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In vitro prostate cancer cell growth inhibition by Brazilian plant extracts

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In the present study, 1220 plant extracts obtained from 352 plants belonging to 73 families that grow in the Amazon and Atlantic rain forests were screened for cytotoxicity against PC-3 prostate cancer cell lines. Extracts were tested in the single dose of 100 μg/mL. Activity was observed in 17 aqueous or organic extracts belonging to Annonaceae, Apocynaceae, Araceae, Capparaceae, Commelinaceae, Flacourtiaceae, Lecythidaceae, Leguminosae, Passifloraceae, Rutaceae, and Violaceae.

1. Introduction

According to the *Instituto Nacional de Câncer*, *Ministério da Saúde*, prostate cancer is the second cause of death caused by cancer among men in Brazil. Approximately 46,330 new cases of the disease are estimated to occur in 2005. Risk fators for the disease include aging and family history. The treatment of prostate cancer consists in surgery, radiotherapy or hormonetherapy, or combination of the previous ones, depending on the stage of the disease.
The Brazilian biodiversity is extremely rich. Approximately 20% of the world's biodiversity can be found in the Brazilian territory (Wilson and Peter 1988), particularly in the Amazon and Atlantic rain forests. Due to the great biodiversity and to the interest in finding new antitumor chemotherapic to be used against prostate cancer, the *in vitro* methodology using the PC-3 prostate cancer cell line should be applied to screen a large amount of extracts, obtained from the plants collected in both areas mentioned, as cytotoxic agents or hormone homologues.
Screening methodologies seem to be the fastest lane for discovering active natural products. The current technique can be used for analyzing both extracts (Lichius et al. 1999) and isolated compounds (Dong et al. 2004).

2. Investigations, results and discussion

The screening procedure was done with 1220 extracts obtained from 352 species native to the Amazon and to the Atlantic rain forests, both classified as hotspots, in terms of conservation, due to their species richness (Myers 2000). Plants were collected in the period spanning from 1997 and 2001. Cytotoxic activity against prostate cell line was observed for 17 extracts. Results described as the percentage of cell growth inhibition are given in the Table. Moreover, an overview of the constituents that are fre-

Table: Relation of plant extracts with inhibitory or lethal properties against prostate cancer cell line PC-3 in the dose of 100 μg/mL. Results are shown as the percentage of growth inhibition

Extract number	Collector number	Organs	Family	Species	% GI	Collect Date
284/A	PS80	AO	Violaceae	*Amphirrox* sp.	−35,48	Apr/97
689/O	AAO3353	WD	Leguminosae	*Abarema* cf. *jupunba*	−32,66	Jan/99
1098/A	AAO3561	FR	Leguminosae	*Swartzia sericea* var. *sericea*	−32,16	Feb/00
1094/A	AAO3562	WD	Annonaceae	*Guatteria riparia*	−31,57	Feb/00
1097/O	AAO3561	FR	Leguminosae	*Swartzia sericea* var. *sericea*	−30,31	Feb/00
719/O	AAO3383	WD, LF	Flacoutiaceae	*Laetia suaveolens*	−30,05	Apr/99
1164/A	AAO3563	AO	Passifloraceae	*Passiflora acuminata*	−27,96	Feb/00
416/A	PS108	WD	Leguminosae	*Taralea oppositifolia*	−27,69	Apr/97
505/O	PS79	WD	Lecythidaceae	*Gustavia augusta*	−26,91	Apr/97
1106/A	AAO3591	WS	Rutaceae	*Adiscanthus fusciflorus*	−24,76	Mar/00
288/A	AAO3296	AO	Capparaceae	*Crataeva tapia*	−23,79	Sep/98
827/O	AAO3481	WD	Leguminosae	*Pentaclethra macroloba*	−22,67	Sep/99
1228/A	AAO3688	WS	Leguminosae	*Swartzia laevicarpa*	−20,65	Jul/01
698/A	AAO3402	WD	Apocynaceae	*Macoubea sprucei*	−20,27	Apr/99
832/A	AAO3423	RT	Araceae	*Philodendron solimoenis*	−19,14	Jul/99
968/A	AAO3476	PL	Commelinaceae	*Commelina diffusa*	−18,93	Sep/99
690/A	AAO3353	WD	Leguminosae	*Abarema* cf. *jupunba*	−15,95	Jan/99

Results are related to analysis done with a single dose of 100 μg/mL of plant extract; %GI = percentage of growth inhibition (mean of six measurements) LF = leaf; WS = stem; FR = fruit; WD = wood, AO = aerial organs; pl = whole plant O = organic extract; A = aqueous extract

quent in the genus or family of each active extract is given, together with reports on the activity of extracts or isolated compounds previously done.

One out of 54 extracts obtained from Annonaceae species showed inhibitory activity against the PC-3 prostate cancer cell line. *Guatteria riparia* was collected three times, in April 1997, January 2000, and February 2000. The aqueous extracts obtained from the wood of the plants collected in February 2000 showed activity but no activity could be detected in the extracts obtained from the wood of the plants collected earlier, meaning that there may be a correlation between the active principles and period of collection. Aporphinic alkaloids that occur in *Guatteria* species against leishmania were previously studied (Montenegro et al. 2003), so were the sesquiterpenoids with antimicrobial activity isolated from *Guatteria* sp. (Zhang et al. 2002).

Apocynaceae is the family of *Catharanthus roseus*, the plant from which vincristin and vinblastin isolated. Studies on this plant and their natural products are being made for more than half a century, due to the importance of the vinca alkaloids to medicine. For this reason, the screening of plants belonging to the Apocynaceae is highlighted for the possible occurrence of indole alkaloids, so our group tested 93 extracts obtained from 20 different species, some of them recollected for comparing purposes. One out of 93 extracts showed activity in the prostate carcinoma cell line assay. Two collections of *Macoubea sprucei* were made in January and April 1999. The aqueous extract from the wood of the plant collected in April showed stronger cytotoxic activity. Previous reports relate the occurrence of indole alkaloids *Macoubea* species, such as vincadifformine and vincadine (Anderson et al. 1985).

The aqueous extract obtained from the roots of *Phylodrendon solimoensis* showed activity. Seven species of *Philodendron* have been traditionally used by Amazon communities (Duke and Vasquez 1994), but no studies related to pharmacological activity or phytochemistry were done. Several plants from South and Central Americas were screened for trypanocydal and tricomonicydal activities, and an extract obtained from a species of *Philodendron* was considered active (Muelas-Serrano et al. 2000).

The aqueous extract from the aerial organs of *Cratavea tapia*, Capparaceae, showed activity. Triterpenoids were isolated from a *Crataeva* species (Shirwaikar et al. 2004; Geetha and Varalakshimi 1999).

The aqueous extract made with *Commelina diffusa* showed activity. A species of *Commelina* has been studied as antidiabetic (Youn et al. 2004), the antibacterial (Cerdeiras et al. 2001) and antidiarrhoeal (Zavala et al. 1998) properties of another two species of the same genus were reported.

One out of 35 plant extracts showed activity among the Flacourtiaceae. The organic extract obtained from stems and leaves of *Laetia suaveolens* showed activity. Clerodane diterpenes were isolated from *Laetia* species (Beutler et al. 2000).

Only one out of eleven extracts of Lecythidaceae showed activity in the assay. An organic extract of the wood of *Gustavia augusta* showed activity against the cancer cell line. Cytotoxic agents, such as gustastatin, betulinic acid and portentol were isolated from *Gustavia hexapetala*, a Brazilian nut tree (Pettit et al. 2004). *Gustavia hexapetala* extracts were tested in the assay and did not show significant cytotoxic results against prostate cancer cell line, under the experimental conditions currently adopted. We could not find activity in the extracts made from *Gustavia hexapetala*.

Seven out of 198 extracts of Leguminosae showed activity against the PC-3 cell line. The aqueous and organcic extracts obtained from the wood of *Abarema* cf. *jupunba* showed cytotoxicity to the prostate cells, *in vitro*. The aqueous extract obtained from the stems of *Swartzia laevicarpa* is cytotoxic, as well as the organic and aqueous extracts obtained from the fruits of *Swartzia sericea* var. *sericea*. *Swartzia madagascariensis* has been studied since the 1950's. Studies related to flavonoids (Bezanger-Beauquesne and Pinkas 1967) and saponin compounds were made (Sandberg 1958), as well as the determination of the molluscicidal (Suter et al. 1986), toxic (Perchman 1978), insecticidal (Minjas and Sarda 1986) and ictiotoxic (Neuwinger 2004) properties. The same activities and group of compounds were found in other *Swartzia* sp. (Magalhães et al. 2003). The aqueous extract obtained from the wood of *Taralea oppositifolia* showed activity against the prostate cancer cell line, as well as the organic extract obtained from *Pentaclethra macroloba*.

The aqueous extract from the aerial organs of *Passiflora acuminata* showed activity in the assay. A cytotoxic and anti-*Pseudomonas* component of *Passiflora tetrandra* was studied (Perry et al. 1991). Passiflora juice was evaluated as anticancer agent (Rowe et al. 2004), and it was suggested that the activity may be related to an induction of caspase-3, which is involved in apoptosis rather than an antiptroliferative action.

The aqueous extract obtained from the aerial parts of *Adiscanthus fusciflorus* showed activity. No reports on the pharmacology or phytochemistry of this species were found, however, inhibition of cell proliferation was found in extracts or isolates from other Rutaceae (Jacquemond-Collet et al. 2002; So et al. 1996)

Only the organic extract obtained from the aerial organs of *Amphirrox* sp. out of eleven extracts of Violaceae showed activity in the assay. The ocurrence of cyclic peptides in Violaceae has been reported previously (Trabi et al. 2004).

The initial evaluation of the cytotoxicity of 1220 plant extracts resulted in 17 active aqueous or organic extracts (1.39% yield) belonging to several different species of plants. The selected extracts are going to be submitted to bioguide-fractination and are going to have their IC_{50} obtained.

3. Experimental

3.1. Plant collection and extract preparation

Plants were collected in the Amazon Rain Forest, in a region near Manaus, AM and in the Atlantic Forest, Iguape, SP. The identification of the plants was done in the field and in the laboratory, with the aid of general keys of identification. Vouchers were deposited in Herbarium UNIP (Universidade Paulista, São Paulo, SP).

During the experiments, 1220 organic and aqueous extracts were obtained from 352 plants belonging to 73 different families occurring in the Amazon and Atlantic rain forests. Different organs of the plants were collected according to the biomass availability of each individual or population, depending on their species and habits, i.e., trees, herbaceous plants, lianas, epiphytes, or shrubs. Each plant material was dried and ground before being submitted to 24-h maceration with methanol : dichloromethane (1 : 1), dried and followed by 24-h maceration with water, resulting in two extracts, each one concentrating polar and non polar substances. Further information on the technique can be found elsewhere (Younes 2000).

3.2. Cell culture technique

Tumor cell line PC-3 (prostate adenocarcinoma) was cultivated in tissue-culture flasks (Coastar), supplemented with RPMI-1640 plus 5% fetal bovine serum (both Cambras) and 1% glutamine (Sigma), and was kept in an incubator (Forma) at 37 °C with 5% CO_2 and 100% relative humidity. Cells were weekly passaged (Trypsin-EDTA, Cambras). Cell densities were

obtained with a haemocytometer chamber, using the trypan blue exclusion method. Tests were carried out in 96-well microplates, and the density of 7,500 cells per well was considered in the screening experiment. Cells are incubated 24 h before the drug/extract was added, and the drug/extract remained in contact with the cells for 48 h in the microculture assay. After that, end points are obtained by the sulforhodamine B (SRB) assay (Monks et al. 1991).

Doxorubicin (DOXO; Sigma) and 5-fluorouracil (5-FU; Sigma) were used as standard drugs in the assays. DOXO concentration in the test was 2.5×10^{-5} M, and 5-FU concentration was 1.86×10^{-5} M. Extracts were tested in one dose of 100 μg/mL, and a percentage of cell lethality < 15 was considered selective in the assays, when compared to cells without treatment.

3.3. SRB assay

Viable cells were fixed in the 96-microplates with cold trichloroacetic acid (TCA) solutions (50 μL/well of 50% TCA). Microplates were washed with water five times until non-viable cells were totally removed. Plates were left to air-dry for 24 h. A hundred μL of SRB/well was added, and the dye was left to react for 10 min. After that period, plates were washed five times with 1% acetic acid until complete removal of unbound SRB. Plates were left to air-dry for 24 h. The stain was resuspended with 100 μL of Trisma Buffer. The amount of viable cells was measured by obtaining the optical densities of the wells in a microplate spectrophotometer reader (Biotek 408x) at 515 nm. The percentage of cell lethality was obtained from the formula 100 X [(T-T0)/(C-T0)], which is the comparison between the control (untreated cells) and test (cells treated with drug/extract) cell growth and time zero growth (which corresponds to the cell growth until addition of extract).

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