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Original article

Cytotoxicity of latex and pharmacobotanical study of leaves and stem of *Euphorbia umbellata* (Janaúba)

Lívia E.C. Luz[a], Katia S. Paludo[b], Vera L.P. Santos[c], Celia R.C. Franco[d], Traudi Klein[a], Rozi Z. Silva[a], Flávio L. Beltrame[a], Jane M. Budel[a,*]

[a] *Departamento de Ciências Farmacêuticas, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[b] *Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[c] *Departamento de Engenharia Saúde e Meio Ambiente, Centro Universitário Internacional, Curitiba, PR, Brazil*
[d] *Departamento de Biologia Celular, Universidade Federal do Paraná, Curitiba, PR, Brazil*

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ABSTRACT

In southern Brazil, the bottled latex of *Synadenium grantii* Hook f., Euphorbiaceae, is popularly used as a treatment of all types of cancer. Similarly, *Synadenium umbellatum* Pax. is used in the central western region of Brazil for the same purpose and in the same manner of use. Both plants are popularly known as *janaúba* or *leitosinha*. The objectives of this study were to use pharmacobotanical analysis to verify whether these two species, which are considered to be distinct, are actually the same to determine anatomical markers; to assist in the identification and differentiation of other *Euphorbia*; and to evaluate the cytotoxic activity of the latex in relation to HeLa and HRT-18 cells. Leaves and stems of the species were collected in Goiânia and Ponta Grossa and were investigated using scanning electron microscopy and optical microscopy techniques. The latex was also collected and analyzed in relation to its cytotoxic effect by employing MTT and NR techniques. The pharmacobotanical study of the specimens in both localities showed that they were the same species, namely *Euphorbia umbellata* (Pax) Bruyns, which is the scientific nomenclature accepted and confirmed by an expert taxonomist who specializes in *Euphorbia*. The pharmacobotanical characteristics highlighted in this study can assist in the identification of the taxon and contribute to the control of the quality of this plant drug. The evaluation of the latex in relation to HRT-18 cells demonstrated action after 48 h of experiment. In contrast, in relation to HeLa cells its induced cytotoxicity in all times and a dose-dependent manner. The IC_{50} values (72 h) observed were 252.58 ± 18.51 µg/ml and 263.42 ± 15.92 µg/ml to MTT experiment and 250.18 ± 19.48 µg/ml and 430.56 ± 19.71 µg/ml to NR experiment for the HeLa and HRT-18 cells, respectively.

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Introduction

Cancer is one of the most common diseases around the word. Statistics show that in 2011 it was the disease with the highest mortality rates (WHO, 2014). In Brazil, different kind of cancers affect the population, but cancer of the colon/rectum, cervix and leukemia are the most prevalent (INCA, 2014).

To find new treatments for this disease, studies of medicinal plants can be an efficient tool. Manosroi et al. (2012) consider medicinal plants as the main sources to obtain new drugs for the treatment of cancer. In the same way, despite the advance of new processes of organic synthesis and biotechnology, medicinal plants play an important role in medical therapy and clearly represent a window of opportunity for the pharmaceutical/chemical industry (Braz Filho et al., 2010).

Euphorbiaceae is one of the largest families of the Angiosperms group and has approximately ten species with recognized promising anticancer activity (Bhanot et al., 2011). *Euphorbia* has about 2000 species, which are predominantly found in arid or semi-arid regions of the tropics and subtropics (Horn et al., 2012).

Euphorbia umbellata (Pax) Bruyns, which is commonly known as *janaúba* or *leitosinha* in Brazil, is the accepted name for *Synadenium grantii* Hook f. and *Synadenium umbellatum* Pax (The Plant List, 2014). The latex of this species is used in folk medicine to treat all types of cancer, allergy, Chagas disease, internal bleeding, sexual impotence, leprosy, obesity, nervous ulcer and menstrual cramps. According to Ortêncio (1997), people prepare the *garrafada* dissolving 18 drops of latex in 1 l of water and drink the recommended

* Corresponding author.
E-mail: jane@uepg.br (J.M. Budel).

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dosage (a glass) three times a day, although this species is not included in the Brazilian Pharmacopeia and do not have indication of use in Brazilian National Form.

Despite the popular use of medicinal plants, anti-cancer action needs to be evaluated, in the first instance, by *in vitro* assays (such as MTT, NR and others) regarding screening and to evaluate cytotoxicity. Previous studies have shown that *E. umbellata* has a cytotoxic effect in relation to Ehrlich's (Mota et al., 2012a), K-562 (Nogueira et al., 2008) and B16F10 (De Oliveira et al., 2013) tumor cells.

In Brazil, a large number of native plants are used as medicines and sometimes there are problems in identifying these plants (Beltrame et al., 2006). Several studies have described the use of the wrong medicinal plants due to similar morphological characteristics between vegetable species and for this reason the frequency of the adulteration is a serious problem (Youssef et al., 2013; Amorin et al., 2014; Camilotti et al., 2014; Araujo et al., 2015).

For this reason, adequate quality control methods, including morpho-anatomical identification, need to be performed for many plants that are not currently described in the Brazilian Pharmacopeia. Consequently, this study is intended to propose a standard of quality control in order to promote a parameter of authenticity for *E. umbellata*, and also to evaluate the preliminary cytotoxicity of the latex of this species in relation to HRT-18 and HeLa cells.

Materials and methods

Plant material

Aerial parts of *Euphorbia umbellata* (Pax) Bruyns, Euphorbiaceae, were collected from specimens grown in open and sunny areas in Ponta Grossa, Paraná, Brazil (altitude: 975 meters, latitude: 25°05′ 38″ S and longitude: 50°09′30″ W) in August 2013, and in Goiânia, Goiás, Brazil (altitude: 787 m, latitude: 16°40′ S and longitude: 49°14′ W) in September 2013. The plant material containing flowers was used to prepare a voucher specimen that was identified by a taxonomist who is a specialist in *Euphorbia*, Inês Cordeiro (Botanical Institute of São Paulo), and it was stored at the Maria Eneida P. Kauffmann Fidalgo Herbarium (Botanical Institute Herbarium of São Paulo) under the number SP 453920. Mature leaves obtained from the sixth node and below, as well as stem fragments from 5 to 15 cm from the shoot, were chemically fixed in FAA70 (Johansen, 1940) and stored in 70% ethanol (v/v) (Berlyn and Miksche, 1976). The fresh latex was collected through transversal cuts in bark of the *E. umbellata*. This latex (1 g, density: 1.22 g/ml) was accurately weight and dissolved in water using a volumetric flask (10 ml). Aliquots of this solution were diluted to give the following stock solutions: 1000, 750, 500, 250, 100, 39 and 19.45 μg/ml that were used in the experiments.

Cell culture

Human cervical adenocarcinoma – HeLa (ATCC CCL-2) – and human ileocecal colorectal adenocarcinoma – HRT-18 (ATCC CCL-244) cells – were obtained from commercial sources (cell bank, Rio de Janeiro, BCRJ). The cells were cultured in a humidity-controlled environment containing 5% CO_2 and a temperature of 37 °C, and fed with RPMI growth media (Roswell Park Memorial Institute, Cultilab, São Paulo, Brazil) supplemented with 10% fetal serum bovine, penicillin and streptomycin (GIBCO, Life technologies, São Paulo, Brazil).

Cytotoxicity assays

The cytotoxic effect of *E. umbellata* latex was determined by a colorimetric assay using MTT ((3-[4,5 dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (Amresco, Solon, USA) and neutral red (NR) (Inlab, São Paulo). MTT assay measures the activity of living cells *via* mitochondrial dehydrogenases. Solutions of MTT are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple formazan crystals, which are insoluble in aqueous solutions (Mossman, 1983). The NR is a vital dye (Basic Red 5, Toluylene red). Viable cells will take up the dye by active transport and incorporate the dye into lysosomes, whereas non-viable cells will not take up the dye (Borenfreund and Puerner, 1985). Briefly the HeLa and HRT cells were seeded (8×10^4 cells per well) into 96-well culture plates and maintained in RPMI containing 10% fetal bovine serum, at 37 °C in a 5% CO_2 atmosphere for 24 h. Then, the cell culture media from each well was removed and replaced with 190 μl of fresh media and 10 μl of *E. umbellata* latex stock solutions (final concentrations): 1000, 750, 500, 250, 100, 39 and 19.45 μg/ml. The cells were incubated at 37 °C in a 5% CO_2 atmosphere for 24, 48 and 72 h. After these times, the media was removed. For the MTT, the cells were rinsed with phosphate-buffered saline (PBS) and 70 μl of MTT solution (0.5 mg/ml) and incubated for 2 h at 37 °C. Subsequently, 100 μl of 0.1 M HCl in anhydrous isopropanol was added and the absorbance was measured at 570 nm. For the NR test, the medium from each well was aspirated and wells were rinsed with PBS. Immediately 200 μl of working NR solution (40 mg/ml of NR in RPMI medium) were added and the plates incubated for 2 h at 37 °C. After this time, NR solution was removed and the cells were treated with 0.5% formaldehyde/1% $CaCl_2$ in PBS for 1 min followed by 100 μl of NR solubilization reagent (50% ethanol, 1% acetic acid in PBS). The absorbance was measured at 520 nm. Negative control was performed using cells incubated only with RPMI medium. The cell viability was expressed in % compared with negative control. Each latex concentration was assayed in triplicate and repeated three times. The IC_{50} values were calculated to MTT and NR 72 h experiment that presented the higher cytotoxicity for both cells lines (Döll-Boscardin et al., 2012; Assaf et al., 2013; Tao et al., 2013). *Probit* regression and the least squares method using *StatPlus* version 5.8.4 statistical software were used to do the determination.

Morphological analysis

HeLa and HRT cells (20×10^4) were added in 24-well plates containing one cover slip (each well) and these were incubated with RPMI medium for 24 h at 37 °C and 5% CO_2. The cell culture media from each well was removed and replaced with 380 μl of fresh media and 20 μl of *E. umbellata* latex stock solutions (final concentrations): 500 and 750 μg/ml (for HeLa cells) and 750 and 1000 μg/ml (for HRT-18) and incubated at 37 °C in a 5% CO_2 atmosphere for 24 and 48 h. After, the cells were washed with PBS they were fixed with 2% formaldehyde for 2 min and stained with May-Grünwald stain. The cytotoxic effects (vacuolization, rounding, loss of adhesion, blebbing, nuclear condensation and fragmentation) were analyzed using a photomicroscope (Olympus BX41 coupled to a Olympus DP71 camera).

Pharmacobotanical study

For the light microscopy, the fixed material was sectioned by hand in transverse and paradermal planes. The sections were stained with toluidine blue (O'Brien et al., 1964) or astra blue and a basic fuchsin combination (Roeser, 1962). The slides were set with glycerol diluted up to 50% (Berlyn and Miksche, 1976). To expose the cell content and cell wall impregnation, the following standard solutions were used in the microchemical tests: Bouchardat reactive for nitrogen compounds (Borio, 1959); hydrochloric phloroglucin to reveal traces of lignin (Sass, 1951); Sudan III for testing lipophilic compounds (Foster, 1949); ferric chloride to test for phenolic substances (Johansen, 1940); methylene blue to test

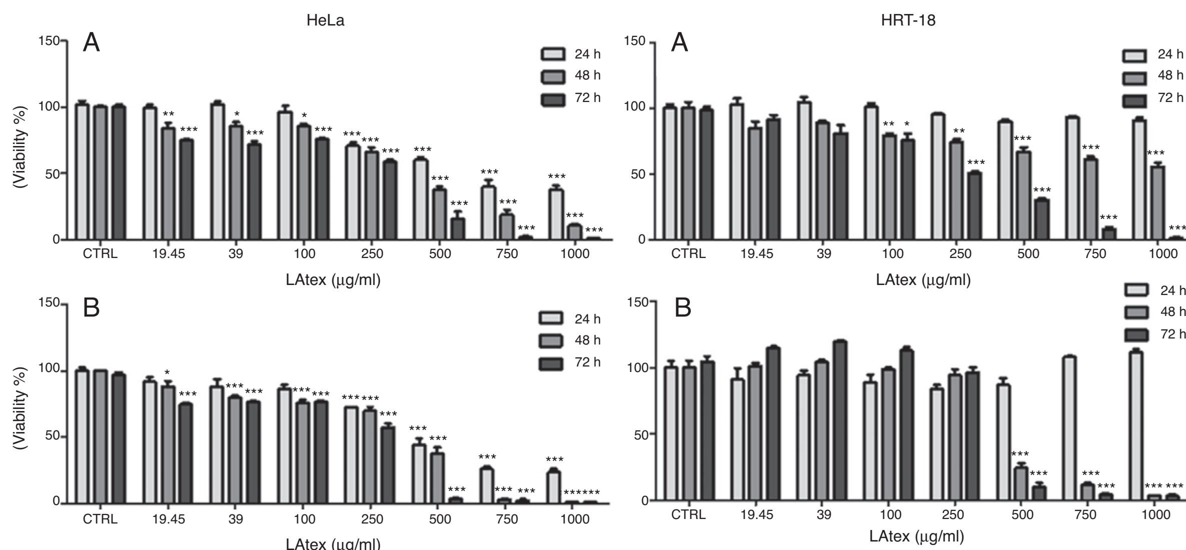

Fig. 1. Cytotoxicity induced by latex of *Euphorbia umbellata* (Pax) Bruyns for HeLa and HRT-18 cells at 24, 48 and 72 h (MTT assay – A, and NR – B). RPMI medium was used as a negative control only. Results were expressed as mean and standard error of the mean. One-way ANOVA followed by Tukey's post-test. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

for mucilage (De Oliveira et al., 2005), and iodine-iodide to test for starch (Berlyn and Miksche, 1976). Some of the photomicrographs were captured using a Olympus CX 31 light microscope equipped with a C 7070 control unit. For the scanning electron microscopy (SEM) analysis (Souza, 1998), the samples fixed in FAA70 were dehydrated in a series of graded ethanol and CO_2 critical point drying in a Bal-Tec CPD-030, coated with gold in Balzers SCD-030 equipment and examined using a Jeol JSM-6360LV microscope.

Results and discussion

Cytotoxicity study

The HeLa cells were responsive to treatment with the latex of *E. umbellata* that induced a dose and time-dependent cytotoxicity. HRT-18 cells showed an initial resistance to latex treatment in 24 h, but at 48 h it was possible to observe reducing cell viability in the concentrations from 100 to 1000 µg/ml and after 72 h at concentration of 1000 µg/ml, latex effectively presented a toxic profile similar to that of the HeLa cells (Fig. 1). The 72 h experiment time was chosen to evaluate the death of 50% of the cells (IC_{50}), according to described by Döll-Boscardin et al. (2012), Assaf et al. (2013) and Tao et al. (2013) resulting in values of 252.58 ± 18.51 µg/ml and 263.42 ± 15.92 µg/ml for the HeLa and HRT-18 cells respectively to MTT experiment and 250.18 ± 19.48 µg/ml and 430.56 ± 19.71 µg/ml to NR experiment.

Previous studies have reported the cytotoxicity of the latex of other species of Euphorbiaceae which corroborate the results obtained in this study. Hsieh et al. (2011) showed the toxicity of

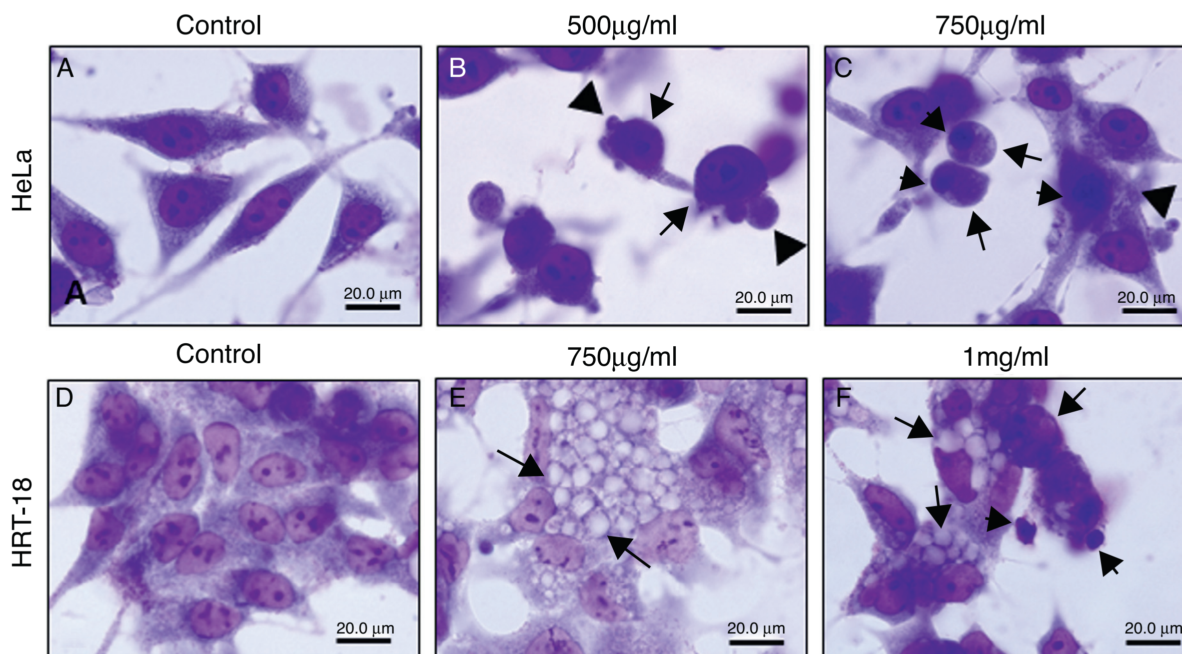

Fig. 2. Morphology of HeLa and HRT-18 cells after 24 h of treatment with the latex of *Euphorbia umbellata* (Pax) Bruyn. (A and D) controls; cells incubated with RPMI only. (B) HeLa cells incubated with 500 and (C) 750 µg/ml of latex. (E) HRT cells incubated with 750 µg/ml and (F) 1000 µg/ml of latex. ↑ cell rounding, ▲ bleb formation, ⮝ chromatin condensation and ➡ intense vacuolization. Magnification = 1000x, bar = 20 mm.

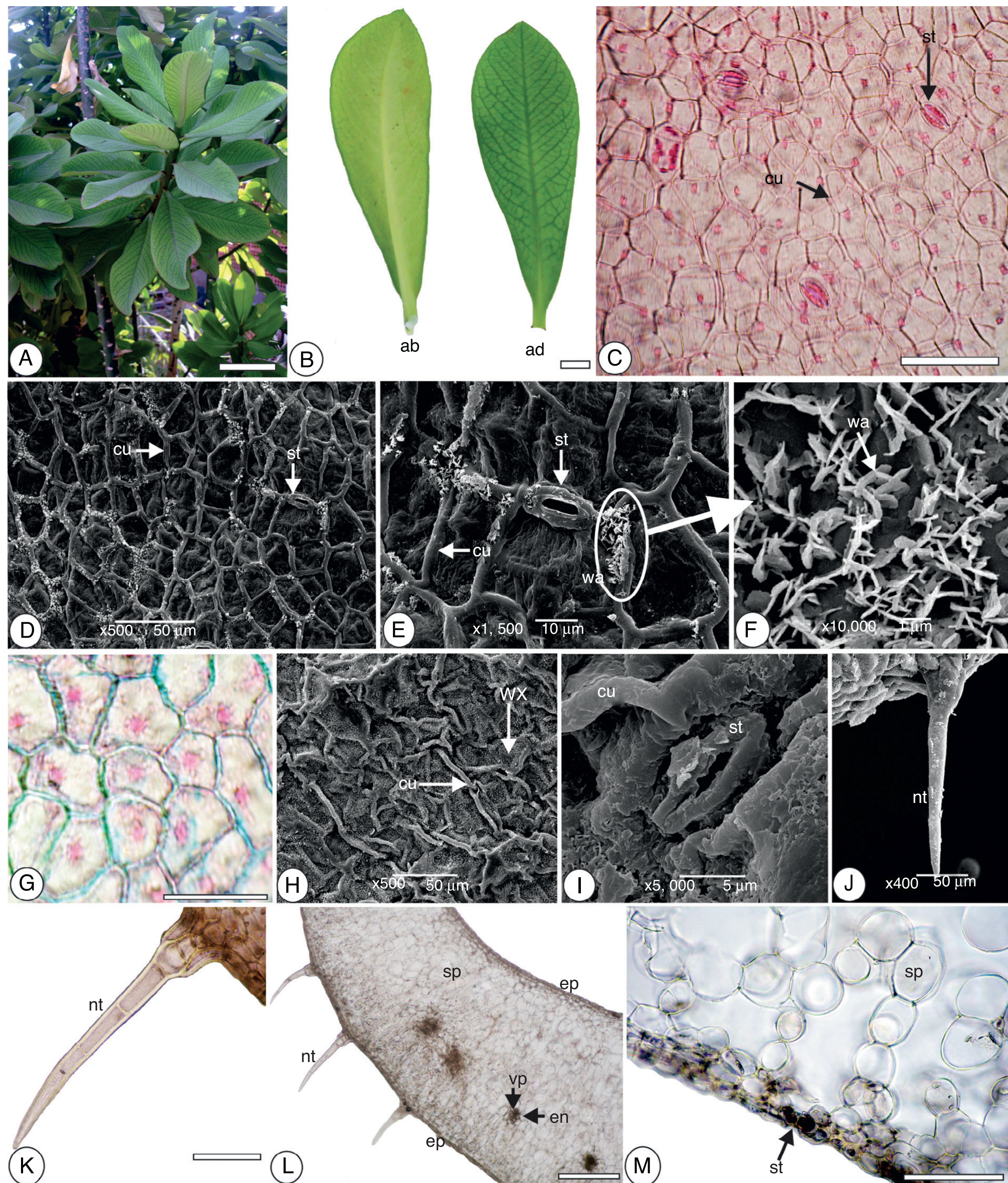

Fig. 3. *Euphorbia umbellata* (Pax) Bruyns. A. Aspect of aerial vegetative organs. B. Aspect of leaves. C–J – Epidermis in surface view. C. Adaxial side, showing anticlinal epidermal cell walls, cuticle and stomata. D. Adaxial side, exhibiting cuticle and stomatum (SEM – scanning electron microscopy). E. Detail of the previous figure, showing cuticle, stomatum and epicuticular wax (SEM). F. Detail of epicuticular wax (SEM). G. Abaxial side, showing anticlinal epidermal cell walls. H. Abaxial side, exhibiting cuticle and epicuticular wax (SEM). I. Abaxial side, presenting cuticle and stomata (SEM). J. Non-glandular trichome (SEM). K. Non-glandular trichome. L. Blade organization, in cross-section, revealing homogeneous mesophyll, minor collateral vascular bundle and parenchymatic sheath. M. Detail of the previous figure, showing stomata, epidermis and spongy parenchyma. ab: abaxial side; ad: adaxial side; cu: cuticle; ep: epidermis; nt: non-glandular trichome; ps: parenchymatic sheath, sp: spongy parenchyma; st: stomatum; vb: vascular bundle; wa: epicuticular wax. Bar = 4 cm (A); 1 cm (B); 25 µm (G); 50 µm (C, K, M); 200 µm (L).

the latex of *Euphorbia antiquorum* L. in relation to HeLa cells, which caused a reduction of cell viability at concentrations of 0.5–3 mg/ml. Similarly, De Oliveira et al. (2013) showed a 64% inhibition of growth of B16F10 cells after incubation with the latex of *E. umbellata*.

Morphological analysis

The latex of *E. umbellata* induced important morphological alterations in the HeLa cells after 24 h, showing the presence of apoptotic events such as loss of adhesion, cell rounding, bleb formation

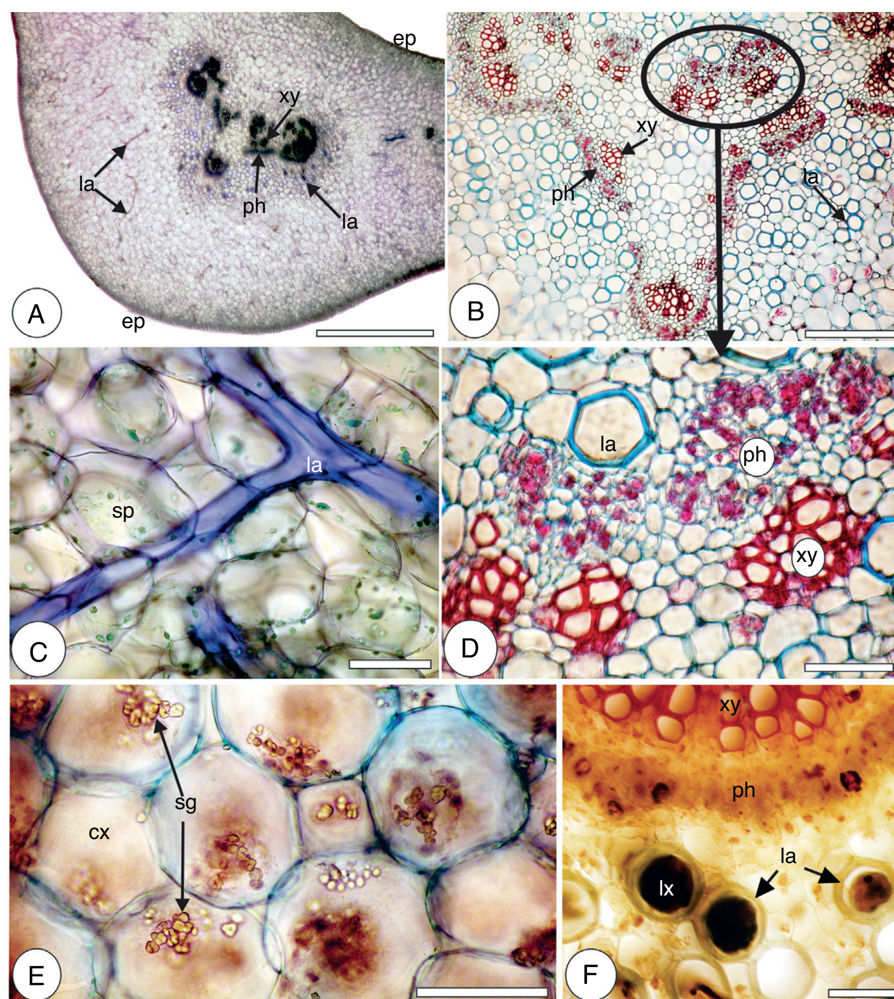

Fig. 4. *Euphorbia umbellata* (Pax) Bruyns – A. Transection of midrib, showing epidermis, vascular system, and laticifers. B. Transection of midrib, indicating vascular system and laticifers. C. Detail of the laticifers showing mucilage, stained blue. D. Detail of vascular system and laticifers of the midrib. E. Midrib in cross-section, showing starch grains in reaction with iodine-iodide. F. Petiole in cross-section, exhibiting laticifers near the vascular bundle. ep: epidermis; gp: ground parenchyma; la: laticifer; lx: latex; ph: phloem; sg: starch grains; sp: spongy parenchyma; xy: xylem. Bar = 50 μm (C, D, E); 100 μm (B); 200 μm (A, F).

and chromatin condensation. After 48 h, more severe toxicity was observed, in addition to the above cited effects (data not shown). The latex also induced morphological changes in the HRT-18 cells such as intense vacuolization, cell rounding and chromatin condensation in high concentrations (Fig. 2). Several species of the genus *Euphorbia* have been extensively studied for their antitumor and cytotoxic activities (Betancur-Galvis et al., 2002; Sadeghi-Aliabadi et al., 2009; Amirghofran et al., 2011; Hsieh et al., 2011; De Oliveira et al., 2013), and apoptosis is always the preferred pathway for therapeutic tumor killing as a form of non-inflammatory cell death.

Events such as the formation of blebbing and chromatin condensation were also observed in K-562 leukemic cells that were treated with ethanol extract of aerial parts of *S. umbellatum* and stained with Giemsa (Mota et al., 2012b). Sumathi et al. (2011) found morphological alterations, such as chromatin condensation, which characterized apoptosis in chick embryo fibloblast cells exposed to *Euphorbia antiquorum* latex. Another study found that *E. antiquorum* latex also induced apoptosis, which was characterized by morphological changes in HeLa cells; an increase in the sub-G1 population and in the levels of caspase-8, 9 and 3, and the suppression of Bcl-2 expression (Hsieh et al., 2011).

Pharmacobotanical study

Synadenium grantii collected in Ponta Grossa (PR) and *Synadenium umbellatum* in Goiânia (GO) – both samples considered two independent species by some scientific botanical websites – showed the same morphological and anatomical characteristics. Therefore, morpho-anatomical description and the figures correspond to the two copies, which in turn belong to *Euphorbia umbellata* as confirmed by the pharmacobotanical studies and by the taxonomist.

Euphorbia umbellata (Fig. 3A) had leaves which measured approximately 12.4 cm long and 4.7 cm wide (Fig. 3A and B). They were simple, green, petiolate, pinnate, spatulate-lanceolate in form with from obtuse to acute apex, attenuate base and entire margins (Fig. 3A and B). The phyllotaxy revealed alternate arrangements (Fig. 3A).

In several *Euphorbia* the shape of the anticlinal epidermal cell walls vary from straight to sinuous (Kakkar and Paliwal, 1974; Gales and Toma, 2006; Essiet et al., 2012; Zahra et al., 2014). In frontal view of the leaves of *E. umbellata*, the anticlinal epidermal cell walls were slightly wavy and thin on abaxial side (Fig. 3G) and straight and thin on adaxial side (Fig. 3C). In cross-section, the single-layer epidermis (Fig. 3L and M) was covered by a cuticle of variable

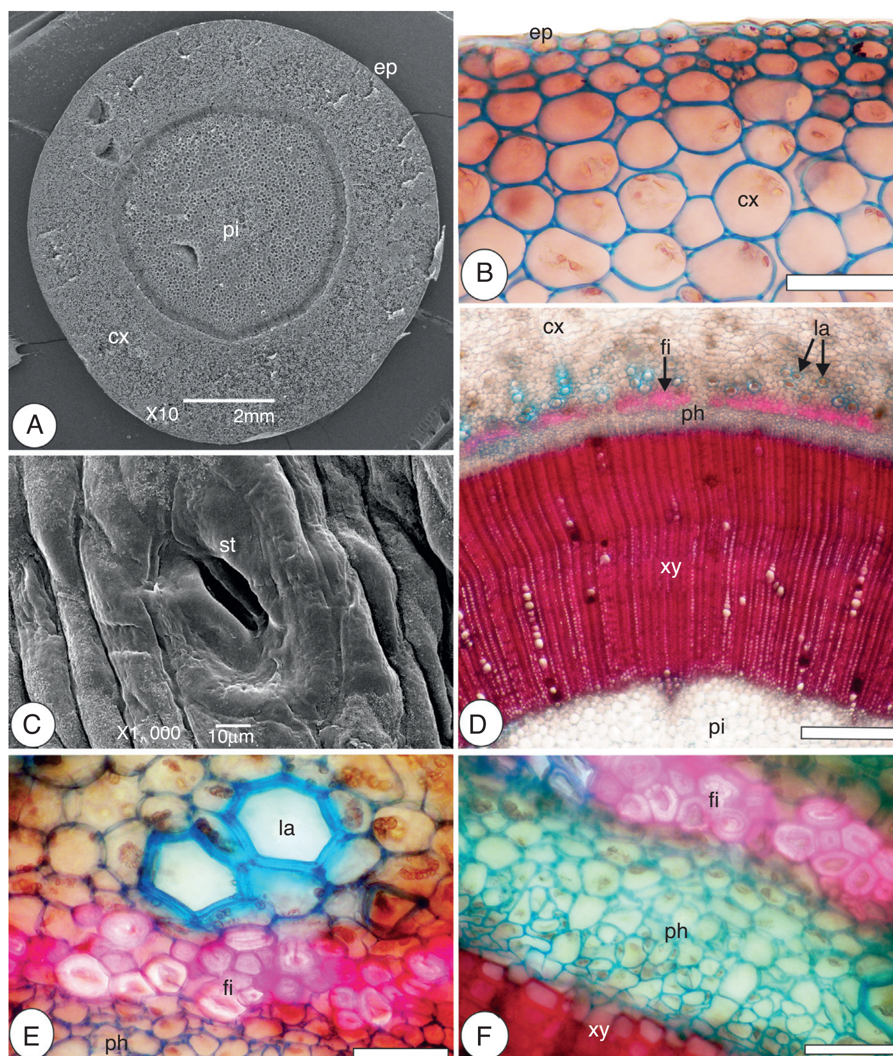

Fig. 5. *Euphorbia umbellata* (Pax) Bruyns – Stem – A. General aspect in cross-section (SEM). B. Cortex region, in cross-section. C. Stomata in frontal view (SEM). D. Cross-section, showing cortex, laticifers, fibers, phloem, xylem and pith. E. Detail of the fibers and laticifers. F. Detail of the vascular system and fibers. cx: cortex, ep: epidermis, st: stomatum, fi: fiber, la: laticifer, pi: pith, ph: phloem, xy: xylem. Bar = 50 µm (B, E, F); 100 µm (D).

thickness, which was striate and with wax that was denser on the adaxial side (Fig. 3E and F). The striate cuticle showed variable thickness and appeared in connected strips that formed polygonal shapes (Fig. 3D, E, H and I). The epicuticular wax type was crystalloid in rosette shapes (Fig. 3E and F).

Various *Euphorbia*, such as *E. pannonica* Host, *E. dobrogensis* Prodán, *E. myrsinites* L., *E. myrsinites* subsp. *litardierei* Font Quer & Garcias Font, *E. nicaeensis* All., *E. characias* L. and *E. bazargica* Prodán have distinct papillae of various shapes and sizes (Kakkar and Paliwal, 1974; Gales and Toma, 2006; Luković et al., 2009; Pinto et al., 2014; Christodoulakis et al., 2015). In *E. umbellata* the papillae was not observed.

According to Kakkar and Paliwal (1974), the stomata in European species of *Euphorbia* are predominantly anomocytic; however anisocytic and anomocytic types can also be found (Kakkar and Paliwal, 1974; Gales and Toma, 2006; Luković et al., 2009; Pinto et al., 2014). Frequently, more than one type of stomata may occur on the same leaf side (Kakkar and Paliwal, 1974). On the other hand, diacytic type stomata have been found in *E. royleana* Boiss., *E. splendens* Bojer ex Hook., *E. peplus* L. (Zahra et al., 2014), while paracityc, staurocytic, brachyparacytic and laterocytic types were observed on both surfaces of *E. hirta* L. and *E. heterophylla* L. (Essiet et al., 2012). In the present study, paracytic stomata occurred on both sides of the leaves (Fig. 3C, D, E and I) and they were located at the same level in the surrounding cells (Fig. 3M).

The presence of the stoma in the leaves is an important feature to characterize and differentiate the species. Amphistomatic leaves have been observed in several *Euphorbia*, such as *E. peplus* L. (Mendivelso et al., 2003); *E. nicaeensis* subsp. glareosa (Luković et al., 2009); *E. amygdaloides* L., *E. cyparissias* L., *E. falcata* L., *E. bazargica* Prodán, *E. carniolica* Jacq. (Gales and Toma, 2006); *E. buxifolia* Lam., *E. corollata* L., *E. radians* Benth (Kakkar and Paliwal, 1974); and *E. hirta* (Pinto et al., 2014). However, hypostomatic leaves were found in *E. taurinensis* All. (Gales and Toma, 2006) and *E. milli* (Essiet et al., 2012). In the present study, *E. umbellata* showed amphistomatic leaves.

Gales and Toma (2006) investigated nineteen species of *Euphorbia* and they verified that most of the investigated taxa had glabrous leaves. However, Kakkar and Paliwal (1974) have mentioned that various species of *Euphorbia* have unicellular and multicellular, uniseriate non-glandular trichomes and that they may have pointed, curved or tapering apices. In *E. umbellata*, the non-glandular trichomes were multicellular and uniseriate, with acute apex and they occurred individually (Fig. 3J, K and L). They were composed of 5–6 cells (Fig. 3K). Considering the histochemical tests, the non-glandular trichomes showed non-lignified cell walls.

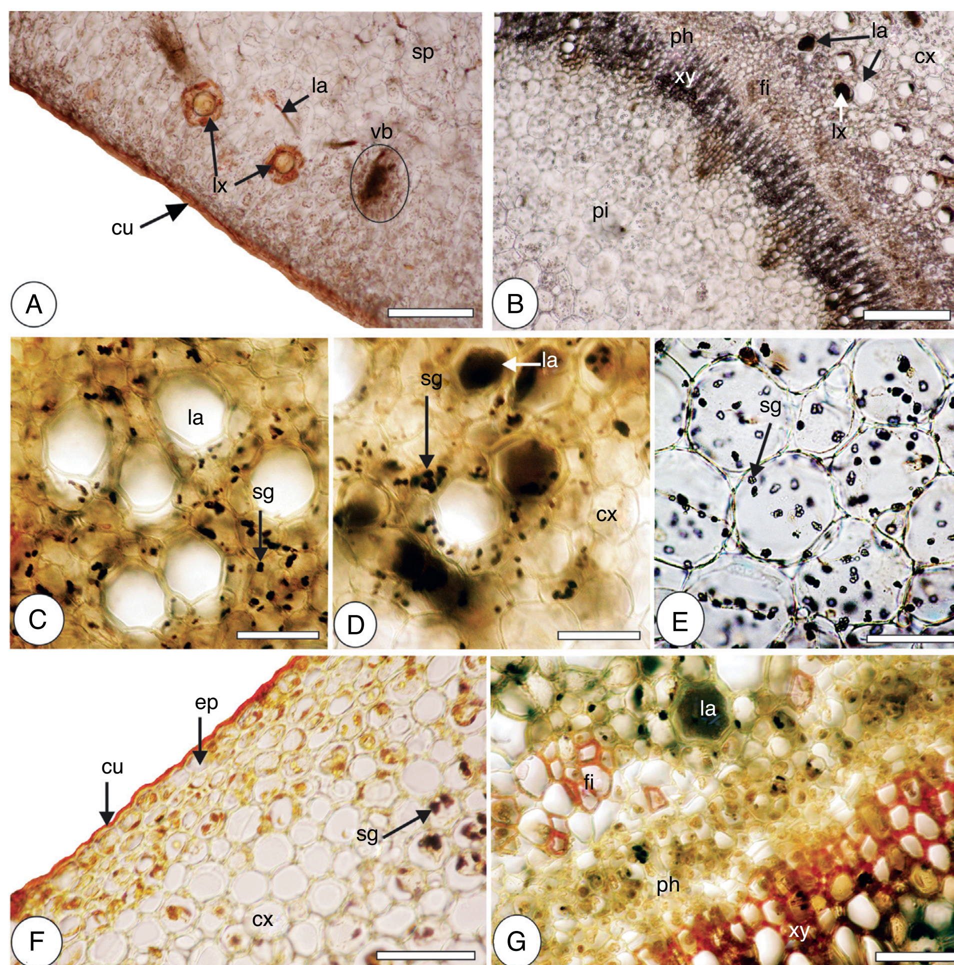

Fig. 6. *Euphorbia umbellata* (Pax) Bruyns. Microchemical tests – in cross-section. A. Blade showing latex and cuticle in reaction with Sudan III. B. Stem indicating xylem and latex in reaction with ferric chloride. C. Stem in reaction with Bouchardat reactive, showing laticifers. D. Stem in reaction with Bouchardat reactive, showing the latex in the laticifers. E. Starch grains reacted with iodine-iodide in the cortex. F. Stem showing cuticle in reaction with Sudan III. G. Stem exhibiting fiber and xylem in reaction with phloroglucin. cu: cuticle, cx: cortex, ep: epidermis, fi: fiber, la: laticifer, lx: latex, pi: pith, ph: phloem, sg: starch grain, xy: xylem. Bar = 50 μm (A, C, D, E, F); 200 μm (B).

Similar trichomes were observed in *E. heterophylla* (Kakkar and Paliwal, 1974; Essiet et al., 2012), *E. pulcherrima* Willd. ex Klotzsch (Zahra et al., 2014) and *E. hirta* (Essiet et al., 2012; Pinto et al., 2014).

Many members of *Euphorbia* present isolateral (Gales and Toma, 2006; Luković et al., 2009) or dorsiventral mesophyll (Gales and Toma, 2006; Pinto et al., 2014; Christodoulakis et al., 2015). Differing from this general observation, in *E. umbellata*, the mesophyll was homogeneous and composed of several layers of spongy parenchyma (Fig. 3L and M). Collateral vascular bundles traversed the spongy parenchyma and were encircled by parenchymatic sheath without visible Casparian strips. They were located in the lower third of the mesophyll (Fig. 3L).

In the midrib of the blade of Euphorbiaceae, the organization of the vascular tissues is variable, initially forming a whole ring and an open arc on the adaxial surface at the ends (Gaucher, 1902). The midrib presents a single vascular bundle in the center of the ground parenchyma in various *Euphorbia* (Gales and Toma, 2006; Luković et al., 2009; Pinto et al., 2014). In transection, the midrib's shape was described as concave-convex in *E. hirta* (Pinto et al., 2014) and as biconvex in *E. characias* (Christodoulakis et al., 2015). In *E. umbellata*, the midrib, in cross-section, was plano-convex (Fig. 4A). This species exhibited several collateral vascular bundles, which were organized in triangle in the ground parenchyma (Fig. 4A and B), where several starch grains were found (Fig. 4E).

Laticifers occur in over twenty families, including some genus of Euphorbiaceae (Demarco et al., 2013), Asteraceae, Moraceae (Esaú, 1974; Hagel et al., 2008) and Campanulaceae (Folquitto et al., 2014). The latex is responsible for the antitumor activity assessed in the *E. umbellata* species, which presents flavonoids, saponins, phorbol esters and terpenes (Bagalkotkar et al., 2006; Costa et al., 2012). Terpenes are described to present cytotoxic activity (Bagavathi et al., 1988) as well as phenolic compounds that induce ROS generation and have anti-tumor activity (Biscaro et al., 2013).

The laticifers differ in size, in the thickness of the walls, in the distribution and their occurrence in the vegetal tissues and organs, and in their presence in specific structures (Gales and Toma, 2007). They are present not only in the pith and primary cortex of the axis, but also near the sheath bundle in the leaf and mesophyll of mature plants (Metcalfe and Chalk, 1957). According to Esaú (1965) and Hagel et al. (2008), the genus *Euphorbia* presents non-articulated, branching and non-anastomosing laticifers.

In accordance with the standard of the *Euphorbia* genus, in the leaves of *E. umbellata*, the laticifers were non-articulated, branching and non-anastomosing; they were also present near the vascular bundles and in all the other parts of the mesophyll and the midrib (Fig. 4A–D). They showed a polygonal shape in the transverse section and the thick cellulosic wall (Fig. 4B and D). Similar features were found in *E. virgata* Waldst. & Kit., however, the laticifers have thin walls and they are analogous to secretory ducts (Gales and Toma, 2007).

In the present study, the petiole in cross-section had a plano-convex shape. The epidermis had the same characteristics as

previous described for the leaves. There were about 6 layers of angular collenchyma adjacent to the epidermis. Approximately 12 free collateral vascular bundles formed a horizontal line in the center of the ground parenchyma; however, in the middle of this line there was a convexity. Laticifers could be observed near the vascular system (Fig. 4F). In *E. hirta*, the petiole in cross-section has an almost round shape. The uniseriate epidermis has showed non-glandular trichomes and the vascular system have presented 4 collateral vascular bundles distributed in an open arc (Pinto et al., 2014).

The stem of *E. umbellata*, in transection, presented a circular shape (Fig. 5A). The epidermis was uniseriate (Fig. 5B). The cuticle was thin, striate and reacted positively to lipophilic compounds (Fig. 6F). In various species of *Euphorbia*, the epidermis cells of the stem have thick periclinal walls covered by a cuticle of variable thickness (Gales and Toma, 2006). In *E. umbellata*, the stomata (Fig. 5C) were found at the same level as the other epidermal cells.

Regarding the trichomes, the stem of some *Euphorbia* taxa are pubescent. The trichomes are unicellular in *E. helioscopia* L., *E. amygdaloides*, *E. agraria*, and *E. glareosa* or multicellular uniseriate in *E. maculata* (Gales and Toma, 2006) and *E. hirta* (Pinto et al., 2014). In this present study, trichomes were not observed in the stem. In most of the *Euphorbia* investigated by Gales and Toma (2006), with the exception of *E. maculata*, variable numbers of layers of collenchyma just beneath the epidermis were tangentially present. In *E. plathyphyllos* L. and *E. helioscopia*, the cortex is formed by angular collenchyma. Additionally, numerous auriferous cavities were observed in the cortex area of *E. myrsinites* subsp. *litardierei*, *E. myrsinites* subsp. *myrsinites* and *E. glareosa*. In the present study, several strata of cortical parenchyma were found in *E. umbellata* (Fig. 5B and D). This tissue was rather compact and collenchyma was not found. In the cortical parenchyma, several starch grains were observed (Fig. 6C–F).

In this study, the vascular cylinder was formed by phloem (outside) and xylem (inside) and perivascular fiber caps that sometimes adjoined the phloem (Fig. 5D–F). The xylem and fibers reacted positively to hydrochloric phloroglucin (Fig. 6G) and the xylem reacted with ferric chloride (Fig. 6B). Perivascular fibers are common in *Euphorbia*, however they were not observed in *E. myrsinites* subsp. *litardierei*, *E. myrsinites* subsp. *myrsinites* and *E. dobrogensis* (Gales and Toma, 2006). Similar laticifers that were encountered in the leaves were commonly observed near the phloem (Fig. 6B, C, D and G) in the stem.

After microchemical treatment with various reagents, it was observed that phenolic compounds (Fig. 6B), mucilage (Fig. 4C), lipophilic components (Fig. 6A) and nitrogen compounds (Fig. 6D) were present in the latex. In order to confirm the presence of the nitrogen compounds, cross-sections of the stems were exposed to specific treatment and to Bouchardat reactive, the results were respectively negative (Fig. 6C) and positive (Fig. 6D).

In some *Euphorbia*, such as *E. glareosa*, *E. myrsinites* subsp. *litardierei*, *E. myrsinites* subsp. *myrsinites*, and *E. cyparissias*, the cells of the pith have numerous aeriferous cavities, which are separated by uniseriate lamellae. Conversely, in *E. taurinensis* and *E. falcate* subsp. *acuminata* the pith is relatively compact. In *E. umbellata*, the pith appeared in the center of the stem, surrounded by the xylem. It was formed by thin-walled parenchymatic cells (Figs. 5D and 6E). These cells were quite compacted and showed the presence of starch grains (Fig. 6E).

It can be concluded from the present study that the main morpho-anatomical features were highlighted in this pharmacobotanical study helping toward the standardization of the *E. umbellata* species in order to improve the quality control of this vegetable material. Results of this study indicated that latex had cytotoxic effects on tumoral cell lines (HeLa and HRT-18 cells). Morphological changes caused by this phytocomplex to HeLa cells are associated with apoptosis.

Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

LECL (Postgraduate student) was responsible for performing the tests presented in the paper. She contributed in collecting and identifying plant samples, performed the voucher, analyzed the data, and drafted the article. KSP was responsible for anti-cancer tests. analyzing the data and drafting the article. VLPS and CRCF were responsible for scanning electron microscopy. TK contributed to running the laboratory works and preparing material for analysis. RZS donated the vegetal material and helped with laboratory support, as well as analysis of results. FLB was responsible for financial support, analysis of the data and drafting the paper. JMB was responsible for morpho-anatomical tests, analysis of the data and drafting the article.

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