (Apocynaceae); *Aristolochia stuckertii* Speg. (Aristolochiaceae); *Eupatoriam buniifolium* Hook. & Arn. var. *buniifolium, Baccharis* sp., *Gaillardia megapotamica* (Spreng.) Baker var. *megapotamica, Thelesperma megapotamicum* (Spreng.) Kuntze, *Zexmenia bupthalmiflora, Hetheroteca latifolia* (Asteraceae); *Acalypha cordobensis, Sebastiania commersoniana* (Baill.) L.B. Sm. & Downs (Euphorbiaceae); *Oxalis erythrorhiza* Gillies ex Hook. & Arn (Oxalidaceae); *Lantana grisebachii*

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(Verbenaceae); *Larrea nitida, Larrea divaricata* (Zygophyllaceae); *Monnina dictyocarpa* Griseb (Polygalaceae).

Preparation of raw extracts.
Plant material (12 g) was thoroughly extracted with ethanol, petroleum ether or dichloromethane (50 ml) by maceration at room temperature for 24 hs under stirring. A supernatant was obtained by centrifugation at 870 *x g* for 20 min, and was further evaporated in a rotatoty evaporator. The solid extract was dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mg dry weight / ml.

***In vitro* viability test.**
MCF 7 cells (cell line from human breast cancer) were *in vitro* cultured according to our experimental model under the following conditions: 37°C in 5% CO_2 and 95% air in a DMEM medium containing 2 mM *L*-glutamine, penicillin (100 IU/ml), streptomycin (100 µg/ml) and 10% fetal bovine serum in the presence or not of plant extract (200 µg/ml). 3,000 cells were added per well (in a 96 wells-plate). After 24 hours, plant extract, and DMSO or DMEM were added, and cells were cultured during 180 min. Cell viability was determined by staining with 0.5% crystal violet in 50% methanol for 15 min. Plate was washed with 50% methanol, and then dried (Mehlen *et al.*, 1995). In each well 200 µ1 of 0.1 M sodium citrate, pH 5.4, in 20% methanol was added, and absorbance was measured at 570 nm with an ELISA reader.

RESULTS AND CONCLUSIONS
To evaluate the cytotoxic effect of organic extracts of the above mentioned species, cell viability was determined on a cell line of human breast tumor. Only eight of the species studied showed cell proliferation inhibitory properties, detecting very lower values than control in extracts obtained with different solvents (Table 1). The extracts of *T. megapotamicum*, *O. erythrorhiza*, and *L. divaricata* showed high inhibitory activity on MCF 7 cell line proliferation. Of the tested species, only *Aspidosperma quebracho-blanco* and *Larrea divaricata* have been previously studied as antitumoral (Soraur *et al.*, 1971; Anesini *et al.*, 1996; Bongiovanni *et al.*, 2006).

Table 1. Cell viability: optical density values obtained by reading at 570 nm, average of two determinations carried out in octuplicate ± SD.

SPECIES	EXTRACTS			
	Ctrol	Petrol Eter	*MeCl2*	EtOH
O. erythrorhiza	286.7 ± 54.7	120.1 ± 61.1	90.7 ± 17.9	245.4 ± 45.1
H. latifolia	873.1 ± 88.9	385.5 ± 62.0	406.5 ± 103.4	674.5 ± 34.0
T. megapotamicum	742.4 ± 83.2	81.6 ± 14.2	97.6 ± 28.9	135.6 ± 27.0
L. divaricata	658.4 ± 60.2		90.7 ± 17.9	120.1 ± 61.1
G. megapotamica	658.4 ± 60.2		287.8 ± 38.1	
L. nitida	658.4 ± 60.2		211.5 ± 71.4	185.1 ± 54.0
A. quebracho blanco	658.4 ± 60.2		211.5 ± 109.1	
Z. bupthalmiflora	437.2 ± 75.1	390.6 ± 96.1	309.2 ± 25.3	

This screening based on alive cells percentage with respect to control, allowed to separate proliferation inhibitory extracts from those that didn't show this activity. These preliminary results justify to continue with the purification of the pure extracts for obtaining active principles with potential antitumoral activity.

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.

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