

**Exploring the chemotherapeutic potential of five plant species through in vitro screening**

Page 1 a 9

*[Explorando o potencial quimioterápico de cinco espécies de plantas por meio de triagem in vitro]*V.F. Neves<sup>1</sup> , B. Soto-Blanco<sup>2</sup> , M.F.C. Oliveira<sup>3</sup> , S.C.S. Oloris<sup>4\*</sup> <sup>1</sup>Graduate, Fundação Ezequiel Dias, Belo Horizonte, MG, Brasil<sup>2</sup>Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil<sup>3</sup>Undergraduate, Fundação Ezequiel Dias, Belo Horizonte, MG, Brasil<sup>4</sup>Fundação Ezequiel Dias, Belo Horizonte, MG, Brasil**ABSTRACT**

Cancer is a group of diseases characterized by uncontrolled proliferation and spread of abnormal cells. Among the commonly used antineoplastic treatments, chemotherapy is one of the most frequent. In searching for new antineoplastic drugs, finding more effective and selective drugs becomes increasingly necessary. Analysis by phenotypic screening can identify promising compounds in cancer chemotherapy. In this work, hydroalcoholic extracts of *Plumbago auriculata* Lam., *Plumbago scandens* L., *Cestrum axillare* Vell., *Jatropha gossypifolia* L. and *Jatropha multifida* L. were screened for cytotoxic effect by MTT on glioblastoma (U-87 MG), colorectal carcinoma (RKO-AS45-1) and lung epithelial cell (WI-26 VA4) cell lines. After exposure to the extracts for 24, 48, and 72 hours, the extract of *P. scandens* exhibited the highest cytotoxicity in all cell lines. The extracts of the other plant species could not reduce cell survival to less than 50% at any cell line at the concentrations and exposure times. In conclusion, hydroalcoholic extract of *P. scandens* was highly cytotoxic to tumor cells, making it a promising tool in cancer chemotherapy. Further studies are needed to explore the isolated compounds as cancer chemotherapeutic agents.

Keywords: antineoplastic drugs; cytotoxicity; *Cestrum axillare*; *Jatropha*; *Plumbago*

**RESUMO**

O câncer é um grupo de doenças caracterizadas pela proliferação e disseminação descontroladas de células anormais. Entre os tratamentos antineoplásicos comumente utilizados, a quimioterapia é um dos mais frequentes. Na busca por novos tratamentos antineoplásicos, encontrar medicamentos mais eficazes e seletivos torna-se cada vez mais necessário. A análise por triagem fenotípica pode identificar compostos promissores na quimioterapia do câncer. Neste trabalho, extratos hidroalcoólicos de *Plumbago auriculata* Lam., *Plumbago scandens* L., *Cestrum axillare* Vell., *Jatropha gossypifolia* L. e *Jatropha multifida* L. foram testados quanto ao efeito citotóxico por MTT em linhas celulares de glioblastoma (U-87 MG), carcinoma colorretal (RKO-AS45-1) e células epiteliais pulmonares (WI-26 VA4). Após exposição por 24, 48 e 72 horas, o extrato de *P. scandens* exibiu a maior citotoxicidade em todas as linhas celulares. Os extratos das outras espécies de plantas não foram capazes de reduzir a sobrevivência celular para menos de 50% em nenhuma linhagem celular nas concentrações e nos tempos de exposição avaliados. Em conclusão, o extrato hidroalcoólico de *P. scandens* foi altamente citotóxico para células tumorais, tornando-o uma ferramenta promissora para a quimioterapia do câncer. Mais estudos são necessários para explorar os compostos isolados do extrato como agentes quimioterápicos do câncer.

Palavras-chave: drogas antineoplásicas, citotoxicidade, *Cestrum axillare*, *Jatropha*, *Plumbago*

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## INTRODUCTION

Neoplasms are among the leading causes of death worldwide, being responsible for almost 10 million deaths in 2022 (Global Cancer Observatory, 2025). In Brazil, the estimate for each year of the 2023-2025 triennium indicates the occurrence of around 704 thousand new cancer cases (Santos *et al.*, 2023). Despite the benefits, treatment with antineoplastic drugs involves different side effects, such as mouth sores, nausea and vomiting, hair loss, diarrhea, sensitive skin, and even infertility. Furthermore, multiple factors can compromise chemotherapy treatment through different mechanisms (Anand *et al.*, 2022). Therefore, searching for new antineoplastic drugs becomes increasingly necessary and aims to find more effective and selective drugs.

About 60% of anticancer compounds available to cancer patients were obtained from plant, marine, and microorganism sources. The positive effect of plants on cancer treatment has been widely studied, and various plant secondary metabolites have shown promising antitumor activity against cancer cell lines (Tauro *et al.*, 2024). The significant success achieved so far with using bioactive natural compounds as chemotherapeutic alternatives has generated a constant increase in the research of new molecules (Elekofehinti *et al.*, 2021).

Species from the Plumbaginaceae family, such as *Plumbago scandens* L. and *Plumbago auriculata* Lam., have been widely explored due to their bioactive compounds. These shrubs with white and purple flowers, respectively, are found mainly in the coastal region of Brazil. Among other compounds, *Plumbago* contains naphthoquinone plumbagin, which has numerous pharmacological properties (Thakor and Janathia, 2022).

*Jatropha multifida* L., Euphorbiaceae family, has been popularly used to treat various disorders, and initial research reveals that this plant species has antioxidants, antibacterial, anti-inflammatory, antifungal, gastroprotective, healing, analgesic, immunomodulatory activities, among others (Dah-Nouvlessounon *et al.*, 2023). In addition to *J. multifida*, secondary metabolites from *Jatropha gossypifolia* L. have been studied for medicinal purposes due to their anti-

inflammatory and anticancer potential (Wu *et al.*, 2019). Another plant with promising therapeutic potential is *Cestrum axillare* Vell. (syn. *Cestrum laevigatum* Schltld.), from Solanaceae family, but its hepatotoxicity must be considered since plants of this genus are responsible for causing acute liver necrosis in ruminants (Marinho *et al.*, 2018).

The present study aimed to evaluate the *in vitro* chemotherapeutic potential of *C. axillare*, *J. gossypifolia*, *J. multifida*, *P. auriculata*, and *P. scandens*, using a phenotypic screening model of colorectal carcinoma, human glioblastoma, and non-tumor cell lines.

## MATERIAL AND METHODS

Fresh leaves of *Cestrum axillare* Vell., *Jatropha gossypifolia* L., *Jatropha multifida* L., *Plumbago auriculata* Lam., and *Plumbago scandens* L. were collected at the Medicinal and Toxic Plants Garden at the Veterinary School, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. The leaves were dried, crushed, and then mixed with absolute ethanol. They were subsequently placed in a sonicator bath for 20 minutes and subjected to maceration for approximately 48 hours. The extraction process was repeated twice, separating the supernatant by filtration. The solvent was removed from the extract under reduced pressure in a rotary evaporator at a maximum temperature of 50°C. The ethanolic residue was solubilized in water, filtered, and fractionated with hexane. The aqueous fraction obtained after fractionation was subjected again to the rotary evaporator to eliminate residual hexane. The residual aqueous portion was filtered through a 0.22µm syringe filter.

The extracts were aliquoted into 15mL polypropylene tubes and lyophilized. The lyophilized contents of the extracts of *P. auriculata*, *J. multifida*, and *J. gossypifolia* were diluted in PBS 1X. The extracts of *C. axillare* and *P. scandens* were diluted in a solution of dimethyl sulfoxide (DMSO) and PBS 1X in a dilution of 1:5. Final concentrations of the extracts in relation to the amount of plant per volume of culture medium were: *C. axillare* – 2.325g/mL; *J. gossypifolia* – 3.145g/mL; *J. multifida* – 3.242g/mL; *P. auriculata* – 2.608g/mL; and *P. scandens* – 2.398g/mL.

For the cytotoxicity assay, cell lines RKO-AS45-1 (RRID:CVCL\_3786) colorectal carcinoma, U-87 MG (RRID:CVCL\_0022) glioma, WI-26 VA4 (RRID:CVCL\_2758) SV40 virus transformed derivative of WI-26 lung fibroblast used as a non-tumor cell (control) were obtained from ATCC® (American Type Culture Collection). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM – GIBCO®, cat. 12100- 046) supplemented with 10% Fetal Bovine Serum (FBS) (GIBCO®, cat. 12657-029) and 1% Pen-Strep solution 100X (10,000U/mL- Penicillin and 10mg/mL- Streptomycin – LGC Biotechnology®, cat. BR30110-01). Incubation was carried out in a humidified incubator at 37°C with 5% CO<sub>2</sub> (Thermo Fisher Scientific, Waltham, MA, USA). The subculture procedure was performed using a 0.25% (w/v) Trypsin-0.53mM EDTA solution in accordance with ATCC guidelines. Cell viability was evaluated with Trypan blue staining before each experiment. Only cell suspensions with >95% cell viability (passage numbers: 3–5) were acceptable for experiments.

The cytotoxicity assessment assay by incorporation of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was used to determine the cell viability and calculate the IC<sub>50</sub> concentration (mean inhibitory concentration). For the assay, 1x10<sup>5</sup> cells per well were cultured in 96-well plates for all cell lines (RKO-AS45-1, U-87 MG, and WI-26 VA4). The plates were incubated in a humidified chamber at 37°C and with 5% CO<sub>2</sub> for up to 24 hours until the cell monolayer reached confluence.

The cells were treated with three different dilutions of the extracts: C1-10<sup>-2</sup>, C2-10<sup>-3</sup>, and C3-10<sup>-4</sup>. Dilutions were performed in DMEM high-glucose medium supplemented with 1% FBS and 1% Pen-Strep solution 100X. Positive, negative, and vehicle controls were used to ensure assay performance. Hydrogen peroxide (1 mmol) was used as the positive control for cytotoxicity. Untreated cells were the negative control (blank). The vehicle control was done by adding 1% of PBS 1X to *J. gossypifolia*, *J. multifida*, and *P. auriculata* extracts and 0.75% of PBS+0.25% DMSO for *C. axillare* and *P. scandens* extracts.

Cells were incubated at 37°C with 5% CO<sub>2</sub> for 24, 48, and 72 hours. Following the exposure periods, the supernatant was aspirated, wells were rinsed with a 1X PBS solution, then MTT solution (0.5mg/mL) was added, and the plates were incubated for an additional 3 hours. At the end of the incubation, the plates were centrifuged at 1,200 rpm for 10 minutes. After removing the supernatant, 50µL of DMSO was added to solubilize the formazan crystals. Subsequently, the plates were analyzed using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 550nm.

Data from cell viability was expressed as mean ±SEM (standard error of the mean) of nine replicates (three replicates repeated three times). The results were normalized by the respective values of vehicle controls (Cao *et al.*, 2018; Zhang *et al.*, 2021) according to the formula:

$$\% \text{ Cell viability} = (\text{average absorbance of treated cells} / \text{average absorbance of vehicle control}) \times 100$$

The IC<sub>50</sub> value represents the dose that inhibits cell viability by 50%. This value was determined using Dr Fit software 1.042 (Di Veroli *et al.*, 2015). The Selectivity Index (TI) was determined by IC<sub>50</sub> against normal cells/IC<sub>50</sub> against tumor cells.

Statistical analysis was conducted using a two-way ANOVA followed by Bonferroni post-test with comparisons between blank and treatments GraphPad Prism 5.0 (GraphPad, San Diego, CA).

## RESULTS

The activities of *P. scandens* extract at concentrations C1, C2 and C3 are shown in Figure 1. The *P. scandens* extract at concentration C1 induced a significant cytotoxic effect across all exposure times (24, 48, and 72 hours) in the cell lines RKO-AS45-1. *P. scandens* extract at concentration C1 induced a significant cytotoxic effect against cell line U-87 MG within 24 hours of treatment.

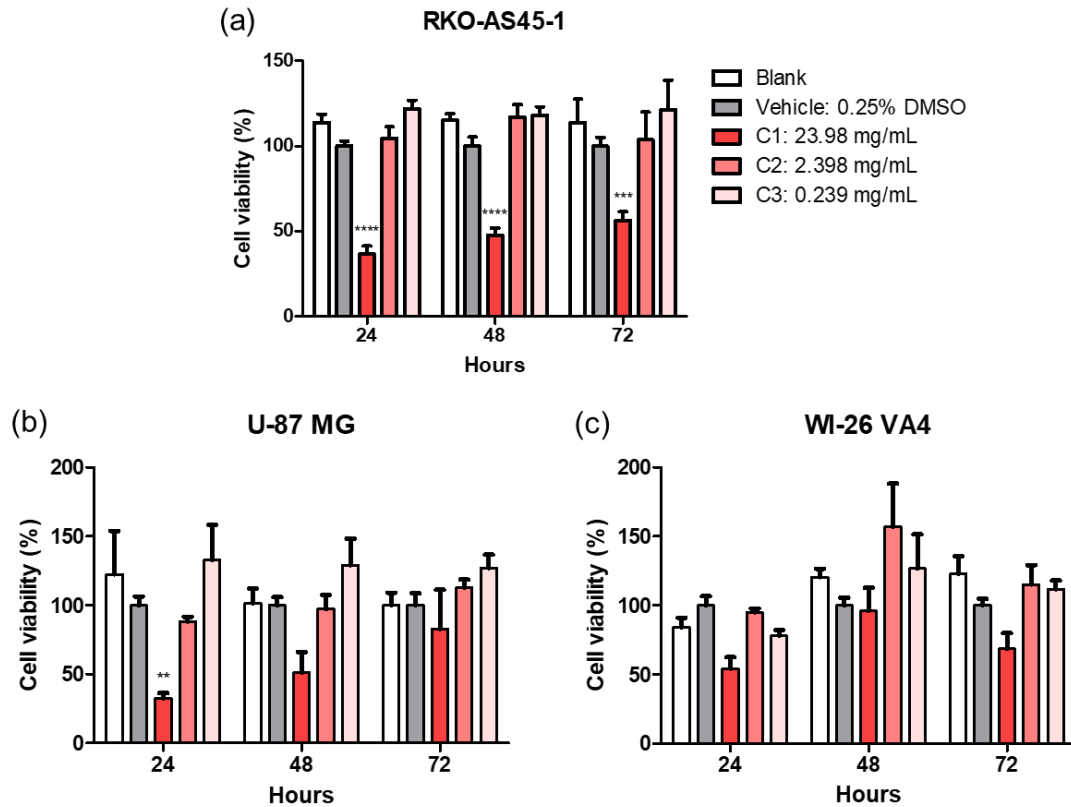


Figure 1. Cytotoxic effect of *Plumbago scandens* extract in the cell lines RKO-AS45-1 (a), U-87 MG (b), and WI-26 VA4 (c) after 24, 48, and 72 hours. Data were normalized to the respective vehicle control values and are presented as the mean  $\pm$  SEM from nine replicates. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

As shown in Table 1, The IC<sub>50</sub> of *P. scandens* extract was determined for all cell lines after 24 hours of treatment. The cytotoxic activity against tumor cell lines was nearly four times higher in RKO-AS45-1 and five times higher in U-87 MG compared to the normal WI-26 VA4 cells, indicating considerable tumor selectivity.

When exposed to the *P. auriculata* extract at all concentrations, the cell line U-87 MG exhibited a significant decrease in survival after 24 hours of exposure (Fig. 2).

Cytotoxic effects of *J. gossypifolia* extract were detected at C1 after 72 hours in the RKO-AS45-1 cell line and at the concentration C1 with 48 and 72 hours in WI-26 VA4. (Fig. 3).

Table 1. Determination of IC<sub>50</sub> and Selectivity Index (SI) of the *Plumbago scandens* extract in the cell lines RKO-AS45-1, U-87 MG e WI-26 VA4

| Cell lines | IC <sub>50</sub> (24 hours) | SI   |
|------------|-----------------------------|------|
| RKO-AS45-1 | 5.16 mg/mL                  | 3.91 |
| U-87 MG    | 4.03 mg/mL                  | 5.01 |
| WI-26 VA4  | 20.2 mg/mL                  | 1.00 |

IC<sub>50</sub>: Inhibitory Concentration of 50%; SI: Selectivity Index

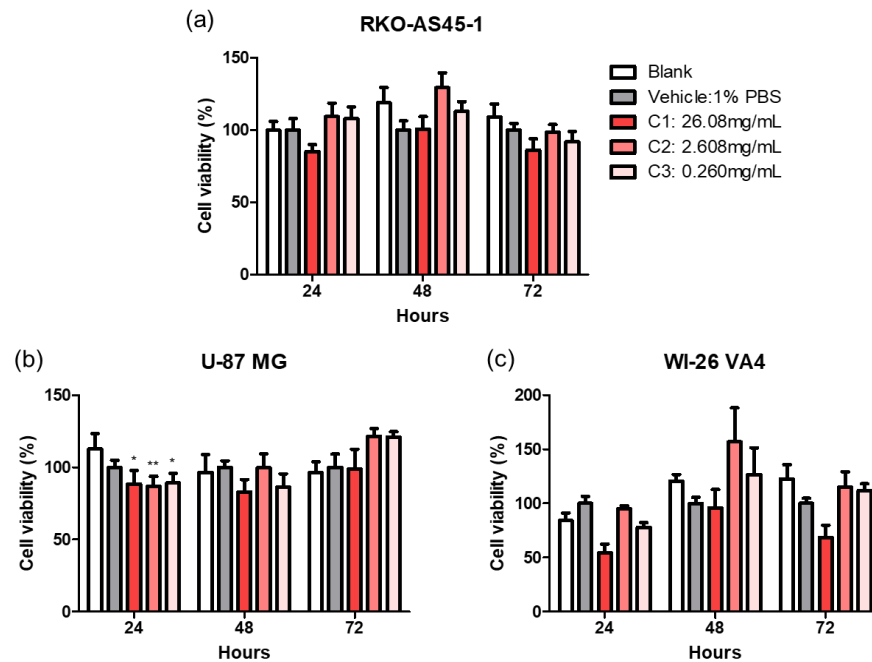


Figure 2. Cytotoxic effect of *Plumbago auriculata* extract in the cell lines RKO-AS45-1 (a), U-87 MG (b), and WI-26 VA4 (c) after 24, 48, and 72 hours. Data were normalized to the respective vehicle control values and are presented as the mean  $\pm$  SEM from nine replicates. \*p<0.05, \*\*p<0.01.

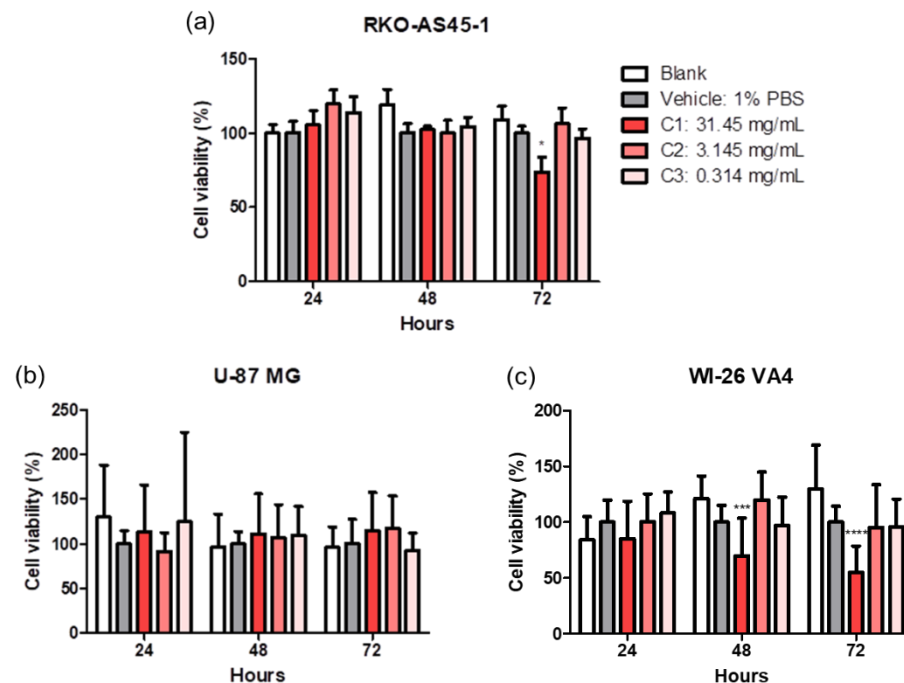


Figure 3. Cytotoxic effect of *Jatropha gossypifolia* extract in the cell lines RKO-AS45-1 (a), U-87 MG (b), and WI-26 VA4 (c) after 24, 48, and 72 hours. Data were normalized to the respective vehicle control values and are presented as the mean  $\pm$  SEM from nine replicates. \*p<0.05, \*\*\*p<0.001, \*\*\*\*p<0.0001.

The *J. multifida* extract was not cytotoxic at any concentration and time points tested in RKO-AS45-1 and WI-26 VA4 cell lines (Fig. 4).

The extract of *C. axillare* induced a significant reduction in cell survival at the concentration C1 in the RKO-AS45-1 and WI-26 VA4 cell lines at 72 hours (Fig. 5).

In summary, high cytotoxicity of the *P. scandens* extract was observed at concentration C1 and at all times evaluated for RKO-AS45-1 and U-87 MG within 24 hours. The *P. auriculata* extract exhibited some cytotoxicity only at concentration C1 and at the 24-hour time point for U-87 MG cell line. The *J. gossypifolia* extract was toxic

RKO-AS45-1 and to the control cell line, and *J. multifida* was cytotoxic only to the U-87 MG cell line. *C. axillare* was cytotoxic to both RKO-AS45-1 and WI-26 VA4 cell lines at concentration C1 within 72 hours of treatment.

Except for *P. scandens* at concentration C1, the extracts from the other plant species were not able to reduce cell viability to less than 50% in any lineage at the tested concentrations and exposure times. The *P. scandens* extract showed selective action, preferentially targeting tumor cells over non-tumorigenic human fibroblasts (Table 1).

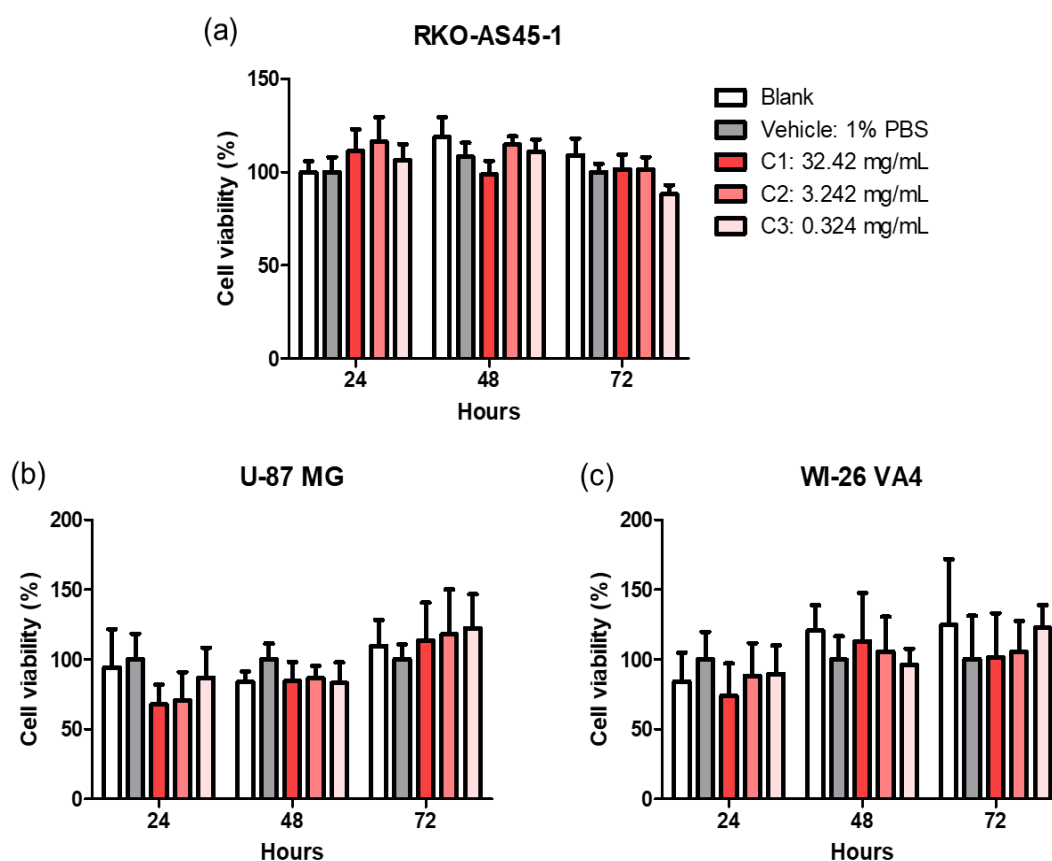


Figure 4. Cytotoxic effect of *Jatropha multifida* extract in the cell lines RKO-AS45-1 (a), U-87 MG (b), and WI-26 VA4 (c) after 24, 48, and 72 hours. Data were normalized to the respective vehicle control values and are presented as the mean  $\pm$  SEM from nine replicates.

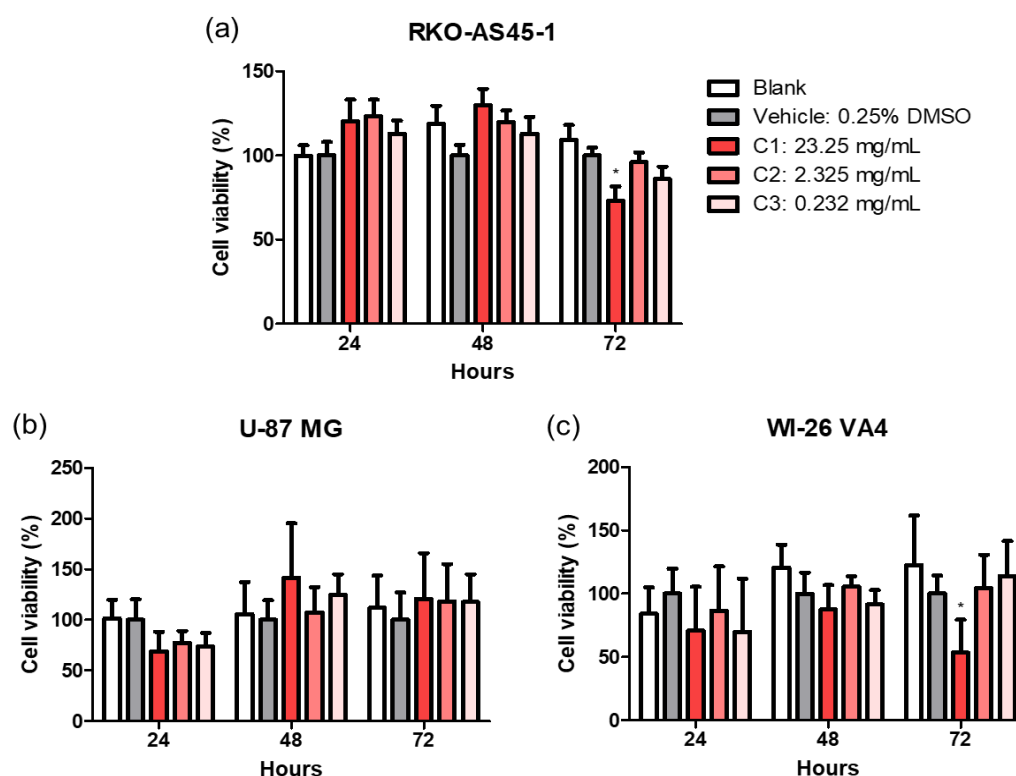


Figure 5. Cytotoxic effect of *Cestrum axillare* extract in the cell lines RKO-AS45-1 (a), U-87 MG (b), and WI-26 VA4 (c) after 24, 48, and 72 hours. Data were normalized to the respective vehicle control values and are presented as the mean  $\pm$  SEM from nine replicates. \* $p < 0.05$ .

## DISCUSSION

In the present study, the cytotoxic activities of hydroalcoholic extracts of *C. axillare*, *J. gossypifolia*, *J. multifida*, *P. auriculata*, and *P. scandens* were evaluated in colorectal carcinoma (RKO-AS45-1), glioblastoma (U-87 MG), and non-tumorigenic lung fibroblast (WI-26 VA4) cell lines.

Among the extracts tested, only *P. scandens* demonstrated cytotoxicity, reducing cell survival by 50% at the C1 concentration in RKO-AS45-1, U-87 MG and WI-26 VA4. This plant species contains a naphthoquinone named plumbagin, which is known to have several pharmacological activities (Thakor and Janathia, 2022). Plumbagin showed high cytotoxic activity in endometrial carcinoma (Zhang *et al.*, 2021), esophageal squamous cell carcinoma (KYSE150 and KYSE450) (Cao *et al.*, 2018), cervical carcinoma (HeLa), colon cancer (LoVo) (Shu *et al.*, 2023), EBV-transformed B leukemia (Raji), lung carcinoma (Calu-1), transformed epithelial

(Wish) cell lines (Lin *et al.*, 2003), human breast cancer (MCF-7) (De *et al.*, 2019), and glioma cell lines (U251, U87, C6, and GL261) (Zhan *et al.*, 2022). Zhan *et al.* (2022) showed that plumbagin inhibited *in vitro* and *in vivo* glioma cell line growth via targeting NQO1/GPX4-mediated ferroptosis. These authors suggest that plumbagin has the potential to be developed as a novel ferroptosis inducer for cancer treatment or an anti-glioma candidate (Zhan *et al.*, 2022). Ferroptosis is an iron-dependent form of non-apoptotic regulated cell death (RCD) caused by excessive lipid peroxidation, resulting in a ruptured plasma membrane (Lee *et al.*, 2021; Gao *et al.*, 2022). The iron accumulated is the typical hallmark distinguishing ferroptosis from other regulated cell death (e.g., apoptosis, necroptosis, pyroptosis, and autophagy) (Gao *et al.*, 2022; Wang *et al.*, 2022).

On the other hand, the *P. auriculata* extract exhibited cytotoxicity only for U-87 MG cell lines in 24h. This lower cytotoxicity observed in the present study can be attributed to the low

concentration of plumbagin compared to *P. scandens*. Although the roots of *P. auriculata* present significant amounts of plumbagin, this substance was either not detected in the leaves (Galal et al., 2013; Teixeira et al., 2024) or found in low concentrations (Mallavadhani et al., 2002).

The *C. axillare* extract C1 showed cytotoxic activity towards colorectal carcinoma (RKO-AS45-1) and non-tumorous lung epithelium (WI-26 VA4) cell lines in 72h treatment. Earlier studies showed that spirostanol glucosides isolated from *C. axillare* showed cytotoxic effects on promyelocytic leukemia (HL-60), glioma (SF-295) (Ribeiro et al., 2016a, 2016b), colorectal adenocarcinoma (HCT-116), and ovarian carcinoma (OVCAR-8) (Ribeiro et al., 2016b) cell lines. However, the pharmacological activities of this plant must be better studied.

*J. gossypifolia* and *J. multifida* contain diterpenes and diterpenoids with recognized antineoplastic activities (Taylor et al., 1983; Asep et al., 2017; Zhu et al., 2017; Zhang et al., 2018a, 2018b; Wu et al., 2019; Shari et al., 2023). In the present study, *J. gossypifolia* extract C1 was cytotoxic to non-tumor lung epithelial cells and colorectal carcinoma. A similar result was reported with *J. gossypifolia* hydroethanol extract, which showed low cytotoxic activity in the human hepatoma HepG2 cell line (Caballero-Gallardo et al., 2023). Diterpenes and diterpenoids were likely present at low levels in the plant extracts.

## CONCLUSIONS

*P. scandens* showed the most significant cytotoxic activities from the five plant species studied, revealing it to be a promising species for future studies aiming to develop new drugs for cancer treatment. Moreover, it exhibits selective action, preferentially targeting tumor cells over non-tumorigenic human fibroblasts. Our results corroborate the literature data regarding the promising antineoplastic activity of *P. scandens*, probably due to the naphthoquinone plumbagin. Further research into isolated compounds and the development of therapeutic strategies is in progress in our group.

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