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Cytotoxicity and genotoxicity of *Vernonia condensata* Baker aqueous extracts in an *Allium cepa* test system and C2C12 cell culture

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Vernonia condensata Baker is a medicinal species widely used by the Brazilian population for its analgesic, hepatoprotective and digestive properties. This study aimed to investigate the cytotoxic and genotoxic effects of *V. condensata* aqueous extracts using *Allium cepa* test system and *in vitro* micronucleus test. Fresh *V. condensata* leaves were collected, dried and ground, and aqueous extracts were prepared at concentrations of 1500; 3000; and 6000 µg/ml of dry extract. In the *A. cepa* test, ten repetitions were used per treatment. Distilled water was used as negative control and sodium azide (2M) as positive control. The onion bulbs were rooted in distilled water for 72 h and then exposed to the extracts for 24 h. The roots were collected and fixed in Carnoy's solution (3:1) for 24 h. Smear slides were prepared of the root apical meristems and 2000 cells/concentration was analyzed, measuring the mitotic index, micronuclei frequency and mitotic abnormalities of *A. cepa*. *In vitro* cytotoxicity and genotoxicity were also performed using the murine myoblast cell line C2C12. *In vivo* data demonstrated that an increase in the concentration of *V. condensata* aqueous extracts caused cytotoxic and genotoxic effects in meristematic cells of *A. cepa*. A concentration of 1500 µg/ml showed no cytotoxic or genotoxic effects, while 3000 µg/ml resulted in mitotic abnormalities and a decline in the mitotic index, indicating cytotoxicity. The 6000 µg/ml aqueous extract showed cytotoxicity and genotoxicity, increasing mitotic abnormalities and micronuclei frequencies in the *A. cepa* test. An IC_{50} value of 15.610 µg/mL was obtained for C2C12 cell line and the doses of 6000 µg/mL and 12000 µg/mL of *Vernonia* extract induced genotoxicity. The data of the present study reveals that *V. condensate* extract should be used with parsimony, once that it can bring unwanted side effects, which can be dangerous.

Keywords: Cell line C2C12, Chromosomal abnormality, Genotoxic effect, *In vitro* micronucleus, Medicinal plants, Mitotic abnormalities

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Plant genetic resources are important components of biodiversity and necessary to maintain the ecosystem. As such, there is great concern about how these resources are applied and efforts must be made to fully understand them and ensure they are correctly used and preserved, thereby contributing to economic and technological development.

Medicinal plants have been present since the earliest civilizations and were the first form of treatment for diseases[1]. Among the many species found to have medicinal properties, *Vernonia condensata* Baker (family Asteraceae), known as 'figatil' or 'necroton', is a cosmopolitan species commonly found in temperate subtropical regions[2].

Plant from the specie *V. condensata* have been used by local inhabitants to treat different diseases, including inflammation, malaria, fever, worms, pain and a variety of gastrointestinal problems. They have also been shown to have hypotensive, antibacterial, anti-inflammatory, immunomodulatory and antihistaminic properties[3]. These pharmacological properties can be largely attributed to the presence of flavonoids and terpenoids, since research

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shows that plants containing high flavonoid levels are used to prevent and treat hyperlipidemia, hypercholesterolemia and atherosclerosis[4].

V. condensata can generally be considered safe for medicinal use. However, there are reports in the literature of adverse effects associated with plants of this species, including abortion in the first three months of gestation if used over extended periods. Additionally, ingesting the plant can cause itching on the tongue and its application in folk medicine to treat skin sores is linked to vomiting and diarrhea[5].

Cytogenetics is the study of chromosomes and chromosomal abnormalities. This field of science encompasses any research related to genetic material in the form of chromosomes, their morphology, arrangement, function, replication, variation and evolution[6]. Karyotyping and genetic and structural chromosomal variations are important because they are the main instrument in cytotaxonomic analyses[7].

In plants, cytogenetic studies can reveal possible chromosomal abnormalities due to the presence of mutagenic agents. These mutations may be caused by the action of chemical, environmental and radioactive compounds and the intrinsic stability of nucleic acids. Mutagenic agents can be cytologically detected by inhibiting the cell cycle, interrupting metaphases, inducing numerical and structural disorders and sister chromatid exchanges, among others[8]. Micronuclei have also been used in *in vitro* genotoxicity assays since they are indicative of irreversible DNA damage[9].

Studies to identify chemical, physical and biological genotoxic agents and their carcinogenic potential have long been used as initial or intermediate indicators in tumor formation. Genetic toxicity is not directly indicative of carcinogenesis, but its presence may suggest the formation of cancer[10,9].

Biotests using *Allium cepa* (onion) assess the cytotoxicity and genotoxicity of biomaterials such as natural extracts of medicinal plants. This *in vitro* technique is simple, relatively inexpensive, versatile and can be carried out under minimal laboratory conditions[11], allowing microscopic analysis of genotoxicity considering chromosomal abnormalities, such as: chromosome bridges, sticky chromosomes, lagging chromosomes, chromosomal losses and the formation of micronuclei.

The assessment of chromosomal aberrations in *A. cepa* roots is validated by the World Health Organization's International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an efficient test for *in situ* analysis and monitoring of cytotoxicity and genotoxicity[12], also regulated by the Brazilian National Health Surveillance Agency (ANVISA) under Resolution 90 of March 16, 2004, governing the use of biomaterials and herbal medicine[13]. In this context, the present study aimed to investigate the cytotoxic and genotoxic aspects of *V. condensata* aqueous extract in *A. cepa* testing.

Methodology

The experiment was conducted at the Laboratório de Genética e Citogenética Vegetal – BioGen Cerrado of Universidade Estadual de Goiás (Ipameri Campus) in July and August, 2016.

Plant material

The plant material was collected in the local community (-17.721944, -48.159722), identified based on the characteristics described by Lorenzi and Matos[14] and confirmed using an identification key[15]. An exsicatti (dried specimen) of the plant sample was deposited in the Herbarium of Universidade Estadual de Goiás (HUEG), recorded under number 11386.

Vernonia condensata Baker leaves were collected, washed and dried in an oven at 45º C until constant weight. Next, they were ground in a Willey grinding mill using 0.85 mm curved screen sieves to increase the contact surface and substance extraction.

Aqueous extracts

The aqueous extracts were obtained by infusion and prepared using the following concentrations: C_0: distilled water (negative control); C_1: 1500; C_2: 3000 and C_3: 6000 µg/ml of dried *V. condensata* extract, as well as 2M sodium azide (positive control). The dried extracts were submitted to infusion in Erlenmeyer flasks containing distilled water at 100ºC. The solution was sealed for 15 minutes and manually shaken, then filtered and left to cool at room temperature.

For cytotoxic and genotoxic analysis, Vernonia extract was prepared according to Roncada et al.[16] methodology with adaptations. An infusion was prepared with the dry extract of Vernonia leaves at a concentration of 48000 g/L in boiling distilled water, keeping the container covered for 5 minutes (without heating). After cooling, the infusion water was used to prepare the RPMi-1640 medium according to the

manufacturer's protocol and the culture medium was sterilized by 0.22 µm membrane filtration.

Allium cepa test system

Medium (40g) and evenly-sized onion bulbs (*A. cepa*) were separated and the abaxial epidermis of the basal plate was scraped to facilitate the emergence of roots. The external cataphylls were removed, washed and dried[17]. After cleaning, they were left to root in 100 ml of distilled water for 72 h, and then exposed to the extracts for 24 h.

The experiment followed a completely randomized design with each bulb tested considered a sample unit, adopting ten repetitions per concentration and per control for a total of fifty individual samples. After the exposure period, ten roots were collected from each bulb, which were fixed and placed in Eppendorf® flasks containing Carnoy's solution (3:1) for 24 h and stored in 70% ethanol.

Histological slides

Smear slides were prepared to observe the nuclei and chromosomes of the meristematic cells, in accordance with Guerra and Souza[18]. The roots were removed from the fixing agent and rinsed in distilled water for 5 minutes, then hydrolyzed in 5N hydrochloric acid solution for 3 minutes and carefully dried with paper towel.

Using a stereomicroscope, clamp and histological needle, three meristems were extracted per slide and macerated in the center of a slide containing a drop of 45% acetic acid. A coverslip was placed over the biological material and pressed down using a histological needle, applying thumb pressure to spread the material. Liquid nitrogen was used to freeze the slides. The coverslips were then removed and the slides placed on paper towel for 15 to 20 minutes to dry.

The slides were stained using 10% Giemsa stain by placing them in a container holding 200 ml of the solution for 15 minutes. They were then rinsed with distilled water to remove excess solution and placed vertically on paper towel for 20 minutes to dry. Two slides were prepared per replicate.

Evaluation of cytotoxic activity by reducing Resazurin method

Cytotoxic activity was performed with the of murine myoblast cell line - C2C12 (2×10^4 cells/well). Cells were plated in a 96-well sterile microplate in 100µl of RPMI-1640 medium containing 10% fetal bovine serum, 25mM HEPES, 1% penicillin-streptomycin (10000 U/ml and 10000 µg/ml) and 2mM L-glutamine. The cells were maintained in a humidified atmosphere of 5% CO_2 at 37 ° C. 100 µL of treatment was added to each well 3h after the end of the plating, with the following concentrations: 100; 500; 750; 1000; 2000; 3000; 4000; 5000; 6000; 8000; 10000; 12000; 16000; 20000 and 24000 µg/ml.

The experiment was performed in quadruplicate and the microplate was incubated at 37ºC with 5% CO_2, for 24 hours after application of the treatments. Then, 20 µL of the resazurin developer (0.1 mg ml^{-1}) was added to each well of the microplate. The plate was read 4 hours after incubation on a spectrophotometer at 570 and 600 nm. Cell viability was calculated using the optical densities (OD) of the wells, compared with the average OD of the control wells (cells incubated in the absence of treatment), using the formula recommended by the test application protocols. The results were evaluated and compared using the GraphPad Prism statistical program, where the percentage of inhibition was related according to the logarithm of the tested concentrations, with a 95% confidence interval ($p < 0.05$).

In vitro genotoxicity assay

Genotoxicity was evaluated using in vitro micronucleus test following the OECD 487 protocol, with some modifications. The C2C12 cells were added in quadruplicate to a 96 wells microplate (0.5×10^4 cells/well) and incubated in a CO_2 chamber (5%) at 37°C for 4 hours before the different concentrations of the extract were added. After testing cytotoxicity by resazurin method, micronucleus test was performed with these same cells at extract concentrations of 1000; 6000 and 12000 µg/mL) present cell viability above 70%. After 24h of treatment, these cells were cultivated for another 24h in the presence of cytochalasin-b (5 µg/mL). After that period, they were fixed in PBS / 10% formaldehyde, stained with the fluorochrome Hoechst 33258 and analyzed in an inverted fluorescence microscope (Evos). A value of 2000 binucleated cells per concentration was counted and only micronuclei present in binucleated cells were considered to determine the micronuclei frequency induced by treatment.

Statistical Procedures

Two hundred cells were counted per repetition (2000 cells per treatment) on an optical microscope at

400x magnification, using the scanning technique. Micronuclei (MN) frequency and mitotic abnormalities (MA) were analyzed according to the percentage of abnormal cells in order to assess the genotoxic effect. To determine the cytotoxic effect, the mitotic index (MI) was calculated based on the number of cells undergoing mitosis. The mitotic index was obtained using the following equation[19]: MI= (m/T) x 100, where MI = mitotic index; m = number of cells undergoing mitosis; T = total number of cells analyzed.

The data obtained were submitted to analysis of variance (Scott-Knott test) to compare the groups and controls and regression analysis to study the significant interactions of treatments C_0 to C_3. Regression analyses were performed using Sisvar v.5.3 software[20], adopting $\alpha = 0.01$. Comparisons between groups of data were performed using the GraphPad Prism 6 program the by One-way analysis of variance test (ANOVA), followed by the Bonferroni post-test. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Allium cepa in vivo analysis

The means of the characteristics assessed showed that concentrations C_2 and C_3 exhibited cytotoxicity, with mitotic indices of 18.43% and 13.22% respectively, when compared to the controls (C_0 = 22.95% and C_4 = 9.1%).Concentration C_3 expressed genotoxicity, with an MN frequency of 9.55% against values of 0% and 13.85% for controls C_0 and C_4, respectively, and an MA frequency of 2.75% compared to C_0 = 0% and C_4 = 32.85% (Table 1).

Given that the average concentration of *Vernonia condensata* Baker typically used is 3000 µg/ml[21-24], the results indicate that care should be taken when using these plants since cytotoxicity was observed even at a normal dose. Excessive doses, such as 6000 µg/ml, also display genotoxic effects as well as the genotoxic agent sodium azide. Under the conditions tested here, a concentration of 1500 µg/ml showed no cytotoxic or genotoxic effects, indicating a certain degree of safety.

A survey conducted by the Ministry of Health demonstrates that *V. condensata* leaves can be efficient against different diseases, including diabetes, malaria, hepatitis, different types of inflammation and gastrointestinal disorders. However, no sources indicate an ideal concentration, dosage or how they should be used[5]. Adegbite and Sanyaolu[25] studied *V. amygdalina* concentrations and suggested that excessive use of the plant could cause genetic damage, while administering low levels with extended time intervals between them may allow safer use of the species.

The mitotic index was negatively affected by an increase in *V. condensata* aqueous extract concentration (Fig. 1) in a dose-dependent way, indicating that rising concentrations produced a cytotoxic effect in *A. cepa* roots, with an average reduction of 10% in MI from 0 to 6 g/L. The Figure 2 shows a graphical representation comparing the treatments.

The significantly low MI reveals abnormalities due to the action of the extract that the organism was exposed to[11]. Pinho *et al.*[26] studied *Baccharis trimera* aqueous extracts and found that concentrations greater

Table 1 — Results of analysis of variance and comparison of means for the different concentrations of *Vernonia condensata* aqueous extracts on *Allium cepa* roots.

Source of variation	Degrees of freedom	Mean Squares		
		MI	MN frequency	MA
Treatments	4	341.669**	0.000424**	0.002384**
Residue	45	6.881	0.000001	0.000005
C.V.%		15.35	23.8	15.29
Treatments		Cytotoxicity	Genotoxicity	
		MI (%)	MN Frequency	MA
C_0 (negative control)		22.95[a]	0.00[a]	0.00[a]
C_1		21.75[a]	0.00[a]	0.00[a]
C_2		18.43[b]	0.00045[a]	0.0137[b]
C_3		13.22[c]	0.00955[b]	0.0285[c]
C_4 (positive control)		9.10[d]	0.01385[c]	0.0328[d]

**significant according to the F test. Means followed by the same letter in the columns do not differ significantly according to the Scott-Knott test at 0.01 probability. MI – mitotic index; MN frequency – micronuclei frequency; MA – mitotic abnormalities.

than 0.2g/ml decreased the MI by around 20%, a response also observed at higher doses.

Iganci *et al.*[27] used extracts of different species locally known as boldo and found that, in addition to influencing germination, the extracts caused a significant variation in the MI of *A. cepa*, exhibiting an allelopathic effect and accelerating initial plantlet development. Moreover, onion proved to be a biomonitor sensitive to these extracts. The mitotic index is used as an indicator of adequate cell proliferation[28] and a decline in MI during the cell cycle when meristematic cells are exposed to the extract is due to its toxic effect[8].

A *V. condensata* extract concentration of 6000 μg/ml increased MN frequency (Fig. 3A) in 19,1% in relation to the negative control, indicating a dose-dependent genotoxic effect at high levels of the extract (Fig. 4). Figure 5 shows a graphical representation comparing the treatments. The appearance of micronuclei is the result of lagging chromosomes or acentric fragments that are not incorporated into the daughter nuclei during the telophase[11] and is a direct indicator of the genotoxicity of the extract under the conditions studied here.

A count of abnormalities in the mitotic cycle confirmed the genotoxic effect of the aqueous extracts in meristematic *A. cepa* roots at high concentrations, also in a dose dependent way (Fig. 6). The presence of different abnormalities was observed, including sticky chromosomes, chromosomal losses, errors during cell division, lagging chromosomes and

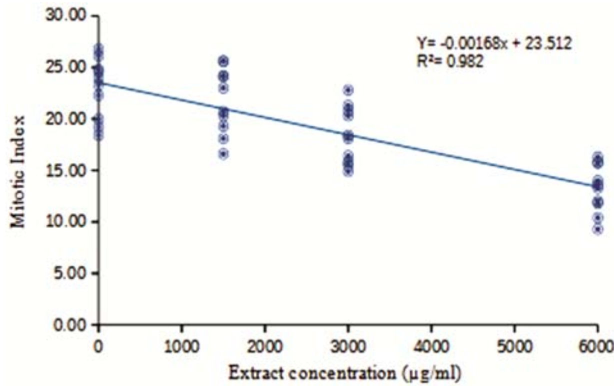

Fig. 1 — Regression analysis of the effect of different concentrations of *Vernonia condensata* aqueous extracts on the mitotic index (MI) in *Allium cepa* roots. Significant regression ($p<0.01$).

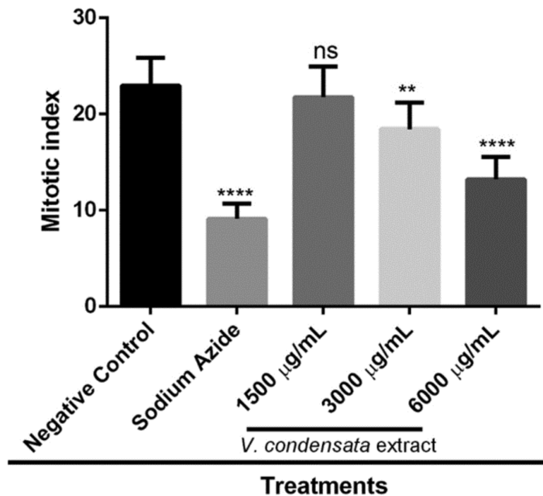

Fig. 2 — Graph representing the frequency of micronuclei found in meristematic cells of *A. cepa* treated with *Vernonia condensata* extract. Data represent the mean ± standard error. *p<0.05. **p<0.01, ***p<0.001, ****p<0.0001 and NS-Not Significant, compared to the negative control using two-way ANOVA, followed by Dunnett´s multiple comparisons post-test.

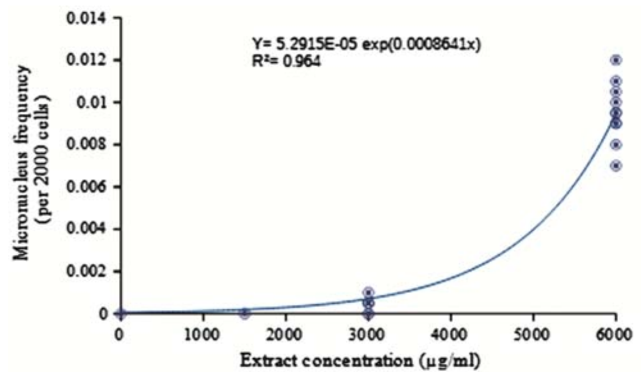

Fig.4 — Regression analysis of the effect of different concentrations of *Vernonia condensata* aqueous extract on MN frequency in *A. cepa* roots. Significant regression ($p<0.01$).

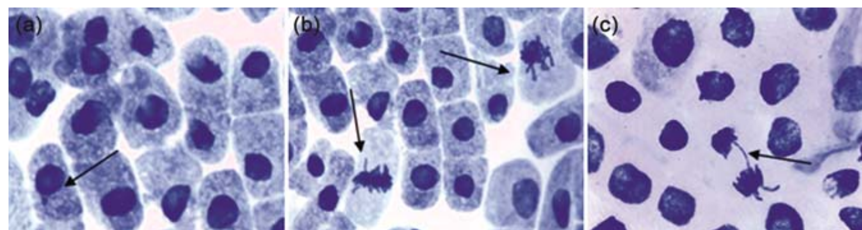

Fig. 3 — Formation of micronuclei in *Vernonia condensata* cells; B – Metaphase with lagging chromosome and disordered metaphase plate; C – Anaphase bridge with lagging chromosome.

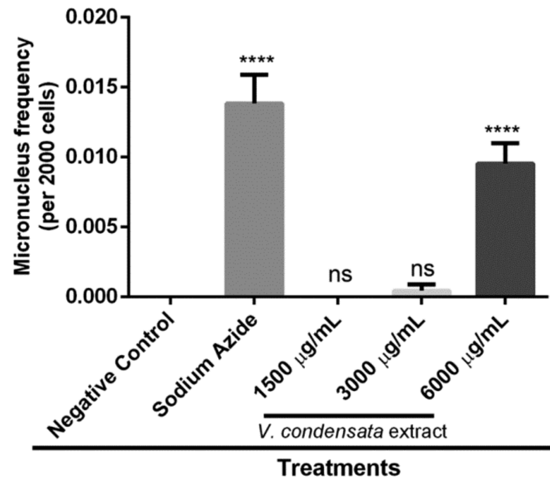

Fig. 5 — Graph representing the frequency of micronuclei found in meristematic cells of *A. cepa* treated with *Vernonia condensata* extract. Data represent the mean ± standard error. *p<0.05. **p<0.01, ***p<0.001, ****p<0.0001 and NS-Not Significant, compared to the negative control using two-way ANOVA, followed by Dunnett´s multiple comparisons post-test.

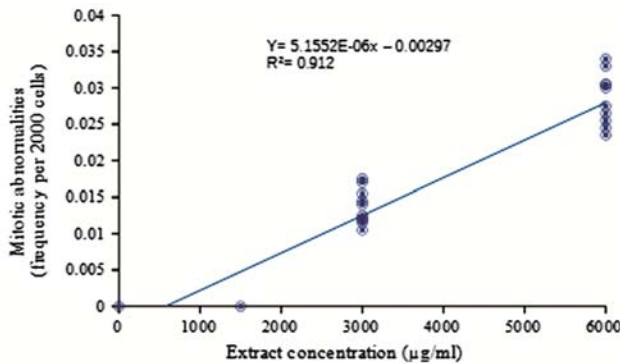

Fig.6 — Regression analysis of the effect of different concentrations of *Vernonia condensata* aqueous extracts on the mitotic abnormalities in *A. cepa* roots. Significant regression ($p<0.01$).

anaphase bridges (Fig. 3B and 3C). A concentration of 6000 µg/ml produced a 23% increase in mitotic abnormalities in relation to 3000 µg/ml and only 14.3% when compared to positive controls. Figure 7 shows a graphical representation comparing the treatments.

The present study demonstrated cytotoxic and genotoxic effects at high concentrations of *V. condesata* aqueous extracts in meristematic *A. cepa* roots, possibly related to the presence of terpenes and alkaloids[29-31].

Terpenes are secondary plant metabolites derived biosynthetically from units of isoprene, representing the largest group of secondary plant products[31]. They are ecologically important for their

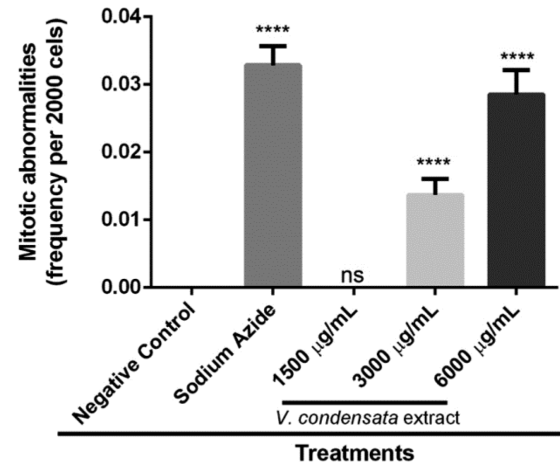

Fig. 7 — Graph representing the frequency of mitotic abnormalities found in meristematic cells of *A. cepa* treated with *Vernonia condensata* extract. Data represent the mean ± standard error. *p<0.05. **p<0.01, ***p<0.001, ****p<0.0001 and NS-Not Significant, compared to the negative control using two-way ANOVA, followed by Dunnett´s multiple comparisons post-test.

well-established role in plant defense. Several monoterpenes have been isolated and assessed for toxicity to different insects[32].These studies involved α-pinene, β-pinene, 3-carene, limonene, mircene, α-terpinene and camphene[33].Some terpenes present in plant essential oils exhibit toxic and hallucinogenic effects[34].

People often confuse *V. condensata* with *Peumus boldus,* the native Chilean boldo, which is less widespread in Brazil. Although the two species are used in the same way in different regions, there are reports of different side effects and indications for *V. condensata*[27], reinforcing the importance of caution when using medicinal plants.

Toxigenic studies are vital in the analysis of phytopharmaceuticals since they point to possible genetic damage, which can cause diseases such as the development of tumors and congenital malformations[35].

Considering its proven properties and activities, *V. condensata* is undoubtedly an alternative in the treatment of a variety of illnesses[5].Recent studies on the species indicate its therapeutic potential, but provide no information on a safe dose. Thus, further research is needed to assess the toxicity and dosage of *V. condensata*. The present study observed cytotoxicity and genotoxicity for the species with increased concentrations of *V. condensata* aqueous extracts. However, this response was not observed at

low concentrations, indicating the need for additional studies to ensure the safe use of the species.

Evaluation of cytotoxic activity by reducing Resazurin method

Resazurin is a blue dye commonly used to measure cell viability because it is weakly fluorescent. In mammalian cells its reduction results in pink and highly fluorescent derived resorufin. Resazurin-based assay has been shown to be adequate and reliable for investigating the toxic effect of various compounds on mammalian cells[36].

Cell viability of the C2C12 cell line remained constant until the concentration of 10000 µg/ml of *V. condensata* extract, after which it was observed to viability decrease, indicating that higher doses induce a cytotoxic effect. The concentration capable of inhibiting 50% (IC50) of cell viability was 15.610 µg/ml (Fig. 8).

In Brazilian folk medicine, many species of the Asteraceae family, in addition to *V. condensata*, have been used for treatment purposes. However, there are several studies that investigate the cytotoxic, genotoxic and mutagenic effects of these species, among which are *Baccharis trimera, Vernonanthura polyanthes, Helianthus annuus, Silybum marianum, Elephantopus scaber*, among others[37-40]. The cytotoxic effect of a plant in another organism, is directly related to the chemical compounds produced by its metabolism, which may produce secondary metabolites as flavonoids and terpenoids, whose production has already been described in the literature for this species. It has already been observed that most flavonoids exhibit a significant cytotoxic effect, as mentioned by Emami *et al.*[41], in their study where they evaluated the cytotoxic activity of thirteen rare and endemic plants from Chaharmahal and Bakhtiari Province in Iran.

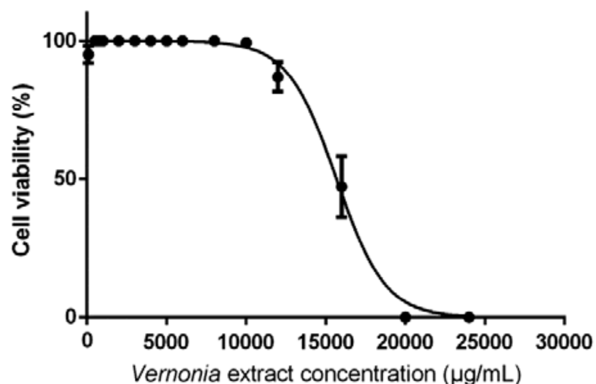

Fig. 8 — Sigmoidal dose response curves of treatment C2C12 cell line with *Vernonia condensata* extract. Each point represents the mean ± standard deviation for n = 4 replicates.

Depending on the metabolite composition present in a plant, it can exhibit or not a cytotoxic effect. Dos Santos *et al*.[40], evaluated the cytotoxicity of the terpenoid trimeroside of the aqueous extract of *Baccharis trimera* in the HepG2 cell line by the MTT assay ((3- (4,5-dimethyl thiazol-2il) -2,5- bromide) diphenyl tetrazolium), and after 3 h of exposure, they did not observed significant cell damage. However, in the cytotoxic and genotoxic studies of extracts and fractions of *Baccharis trinervis* (Lam, Persoon), from Brazil and Colombia in Chinese hamster ovary (CHO) cells, Jaramillo-García *et al.*[42], observed dose-dependent cytotoxic effects in CHO cells treated with *B. trinervis*. Cruceriu *et al.*[43], studied the extracts of *Calendula officinalis* and showed cytotoxic activity for different cancer cell lines. However, its cytotoxic activity is related to few bioactive compounds, having multiple roles both in the activation of pro-apoptotic proteins and in the reduction of the expression of proteins that inhibit cell death. In this context, even that plant extracts are from natural origin, it should be used with parsimony, once that it can brings unwanted side effects, which can be dangerous.

***In vitro* genotoxicity assay**

Genotoxicity analysis were carried out by the micronucleus test, in which 2000 binucleated cells per concentration were evaluated and only binucleated cells were considered in determining the frequency of micronuclei (Fig. 9A). Vernonia extract increased the presence of micronuclei in the binucleated cells at concentrations of 6000 and 12000 µg/mL (Table 2). The Figure 9B represents a regression analysis of the effect of different concentrations of *V. condensata* aqueous extract on MN frequency in C2C12 cells, showing a dose-dependentin mode of action tendency. Although there was a cytotoxic effect on the strain under study at doses greater than 10000 µg/mL, genotoxic analysis indicates that even at dosages below this concentration, DNA damage occurs (Fig. 10).

In the Asteraceae family, the study of species with different popular uses is quite varied. The cytotoxic, genotoxic, antigenotoxic and antifungal effects of the aqueous extract of the leaves of *Vernonanthura polyanthes* (Spreng.), Known as “assa-peixe”, in somatic cells of Drosophila melanogaster were investigated. They found that the aqueous extract of the plant did not show cytotoxic, genotoxic and antigenotoxic activity in the experimental conditions tested using the somatic mutation and recombination test in wing (SMART-wing)[44].

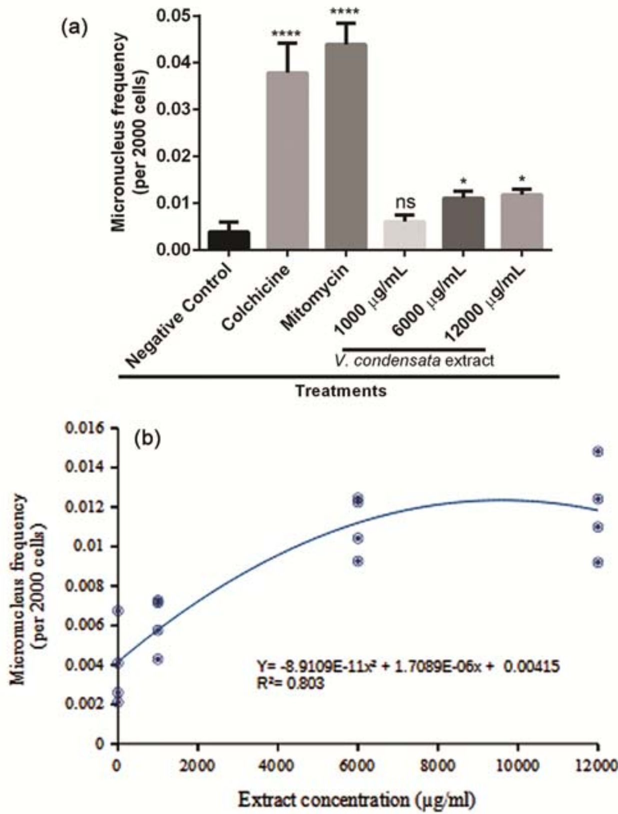

Fig. 9 — A) Graph representing the frequency of micronuclei found in C2C12 cells treated with *Vernonia condensata* extract. Data represent the mean ± standard error. *p<0.05. **p<0.01, ***p<0.001, ****p<0.0001 and NS-Not Significant, compared to the negative control using two-way ANOVA, followed by Dunnett´s multiple comparisons post-test. B) Regression analysis of the effect of different concentrations of *Vernonia condensata* aqueous extract on MN frequency in C2C12 cells. Significant regression (p<0.01).

Table 2 — Results of analysis of variance and comparison of means for the frequency of micronuclei found in C2C12 cells treated with *Vernonia condensata* extract *in vitro* genotoxicity assay.

Source of variation	Degrees of freedom	Mean Squares
		MN frequency
Treatments	5	0.001186**
Residue	18	0.000013
C.V.%	18.52	
Treatments		MN frequency
Negative control		0.00387^{A}
Extract 1000 µg/ml		0.00615^{A}
Extract 6000 µg/ml		0.01107^{B}
Extract 12000 µg/ml		0.01185^{B}
Colchicine 0.025 µg/ml		0.03785^{C}
Mitomycin 0.04 µg/ml		0.04390^{D}

**significant according to the F test. Means followed by the same letter in the columns do not differ significantly according to the Scott-Knott test at 0.01 probability. MN frequency – micronuclei frequency per 2000 cells.

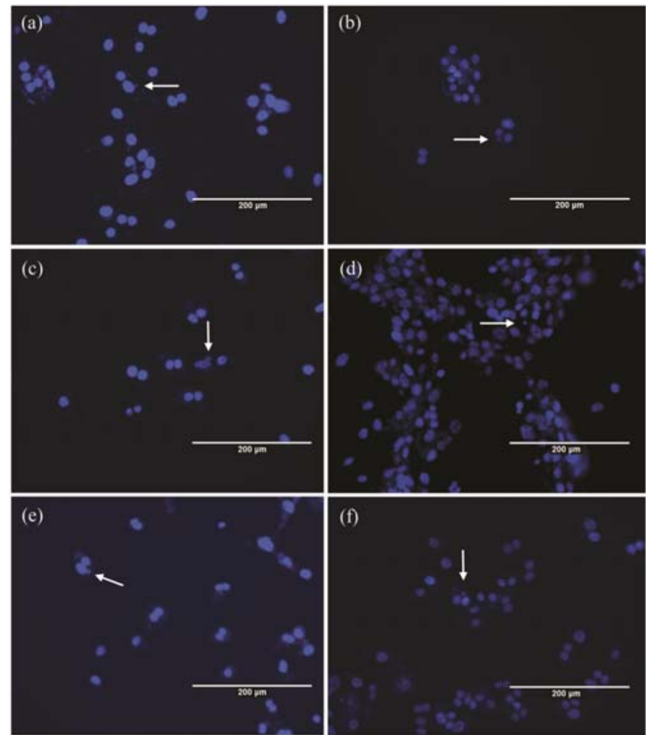

Fig. 10 — Micronucleus found in C2C12 cells treated with *Vernonia condensata* extract by *in vitro* genotoxicity assay. A - Negative control, B, C and D - extract concentrations of 1000; 6000 and 12000 µg/mL, respectively, E - Colchicine 0.025 µg/mL and F - Mitomycin 0.04 µg/mL. Arrows indicate micronucleus.

In order to assess the genotoxic potential of *Helianthus annuus* L. seed oil using the Ames test, Oliveira *et al*.[38], observed that sunflower oil is not genotoxic, as indicated by frameshift mutations and base pair substitutions regardless of the treatment dose, but shows dose-dependent toxicity.

Investigating the toxicological effects of the terpenoid trimeroside from the aqueous extract of aerial parts of *Baccharis trimera,* using the micronucleus test in HepG2 cells, Dos Santos *et al.*[40], observed a reduction of micronucleus in binucleated cells, but it was not statistically different from the negative control, indicating that it has no genotoxic effect.

The cytotoxicity and genotoxicity of flavonolignans, which are the main components of silymarin, extracted from the thistle fruit (*Silybum marianum* L.), were evaluated in different human cell lines revealing that the three main flavonolignans found in silymarin (silybin, silicristine and silidianine) do not have a cytotoxic or genotoxic effect in concentrations up to 100 µM[39].

The cytotoxic and genotoxic effect of Vernonia extract observed in C2C12 myoblasts in the present study, open perspectives for further research, including in tumor cells, once that many Asteraceas as *Elephantopus scaber*, studied by Beeran *et al*.[37], shows antitumor properties. The authors investigated the anticancer effects of *E. scaber* on human epithelial cancer cells, observing that an enriched fraction of this plant possessed cytotoxic effects, triggering genotoxicity and apoptosis in human epithelial cancer cells.

Conclusion

The data obtained in the present study demonstrate that an increase in the concentration of *Vernonia condensata* Baker aqueous extracts caused cytotoxic and genotoxic effects in meristematic *Allium cepa* cells. A concentration of 1500 µg/ml showed no cytotoxic or genotoxic effects, while 3000 µg/ml resulted in mitotic abnormalities and a decline in the mitotic index, indicating cytotoxicity. The 6000 µg/ml aqueous extract showed cytotoxicity and genotoxicity, evident in mitotic abnormalities and the presence of micronuclei in the *A. cepa* test. Doses greater than 6000 µg/mL of *V. condensata* extract showed cytotoxic and genotoxic effects in the murine myoblast C2C12.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

WCS; PHAM; BSS; TPR; RJOJ; ASA conceived and designed the study. WCS; PHAM, ECR; RJOJ; ASA performed the experiments and analyzed the data; WCS; ECR; RJOJ; ASA participated in the figures production and drafted the paper. WCS, PHAM, BSS, TPR, ECR, AC, SM, RJOJ; ASA revised the manuscript.

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