ABSTRACT

This study assessed the antiproliferative and cytotoxic potential against tumor lines of ethanolic seed extracts of 21 plant species belonging to different families from Northeastern Brazil. In addition, some underlying mechanisms involved in this cytotoxicity were also investigated. Among the 21 extracts tested, the MTT assay after 72 h of incubation demonstrated that only the ethanolic extract obtained from Myracrodruon urundeuva seeds (EEMUS), which has steroids, alkaloids and phenols, showed in vitro cytotoxic activity against human cancer cells, being 2-fold more active on leukemia HL-60 line \([IC_{50} \text{ value of } 12.5 (9.5-16.7) \mu g/mL}] than on glioblastoma SF-295 \([IC_{50} \text{ of } 25.1 (17.3-36.3) \mu g/mL}] and Sarcoma 180 cells \([IC_{50} \text{ of } 38.1 (33.5-43.4) \mu g/mL}]. After 72h exposure, flow cytometric and morphological analyses of HL-60-treated cells showed that EEMUS caused decrease in cell number, volume and viability as well as internucleosomal DNA fragmentation in a dose-dependent way, suggesting that the EEMUS triggers apoptotic pathways of cell death.

Key words: antiproliferative potential, Northeastern Brazilian plants, Myracrodruon urundeuva, sarcoma 180 tumor, seed extracts.

INTRODUCTION

There is considerable scientific and commercial interest in discovering new anticancer agents from natural product sources (Kinghorn et al. 2003). The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI) and since then several studies have given valuable contributions to the discovery of new naturally occurring anticancer agents. In the 1980s, the development of new screening technologies led the research for new anticancer agents in plants and other organisms, focusing on the tropical and sub-tropical regions of the world (Cragg and Newman 2005).

Brazil possesses the largest diversity of plant species in the world, but less than 10% have been evaluated with respect to their biological characteristics, and fewer than 5% have been subjected to detailed phytochemical studies (Luna et al. 2005). In Northeastern Brazil, a region with approximately 1,539,000 km², with warm and dry climate, grows the peculiar xerophytic “Caatinga” vegetation (dry land vegetation). In Caatinga flora, there are almost 1000 vascular plant species and due to the extreme climate conditions most species are endemic (Lemos and Rodal 2002, Sampaio 2002,
Despite this great biodiversity, Northeastern Brazilian plants are relatively under-exploited with regard to discoveries of active biological substances (Luna et al. 2005).

Seeds, especially from legumes, are recognized by their nutritional value, rich in proteins, carbohydrates and oil, though they are not merely a site to accumulate organic materials. They need physical and chemical mechanisms for protection and/or defense for the developing embryo. The compounds involved in chemical defense include lectins, protease and amylase inhibitors, toxins and low molecular mass compounds (secondary metabolites) (Xavier-Filho 1993, Sampaio et al. 1992, Ferreira et al. 2009). According to some reports many seeds and other parts from Northeastern Brazilian plants are exploited in popular medicine and many of these present important pharmacological properties. The ethnomedical data and some pharmacological activities of the studied plants are shown in Table I. Nevertheless, these plants, especially their seeds, are scarcely studied concerning cytotoxicity on tumor cell strains. Thus, the aim of the present study was to assess the antiproliferative potential of ethanolic seed extracts of twenty-one plant species belonging to different families from Northeastern Brazil on tumor cells and study some underlying mechanisms involved in this cytotoxicity.

**MATERIALS AND METHODS**

**ANIMALS**

Adult Swiss mice (*Mus musculus* Linnaeus, 1758) were obtained from the animal facilities of the Universidade Federal do Ceará, Fortaleza, Brazil. They were kept in well ventilated cages under standard conditions of light (12 h with alternative day and night cycles) and temperature (27 ± 2°C) and housed with access to commercial rodent stock diet (Nutrilabor, Campinas, São Paulo, Brazil). All procedures are in accordance with COBEA (*Colégio Brasileiro de Experimentação Animal* (Process no. 102/2007) and International Standard on the care and use of experimental animals (EEC Directive of 1986, 86/609/EEC).

**PLANT MATERIAL**

Harvest of the plant materials was carried out in three different localities in Ceará State, Northeastern Brazil: the Caatinga forest (dry land vegetation), Araripe National Forest (rain forest), and in the coastal zone. Mature wild seeds (at least 500 g) of each plant species were collected during the dry period (from January 2005 to November 2007), with help of native people. Plants were identified and voucher specimens were deposited at Herbarium Prisco Bezerra – EAC, Universidade Federal do Ceará (Fortaleza, Ceará, Brazil). Table I described all the species studied in this work, their voucher numbers, common names, harvest data and medicinal applications.

**PREPARATION OF CRUDE EXTRACTS**

Seeds of freshly collected plant material were separated, immediately air dried and finally ground in a laboratory mill (Quimis, Campinas, São Paulo, Brazil) to a moderately-fine powder (mesh size 0.5 mm). Powdered material (500 g) was submitted to extraction with 99% ethanol (1.5 L) at room temperature (25-27°C) for 3 days and filtered. The residue was re-extracted twice in a similar manner. The extracts were evaporated and bulked under reduced pressure in a rotary evaporator. Crude extracts were stored in a freezer at –20°C until required. A stock solution containing 10 mg/mL of each crude extract was prepared by suspending 10 mg of extract in 1 mL of sterile dimethylsulphoxide (DMSO, Sigma Aldrich) (Torres et al. 2005, Costa et al. 2008, Buriol et al. 2009, Magalhães et al. 2010), mixed by sonication (Bandelin, model RK-100, Berlin, GER) for 20 min.

**CYTOTOXICITY AGAINST HUMAN TUMOR CELL LINES**

The antiproliferative potential of the seed extracts was evaluated by the MTT assay (Mosmann 1983) against 4 human tumor cell lines: HL-60 (leukemia), SF-295 (glioblastoma), HCT-8 (colon) and MDA/MB-435 (melanoma), all obtained from the National Cancer Institute (Bethesda, MD, USA). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37°C with 5% CO2. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product. Briefly, cells were plated in 96-well plates [0.7 × 10^5 cells/well for adherent cells

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### Antiproliferative Potential of Northeastern Brazilian Seeds

#### Table I

<table>
<thead>
<tr>
<th>Family</th>
<th>Botanical name</th>
<th>Common name</th>
<th>Yield (%)</th>
<th>Traditional medicinal use</th>
<th>Pharmacological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANACARDIACEAE</td>
<td>Myracrodruon urundeuva Fr. All.</td>
<td>Aroeira-do-sertão</td>
<td>25.2</td>
<td>Cutaneous and gynecological affections, kidney and respiratory problems; anti-inflammatory, anti-ulcer, healing (Sousa et al. 2004); antimicrobial (Matos 2000); analgesic, anti-diarrheal (Maia 2004)</td>
<td>Anti-colitis (Rodrigues et al. 2002); anti-inflammatory, analgesic (Viana et al. 2003); anti-ulcer (Souza et al. 2007); anti-periodontitis (Botelho et al. 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Araripe National Forest: 01/07]</td>
<td>34,865</td>
<td></td>
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</tr>
<tr>
<td>SCHINOPSEI属</td>
<td>brasiliensis Engl.</td>
<td>Braúna</td>
<td>2.88</td>
<td>Cause nervosism and hysteria, analgesic (Maia 2004)</td>
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<td>CARYOCARACEAE</td>
<td>Caryocar coriaceum Wittm.</td>
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<td>CHRYSOBALANACEAE</td>
<td>Licania tomentosa Benth.</td>
<td>Oiti</td>
<td>4.4</td>
<td>Not described</td>
<td>Anti-viral (Miranda et al. 2002); cytotoxic (Fernandes et al. 2003)</td>
</tr>
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<td>[Coast zone: 01/08]</td>
<td></td>
<td></td>
<td>40,215</td>
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<td></td>
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<tr>
<td></td>
<td>Licania rigida Benth.</td>
<td>Oiticica</td>
<td>28.1</td>
<td>Used to treat diabetes, anti-inflammatory (Maia 2004)</td>
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<td>[Coastal zone: 12/07]</td>
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<td>CONNARACEAE</td>
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<td>Cabelo-de-negro</td>
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<td>34,733</td>
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<td>FABACEAE</td>
<td>Adenanthera pavonina L.</td>
<td>Falso-sândalo</td>
<td>6.12</td>
<td>Not described</td>
<td>Anti-inflammatory, analgesic (Olajide et al. 2004)</td>
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<td></td>
<td>38,389</td>
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<tr>
<td></td>
<td>Amburana cearensis (All.) A.C. Smith</td>
<td>Cumaru, amburana</td>
<td>6.43</td>
<td>To treat rheumatism, cold, synustes, antispasmodic, healing, anti-inflammatory (Maia 2004, Sousa et al. 2004)</td>
<td>Hepatoprotection (Leaf et al. 2008); broncodilatation (Leaf et al. 2006); neuroprotection (Leaf et al. 2005); cytotoxic (Costa-Lotufo et al. 2003); anti-inflammatory, muscle relaxant (Leaf et al. 2003)</td>
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<td>[Caatinga forest: 11/07]</td>
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<tr>
<td></td>
<td>Anadenanthera macrocarpa (Benth.) Brenan</td>
<td>Angico vermelho</td>
<td>9.35</td>
<td>To treat respiratory diseases, gonorrhea, diarrhea; adstringent, anti-inflammatory, sedative, healing (Maia 2004)</td>
<td>Antioxidant (Desmarchelier et al. 1999)</td>
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<td>[Caatinga forest: 07/05]</td>
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<td>38,697</td>
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<td></td>
<td>Diospyros guianensis Rolfe</td>
<td>Mucunã, olho-de-bai</td>
<td>2.40</td>
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<td>Not described</td>
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<td></td>
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</table>

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### TABLE I (continuation)

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<tr>
<th>FAMILY</th>
<th>Botanical name</th>
<th>Common name</th>
<th>Yield (%)</th>
<th>Traditional medicinal use</th>
<th>Pharmacological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>FABACEAE</td>
<td><em>Enterolobium contortisiliquum</em> (Vell.) Morong</td>
<td>Orelha-de-negro</td>
<td>2.80</td>
<td>Not described</td>
<td>Antimicrobial (Shahat et al. 2008); cytotoxic (Mimaki et al. 2003); anticoagulant (Sampaio et al. 1992); pro-inflammatory (Castru-Faria-Neto et al. 1991); hemolytic (De Sousa and Morhy 1989)</td>
</tr>
<tr>
<td></td>
<td><em>Hymenaea courbaril</em> L.</td>
<td>Jatobá</td>
<td>9.50</td>
<td>Not described</td>
<td>5-lipoxygenase inhibition (Braga et al. 2000); antiplasmodial (Köhler et al. 2002)</td>
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<tr>
<td></td>
<td><em>Hymenaea courbaril</em> L.</td>
<td>Ingá</td>
<td>10.60</td>
<td>Not described</td>
<td>Anti-inflammatory, antimicrobial (Alencar et al. 2005); cytotoxic (Cunha et al. 2003)</td>
</tr>
<tr>
<td></td>
<td><em>Lueczelburgia auriculata</em> (Allemão) Ducre</td>
<td>Pau-mosó</td>
<td>8.40</td>
<td>Not described</td>
<td>Not described</td>
</tr>
<tr>
<td></td>
<td><em>Parkia platycephala</em> Benth.</td>
<td>Visgueiro</td>
<td>17.50</td>
<td>Not described</td>
<td>Not described</td>
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<td></td>
<td><em>Piptadenia moniliformis</em> Benth.</td>
<td>Catanduva</td>
<td>6.18</td>
<td>Not described</td>
<td>Not described</td>
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<td></td>
<td><em>Senna obtusifolia</em> (L.) H.S.Irwin &amp; Bameby</td>
<td>Mata pasto</td>
<td>5.70</td>
<td>Not described</td>
<td>Not described</td>
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<tr>
<td>POLYGONACEAE</td>
<td><em>Triplaris gardneriana</em> Wedd</td>
<td>Pajéu</td>
<td>26.0</td>
<td>Not described</td>
<td>Not described</td>
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<td></td>
<td><em>Ziziphus joazeiro</em> Mart</td>
<td>Juá, juazeiro</td>
<td>7.22</td>
<td>Expectorant, antipyretic, used to treat skin, blood, stomach and liver diseases, ulcer, antimicrobial (Maia 2004)</td>
<td>Antifungal (Cruz et al. 2007); antioxidant, antibacterial (Alviano et al. 2008)</td>
</tr>
<tr>
<td>SAPINDACEAE</td>
<td><em>Talisia esculenta</em> (A. St-Hill) Radlk</td>
<td>Pitomba</td>
<td>1.50</td>
<td>Not described</td>
<td>Not described</td>
</tr>
<tr>
<td></td>
<td><em>Sapindus saponaria</em> L.</td>
<td>Sabonete</td>
<td>10.7</td>
<td>Not described</td>
<td>Antifungal (Tsuzuki et al. 2007); antilúcer (Albiero et al. 2008)</td>
</tr>
</tbody>
</table>
(SF295, MDA/MB-435, HCT-8) and 0.3 × 10^5 cells/well for suspended cells (HL-60) and extracts (50 μg/mL) were added to each well. After 72 h of incubation, the supernatant was replaced by fresh medium containing MTT (0.5 mg/mL), the formazan product was dissolved in 150 μL DMSO and absorbance was measured at 595 nm (DTX 880 Multimode Detector, Beckman Coulter). Doxorubicin (0.3 μg/mL, Sigma Aldrich) was used as positive control.

**Hematoxylin-eosin stain**

HL-60 cells treated or untreated with the ethanolic extract of *Myracrodruon urundeuva* seeds (EEMUS) (6.25, 12.5 and 25 μg/mL) were examined for morphological changes by light microscopy (Metrimpex Hungary/PZO-Labimex Modelo Studar lab) after 72 h exposure. Cells were harvested, transferred to cytopsin slides, fixed with methanol for 1 min and stained with Hematoxylin-Eosin (H&E). Doxorubicin (0.3 μg/mL) was used as positive control.

**Flow cytometry analyses**

All cytometry analyses were determined in a Guava EasyCyte Mine (Guava Express Plus software). Five thousand events were evaluated per experiment and cellular debris was omitted from the analysis. Experiments were performed in triplicate using HL-60 cells and analyzed after 72 h of incubation with EEMUS (6.25, 12.5 and 25 μg/mL).

**Cell number and membrane integrity**

Cell membrane integrity was evaluated by the exclusion of propidium iodide (50 μg/mL, Sigma Aldrich Co. – St. Louis, MO/USA). Briefly, 100 μL of treated and untreated cells were incubated with propidium iodide (50 μg/mL). The cells were then incubated for 5 min. Fluorescence was measured and cell number and membrane integrity were determined (Darzynkiewicz et al. 1992).

**Internucleosomal DNA fragmentation**

Internucleosomal DNA fragmentation was evaluated by the incorporation of propidium iodide (50 μg/mL). Briefly, HL-60 cells were treated and then incubated at 25°C for 30 min, in the dark, in a lysis solution containing 0.1% citrate, 0.1% Triton X-100 and 50 μg/mL propidium iodide. Fluorescence was measured and DNA fragmentation was analyzed according to Nicoletti et al. (1991).

**Measurement of mitochondrial transmembrane potential**

Mitochondrial transmembrane potential was determined by the retention of rhodamine 123 dye. Aliquots removed from wells were incubated with 200 μL of rhodamine 123 in the dark for 15 min and centrifuged at 2000 rpm/5 min. Subsequently, cells were harvested and incubated in PBS solution for 30 min at 25°C (Cury-Boaventura et al. 2003).

**In vitro Antiproliferative Activity on Sarcoma 180 Cells**

In order to predict activity of EEMUS towards an in vivo cancer model, ascite-bearing female mice between 7 and 9 days postinoculation were sacrificed by cervical dislocation and a suspension of Sarcoma 180 cells was harvested from the intraperitoneal cavity under aseptic conditions. The suspension was centrifuged at 500 X g for 5 min to obtain a cell pellet and washed three times with RPMI medium. Cell concentration was adjusted to 0.5 × 10^6 cells/mL in RPMI 1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, plated in a 96-well plate and incubated with increasing concentrations of EEMUS (1.56-100 μg/mL). Cell proliferation was determined by the Alamar Blue assay after 72 h extract exposure according to Ferreira et al. (2010), with some modifications. Eight hours before the end of the incubation, 10 μL of stock solution (0.312 mg/mL) of Alamar Blue (Resazurin, Sigma Aldrich Co) were added to each well. The absorbance was measured at 570 nm and 595 nm using a multiplate reader (DTX 880 Multimode Detector) and the drug effect was quantified as the percentage of the control. Doxorubicin (0.3 μg/mL) was used as positive control.

**Phytochemical Study of Ethanolic Extracts**

Phytochemical tests to detect the presence of secondary metabolites in EEMUS such as phenols, tannins, leucoanthocyanidins, flavonoids, steroids, triterpens and alka-

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Photochemical compounds were performed according to Matos (2000). These tests are based on visual observation of color modification or precipitate formation after addition of specific reagents.

**Statistical Analysis**

For cytotoxicity assays, the IC$_{50}$ and EC$_{50}$ values and their 95% confidence intervals were obtained by non-linear regression using the Graphpad program (Intuitive Software for Science, San Diego, CA). In order to determine differences, data (mean ± standard error mean) were compared by analysis of variance (ANOVA) followed by Newman-Keuls test (P<0.05).

**RESULTS AND DISCUSSION**

Drug discovery from medicinal plants has played an important role in the treatment of cancer and most new clinical applications of plant secondary metabolites and their derivatives have applied towards combating cancer (Butler 2004, Cragg and Newman 2005). In this work, of the 21 ethanolic extracts tested, the analyses by MTT assay showed that only the ethanolic extract obtained from *M. urundeuva* seeds showed cytotoxic potential against cancer cells (Table II), given that it was the sole extract that caused cell proliferation inhibition higher than 90% (Torres et al. 2005). Subsequently, we determined its IC$_{50}$ values on tumor lines by MTT assay in a similar way as described above with the bioactive extract concentration ranging from 0.09 to 50 µg/mL. According to the American National Cancer Institute, the IC$_{50}$ limit to consider a promising crude extract for further purification is a value lower than 30 µg/mL (Suffness and Pezzuto 1990). As seen in Table III, EEMUS was inactive against *in vitro* colon and melanoma tumors, while it demonstrated moderate activity on glioblastoma [SF-295, IC$_{50}$ of 25.1 (17.3-36.3) µg/mL] and especially on leukemia [HL-60, IC$_{50}$ of 12.5 (9.5-16.7) µg/mL] cells. On the other hand, the positive control doxorubicin presented high cytotoxicity against all cell lines (Table III).

*Myracrodruon urundeuva* Fr. Allemao, 1881 (Anarcardiaceae), an endemic tree in Northeastern Caatinga, is a folk medicinal plant known as “aroeira do serlão” very used for treating bleeding gums and gynecological disorders (Viana et al. 2003, Monteiro et al. 2006). Different parts of *M. urundeuva* also possess hepatoprotective, anti-diarrheal, anti-ulcer, cicatrizing, colonic anastomotic wound healing properties and larvicidal activity (Viana et al. 2003, Goes et al. 2005, Souza et al. 2007, Sá et al. 2009). Recently, Sá et al. (2008) isolated a termiticidal lectin from *M. urundeuva* heartwood resistant to enzyme degradation to elucidate the resistance and durability of its wood to biodegradation by termites, a plague that has caused damages in several wooden parts in buildings and serious economical issues. Despite its diverse pharmacological applications, this is the first study showing a directed anticancer potentiality of this plant.

In an attempt to envisage an antitumor action upon *in vivo* assessments, it was determined the EEMUS activity on Sarcoma 180 cells using the Alamar Blue assay. Here, we also found antiproliferative action of EEMUS against these malignant cells, exhibiting an IC$_{50}$ of 38.1 (33.5-43.4) µg/mL, an additional findings that stimulates further investigation to understand its mechanism of action.

Cell type antiproliferative specificity is observed in some plant extracts and this is probably due to the presence of different classes of compounds (Cragg et al. 1994). Hence, the use of more than one cell line is considered necessary for detection of cytotoxic compounds. In the present work, EEMUS showed activity against leukemia line 2-fold higher than against melanoma and Sarcoma 180 cells (P<0.05). Then, we chose the HL-60 line to study underlying mechanisms involved in the *in vitro* cytotoxicity. The human HL-60 cell line, acute promyelocytic leukemia with prevailing of neutrophilic promyelocytes, is commonly used in the research for novel cytotoxic substances (Costa et al. 2008, Ferreira et al. 2010, Magalhães et al. 2010).

Extract-induced morphological alterations in HL-60 treated and untreated cells were examined following 72 h of treatment and staining by H&E. In comparison with the control cells and treated cells at the lowest concentration (6.25 µg/mL), both exhibiting a typical non-adherent morphology and dividing cells (Fig. 1A), EEMUS-treated cells at 12.5 µg/mL presented chromatin condensation and shrinking (Fig. 1D) while damage on plasmatic membrane was mainly seen at 25 µg/mL (Fig. 1E). Doxorubicin also induced reduction in cell volume, besides nuclear disintegration and chromatin condensation (Fig. 1B).
**TABLE II**

Tumor cell proliferation inhibition (%) of ethanolic seed extracts of twenty-one plant species belonging to different families from Northeastern Brazil determined by MTT assay after 72h of incubation at the concentration of 50 μg/mL.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cell proliferation inhibition (%)</th>
<th>HCT-8</th>
<th>SF-295</th>
<th>MDA/MB-435</th>
<th>HL-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myracrodruon urundeuva</td>
<td>69.2 ± 0.8</td>
<td>101.7 ± 1.4</td>
<td>17.2 ± 0.3</td>
<td>100.2 ± 0.4</td>
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<tr>
<td>Schinopsis brasiliensis</td>
<td>16.7 ± 12.7</td>
<td>22.5 ± 1.9</td>
<td>-19.1 ± 5.8</td>
<td>-15.8 ± 16.7</td>
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</tr>
<tr>
<td>Caryocar coriaceum</td>
<td>8.5 ± 5.9</td>
<td>19.6 ± 1.5</td>
<td>-3.2 ± 8.7</td>
<td>23.9 ± 9.8</td>
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<tr>
<td>Licania tomentosa</td>
<td>3.0 ± 12.7</td>
<td>11.7 ± 2.6</td>
<td>32.5 ± 0.2</td>
<td>-5.9 ± 0.1</td>
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<tr>
<td>Licania rigida</td>
<td>21.6 ± 3.5</td>
<td>29.5 ± 0.2</td>
<td>40.0 ± 7.3</td>
<td>67.9 ± 16.3</td>
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<tr>
<td>Connarus detersus</td>
<td>6.5 ± 4.2</td>
<td>23.2 ± 3.5</td>
<td>6.4 ± 5.0</td>
<td>-29.2 ± 20.3</td>
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<tr>
<td>Adenanthera pavonina</td>
<td>30.8 ± 5.2</td>
<td>23.7 ± 3.2</td>
<td>4.5 ± 2.4</td>
<td>1.2 ± 13.2</td>
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<td>Ambarana ceorenis</td>
<td>12.8 ± 3.8</td>
<td>29.0 ± 2.8</td>
<td>2.1 ± 10.8</td>
<td>16.6 ± 0.1</td>
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<tr>
<td>Anadenanthera macrocarpa</td>
<td>5.5 ± 6.8</td>
<td>23.1 ± 1.9</td>
<td>-4.7 ± 1.3</td>
<td>-37.2 ± 7.0</td>
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<td>Diolea megacarpa</td>
<td>-0.8 ± 2.2</td>
<td>29.1 ± 1.6</td>
<td>8.8 ± 0.6</td>
<td>1.7 ± 6.6</td>
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<td>Enterolobium contortisiliquum</td>
<td>-5.9 ± 3.0</td>
<td>36.0 ± 1.2</td>
<td>12.6 ± 3.8</td>
<td>-33.3 ± 4.2</td>
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</tr>
<tr>
<td>Hymenea courbari</td>
<td>30.9 ± 1.0</td>
<td>27.5 ± 1.3</td>
<td>7.9 ± 1.0</td>
<td>68.4 ± 6.4</td>
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</tr>
<tr>
<td>Lonchocarpus sericeus</td>
<td>11.0 ± 3.5</td>
<td>43.0 ± 0.1</td>
<td>22.3 ± 10.9</td>
<td>-16.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Luethelburgia auriculata</td>
<td>-1.1 ± 0.1</td>
<td>24.3 ± 7.3</td>
<td>3.0 ± 7.3</td>
<td>-25.5 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Parkia platycaphala</td>
<td>27.4 ± 5.7</td>
<td>27.5 ± 3.9</td>
<td>0.7 ± 3.3</td>
<td>-3.7 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>Piptadenia moniliformis</td>
<td>12.3 ± 5.7</td>
<td>30.6 ± 0.6</td>
<td>-15.7 ± 2.1</td>
<td>0.8 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>Senna obtusifolia</td>
<td>18.6 ± 0.5</td>
<td>33.5 ± 1.9</td>
<td>-3.2 ± 3.2</td>
<td>14.8 ± 26.3</td>
<td></td>
</tr>
<tr>
<td>Triplaris gardneriana</td>
<td>12.6 ± 0.4</td>
<td>34.6 ± 0.2</td>
<td>10.9 ± 9.3</td>
<td>5.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Ziziphus joazeiro</td>
<td>-22.0 ± 4.6</td>
<td>25.3 ± 6.5</td>
<td>12.3 ± 4.5</td>
<td>-9.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Talissa escalenta</td>
<td>-0.9 ± 3.9</td>
<td>25.2 ± 2.5</td>
<td>16.7 ± 3.4</td>
<td>-22.4 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Sapindus saponaria</td>
<td>-2.1 ± 5.7</td>
<td>23.6 ± 3.6</td>
<td>-2.1 ± 1.4</td>
<td>-15.8 ± 16.7</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>89.6 ± 0.6</td>
<td>98.3 ± 2.5</td>
<td>99.1 ± 2.2</td>
<td>97.2 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± standard error mean (S.E.M.) from two independent experiments for leukemia (HL-60), melanoma (MDA/MB-435), glioblastoma (SF-295) and colon (HCT-8) human cancer cells. All cell lines were plated with RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37°C with 5% CO₂. Doxorubicin (0.3 μg/mL) was used as positive control.

**TABLE III**

In vitro cytotoxic activity of the ethanolic extract of *Myracrodruon urundeuva* seeds (EEMUS) against human tumor cell lines determined by MTT assay after 72 h exposure.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>I&lt;sub&gt;C&lt;/sub&gt;&lt;sup&gt;50&lt;/sup&gt; (μg/mL)*</th>
<th>HCT-8</th>
<th>SF-295</th>
<th>MDA/MB-435</th>
<th>HL-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>0.02</td>
<td>0.23</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01–0.02</td>
<td>0.34–0.66</td>
<td>0.19–0.25</td>
<td>0.01–0.02</td>
<td></td>
</tr>
<tr>
<td>EEMUS</td>
<td>12.5</td>
<td>25.1</td>
<td>&gt;50</td>
<td>17.3–36.3</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

*Data are presented as I<sub>C</sub><sup>50</sup> values and 95% confidence intervals for leukemia (HL-60), melanoma (MDA/MB-435), glioblastoma (SF-295) and colon (HCT-8) cells. All cell lines were plated with RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37°C with 5% CO₂. Doxorubicin (0.009-5 μg/mL) was used as positive control. Independent experiments were performed in triplicate.

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Fig. 1 – Morphological analysis of 72 h-untreated (A) or treated leukemia HL-60 cells with ethanolic extract of *Myracrodruon urundeuva* seeds (EEMUS) 6.25 (C), 12.5 (D) and 25 μg/mL (E), stained by Hematoxylin-Eosin (H&E) and examined by light microscopy. Doxorubicin (0.3 μg/mL) was used as positive control (B). Negative control (A) was treated with the vehicle used for diluting the tested substance. Black arrows: chromatin condensation; white arrows: cell volume reduction; black dashed arrow: plasmatic membrane disruption. Magnification, 400×.

Growth-inhibitory effects of the EEMUS were also analyzed in HL-60-treated cells by flow cytometry. The propidium iodide intercalation test showed that EEMUS caused a decreasing in the cell number in a dose-dependent manner after 72 h of exposure (Fig. 2A), starting at the concentration of 12.5 μg/mL (IC₅₀ value). These results are consistent with the MTT findings and with reduction of cell viability (Fig. 2B) at 25 μg/mL (71.9 ± 2.4%) in comparison to the negative control (95.1 ± 1.1%) (P<0.01). Internucleosomal DNA evaluations showed that EEMUS led to a significant and dose-dependent increase in the DNA fragmentation (P<0.01), with fragmentation percentages of 27.7 ± 2.4% and 45.5 ± 2.0%, for the concentrations of 12.5 and 25 μg/mL, respectively (Fig. 3). On the other hand, statistically significant cellular mitochondrial depolarization (Fig. 2B) was detected only at the highest concentration 27.6 ± 4.5% (P<0.01), suggesting that cell death should be initially triggered by a mitochondrial independent pathway or it could be an apoptosis resulting from an early DNA fragmentation (Wang et al. 1999).

Morphological and flow cytometric studies revealed a concomitant deoxyribonucleic acid disintegration (increasing sub-G1 population cells), chromatin condensation with membrane integrity at 12.5 μg/mL and membrane disruption at higher concentrations. These results suggest dose-dependent apoptotic cell death activation by EEMUS, though other biochemical and
Fig. 2 – Effects of the ethanolic extract of *Myracrodruon urundeuva* seeds (EEMUS) on HL-60 cells analyzed by flow cytometry after 72 h exposure. A – Total of cells; B – Cell membrane integrity. Analyses were determined by exclusion of propidium iodide. Negative control (C) was treated with the vehicle used to dilute the tested substance. Doxorubicin (0.3 μg/mL) was used as positive control (D). Results are expressed as mean ± standard error of measurement (S.E.M.) from three independent experiments. *P < 0.01 compared to control by ANOVA followed by Student Newman-Keuls test.

morphological examinations are necessary to confirm it, such as phosphatidylserine externalization and caspase determinations (Strasser et al. 2000).

The phytochemical analyses detected the presence of steroids, alkaloids and phenols in EEMUS (*data not shown*). Some steroids, flavonoids and other phenolic substances are frequently associated with the aging process of the human body, production of free radicals due
Fig. 3 – DNA fragmentation on HL-60 cells determined by flow cytometry after 72 h of incubation with ethanolic extract of *Myracrodruon urundeuva* seeds (EEMUS). All evaluations were performed by nuclear fluorescence using propidium iodide, triton X-100 and citrate. Negative control (C) was treated with the vehicle used for diluting the tested substance. Doxorubicin (0.3 μg/mL) was used as positive control (D). Results are expressed as mean ± standard error of measurement (S.E.M.) from three independent experiments. *P* < 0.01 compared to control by ANOVA followed by Student Newman-Keuls test.

To metabolic processes, initiation and promotion of cancer and tissue injury by free radicals, which has induced the intake of antioxidant products as chemical factors that prevent the onset of diseases (Núñez-Sellés 2005). Previously, it was demonstrated that hydroalcoholic stem bark extracts from *M. urundeuva* exert anti-inflammatory effects attributed to chalcones (Viana et al. 2003, Souza et al. 2007), a compound belonging to the group of flavonoids naturally found in fruits, flowers, vegetables, teas and wines (Abdulla and Gruber 2000, Ferreira et al. 2008). Besides the traditional antioxidant properties attributed to the flavonoids, some chalcones and their derivatives have reported to be potent cyclooxygenase inhibitors (Hsieh et al. 1998). This is an important approach to some kinds of cancers, since COX-2 blockade avoids the expression of NF-κB activation, a key nuclear transcription factor involved in controlling inflammation and tumorigenesis (Surh et al. 2001), since inflammation has been frequently found in premalignant lesions (Dranoff 2004).

This study displays the antiproliferative action of the ethanolic extract of *Myracrodruon urundeuva* seeds on leukemia cells by death suggestive of apoptosis and also showed its potential against experimental in vivo tumors. Further studies to support these discoveries are in progress as well as phytochemical and molecular investigations to identify the bioactive compound(s) responsible for this cytotoxic activity.
ACKNOWLEDGMENTS

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RESUMO

Este estudo avaliou o potencial antiproliferativo e citotóxico contra linhagens de células tumorais de extratos etanólicos de sementes de vinte e uma espécies vegetais pertencentes a diferentes famílias do Nordeste brasileiro. Além disso, alguns mecanismos subjacentes envolvidos nesta citotoxicidade também foram investigados. Dentre os 21 extratos testados pelo ensaio do MTT após 72 h de incubação, apenas o extrato etanólico obtido a partir de sementes de Myracrodruon urundeuva (EEMUS), o qual apresentou traços de esteróides, alcaldóis e fenóis em sua composição, demonstrou atividade citotóxica in vitro contra células tumorais humanas, sendo 2 vezes mais ativo sobre a linhagem leucêmica HL-60 [IC50 valor de 12,5 (9,5-16,7) µg/mL] do que sobre células de glioblastoma SF-295 [IC50 de 25,1 (17,3-36,3) µg/mL] e de sarcoma 180 [IC50 de 38,1 (33,5-43,4) µg/mL]. Após 72 h de exposição, as análises morfológicas e por citometria de fluxo de células HL-60 tratadas com EEMUS mostraram diminuição no número de células, seu volume e viabilidade, assim como fragmentação internucleosomal do DNA de forma dose-dependente, sugerindo que a ação antiproliferativa de EEMUS pode ser ativada por vias apoptóticas.

Palavras-chave: potencial antiproliferativo, plantas do Nordeste Brasileiro, Myracrodruon urundeuva, tumor sarcoma 180, Extratos de sementes.

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