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Evaluation of cytotoxicity, mutagenicity, and genotoxicity of *Tarenaya aculeata* (Cleomaceae) using *Allium cepa* L.

Avaliação da citotoxicidade, mutagenicidade e genotoxicidade de *Tarenaya aculeata* (Cleomaceae) utilizando *Allium cepa* L.

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Tarenaya aculeata is a species belonging to the Cleomaceae family, which has been gaining prominence in medicinal studies. In this study, the effect of aqueous extracts and fractions obtained using XAD-2 amberlite resin from the leaves, stems and roots of *T. aculeata* on the germination and cell proliferation of *Allium cepa* L. (Amaryllidaceae) seeds was analyzed. A Principal Component Analysis (PCoA) analysis was carried out to observe similarities and dissimilarities in the responses of the relative germination index and mitotic index between the samples, and the chromatograms of the samples were obtained using a high-performance liquid chromatograph (UHPLC-ESI-MS/MS). Leaf extracts (LE) and fractions (LF), in the concentration of 100 µg/mL, showed a reduction in germination index compared to the control, with values of 81.66 ± 6.02 for LE and 80.26 ± 6.87 for LF. Concerning the mitotic index, seeds treated with all the extracts and fractions of *T. aculeata* acted in a dose-dependent manner. The results obtained about the germination and mitotic index indicated an allelopathic effect of the samples. In the cell proliferation analysis, the steam extracts (SE) and fractions (SF) were the most cytotoxic, presenting a mitotic index value of 9.0 ± 0.2 for SE and 8.4 ± 0.5 for SF, at 1000 µg/mL. The PCoA analysis allowed us to observe a significant difference in the stems and roots compared to the leaves, which were also observed in the chromatograms generated from the different samples. The extracts and fractions did not show genotoxicity and mutagenicity.
Keywords: mitotic index, allelochemicals, plant bioassays.

Tarenaya aculeata é uma espécie pertencente à família Cleomaceae, que vem ganhando destaque nos estudos medicinais. Neste estudo foi analisado o efeito de extratos aquosos e frações obtidos com resina amberlite XAD-2 de folhas, caules e raízes de *T. aculeata* na germinação e proliferação celular de sementes de *Allium cepa* L. (Amaryllidaceae). Uma análise de Componentes Principais (PCoA) foi realizada para observar semelhanças e dissimilaridades nas respostas do índice de germinação relativo e índice mitótico entre as amostras, e os cromatogramas das amostras foram obtidos utilizando um cromatógrafo líquido de alta eficiência (UHPLC-ESI-MS/MS). Os extratos das folhas (LE) e frações (LF), na concentração de 100 µg/mL, apresentaram redução no índice de germinação em relação ao controle, com valores de 81,66 ± 6,02 para LE e 80,26 ± 6,87 para LF. Em relação ao índice mitótico, sementes tratadas com todos os extratos e frações de *T. aculeata* agiram de maneira dose-dependente. Os resultados obtidos referentes aos índices de germinação e mitótico indicaram um efeito alelopático das amostras. Na análise de proliferação celular os extratos dos caules (SE) e frações (SF) foram os mais citotóxicos, apresentando valor de índice mitótico de 9,00 ± 0,2 para SE e 8,4 ± 0,5 para SF, a 1000 µg/mL. A análise do PCoA permitiu observar uma diferença significativa nos caules e raízes comparados às folhas, que foram também observadas nos cromatogramas gerados a partir das diferentes amostras. Os extratos e frações não apresentaram genotoxicidade e mutagenicidade.
Keywords: índice mitótico, aleloquímicos, bioensaios com plantas.

1. INTRODUCTION

To protect themselves against predators and pathogens, plants can produce a range of secondary metabolites, which in turn can cause metabolic changes to the host plant [1]. When the release of these metabolites interferes with both stimulatory and inhibitory effects in the plant process, they are known as allelochemicals [2]. These allelochemicals can act positively or negatively in the plant producing the compound [3]. Positively, it can be used as a bioherbicide [4].

Tarenaya aculeata (L.) Soares Neto & Roalson has a global distribution that can be invasive in plantations, being previously known as *Cleome aculeata* [5]. The weeds are unwanted plants that appear in plantations, influencing the yield of food production due to the release of allelochemicals [6], but can have ethnobotanical and ethnopharmacological uses [7].

Some classes of secondary metabolites have already been described in *T. aculeata* [8, 9], such as the presence of phenolic compounds and tannins in the extracts and fractions of the leaves [9] of *T. aculeata*, and the alkaloids, phenolic compounds, and tannins in extracts and fractions of its steams and roots [8]. In traditional medicine, it is known as "cecê" and "mussambê" and there are reports of the use of teas from its leaves and roots to treat fever, intestinal and stomach infections, and as an antipruritic [10]. Leaf teas can also be used for body aches [11], and tea from the whole plant for the treatment of diabetes, high blood pressure [12], inflammation, and bladder problems [13].

Some weed species have already been cited in the literature for use in controlling other weed species, as is the case of *Tarenaya spinosa* (Jacq.) Raf., which has proven to be efficient in controlling *Nicotiana glauca* Graham (Solanaceae), due to its allelopathic potential, in a field experimental model [14]. The species belongs to the same genus as *T. aculeata*.

The *Allium cepa* (L.) model has been used as an efficient biological model to obtain preliminary answers about the cytotoxic, genotoxic, and mutagenic potential of different plant-based compounds and preparations [15-18]. This test has been widely used to evaluate the toxicity of tea preparations [19].

Based on what has been reported, this study is a continuation of the studies listed above and evaluates the behavior of this species using a plant model. In this sense, the objective of this study is to evaluate the cytotoxicity, mutagenicity, genotoxicity, and allelopathy of aqueous extracts and fractions of the leaves, stems, and roots of *T. aculeata* in the *A. cepa* meristem cells.

2. MATERIAL AND METHODS

2.1 Plant material, post-harvest processing and extraction

T. aculeata specimens were collected in Dourados city, Mato Grosso do Sul, Brazil. The collections were registered under the SISGen code number AD26007, and a specimen voucher was deposited at the Federal University of Grande Dourados – MS under code DDMS 7618. The *T. aculeata* species was identified by Dr. Raimundo Luciano Soares Neto, a specialist in the *Tarenaya* genus.

To prepare the extracts, the different parts of the plant were dried at 50 °C for 72 h in an oven and subsequently crushed in a mill, with a particle size of 10 mesh (Marconi, Brazil), separately obtaining, the plant materials from leaves, stems and roots. To prepare the different extracts and fractions, the decoction methodology was used, described by Duarte et al. (2023) [9], obtaining the aqueous extracts of leaves (LE), stems (SE) and roots (RE), and the fractions of leaves (LF), stems (SF) and roots (RF).

2.1 The *A. cepa* germination test

Pesticide-free *A. cepa* seeds of the Crioula type (2n = 16 chromosomes, Bleuline) were used. A total of 80 *A. cepa* seeds were placed in a petri dish for seed germination, and incubated with different concentrations of LE, SE, RE, LF, SF and RF, under controlled conditions (12/12 h

photoperiod, light-dark cycle with temperatures of 23 ± 3 ºC for 120 h), using a germination chamber (Quimis, Brazil). Extracts and fractions were tested at concentrations of 100, 500 and 1000 μg/mL. For each of the concentrations tested, it was accomplished 3 repetitions. Additionally, 2 more Petri dishes were used for the controls. Distilled water was used as the negative control and Trifluralin 0.84 μg/mL as the positive control. At the end of the test, the germinated seeds were counted and the roots were separated for analysis under the microscope.

2.3 Analysis of meristem cells

The roots used for cellular analysis were immediately fixed in a 3:1 ethanol:acetic acid solution. Soon after, hydrolysis was carried out with a 5-minute wash in distilled water followed by immersion in 1 mol/L hydrochloric acid at 60 °C for 10 min and 3 consecutive washes in distilled water. After separating the meristem from the rootlets, the staining process was carried out using Schiff's reagent, with an immersion time of 1 hour and 30 minutes. The analysis was carried out using an optical microscope (Nikon, Japan) at 400x magnification. Relative percentage index (RGI), mitotic index (MI), chromosomal alteration index (CAI), and mutagenicity index (MTI) were calculated as described by Francisco et al. (2018) [20].

2.4 UHPLC-ESI-MS/MS

To obtain molecular chromatograms of LE, SE, RE, LF, SF and RF, 1.0 mg of each sample was resuspended in CH3CN–H2O and analyzed in positive ionization mode via ultra-high performance liquid chromatography–electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) using an Agilent 6545 Q-TOF LC/MS system. The samples were analyzed using an Agilent Zorbax Eclipse Plus C18 column (Rapid Resolution HD 2.1 × 50 mm, 1.8 μm), with a flow rate of 0.3 mL/min, column temperature of 33 ◦C, and injection volume of 3 μL. The mobile phase comprised of water containing 0.1% (v/v) of formic acid (A) and acetonitrile (B). The elution program involved an initial linear gradient (0–14 min, 5–100% B) followed by isocratic elution (14–16 min, 100% B). The operating parameters were as follows: capillary voltage of 3,000 V; skimmer voltage of 65 V; dry gas temperature of 320 ◦C; drying gas of 12 L/min; nebulizer gas pressure of 35 psi; sheath gas flow of 10 L/min, and sheath gas temperature of 300 ◦C. MS1 data were acquired in the range of m/z 100 to 1.500. LC-MS/MS data were processed using MassHunter B.08.00 (Agilent) and MZmine 2.53 software.

2.5 Statistical analysis

The statistics were performed using the R language (R Core Team) [21]. The homogeneity of variances was tested using the Levene test using the Levene Test function from the car package [22] and normality using the Shapiro-Wilk test using the native function shapiro.test. Normality and homogeneity of the data were verified, with results presented as mean and standard deviation. Sequentially, the analysis of variance (ANOVA) was applied using the native function aov where there was a significant difference between treatments and between days ($p < 0.05$). Based on this, the Tukey test was used using the native TukeyHSD function and the multcompLetters4 function from the multcompView package was used to visualize the data, considering 5% significance [23].

Principal Component Analysis (PCoA) was carried out to evaluate similarities and dissimilarities of extracts and fractions, and in the different parts of the plant used, based on the results obtained from the RGI and MI. The analysis was carried out using the “vegan” package [24], using the R environment [21].

3. RESULTS AND DISCUSSION

Regarding the IGR and IM results, we observed that the extracts and fractions of stems and roots did not present changes in root growth at the different concentrations tested (Figure 1A).

However, leaf extracts and fractions showed significant differences in the concentrations of 100 and 1000 µg/mL. Regarding the results regarding MI, a significant difference was observed in all the tested samples, when compared to the control. It was also noted that the extracts and fractions of stems and roots showed the greatest differences.

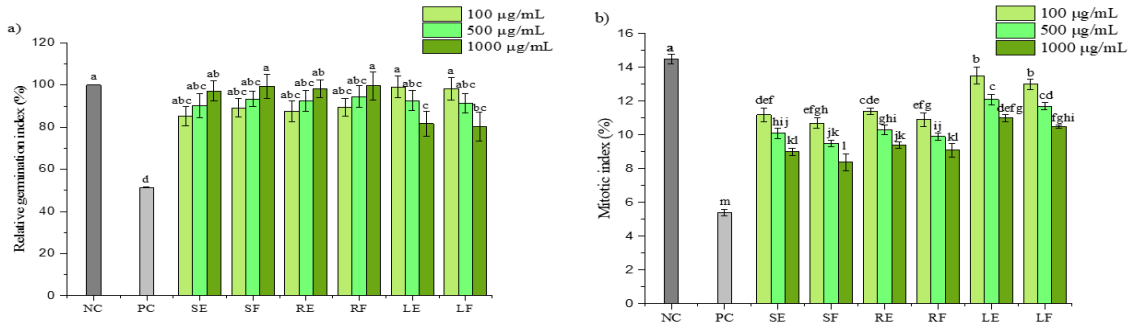

Figure 1. Relative germination index and mitotic index of Allium cepa *seeds in the presence of different concentrations of the aqueous extracts and fractions of* Tarenaya aculeata*. NC: Negative control; PC: Positive control; SE = Steam extract; SF = Steam fraction; RE = Root extract; RF = Root fraction; LE = Leaf extract; FL = Leaf fraction. The bars represent the standard deviation.*

Principal component analysis (PCoA) was applied to analyze the effects caused by the aqueous extracts and the fractions in different parts of the plant, according to the results of RGI and MI. The analysis generated a well-defined separation between the leaf samples, which were more distant from the roots and stems, enabling us to say that the aqueous extracts and fractions of stems and roots present greater similarity to the leaf samples. (Figure 2A). Regarding the type of preparation, the fractionation of the samples showed similarity when compared to the results of the extracts (Figure 2B), highlighting the similarity of these samples.

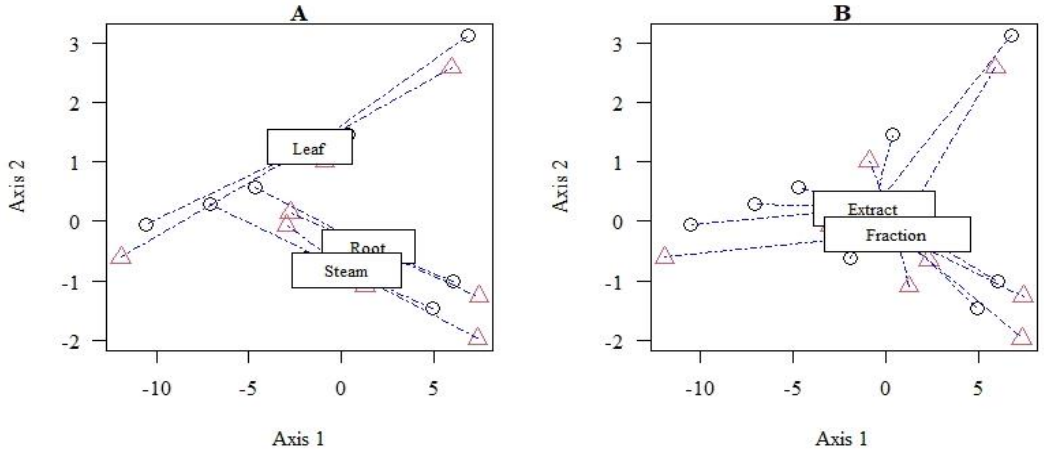

Figure 2. Principal Coordinate Analysis (PcoA), based on the Euclidean distance. The extracts were grouped by the part of the plant (A) and aqueous or fractionated extract (B). Extracts are represented by circles, and fractions by triangles.

In this sense, the extracts and fractions did not show substantial differences according to the PCoA analysis. If we consider the expense of organic solvent used to obtain the fractions produced, the costs of the column, and the excessive preparation time to obtain them, the fractions are the least attractive option. Furthermore, choosing a single step with only an aqueous solvent is the most eco-friendly option, resulting in less environmental damage. In this regard, we can attribute the best results of allelopathy to the aqueous extracts.

The PCoA graph, together with the data obtained through Figure 1A, allows us to say that the extracts and fractions of the steams and roots presented the greater cytotoxic effects, being the concentration of 1000 µg/mL the one that showed the greatest difference compared the control, with the MI value equal to 9.0 ± 0.2 for SE, 8.4 ± 0.5 for SF, 9.4 ± 0.2 for RE and 9.1 ± 0.4 for RF. However, the extracts and fractions of the leaves were the most toxic, with the concentration of 100 µg/mL being the only one to present significant differences in the IGR values, compared to the control. The IGR values for leaf extracts and fractions were equal to 81.66 ± 6.02 for LE and 80.26 ± 6.87 for LF. These changes can be due to differences concerning the profile of secondary compounds in the different samples. Figure 3 allows us to observe differences in the chromatographic profiles of LE and LF, compared to the rest of the samples.

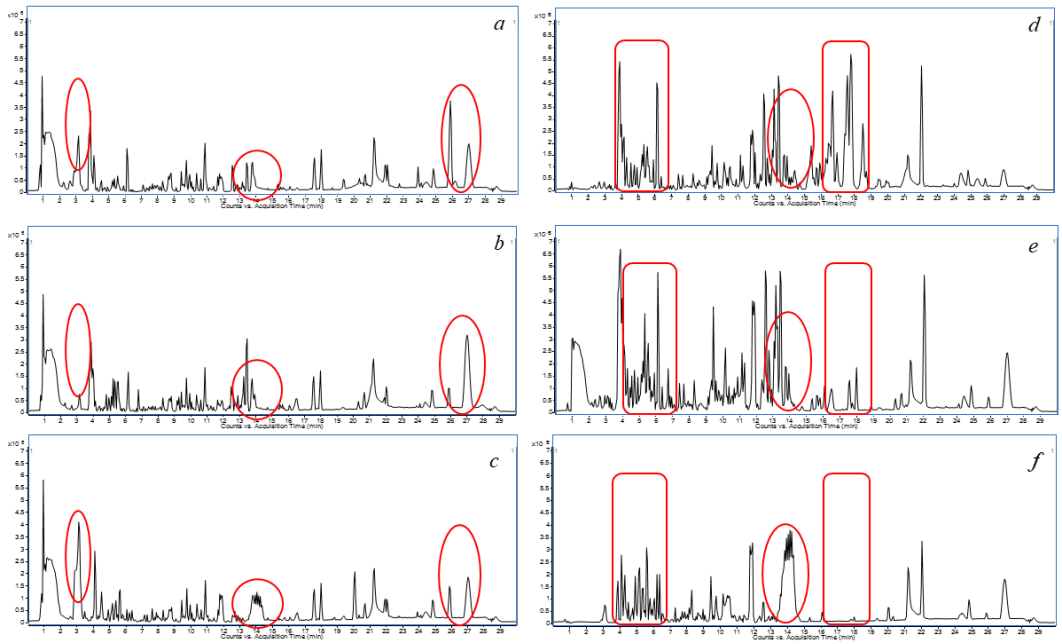

Figure 3. UHPLC-ESI-MS/MS profile of a)SE, b)RE, c)LE, d)SF, e)RF and f)LF of Tarenaya aculeata. *SE = Steam extract; SF = Steam fraction; RE = Root extract; RF = Root fraction; LE = Leaf extract; FL = Leaf fraction.*

Differences in chromatographic profiles result from changes in the chemical composition of plant extracts and fractions. In this context, it can be seen in Figure 3 that the extracts and fractions obtained by different preparation methods showed variations in their chemical composition. These variations can be attributed to the insensitivity of the peaks present in the chromatograms, especially in preparations using leaves (Figures 3C and 3F), which in some cases are presented in lower or higher insensitivity compared to the other obtained extracts and fractions (Figures 3A, 3B, 3D and 3E).

When a plant extract is capable of causing changes in the germination pattern and mitotic index, we can say that this plant has cytotoxic properties [20]. Silva et al. (2022) [18], when studying the cytotoxic nature of *Campomanesia sessiliflora* (O. Berg) Mattos and *Campomanesia guazumifolia* (Cambess) O. Berg (CG), observed changes in the germination patterns and root size of the samples and associated them with allelopathic effects in the extract. In the study by Silva et al. (2023) [25], when evaluating infusions obtained from rhizomes of Turmeric (*Curcuma longa* L.), they attributed the cytotoxic character of the extract to changes in the mitotic index and the concentrations of 20 and 40 mg/mL showed antiproliferative activity because they presented a significant reduction in the mitotic index.

The Mitotic Index may be related to an alteration in the mechanisms of mitosis resulting from cytotoxic effects [25, 26]. In this sense, our RGI and MI results suggest that both extracts and fractions may have negatively affected root growth and cell proliferation of the seeds of *A. cepa*, causing an allelopathic and cytotoxic effect of the samples.

T. aculeata is popularly known as a weed. Weed species are known as such due to their undesirability concerning human activity, with competition being their best-known form of interference with other plants, which ends up resulting in allelopathic damage to other crops, modifying their patterns of growth, development, and productivity [27]. Changes in the growth patterns caused by plant species are generally related to the allelopathic properties present in the plant, often associated with its constituent allelochemicals. A good application for the use of allelopathic extract is in the control of invasive plants [28, 29]. Some studies have shown that plant species can be used to control invasive plants, as is the case of *T. spinosa*, and some species of *Amaranthus*, species considered to be weeds, but also used in popular medicine [14, 30].

Extracts of the leaves, stems and roots of *T. spinosa* showed significant differences in the growth and germination patterns against *Nicotiana glauca*, in a field experimental model. The aqueous extract of the stem did not show any root germination in none of the concentrations tested, while the root extract showed a significant reduction from the 5% concentration, compared to the control [14].

In another experimental model, different species of *Amaranthus* (*Amaranthus spinosum* L. (spiny amaranth), *Amaranthus viridis* L. (Amaranthaceae), *Amaranthus deflexus* L. (Amaranthaceae), *Amaranthus hybridus* L. (Amaranthaceae), and *Amaranthus retroflexus* L. (Amaranthaceae) showed a reduction in root germination in a dose-dependent manner at all concentrations tested (0.25-4.9 $g.L^{-1}$) using the plant model *Lactuca sativa* L. (lettuce), characterizing its allelopathic effect [30].

The cytotoxic character may be due to the presence of allelochemicals in the samples. Previous studies describe the presence of phenolic compounds and tannins in LE, SE, RE, LF, SF, and RF [8, 9]. The allelopathic effect of phenolic compounds may be associated with the plant's defensive system, affecting its physiological mechanisms, such as growth, development, and protection, in addition to influencing the metabolite mechanism of other plants [31]. About the tannins, their metabolites tend to defend their leaves against herbivorous insects through their deterrence and/or toxicity [32].

Plants produce allelochemicals in response to biotic and abiotic stress, and they range from simple hydrocarbon to complex polycyclic aromatic compounds [2]. In fact, most of these compounds, such as alkaloids, phenolic compounds, flavonoids, glycosides, saponins, and tannins have already been reported as present in a phytochemical screening for extracts and fractions of stems and roots of *T. aculeata*, and can be responsible for the cytotoxicity of these extracts [8].

However, differences also occur due to the part of the plant used, we saw previously that the leaves, stems and roots of *T. spinosa* responded in different ways when used to control a weed [14]. Our results present results similar to those of de Castro et al. (2017) [14]. Although the stem extract was considered the most allelopathic, in our study, this was the extract with the greatest cytotoxic effect, followed by the root extract. Also, in both experiments, the extract of the leaves showed a reduction in germination percentages with increasing concentration in the samples.

The analysis of chromosomal alterations serves as a mutagenicity test and is one of the few direct methods to measure damage in systems exposed to possible mutations or carcinogenesis [19]. In this sense, the mutagenicity index (MTI) and chromosomal alteration index (CAI) showed no differences compared to the control, leading to the inference that the leaves, stems, and roots of *T. aculeata*, regardless of whether it was a crude extract or fraction, showed an absence of mutagenicity and genotoxicity.

The absence of genotoxicity and mutagenicity in extracts and fractions of *T. aculeata* can be considered a positive factor since this species is popularly used as a tea, making it possible to ensure that the species does not tend to present DNA damage. Other applications can be the uses of this species as alternatives for commercial use and even products that can be manufactured, such as cosmetic products and bioherbicides.

4. CONCLUSION

The extracts and fractions of *T. aculeata* showed allelopathic potential against *A. cepa* at all tested concentrations (100, 500, and 1000 µg/mL) for leaves, stems, and roots.

The extracts and fractions of the leaves affected the germination of the *A. cepa* seeds, and the relative germination index decreased concerning the concentration increased, being the concentration of 1000 µg/mL the most harmful to the *A. cepa* plant system. However, the extracts and fractions of the stems and roots didn't affect the seed germination, presenting no significant difference in the concentrations.

The results from MI showed that all extracts and fractions affected seed cell proliferation and that this reduction was dose-dependent. The extracts and fractions of the stem presented the highest MI values, presenting greater cytotoxicity.

Regarding the germination index results, the extracts and fractions are considered toxic since they altered the germination pattern of *A. cepa* seeds, compared to the control.

T. aculeata did not present genotoxicity and mutagenicity in the *A. cepa* model and has increasingly shown itself to be a promising species for future studies that analyze its potential allelopathic, against other crops, and a possible bioherbicidal effect.

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