

Anti-tumor potential and acute toxicity of *Jacaranda puberula* Cham. (Bignoniaceae)

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Abstract: Cancer chemotherapy is an important strategy to treat this leading cause of death worldwide and plants may constitute a source of new antineoplastic agents. This work fractionated the ethanolic extract of *Jacaranda puberula* leaves and studied the *in vitro* antitumoral action and some toxicological effects of the most bioactive fraction. Cell lines related to worldwide cancers were used. The Dichloromethane (DCM) and PP fractions were the most bioactive ones. The anti-tumoral action of the DCM fraction was higher than that of the crude EtOH extract while that of PP fraction was higher than the original one (DCM) for both breast (MCF-7), prostate (PC3) and lung (A549) tumor cells, chronic leukemia cells. The K562 cells were the most sensitive cell line. The PP fraction (20 µg/ml) cytotoxicity for these cells was similar to that of the ursolic acid triterpene or the antineoplastic ethoposide. The PP fraction inhibited K562 cell proliferation without cell cycle arrest in a specific phase or apoptosis. PP increased the mitochondrial reduction activity of lymphocytes. After a single dose by oral route, PP fraction did not induce intrinsic acute toxicity or animal death. This work demonstrated that the *J. puberula* fraction (PP) present high *in vitro* anti-tumoral effect with no cytotoxicity for immune system cells or oral acute toxicity, improving the *Jacaranda puberula* ethnopharmacology and reporting new biological effects for the genus *Jacaranda*.

Keywords: *Jacaranda puberula*; Antitumor; Acute toxicity; Pentacyclic triterpenes.

INTRODUCTION

Cancer is the second leading cause of death in both developed and developing countries and is therefore of worldwide concern (Reddy *et al.*, 2003). World Health Organization (WHO) estimates that new cases of cancer will increase from 12.7 (2008) to 21.4 million cases (2030) and the leading sites of cancer deaths are: lung, stomach, liver, colorectal, breast, cervix and prostate, in this order (Beaglehole *et al.*, 2011). Analysis of new anticancer drugs between 1981 and 2006 revealed that 47.1% of 155 clinically approved anticancer drugs were natural products, natural product derivatives or synthesized molecules based on natural product compound pharmacophores (Pan *et al.*, 2010).

Brazil is a rich source of medicinal plants and several plant extracts have been used against diseases in folk medicine, but only a few ones have been scientifically investigated (Kwiecinski *et al.*, 2008). Plant derived natural products such as polyphenol compounds, alkaloids, terpenes and so on play important roles nowadays due to their diverse pharmacological properties, including cytotoxic and cancer chemopreventive effects (Kwiecinski *et al.*, 2008; Ravi *et al.*, 2011).

Jacaranda puberula Cham. (Bignoniaceae), popularly known as “caroba” and “carobinha”, is a tree originally from Brazil which is distributed in the tropical forest of South and Southeast regions (Lorenzi, 2002; Gachet and Schühly, 2009). It is popularly used as a water decoction from the leaves in baths (three full handfuls boiled in one liter of water) for the treatment of frostbite (Lorenzi, 2002; Gachet and Schühly, 2009). The knowledge about *J. puberula* chemical composition is still poor. Santos *et al.*, (Santos *et al.*, 2010) isolated and identified polyphenol compounds, such as verbascoside and cis-caffeoyl aldehyde, from the leaves polar extract of this plant. In the other hand, there is no pharmacological effect scientifically demonstrated for *J. puberula*. As the anti tumor action has been reported for the *J. minosifolia* (Mam one *et al.*, 2011) and *J. caroba* (Endringer *et al.*, 2010), this work evaluatead the antitumor activity of *J. puberula* leaves extracts against cell lines related to worldwide cancers. The *in vitro* toxic effects on normal cells and the mice acute toxicity of *J. puberula* were also evaluated.

MATERIAL AND METHODS

Plant material and extract preparation

The *J. puberula* species was collected in March 2004 in

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Petrópolis city, Rio de Janeiro State, Brazil, and a voucher specimen (No.103011) was deposited in the herbarium of the Biological Institute of the Federal University from Rio de Janeiro, Brazil. The dried and ground leaves of *J. puberula* (735 g) were extracted by maceration in EtOH (10 liters) for 21 days at room temperature (r.t.). After concentration under reduced pressure, the crude EtOH extract (117 g) was successively fractionated by liquid-liquid partition between water and the following solvents: n-hexane (HX), dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BU). The samples were evaporated to give the respective dried fractions (5.7g, 11.9g, 10.3g, 5.5g and 5.7g). A green powder, corresponding to precipitated fraction (PP, 7.4g), was formed between water and n-hexane solvent.

Animals

Female Swiss webster (SW) mice, 6 months old, 20-30 g body weight (b.w.) were used to evaluate *in vitro* murine lymphocyte proliferation and *in vivo* acute toxicity. Mice were housed under standard laboratory conditions, in a 12-h light/12-h dark cycle, and were kept with free access to water and food. All experiments were performed under the consent and surveillance of the Ethical Committee for animals use in research of Biomedical Center, Rio de Janeiro State University, Brazil (CEUA-IBRAG/05/2009).

Cell culture

The antitumor potential of the samples was evaluated by studying human cell lines of cancers of high prevalence in the world: lung adenocarcinoma cells (A549), chronic myeloid leukemia (K562), prostate adenocarcinoma cells (PC3) and breast adenocarcinoma cells (MCF-7). The cells were obtained from Genetic Department of Rio de Janeiro State University or from the Basic Research Department of National Cancer Institute, Rio de Janeiro, Brazil. The cells were maintained cryopreserved in liquid nitrogen and, after thawing, they were grown in RPMI 1640 (Sigma Chemicals®) supplemented with 10% fetal bovine serum (Cultilab®). Cells were incubated in a humidified atmosphere of CO₂ 5%, at 37°C, in absence of antibiotic, being sub-cultured twice a week.

MTT assay

The mitochondrial reduction activity (MRA), which is proportional to cell viability or cytotoxicity, was determined by the MTT test (Mosmann, 1983). The tumor cells lines (5x10⁴ cells/ml) were incubated (96-microwell plates) for 24 h with different sample concentrations, antineoplastic control drugs or supplemented medium (control), final volume of 100 µl. The plant samples were tested at final concentrations of 20, 40, 50 and 100 µg/ml. The triterpenes ursolic and oleanolic acids, already described in the Jacaranda Genus (Gachet and Schühly, 2009), were also tested. Adherent cells (PC3, A549 and MCF-7) were cultured for 24 h to adhere before sample addition. Four hours before the end of culture 10 µl of MTT (Methylthiazolyldiphenyl-tetrazolium bromide,

Sigma®) 5 mg/ml in phosphate buffered saline pH 7.4 (PBS) were added to each well and the plates were further incubated for 4 h at 37°C and 5% CO₂. Afterwards the plates were centrifuged at 400xg for 15 min, the supernatant was removed and 200 µl of DMSO (Dimethyl sulfoxide, Sigma®), were added to each well to solute the formazan crystals. The plates with K562 cells were not centrifuged, they received 100 µl of SDS 10% (Sodium dodecyl sulfate, Sigma®), with HCl 0.01 N (v/v) (Merck®) to solute the formazan crystals. The absorbance was measured at 570 nm on a microplate reader (µQuant, Bio-Tek Instruments Inc., EUA). Results were expressed as the percentage of control considering the values obtained in the control culture as 100%, after deducting the sample blank values.

Cell cycle analysis

The effects of the most active PP extract and ursolic and oleanolic acids (20 µg/ml) on inhibition of K562 cell cycle were assessed by flow cytometry after staining the cells with propidium iodide (PI - Sigma Chemical®), according to Dalmau *et al.*, 1999. Cells (5 x 10⁴ cells/ml) were cultured for 36 h with plant samples or ursolic or oleanolic acids at 20 µg/ml at 37°C and 5% CO₂. Afterwards, 1x10⁵ cells of each cell culture were suspended in 500 µl of 50 µg/ml PI diluted in 43 mM citrate buffer, pH 8.2, containing 0.3% Triton X-100 and incubated at room temperature for 15 min in the dark. Then, 500 µl of 100 µg/ml RNase (Sigma Chemical®) solution, prepared in 43 mM citrate buffer, pH 8.2) were added and incubated at room temperature for another 15 min in the dark. A control culture was also prepared. DNA content was determined by the PI fluorescence (FL-2 channel - 585±15 nm) acquired on a FACScalibur flow cytometer (Benckton & Dickinson). Data on a minimum of 1x10⁵ cells was analyzed using WinMDI 2.8 software.

Annexin V-FITC binding assay

K562 cells were cultured for 36 h in the same conditions as for cell cycle assay and afterwards they were stained with annexin V-fluorescein isothiocyanate (FITC) propidium iodide (PI) kit (BD Pharmingen, San Diego, CA, USA) for apoptosis analysis. Cells (1x10⁵) were incubated with 5 µL of annexin V-FITC/PI solution at room temperature for 15 min in the dark. Annexin V bind to phosphatidylserine in the outer leaflet of the apoptotic cells plasma membrane. Annexin-FITC fluorescence was acquired in the FL-1 channel (535±15 nm) of a FAC Scalibur flow cytometer. Debris were discarded from analysis. The cell acquisition was performed with the CELLquest-Pro software and data analyzed with the Win MDI 2.8. software.

Lymphocyte isolation and culture

Spleen cells were isolated from mice, according to Pinto *et al.*, (Pinto *et al.*, 2007). Briefly, the spleen was aseptically removed, the cells were dissociated in RPMI medium diluted (1:1 v/v) with PBS, treated with 10 ml of

hypotonic solution (145 mM ammonium chloride, 10 mM potassium bicarbonate and 2 mM ethylenediaminetetraacetic acid (EDTA), centrifuged for 3 minutes at 400 x g, washed twice with RPMI medium containing 2 mM EDTA and suspended in completed medium. The spleen cells (2×10^6 cells/ml) were incubated with Concanavalin A (10 $\mu\text{g/ml}$) for 24 h and afterwards treated with the plant samples (50, 100, 150 and 200 $\mu\text{g/ml}$) or not (control) for 24 h, at 37°C and 5% CO_2 , final volume of 200 μl . At the end of culture the cell supernatant was removed and the cytotoxicity determined by the MTT assay (Mosmann, 1983). The formazan crystals were soluted in DMSO (200 μl) and the absorbance measured at 570 nm on a microplate reader (μQuant , Bio-Tek Instruments Inc., EUA). Results were expressed as the percentage of control considering the control culture as 100%, after deducting the sample blank values.

Acute toxicity evaluation

The test was carried out in SW mice treated with the PP fraction, according to Sabino *et al.*, 1999. The animals were divided into six groups (n=5), five of them received a single oral dose of 100, 500, 1000, 3000 or 5000 mg/kg b.w., respectively, and a control group received only the vehicle (sterile water). The animals, with free access to food and water, were kept under observation for mortality or clinical signs of toxicity (gastrointestinal, cardiac, respiratory, diuresis, vascular, ocular, muscle tone, mobility) three times a day during one week and then sacrificed in CO_2 chamber.

STATISTICAL ANALYSIS

The *in vitro* assays were performed in triplicate and the results expressed as mean \pm SEM of three independent experiments. The data comparison was performed by Student's *t* test, using the GraphPad Prism[®] software, considering the differences statistically significant when $p < 0.05$.

RESULTS

Cytotoxic effect on tumor cells lines

The toxicity of *J. puberula* samples, triterpenic standard compounds and paclitaxel on the human lung tumor cell line A549 mitochondrial reduction activity (MTT reduction) are shown in fig. 1 The inhibition of this activity is proportional to cytotoxicity level. The DCM and PP fractions showed higher inhibitory action than the other vegetal samples, inhibiting up to 64% and 95% ($p < 0.001$) of tumor cells reduction activity, respectively, at 100 $\mu\text{g/ml}$. Oleanolic acid (OA) only showed significant inhibition (65%) at 100 $\mu\text{g/ml}$, while ursolic acid (UA) reduced 67% and 98% ($p < 0.001$) of A549 cell reduction activity at 20 and 40 $\mu\text{g/ml}$, respectively, reaching inhibition index of 100% at 100 $\mu\text{g/ml}$. Paclitaxel inhibited 25% and 62% of A549 mitochondrial reduction activity at 10 and 20 $\mu\text{g/ml}$, respectively ($p < 0.001$).

The effects of *J. puberula* samples, triterpenic standard compounds and chemotherapeutic control drug on MRA of breast cancer (MCF-7) cells are shown in fig. 2 Increased cytotoxic effects ($p < 0.001$) were observed in MCF-7 cells treated only with DCM and PP fractions of *J. puberula*, and in a concentration dependent manner (fig. 2), with inhibition indexes of 57% and 95%, respectively, at 100 $\mu\text{g/ml}$. Oleanolic and ursolic acids also inhibited ($p < 0.01$ and $p < 0.001$) the mitochondrial reduction activity of this cell line in a concentration dependent manner, with inhibition of 87% and 100%, respectively, at the same concentration. MCF-7 cells treated with 1 $\mu\text{g/ml}$ Doxorubicin showed inhibition of 66%.

The profiles of PC3 mitochondrial reduction activity to *J. puberula* samples (fig. 3) were similar to those of A549 (fig. 1) and MCF-7 (fig. 2), except for its crude EtOH extract and hexanic fraction (fig. 3), PC3 being more sensible to these samples than the other two cell lines. The crude EtOH extract reduced 86% of this cell MRA at 100 $\mu\text{g/ml}$, while the hexanic fraction inhibited it 31%, 47%, 64% and 85% at 20, 40, 50 and 100 $\mu\text{g/ml}$, respectively. Higher inhibitory action of DCM and PP fractions than the other samples on PC3 cell MRA can also be observed in the fig. 3. These samples inhibited the MTT reduction in 19% and 84%, at 50 $\mu\text{g/ml}$, and 84% and 100% at 100 $\mu\text{g/ml}$, respectively (fig. 3). Ursolic acid inhibited 84% of PC3 MRA while oleanolic induced mild cytotoxic effects. Paclitaxel markedly reduced PC3 cell mitochondrial reduction activity at all tested concentrations, reaching to 96% at 25 $\mu\text{g/ml}$.

The fig. 4 shows the effect of *J. puberula* extracts, triterpenic standard compounds and etoposide on K562 cell MRA. All samples inhibited this cell viability with the DCM and PP fractions also showing the most marked effects. DCM inhibited ($p < 0.001$) 90% of cell viability at 100 $\mu\text{g/ml}$ and PP inhibited ($p < 0.001$) 94% of that with just 50 $\mu\text{g/ml}$ and 96% at 100 $\mu\text{g/ml}$. As observed with the other cell lines (fig. 1-3), the ursolic acid has also shown higher activity than oleanolic acid, presenting 99% ($p < 0.001$) and 14.5% ($p < 0.05$) of inhibition at 40 $\mu\text{g/ml}$, respectively. Etoposide inhibited 83% and 90% of mitochondrial reduction activity of K562 cell at 10 and 20 $\mu\text{g/ml}$, respectively.

Comparing the cytotoxic activity of *J. puberula* samples on different tumor cell lines, it can be observed that K562 cells showed higher sensitivity for *J. puberula* samples and UA than the other ones. The lowest IC_{50} values were found with DCM (38 $\mu\text{g/ml}$) and PP (11 $\mu\text{g/ml}$) fractions of this plant, and with UA triterpene (19 $\mu\text{g/ml}$). On the other hand, PP fraction was the most active *J. puberula* fraction on all tested tumor cell lines, presenting IC_{50} values of 28 $\mu\text{g/ml}$ (A549), 11 $\mu\text{g/ml}$ (K562), 36 $\mu\text{g/ml}$ (MCF-7) and 37 $\mu\text{g/ml}$ (PC3). Based on these results the following studies were performed only with K562 cells.

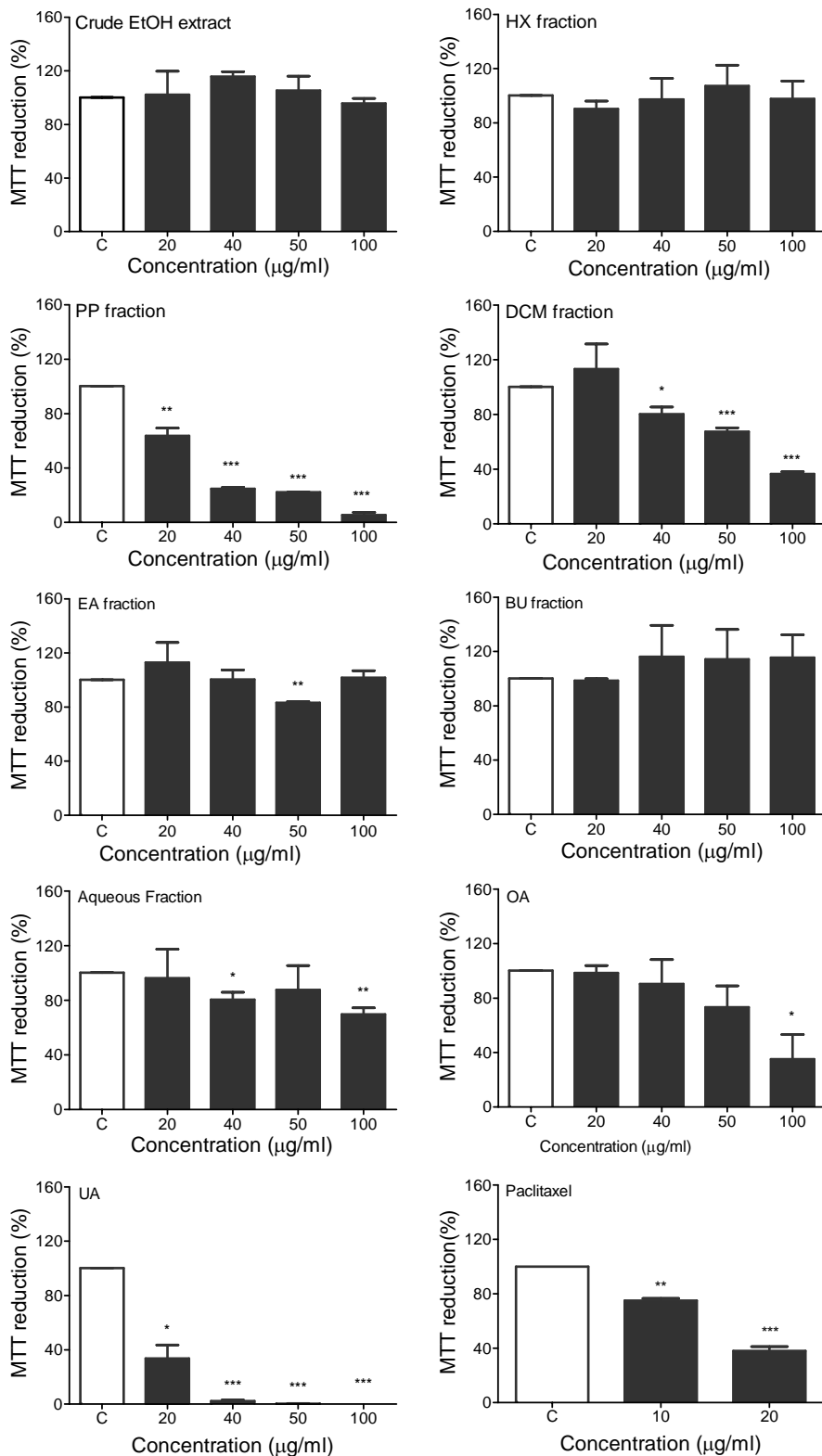


Fig. 1: Cytotoxic effects of *J. puberula* and triterpenes on A549 lung tumor cell line. Cells (5×10^4 /ml) were cultured for 24 h either in the absence (control) or presence of *J. puberula* samples, oleanolic acid, ursolic acid or paclitaxel. Cytotoxicity was determined by MTT assay. Control absorbance at 570 nm was 0.507. Results represent the mean \pm SEM. *p<0.05; **p<0.01; ***p<0.001 versus control, by Student's *t* test.

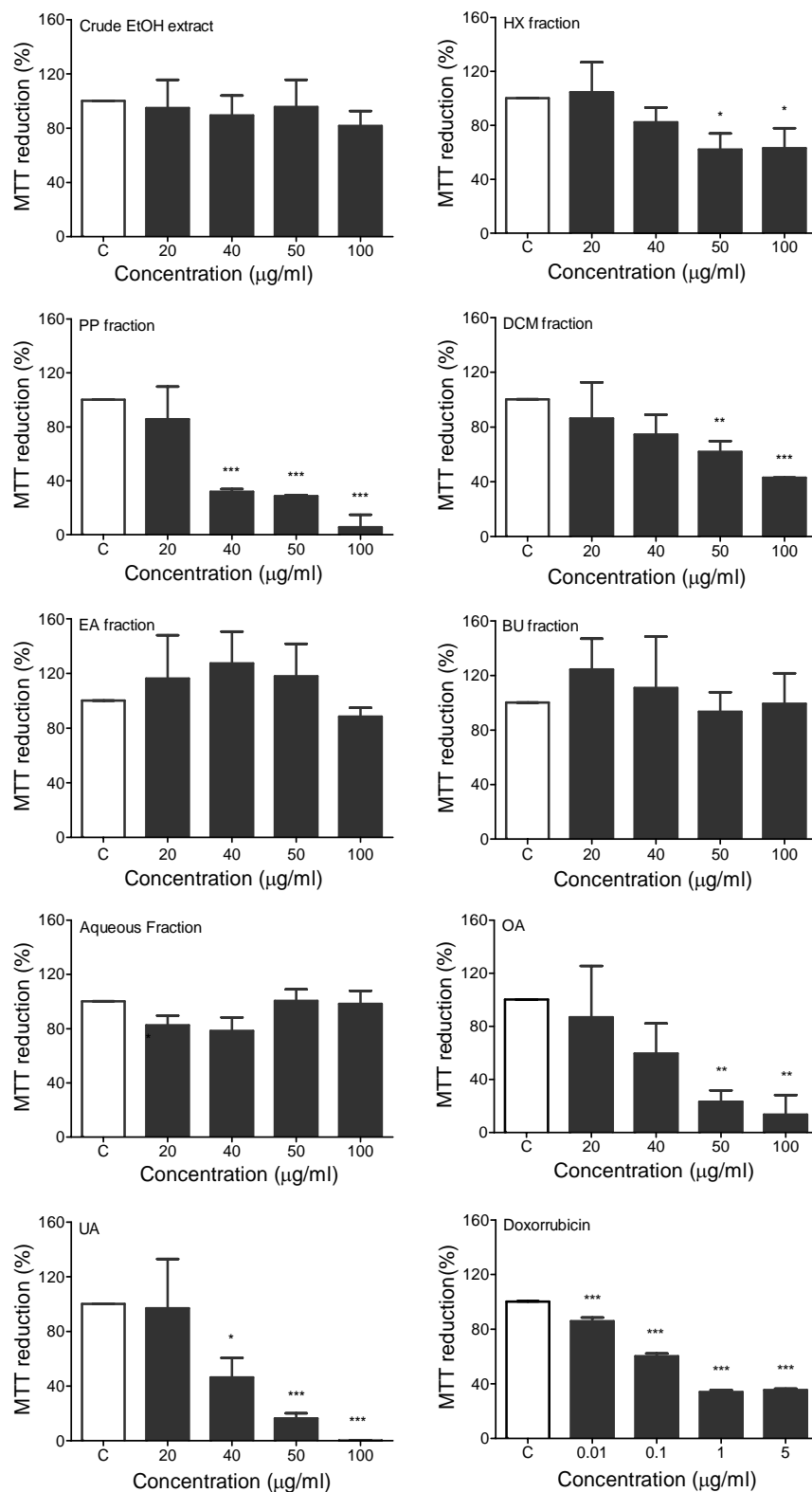


Fig. 2: Cytotoxic effects of *J. puberula* and triterpenes on MCF7 breast tumor cell line. Cells (5×10^4 /ml) were cultured for 24 h either in the absence (control) or presence of *J. puberula* samples, oleanolic acid, ursolic acid or doxorubicin. Cytotoxicity was determined by MTT assay. Control absorbance at 570 nm was 0.845. Results represent the mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control, by Student's *t* test.

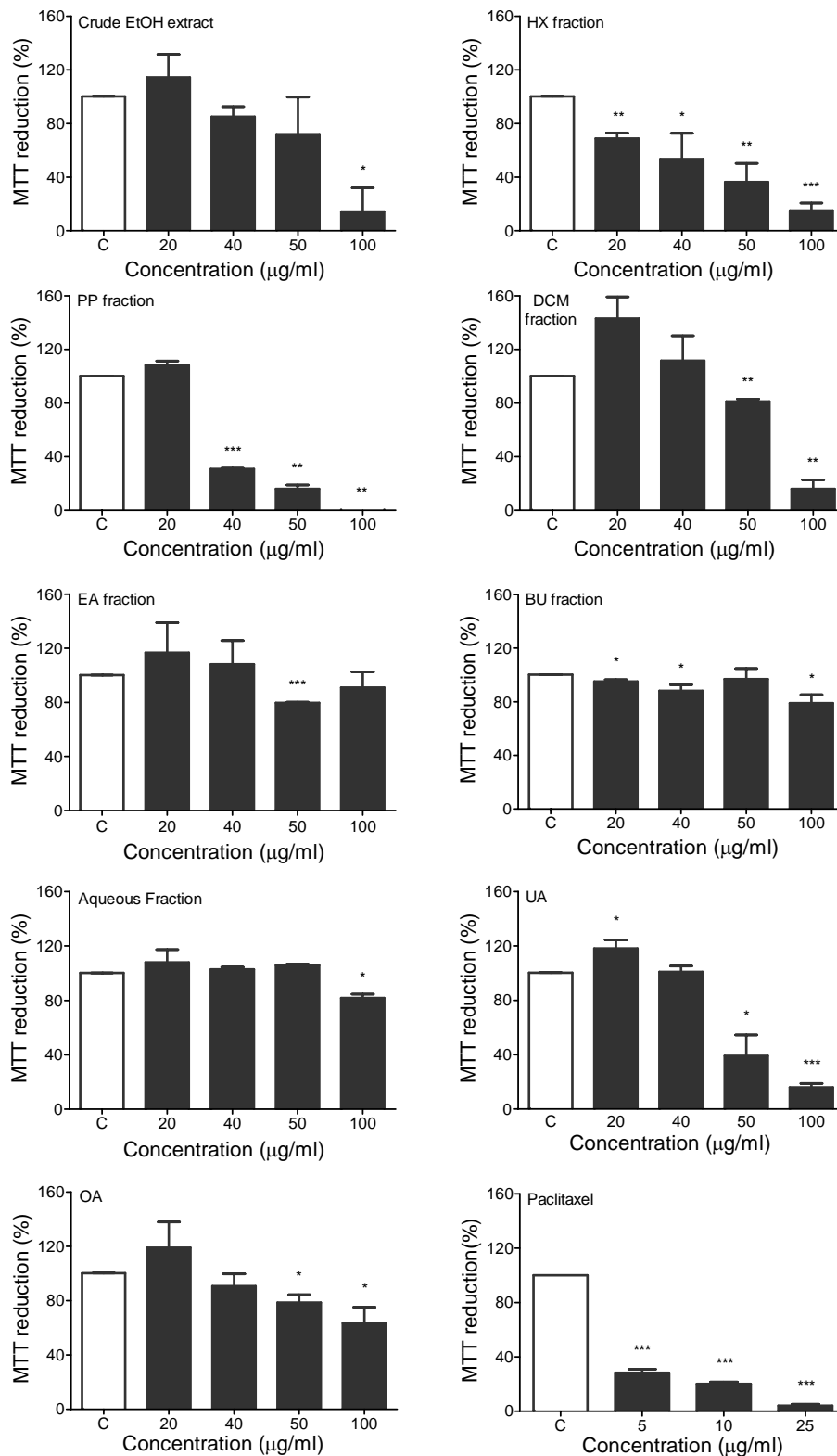


Fig. 3: Cytotoxic effects of *J. puberula* and triterpenes on PC3 prostate tumor cell line. Cells (5×10^4 /ml) were cultured for 24 h either in the absence (control) or presence of *J. puberula* samples, oleanolic acid, ursolic acid or paclitaxel. Cytotoxicity was determined by MTT assay. Control absorbance at 570 nm was 0.319. Results represent the mean \pm SEM. *p<0.05; **p<0.01; *** p<0.001 versus control, by Student's *t* test.

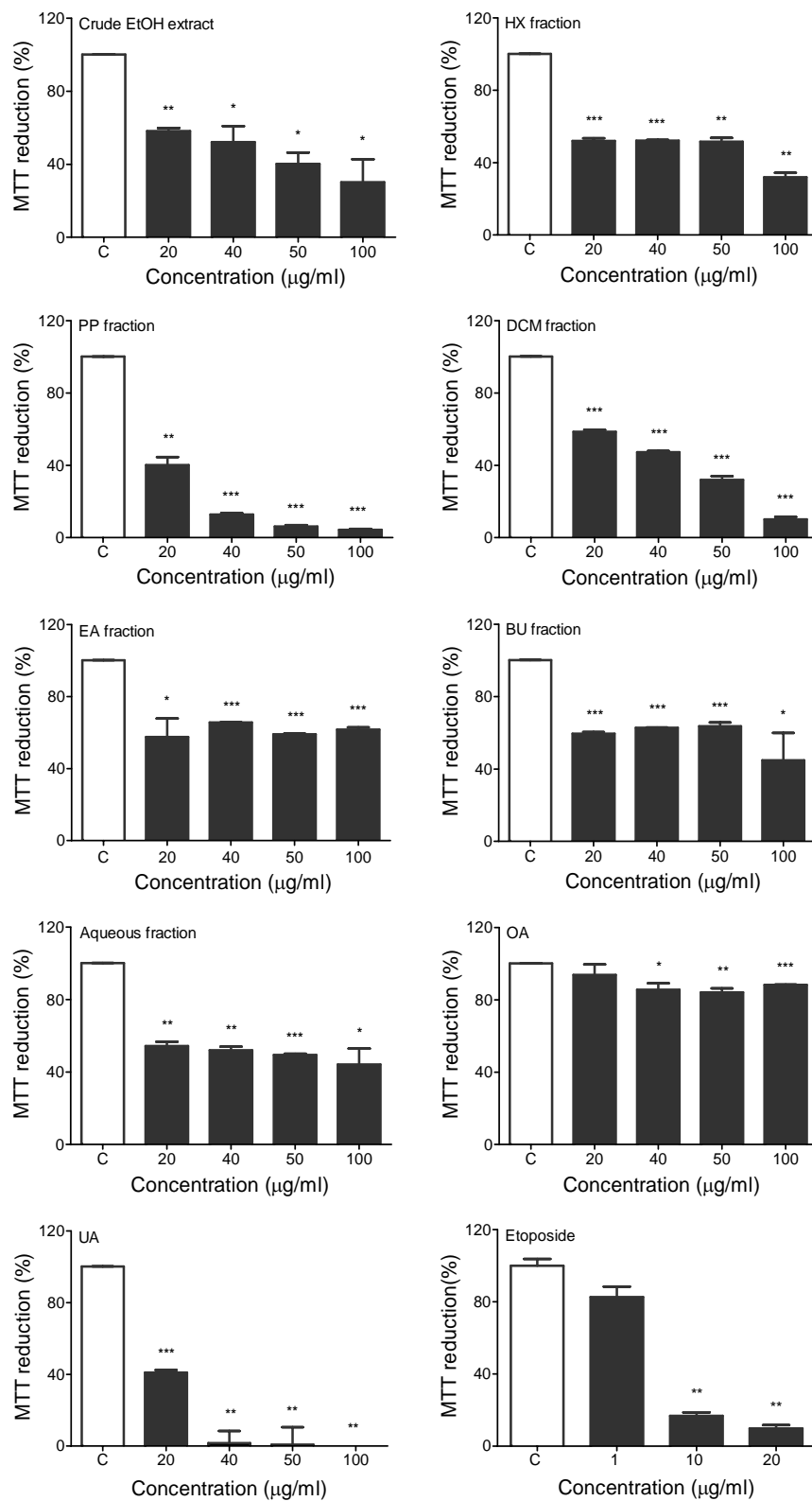


Fig. 4: Cytotoxic effects of *J. puberula* and triterpenes on K562 leukemia cell line. Cells (5×10^4 /ml) were cultured for 24 h either in the absence (control) or presence of *J. puberula* samples, oleanolic acid, ursolic acid or etoposide. Cytotoxicity was determined by MTT assay. Control absorbance at 570 nm was 0.398. Results represent the mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control, by Student's *t* test.

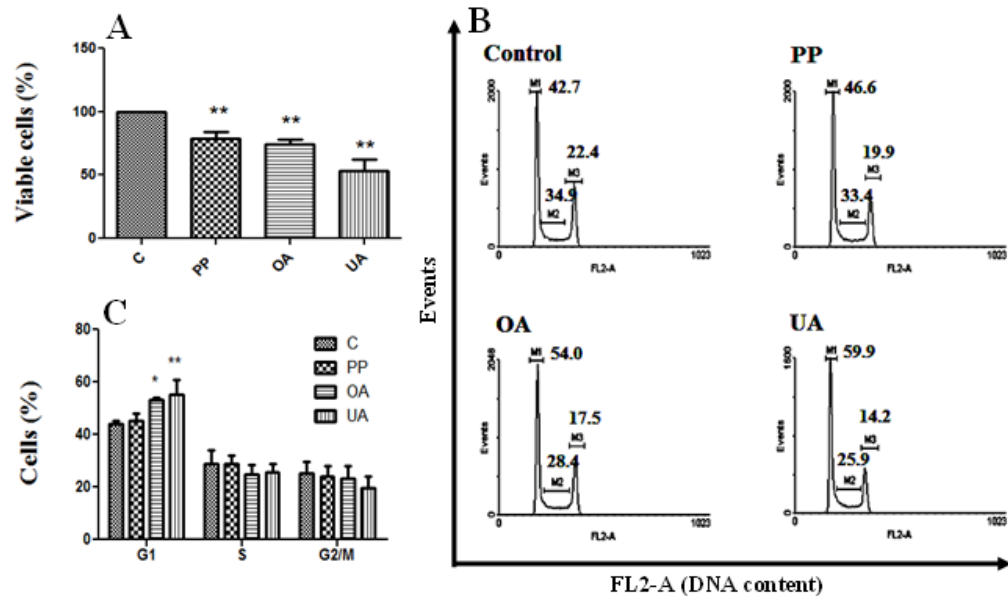


Fig. 5: Effects of *J. puberula* PP fraction and triterpenes on K562 leukemia cell proliferation. A- Number of viable cells. B- Cell cycle analysis (representative experiment). C- Relative number of cells in cell cycle phase. Cells ($5 \times 10^4/\text{ml}$) were cultured for 36 h either in the absence (control) or presence of PP fraction, oleanolic acid or ursolic acid ($20 \mu\text{g}/\text{mL}$). Propidium iodide fluorescence (proportional to DNA content) was determined by the FL2-width channel. Hypodiploid nucleus and debris were discarded from cytofluorimetric analysis. Data represent the mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$ versus control, by Student's *t* test.

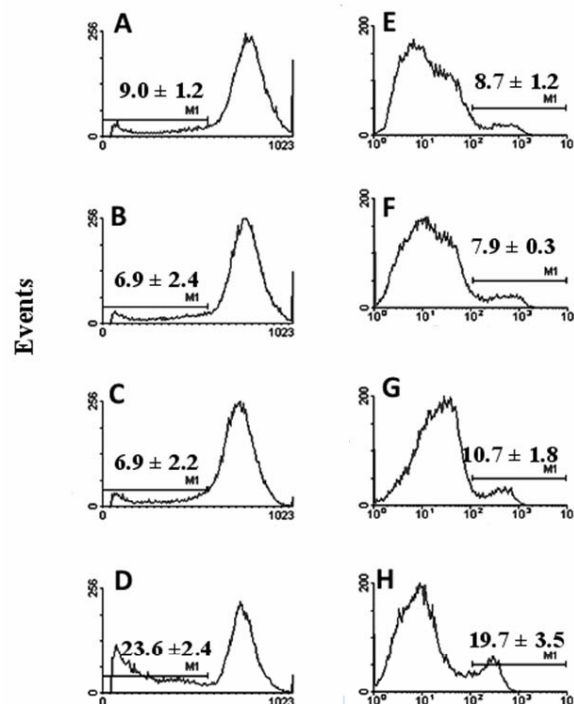


Fig. 6: Effects of *J. puberula* PP fraction and triterpenes on K562 leukemia apoptosis by flow cytometry. A-D) Cell size (FSC parameter) histograms; E-H) Annexin-V-FITC staining (FL-1 channel fluorescence). Cells ($5 \times 10^4/\text{ml}$) were cultured for 36 h either in the absence (control; A, E) or presence of *J. puberula* PP fraction (B, F), oleanolic acid (C, G) or ursolic acid (D, H), both at $20 \mu\text{g}/\text{ml}$. Data represent the mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$ versus control, by Student's *t* test.

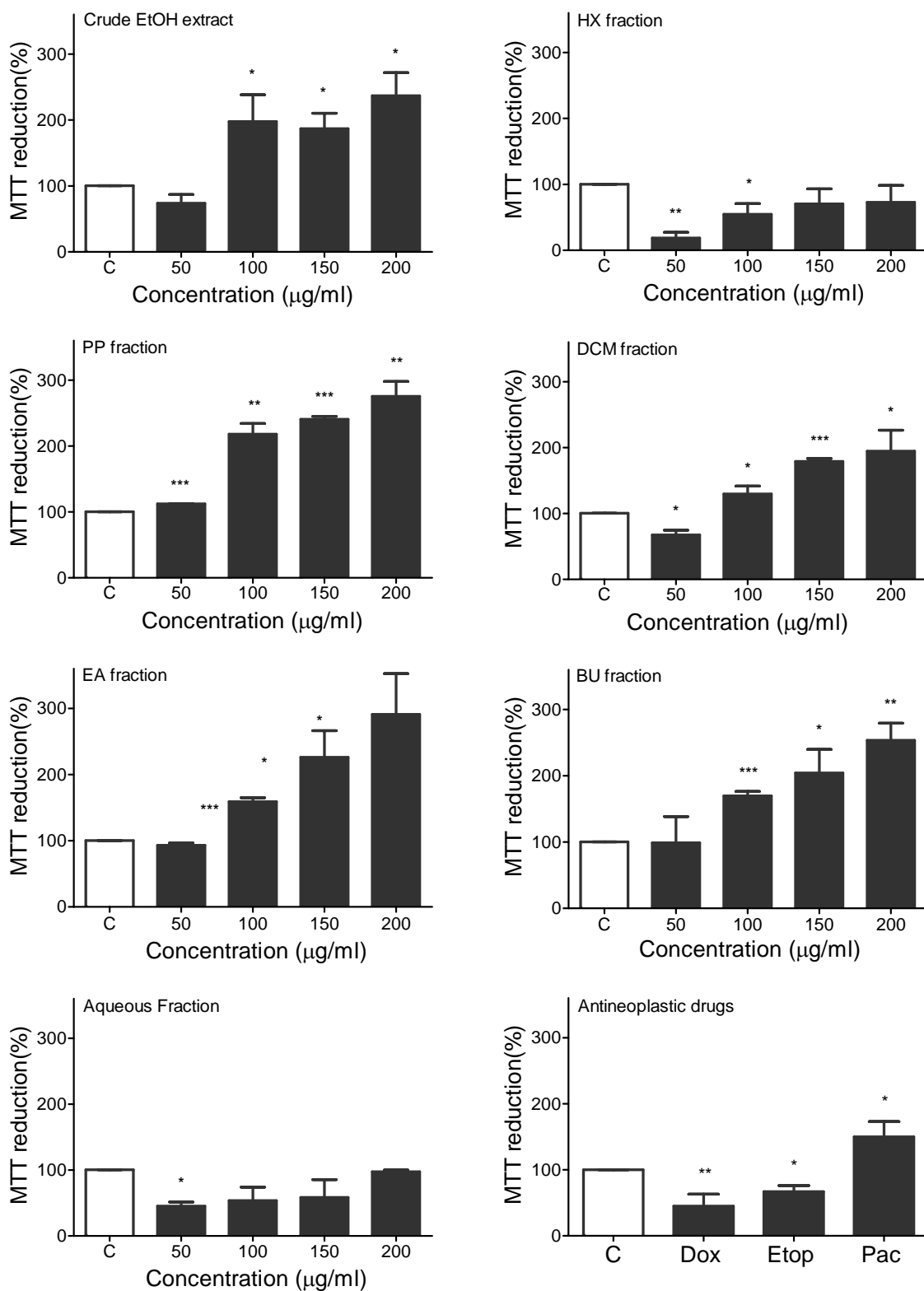


Fig. 7: Effects of *J. puberula* on mitochondrial reduction activity of normal lymphocytes. Cells (2×10^6 /mL) were stimulated with Con A ($10 \mu\text{g/mL}$) in the absence (control) or presence of *J. puberula* samples and the mitochondrial reduction activity determined by MTT assay, as described in methods. Control absorbance was 0.371. Data represent the mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control, by Student's *t* test.

K562 cell proliferation

The number of viable K562 cells when treated for 24 h with PP fraction, oleanolic acid or ursolic acid at 20 µg/ml is shown in fig. 5A, with significant reduction of 22%, 25% and 49%, respectively ($p < 0.01$). The histograms (fig. 5B) show the effects of PP, OA and UA in the K562 cell cycle of a representative experiment. Abscissas axis is a function of DNA content (PI fluorescence), indicating the percentage of cells in each cell cycle phase. In each histogram, the peak on the left (M1) represents the DNA content of a cell in G0/G1 phase; the intermediate region (M2) represents the nuclei in S phase, while the peak on the right (M3) represents nucleus of cells in G2/M phase with duplicated DNA contents. UA and OA both increased ($p < 0.05$) the cells in the G1 phase (40.6% and 26.4%, respectively), compared to control culture. PP did not altered significantly any phase of cell cycle. The increase of K562 cells in the G1 phase by treatment with OA and UA (21.2% and 26%, respectively) was confirmed by the calculated means (fig. 5C).

K562 cell apoptosis assay

The effects of PP fraction and triterpenes on K562 cell apoptosis are shown in fig. 6. Only UA (fig. 6D) increased significantly (162%) the number of cells with reduced size compared to control culture (fig. 6A), which was not noted for OA (fig. 6C) or PP fraction (fig. 6B). The histograms shown in fig 6E-6H illustrate the cell distribution according to annexin V-FITC (FITC fluorescence detected by FL-1 channel) staining. The control culture (fig. 6E) presented $8.7 \pm 1.2\%$ of apoptotic cells (high annexin-V fluorescence) and the treatment with UA (fig. 6H) increased it significantly (126%). PP fraction (fig. 6F) and OA (fig. 6G) did not induce significant alterations.

***J. puberula* effects on normal lymphocytes**

The samples effects at high concentrations were studied on immune system cells of healthy mice by the MTT assay. It can be seen in the fig. 7 an increase of MRA of lymphocytes when treated with almost all the samples, except with HX and aqueous fraction, compared to control culture. The EA fraction presented the highest stimulating effect, increasing up to 191% ($p < 0.05$) the MTT reduction by lymphocytes at 200 µg/ml. The crude EtOH, BU and PP fractions showed significant stimulating effect, increasing up to 137%, 153% and 175% the lymphocytes MRA at 200 µg/ml, respectively. Although the DCM fraction had stimulated these cells MRA at higher concentrations (up to 94%, $p < 0.05$), it was inhibited (33%, $p < 0.05$) at 50 µg/ml. The HX and aqueous fractions inhibited ($p < 0.05$) 46% and 55% of lymphocyte MTT reduction at 100 µg/ml and 50 µg/ml, respectively.

Acute toxicity evaluation

The single oral treatment of mice with PP fraction up to

5000 mg/kg b.w. did not induce toxic effects. No lethal dose or LD₅₀ could be determined since the highest dose tested caused no mortality. All test and control groups did not show any of those clinical signs of toxicity mentioned in methods.

DISCUSSION

Evaluation of *J. puberula* anti-tumoral effects indicated that the fractioning of its crude extract was really effective, showing higher anti-tumoral action for the DCM fraction than the original crude EtOH extract, and higher effect for PP fraction than the original one (DCM). The presence of other compounds in the DCM fraction may be contributing negatively for its bioactivity, comparing to PP. These plant samples induced cytotoxicity for all tumor cell lines tested in a concentrate independent manner. The triterpenes UA and OA also induced cytotoxicity on tumor cells in this work, as have already been described (Shan *et al.*, 2011; Hsu *et al.*, 2004; Urech *et al.*, 2005). Considering that UA and OA have already been described in the *Jacaranda* genus (Gachet and Schühly, 2009), the high cytotoxic effects here demonstrated for both DCM or PP fraction and triterpenes, and the apolar characteristics of both samples and triterpenes, maybe the cytotoxic effects of DCM or PP fractions of *J. puberula* on tumor cells can be related to these triterpenes.

The *in vitro* anti-tumoral action determined by mitochondrial reduction activity also showed that cytotoxicity of PP fraction (36%) at 20 µg/ml was similar to that of paclitaxel, a traditional antineoplastic drug, at 10 µg/ml (39%) for lung tumor cells. The PP fraction cytotoxicity for breast tumor cells (68%) was higher than that of the ursolic acid (54%) at 40 µg/ml, and also for prostate tumor cells (84%), ursolic acid showing MRA inhibition index of 74% at 50 µg/ml.

Both DCM, PP and ursolic acid were more active against K562 leukemia cells than the other tumor cell lines, the PP fraction showing cytotoxicity level (60%) similar to that of ursolic acid (59%) or the antineoplastic drug ethoposide (63%), at 20 µg/ml. Although these isomeric triterpenes OA and UA have similar chemical structures, differing only in the position of one methyl group on their E ring, their antitumor effects are not identical (Yan *et al.*, 2010), being the UA more potent than OA in their anti-tumoral effects (Sun *et al.*, 2006; Shan *et al.*, 2011), as observed in this study.

The most cytotoxic fraction of *J. puberula* (PP) on tumor cells and the triterpenic standard compounds also inhibited K562 cell proliferation, but only UA and OA accumulated cells in a specific phase of cell cycle (G1). This arrest in G1 phase has been described for other cell lines by treatment with UA (Hsu *et al.*, 2004) or OA

(Allouche *et al.*, 2001). PP fraction reduced K562 cells proliferation without inhibiting a specific phase of cell cycle.

The K562 cell apoptosis was also evaluated after treatment with PP fraction and triterpenes since it is one destination when the cell cycle is arrested. Only the ursolic acid induced K562 cells into apoptosis in the used conditions, which was suggested by cell shrinkage (reduction of cell size - FSC) and confirmed by externalization of phosphatidylserine in the plasma membrane (high fluorescence of annexin-V-FITC). PP fraction inhibited K562 cell proliferation without apoptosis induction. Increased number of annexin V positive cells has been described in A549 cells cultures treated with UA (Hsu *et al.*, 2004) while OA inhibits MCF-7 cells proliferation without apoptosis induction (Allouche *et al.*, 2011). Apoptosis of leukemia cells (K562, HL60) by OA has been described only at higher concentrations (Urech *et al.*, 2005) than those used in this work.

A common adverse effect of traditional antineoplastic chemotherapy is their cytotoxicity to non-tumoral tissues. The toxicity of *J. puberula* samples on lymphocytes of healthy mice was investigated since they are more sensitive than human cells (Mamone *et al.*, 2011). This work demonstrated for the first time in the genus Jacaranda a potential stimulatory action on immune system cells, which can contribute for the anti-tumor activity of *J. puberula* species. All the plant samples, except HX and aqueous fraction, stimulated lymphocytes mitochondrial reduction activity at 100 µg/ml (effective anti-tumor effect) or higher concentration, specially the crude EtOH extract (97%) and PP fraction (118%).

The acute toxicity is defined by the Organization of Economic Cooperation and Development (OECD) Guidelines as the adverse effects occurring within a short time after oral administration of a single dose of a substance or multiple doses given within 24 h. In this work the acute toxicity studies of PP fraction, determined after a single dose, did not present any significant toxic effect or death, even at the maximum dose recommended (5 mg/kg b.w), indicating that the substances present in the PP fractions have no intrinsic acute toxicity by the oral route (Chan and Hayes, 1994).

CONCLUSION

This work demonstrated an important *in vitro* anti-tumor action for the PP fraction of *J. puberula* with no *in vitro* toxicity for immune system cells and no oral acute toxicity, improving and encouraging the ethnopharmacological study of this species.

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