



Chemical composition and cytotoxicity activity of the essential oil of *Pterodon emarginatus*

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Article

Received 10 Sep 2011

Accepted 19 Jan 2012

Available online 3 Apr 2012

Abstract: *Pterodon emarginatus* Vogel, Fabaceae, is a native aromatic tree distributed by central region of Brazil. Hydroalcoholic infusions of the seeds are used in folk medicine for their anti-rheumatic and anti-inflammatory properties. The objective of this work was identified the chemical components and verify the cytotoxic effect of the essential oil (EO) from *P. emarginatus* seeds. Thus, the EO of *P. emarginatus* seeds was analyzed by GC/MS analysis followed by brine shrimp lethality test and cytotoxic activity against tumor cell lines and human peripheral mononuclear blood cells (PBMC). The cancer cell lines tested were C₆ (rat glioma), MeWo (human melanoma), CT₂₆.WT (mouse colon carcinoma), MDA (human breast cancer), A₅₄₉ (human lung carcinoma), B₁₆-F₁ (mouse melanoma), CHO-K1 (hamster ovary cell) and BHK-21 (hamster kidney fibroblast). Eleven compounds were identified by GC and CG/MS analyses. The main compounds with concentrations higher than 5% were β -elemene (15.3%), *trans*-caryophyllene (35.9%), α -humulene (6.8%), germacrene-D (9.8%), bicyclo germacrene (5.5%) and spathulenol (5.9%). The EO of *P. emarginatus* seeds showed toxicity to *Artemia salina* (LC50 1.63 μ g/mL) and was active against all the cell lines tested. The potent cytotoxic activity had IC50 values ranging from 24.9 to 47 μ g/mL. However, EO (1-100 μ g/mL) had less cytotoxicity in PBMCs isolated from a healthy subject. In summary, the present study showed the potential antiproliferative of the EO of *P. emarginatus* seeds.

Keywords:

trans-caryophyllene
cytotoxic activity
 β -elemene
essential oil
Pterodon emarginatus
Leguminosae

ISSN 0102-695X

<http://dx.doi.org/10.1590/S0102-695X2012005000042>

Introduction

Medicinal plants have been a useful source for the research of new biologically active compounds. There have been worldwide efforts to discover new antitumoral compounds from plants (Kamuhabwa et al., 2000). Different approaches are used to select a plant for research, specially the ethnomedical data. However, ethnomedical data obtained of a plant used to treat cancer might not be reliable, since it is difficult to be well diagnosed (Cragg et al., 1994). Apart from the medicinal effects of traditional herbs, exploratory researches have been made and a wide variety of new biological activities from traditional medicinal plants have recently been reported, including anticancer activity (Yoo et al., 2007).

Pterodon emarginatus Vogel, Fabaceae, known in Brazil as “sucupira-branca” or “faveiro”, is a native aromatic tree reaching the height of 5-10 m and distributed by all central region of Brazil (Goiás, Minas Gerais and São Paulo) (Lorenzi & Matos, 2002). Hydroalcoholic infusions of the seeds are used in folk medicine for their anti-rheumatic, analgesic and anti-inflammatory properties (Brito & Brito, 1993; Duarte et al., 1996). Chemical studies on genus *Pterodon* have shown the presence of alkaloid compounds in the bark (Torrenegra et al., 1989), isoflavone and some triterpenes in the wood (Marques et al., 1998), diterpenes (Fascio et al., 1976; Arriaga et al., 2000) and isoflavones in seed oil (Braz-Filho et al., 1971). The diterpene 14,15-epoxygeranylgeraniol and some derivatives isolated of *P. pubescens* have been associated with protective activity against the penetration

of cercarias of *Schistosoma mansoni*, a typically tropical disease (Dos Santos Filho et al., 1972). The furane diterpene 6 α , 7 β -dihydroxyvouacapan-17-oate sodium, isolated from the oil of the fruits of *P. polygalaeiflorus* Benth, presented anti-inflammatory activity on the test in rats leg edema induced by carrageenan (Silva et al., 2004).

Recently, Vieira et al. (2008) showed the crude ethanolic extract and furane diterpene, isolated from *P. pubescens* seeds, inhibited human melanoma cell line SK MEL cell growth. In this same study, the subfraction A (fractionation by gradient elution with hexane/ethyl acetate-80:20) caused morphological features and internucleosomal DNA fragmentation pattern of apoptosis (Vieira et al., 2008). These results have led authors to conclude that diterpenoids vouacapan-type skeleton could be used as a model to develop new anticancer agents. This evidence has been further extended by recent results showing that 6 α , 7 β -hydroxyvouacapan-17 β -lactone inhibited the growth of human cancer cells (Euzébio et al., 2009). These findings were recently confirmed by Pereira et al. (2011), who have demonstrated that methyl-6 α -acetoxy-7 β -hydroxyvouacapan-17 β -oate (SF5) significantly induced cytotoxic and anti-proliferative effects on K562 cells. Moreover, down-regulation of DNA (cytosine-5)-methyltransferase 1 (DNMT1) gene transcription and over-expression of apoptotic protease activating factor 1 (Apaf-1) mRNA suggested that SF5 may be inducing apoptosis of K562 cells by epigenetic up-regulation of pro-apoptotic proteins involved in the mitochondrial intrinsic pathway (Pereira et al., 2011).

However, there have been no reports about the effects of EO from *P. emarginatus* on the growth of human and/or non-human cancer cells. In the present study, we sought to investigate the cytotoxicity activity of the EO of *P. emarginatus* seeds and attempted to elucidate some of the mechanisms through which EO modulates the growth of cancer cells. For this reason, the EO chemical composition, brine shrimp lethality test and *in vitro* cytotoxic activity against some cancer cell lines were evaluated.

Material and Methods

Plant material and EO isolation

Plant material (seeds) of the species *Pterodon emarginatus* Vogel, Fabaceae, was collected in September 2006 at the city of Três Marias (Minas Gerais, Brazil) and authenticated by Dr. Fátima Regina Gonçalves Salimena from the Departamento de Botânica, Universidade Federal de Juiz de Fora (UFJF), Brazil. A voucher specimen (N° 48.077) has been deposited at Herbarium CESJ of the Universidade Federal de Juiz de Fora (Minas Gerais, Brazil). The seeds of *P. emarginatus* were triturated (30

g) and hydrodistilled in a Clevenger-type apparatus. After 2 h distillation, the EO was collected as a film floating on the surface of water. The samples were sealed and kept in dark glass vials in the refrigerator at 4 °C for further analysis.

Chemical composition of EO

Gas chromatography (GC)

The essential oils were analyzed using a gas chromatograph (model 8700, PerkinElmer, Norwalk, CT, USA) equipped with flame ionization detector (FID) and HP-5 MS capillary column (30 m \times 0.25 mm, film thickness 0.25 μ m). Injector and detector temperatures were set at 220 and 290 °C, respectively. Column oven temperature was programmed from 80 to 240 °C at a rate of 4 °C/min; initial and final temperatures were held for 3 and 10 min, respectively. Helium was used as carrier gas with a flow rate of 1.5 mL/min. A sample of 1.0 μ L was injected using the split mode (split ratio 1:100). All quantification was done using a built-in data-handling program provided by the manufacturer of the gas chromatograph (PerkinElmer). The composition was reported as relative percentage of the total peak area.

Gas chromatography-mass spectrometry (GC-MS) and compound identification

GC-MS analysis of the essential oils was performed using an Agilent Technologies (Little Falls, CA, USA) 6890N network GC system, equipped with an Agilent Technologies 5975 Inert XL mass selective detector and 7683B series auto-injector. Compounds were separated on an HP-5 MS capillary column (30 m \times 0.25 mm, film thickness 0.25 μ m). A 1.0 μ L sample was injected in the split mode, with a split ratio of 1:100. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Column oven temperature programmed was the same as in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. Mass range was 50-550 *m/z*, while the injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Most constituents were identified by gas chromatography by comparison of their GC retention indices with those of the literature or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature (Adams, 1995).

Brine shrimp lethality test

The assay was carried out according Meyer et al. (1982). *Artemia salina* Leach eggs were cultured in artificial sea water: NaCl 24.0 g/L, CaCl₂·2H₂O 1.5 g/L, KBr 0.1 g/L, KCl 0.7 g/L, Na₂SO₄ 4.0 g/L, NaHCO₃ 0.3 g/L, MgCl₂·6H₂O 11.0 g/L. EO was dissolved in Tween 80 and dimethyl sulfoxide (DMSO) (1:2, v/v), and then in artificial sea water. Ten brine shrimp (*A. salina*) were transferred for test tubes containing the following concentrations of the EO: 0.5, 1, 5, 10 and 50 µg/mL (n=4). The tubes were kept in constant illumination and thymol was used as standard control. Living nauplii were counted after 24 h of exhibition to the oil. LC50 and 95% confidence intervals were from the 24 h counts using the probit analysis method (Litchfield & Wilcoxon, 1949). The final volume used of the solution of Tween 80 and DMSO (1:2, v/v) was also tested.

Cell viability assay

The following cell lines were used in this study: C₆ (rat glioma), MeWo (human melanoma), CT₂₆-WT (mouse colon carcinoma), MDA-MB 231 (human breast cancer), A₅₄₉ (human lung carcinoma), B₁₆-F₁ (mouse melanoma), CHO-K1 (hamster ovary cell) and BHK-21 (normal hamster kidney fibroblast). All cell lines used were obtained from the American Type Culture Collection (ATCC, Rockville MD). Cells were grown in RPMI 1640 medium (Sigma Chemical Co.) supplemented with 10% fetal bovine serum (FBS-Gibco). Cells were maintained in humidified 5% CO₂ atmosphere at 37 °C. The cytotoxic potential of the EO was evaluated using an MTT assay according to Denizot & Lang (1986). An EO stock solution (5 mg/mL) in 0.05% DMSO and purified water was prepared, filtered in 0.22 µm filter and stored at -20 °C till use. Before the experiments, this stock solution was diluted with supplemented RPMI 1640 medium to obtain final test concentrations. Cells (10³ cells/well) were seeded in supplemented medium in 96-well culture plates (Falcon, NJ, USA) and were incubated in a humidified atmosphere with 5% CO₂, 37 °C, for 24 h until total adhesion to surface. The medium was replaced with fresh supplemented medium containing different concentrations of EO (20-50 µg/mL). Cells were then incubated at 37 °C for 48 h. After this time, the medium was newly refreshed with the same concentration of EO and the plate was incubated again for 72 h. The time of analysis was chosen according to the method previously described (Qu et al., 2007; Huang et al., 2011; Rivest et al., 2011). After incubation time, 10 µL of a 5 mg/mL stock solution of MTT in PBS was added to each well containing the cells and incubated again for 4 h. Then, the supernatant without cells was aspirated from each well and 100 µL of dimethyl sulfoxide (DMSO-Sigma Chemical Co.) was added to dissolve the dark blue formazan crystals resulting from MTT reduction

by homogenization in plate shaker. The extent of MTT reduction to formazan within cells was measured by absorbance at 600 nm using a scanning microplate reader (Stat Fax-2100, Awareness Tecn.). The final volume used to dissolve the EO was also tested. Cultures used as controls did not receive extract.

Mononuclear cell isolation

Peripheral blood mononuclear cells (PBMC) were isolated from the peripheral blood of healthy volunteers, and were immediately isolated by gradient centrifugation in Ficoll-Paque™ Plus (Amersham Biosciences, USA). The mixture was centrifuged under at 400×g for 40 min at 18-20 °C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with three volumes of balanced salt solution for twice and resuspended RPMI 1640 medium supplemented with 10% v/v fetal bovine serum, HEPES (20 nM), 2-mercapto ethanol, penicillin (100 U/mL) and streptomycin (100 µg/mL). The PBMC cells were incubated with different concentrations of EO (1-100 µg/mL) for 12, 24 and 48 h at 37 °C in a humidified 5% CO₂ atmosphere. The cell survival fraction was determined with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye-reduction assay, as described above.

Statistical analysis

Each experiment was performed at least in triplicate. The results were expressed as mean±SEM for the number of experiments. The percentages of inhibition of cell viability were calculated with the values of viability of cell exposed to the EO and with non-exposed cells, using the *software* GraphPad Prisma 4.0 (GraphPad Software, Inc.). Statistical significance was compared between each treated group and control by the Student's t-test. Results with *p*<0.05 were considered significant from control group. The 50% inhibitory concentrations for cellular population (IC₅₀) were calculated by linear regression (SigmaPlot 10.0, Systat Software, Inc.) in the interval of the corresponding concentrations to the curve of MTT metabolization vs. log of the used concentrations.

Results and Discussion

EO has been found widely applications in many areas such as medicine and pharmaceutical, cosmetic, agricultural and food industries (Bakkali et al., 2008; Lukas et al., 2009). Therefore, nowadays the investigation of biological activities of EO from a range of aromatic plants has been one of the attractive research areas. This study has evaluated the chemical composition

and cytotoxicity activity of EO of *Pterodon emarginatus* Vogel, Fabaceae, seeds commonly consumed in Brazil and elsewhere in the world. Here, a light white EO of *P. emarginatus* seeds was obtained in a yield of 3.9% (w/w) fresh weight. The results of the GC and GC-MS analysis of the oil identified eleven compounds, as monoterpenes and sesquiterpenes, such as: δ -elemene (4.8%; Retention time (RT), 15.64; Kovats index (KI), 1327), β -elemene (15.3%; RT, 15.67; KI, 1380), *trans*-caryophyllene (35.9%; RT, 18.16; KI, 1411), β -gurjunene (4.6%; RT, 18.49; KI, 1423), α -humulene (6.8%; RT, 19.23; KI, 1449), γ -muurolene (2.7%; RT, 19.44; KI, 1462), germacrene-D (9.8%; RT, 19.97; KI, 1475), bicyclo germacrene (5.5%; RT, 20.44; KI, 1492), spathulenol (5.9%; RT, 22.53; KI, 1570), caryophyllene oxide (3.8%; RT, 22.72; KI, 1577) and *cis*-farnesyl acetate (4.9%; RT, 26.34; KI, 1815). Importantly, our results confirm and extend some previously published data (Dutra et al., 2009b). A predominance of sesquiterpene was found analyzing the chemical composition of EO from *P. emarginatus* seeds, being *trans*-caryophyllene the one in highest concentration. These findings are in agreement with those from Carvalho (2004), however the presence of α -caryophyllene, diterpenes and isoflavones was not verified in the EO of the seeds of *P. emarginatus*, as previously described (Braz-Filho et al., 1971; Fascio et al., 1976; Arriaga et al., 2000).

The EO of *P. emarginatus* seeds showed a potent toxicity in *Artemia salina* with LC50 1.63 (1.34-1.99) $\mu\text{g/mL}$ (Table 1). It was about 250 times more toxic than thymol (LC50 480.20 $\mu\text{g/mL}$), which was used as a reference substance. In agreement with our data, a number of works indicates that thymol showed several biological activities, such as cytotoxicity (Buyukleyla & Rencuzogullari, 2009), antifungal, genotoxic (Stammati et al., 1999) and antileishmanial (De Medeiros et al., 2011) effects. Moreover, recent published data showed that thymol significantly increased the sister chromatid exchange, especially at the lower concentrations (75 and 100 $\mu\text{g/mL}$), induced both the chromosome aberration (CA) and frequency of micronucleus (MN), as well as dose-dependently decreased the nuclear division index (NDI) in human peripheral lymphocytes (Buyukleyla & Rencuzogullari, 2009). Taken together, our data confirms that thymol is an adequate positive control and that the EO obtained from the seeds of *P. emarginatus* could be cytotoxic to *Artemia salina* probably by modification the structure of DNA, as well as thymol. However, further studies are necessary to investigate this hypothesis.

A growing amount of evidences point out *A. salina* assay as crucial for antiproliferative effect of many natural products (Pimenta et al., 2003). Furthermore, among the bioassays that seeks guidance for drug discovery the *A. salina* assay is considered efficient, fast, cheap, and requires only 2 to 20 mg of compounds (Pimenta et al.,

2003; Ajaiyeoba et al., 2006). The reference methodology for the *A. salina* bioassay which indicates the concentration to inhibit 50% of cell growth (IC50) as being a tenth of the effective dose to kill 50% of the brine shrimp in its nauplii stage (ED50) was initially established by McLaughlin & Rogers (1998). In fact, studies conducted with naturally extracts and substances purified confirm and further extend the proposition that *A. salina* is a suitable experimental model to assess cytotoxicity. For instance, a recent study has demonstrated that methanol extract of *Vitex trifolia purpurea* showed a significant cytotoxic activity in brine shrimp bioassay, with LC50 value 173 $\mu\text{g/mL}$ (El-Sayed et al., 2011). Other studies have confirmed and extended our data. It has been shown that *Tamarindus indica* crude extract and fractions showed brine shrimp death percentages ranging from 86.70% to 3.30% (Nwodo et al., 2011). Moreover, a very interesting study conducted by Boehme et al. (2008) suggested that the leaf EO of *Zanthoxylum rhoifolium* and *Zanthoxylum setulosum* showed activity against *A. salina* (Boehme et al., 2008). Herein, our data further extended these studies and together we reported for the first time that EO obtained from *P. emarginatus* markedly showed a potent cytotoxic activity in brine shrimp lethality bioassay.

Table 1. *In vitro* toxicity of the seeds oil of *Pterodon emarginatus* in *Artemia salina*.

Compound	Concentration ($\mu\text{g/mL}$)	LC50 ($\mu\text{g/mL}$)	95% confidence interval
essential oil	0.5-50	1.63	1.34-1.99
thymol	10-1000	480.20	337.3-683.5

Data are presented as mean (n=4/concentration) and are representative of four independent experiments.

EO has been shown to exhibit cytotoxic activity generally without being mutagenic in various organisms (Bakkali et al., 2008; Stammati et al., 1999). Since the differential cytotoxicity is also a useful feature for potential antitumor agents, the antiproliferative effect of the EO from *P. emarginatus* was evaluated in both cancer and normal cell line by MTT assay based on cell viability. Moreover, the MTT assay is a sensitive, simple and reliable approach used to evaluate the cytotoxicity of plant-based products. The inhibitory effects of EO (IC50 values) are presented in Table 2, indicating that the tested *P. emarginatus* EO showed prominent cytotoxic activity against all cancer cell lines. The lowest value for IC50 was obtained for C₆ cell line (24.9 $\mu\text{g/mL}$) while the highest value for IC50 was obtained for A₅₄₉ cell line (47.0 $\mu\text{g/mL}$). On the other hand, the EO of *P. emarginatus* seeds did not display strong cytotoxic effect on freshly isolated normal PBMC with viability of 94 \pm 1.5%, 93 \pm 3.5% and 95 \pm 3.3%, during 12, 24 and 48 h, at 100 $\mu\text{g/mL}$, respectively (Table 3). Here,

doxorubicin (DOXO) used as a positive control in this experiment, had the strong cytotoxic effect on PBMC cells (Table 3). Doxorubicin, a broad-spectrum antitumor antibiotic, has been one of the most extensively used agents in the chemotherapy regimens of cancer patients for the past thirty years (Weiss, 1992; Singal & Iliskovic, 1998). Moreover, DOXO stabilize a transient DNA-topoisomerase II complex in which DNA strands are cut and covalently linked to the enzyme subunits (Burden & Osheroff, 1998). Consequently, the stabilized complex results in DNA damage that is related to the cytotoxic effect (Kaufman, 1998; Brown & Wouters, 1999). Based on these results, it is tempting to speculate that EO of *P. emarginatus* seeds could modulate the DNA damage of tumor cells without affecting normal cells. Nonetheless further studies are needed to better understand whether EO had effects on DNA damage (such as p53, Bcl-2, Bax, cytochrome c, and caspases) or protein damage (such as thioredoxin and glutathione) in cancer cells.

Table 2. *In vitro* cell viability assay of EO of *Pterodon emarginatus* seeds on different cell lines.

Cell lines	IC50 ($\mu\text{g/mL}$)	Taxol [®] ($\mu\text{g/mL}$)
B ₁₆ F ₁	43.4	3.55x10 ⁻⁶
MDA-MB 231	33.5	6.71x10 ⁻⁶
C ₆	24.9	3.43x10 ⁻⁶
MeWO	25.5	6.42x10 ⁻⁶
CT ₂₆ -WT	32.0	3.26x10 ⁻⁶
A ₅₄₉	47.0	6.03x10 ⁻⁶
BHK-21	38.0	1.06x10 ²
CHO-K1	25.2	3.04x10 ³

C₆ (rat glioma), MeWo (human melanoma), CT₂₆-WT (mouse colon carcinoma), MDA-MB-231 (human breast cancer), A₅₄₉ (human lung carcinoma), B₁₆-F₁ (mouse melanoma), CHO-K1 (hamster ovary cell) and BHK-21 (normal hamster kidney fibroblast). Data are presented as mean (n=3/concentration) and are representative of three independent experiments.

According to published guidelines, IC₅₀<10 $\mu\text{g/mL}$ represents potentially 'very toxic'; IC₅₀ 10-100 $\mu\text{g/mL}$ represents 'potentially toxic'; IC₅₀ 100-1000 $\mu\text{g/mL}$ represents 'potentially harmful' and IC₅₀>1000 $\mu\text{g/mL}$ represents 'potentially nontoxic' (Gad-Shayne, 2009). Based on these data, it is possible to infer that EO obtained from *P. emarginatus* may be considered very to potentially toxic. Nowadays, an increasing body of evidence has emerged indicating that pure compounds obtained from plant extracts are used in cancer treatment, such as camptothecin. Furthermore, medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, such as new anticancer molecules (Calixto et al., 2004; Costa et al., 2008). In fact, Kaileh et al. (2007) evaluated the potentiality against cancer cell lines of 24 plants from Palestine, and found IC₅₀ values varying from 55 to 2000 $\mu\text{g/mL}$. More recently, Fiore et al. (2006) have analyzed six different species of *Salvia* and found moderate activity ranging from 89.6 to 405.1 $\mu\text{g/mL}$. Also of interest are the results from the other group showing that pure substances show low values in MTT cytotoxic assay, ranging from 0.1 to 10 $\mu\text{g/mL}$, for instance, jacaranone, a quinoid isolated from *Senecio ambiguus* had values of IC₅₀ from 4.32 to 7.39 $\mu\text{g/mL}$ (Loizzo et al., 2007). In addition, another recent and relevant publication has shown that berberine and coptisine isolated from *Coptis chinensis* showed IC₅₀ values ranged from 0.6 to 15.2 $\mu\text{g/mL}$ (Lin et al., 2004). A previous report showed the toxicity of seed's oil of a synonymy species *P. pubescens* on PBMC, and the authors found a cytotoxic effect with IC₅₀ 2.0 $\mu\text{g/mL}$ for these cells (Sabino et al., 1999). Very recently, an interesting study conducted by Vieira et al. (2008) has demonstrated the cytotoxicity, morphological and biochemical alterations on human melanoma cells (cell line SK MEL 37) induced by vouacapan-derived compound isolated from EO of *P. pubescens* seeds. These results have led authors to conclude that diterpenoids vouacapan could be used as a model to develop new anticancer agents. This evidence has

Table 3. *In vitro* cell viability assay of EO of *Pterodon emarginatus* seeds in peripheral blood mononuclear cells (PBMC).

Treatment	Concentration ($\mu\text{g/mL}$)	Viability (%)		
		12 h	24 h	48 h
Control (Medium)	-	100±5.5	100±4.7	100±3.5
EO	1	97±2.5	98±1.5	96±3.2
	10	96±1.3	95±2.9	97±2.2
	100	94±1.5	93±3.5	95±3.3
DOXO	1	50±3.8**	68±4.3**	44±4.5**
	10	16±8.1**	23±5.7**	17±4.8**
	100	7±2.1**	4±2.3**	5±3.2**

PBMC (1×10^5 cells/mL) were treated with different concentrations of EO (1-100 $\mu\text{g/mL}$) and the cells were tested for viability by MTT assay after the treatment of EO and Doxorubicin (DOXO, 1-100 $\mu\text{g/mL}$) for 12, 24 and 48 h. The value represented the mean±S.D from two independent experiments. ** $p < 0.01$ significantly different from the control value

been further extended by recent results showing that 6α , 7β -hydroxyvouacapan- 17β -lactone inhibited the growth of human cancer cells (Euzébio et al., 2009). Herein, our data showed that EO obtained from *P. emarginatus* seeds markedly induced *in vitro* cytotoxicity to a number of human/rodents tumour cell lines.

In fact, our data extends the results of recent reports which indicated that bicyclic sesquiterpenoids, such as (+)-spathulenol, α -pinene, globulol, viridiflorene and eremophilanes exhibited cytotoxicity against P388D-1 mouse lymphoblast cells *in vitro* (Beattie et al., 2011). Another recent and relevant publication has shown that the leaf EO of *Oreopanax nubigenus* and *Schefflera rodrigueziana* was dominated by the sesquiterpene hydrocarbons germacrene-D (70.1 and 27.6%) and trans-caryophyllene (11.8 and 12.2%), respectively. Moreover, both *O. nubigenus* and *S. rodrigueziana* leaf oils showed notable *in vitro* cytotoxicity on MDA-MB 231 cells, which have led authors to conclude that antiproliferative effect presented by EO may be attributable to the relatively high concentrations of germacrene-D and trans-caryophyllene in those oils (Richmond et al., 2009). A very interesting report has shown that α -humulene and trans-caryophyllene, constituents of *P. emarginatus* EO, were found in other plants (Saroglou et al., 2006). However, Loizzo et al. (2007) identified in their extract the presence of α -humulene, suggesting it to be one (but not the only) molecule responsible for its cytotoxic activity in rectal and prostate carcinoma. Here, we demonstrated that EO administration suppressed, mainly, rat glioma (C₆) and human melanoma (MeWo), among other cells. Therefore, it is tempting to suggest that sesquiterpene compounds, such as trans-caryophyllene, β -elemene, germacrene-D and α -humulene present in the EO may, at least in part, explain how EO attenuates growth of tumor cells *in vitro*. However, whether these individual compounds inhibit the growth of tumor cells *in vitro* or *in vivo* requires further investigation. In addition, our data further extend the notion that EO obtained from *P. emarginatus* seeds have significant pharmacological activities, such as antinociceptive, antimicrobial and anti-inflammatory activities (Dutra et al., 2008; Dutra et al., 2009a; Dutra et al., 2009b).

Conclusion

In summary, the data obtained in this study revealed that the EO obtained from *P. emarginatus* seeds showed lower cytotoxicity in normal cells, but it exerted higher cytotoxic activity toward the cancer cells. Our data also showed the chemical composition of EO, as well as preliminary toxicity in *A. salina*. Moreover, the EO may be acting at least partially by inhibits the apoptosis pathway. Therefore, it seems reasonable to suggest that EO obtained from *P. emarginatus* seeds or its constituents

may represent, in the near future, a new therapeutic option for the development of new drug for cancer treatment. Further studies aiming to investigate which compounds in the EO are responsible for the described antiproliferative activity and the precise mechanisms of action are underway.

Acknowledgements

This work was supported by grants from the CNPq and the CAPES. RCD, DDJ and RM are D.Sc. students in pharmacology with a scholarship from CNPq.

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