Antiproliferative analysis of aqueous extracts of cabreúva (*Myrocarpus frondosus*) on the *Allium cepa* cell cycle

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Abstract: *Myrocarpus frondosus* is widely used in Brazilian folk medicine for bronchitis, gastritis, ulcers and wound asepsis. However, there is a scarcity of scientific data proving the safe use of tea of this species or if there is any toxic effect on the human organism. This study aimed to evaluate the antiproliferative effect of aqueous extracts of cabreúva on the *Allium cepa* cell cycle. The aqueous extracts were prepared from leaves, bark and roots (dried material) of the species in two concentrations: 2.5 and 5.0 grams in 250 mL of distilled water. The aqueous extract of the leaves was obtained by infusion and the aqueous extract of the bark and roots by decoction. Distilled water was used as negative control and glyphosate 2% as positive control. Eight groups of four onion bulbs were evaluated and each group corresponded to one treatment and, in each group, 4000 cells were analyzed and the mitotic index was calculated. The results demonstrated reduction of mitotic indices in all treatments when compared to the negative control in water. The aqueous extracts of cabreúva in the studied concentrations have antiproliferative action on the *Allium cepa* cell cycle, ensuring the safe use of tea of this medicinal species.

Keywords: *Myrocarpus frondosus*; medicinal plant; mitotic index

1. Introduction

Plants with medicinal potential have been widely used to treat various diseases and are often the only medicine available to the population (Fachinetto and Tedesco, 2009). However, indiscriminate use coupled with lack of knowledge about the medicinal species can cause harm to health (Frescura et al. 2012). The genus *Myrocarpus* is exclusively South American, being *Myrocarpus frondosus* Allemão the only species recorded in southwestern Paraguay, northern Argentina and southern, southeastern and northeastern Brazil (Lorenzi and Matos, 2008).

The species *M. frondosus* belongs to the family Leguminosae, is characterized by being a large tree plant and popularly known as cabreúva (Cabrera et al. 2012). Cabreúva is considered a relevant species because, besides being used as a medicinal plant, it is also used in reforestation of degraded areas (Sccoti et al. 2011; Santi et al. 2017). It is popularly used for diarrhea, gastritis, ulcers, wound asepsis, has expectorant effect, anti-inflammatory and antimicrobial activity (Pereira Junior et al. 2014; Santi et al. 2017). Despite its widespread use in folk medicine, there are no scientific studies to prove its effectiveness and / or rule out possible unwanted or adverse effects from its consumption. Thus, this fact highlights the need for studies that evaluate the action of aqueous extracts of the species *Myrocarpus frondosus* on organisms.

The effects of aqueous extracts can be assessed by testing with bioindicators, which generally include subsystems of a complete organism used to identify a specific target (Leme and Marin-Morales, 2009). From the results obtained, the mitotic index is calculated. Mitotic index values are used as indicative of proper cell proliferation and measured by the *Allium cepa* plant test system. This test has been widely used as a genotoxicity bioindicator (Tedesco and Laghinghouse, 2012). The efficiency of the *Allium cepa* system is due to its proliferation kinetic characteristic, rapid root growth, large number of dividing cells, high tolerance to different cultivation conditions, permanent availability, easy handling, reduced chromosome number (2n = 16) and easy viewing under the microscope (Caritá and Marin-Morales, 2008). And from this test it is possible to monitor the effects of medicinal plant extracts, ensuring their safe use by the population in the treatment of diseases and sporadic consumption (Ubessi et al. 2019).

Considering the medicinal importance of the species *Myrocarpus frondosus* and the lack of information regarding its antiproliferative activity, the effect of aqueous extracts from leaves, bark and roots on the *Allium cepa* cell cycle was evaluated.

2. Material and Methods

2.1. Obtaining plant material

The plant material of the species *Myrocarpus frondosus* was collected from a population located in the west of Santa Maria, Rio Grande do Sul, Brazil, under the coordinates 29°41'23.6"S 53°50'45.3"W. The experiment was carried out at the Plant Cytogenetics and Genotoxicity Laboratory at the Federal University of Santa Maria (UFSM).

2.2. Preparation of aqueous extracts

For the preparation of aqueous extracts leaves, bark and roots (dried material) were used in two concentrations: 2.5 and 5.0 grams (g) in 250 mL of distilled water. The leaves were placed in a container containing boiling water, remaining infused for 10 minutes. The extracts of the bark and roots of cabreúva were prepared by decoction in a period of 10 minutes. All extracts after 10 minutes were strained and stored until room temperature.

2.3. *Allium cepa* test

The *Allium cepa* test was developed at the Plant Cytogenetics and Genotoxicity Laboratory (UFSM) and organized into eight groups of four onion bulbs, which were placed for rooting in distilled water for a period of 72 hours. Distilled water was used as negative control and glyphosate 2% as positive control. The evaluated treatments are described in Table 1.

After rooting, the bulbs remained in contact with the treatments described in Table 1 for a period of 24 hours. Time lapse mentioned, the roots were detached from the bulbs and fixed in Carnoy 3:1 (ethanol: acetic acid) for 24 hours at room temperature. Soon after, the roots were placed in 70% ethanol and stored under refrigeration until blades preparation.

2.4. Preparation of blades

Two blades per bulb were made, and 500 cells per blades were analyzed, totaling 4000 cells per treatment. The preparation of the blades was performed according to the crushing technique (Guerra and Souza, 2002). In this procedure the roots were washed in distilled water and hydrolyzed for 5 minutes in HCL 1N at room temperature. They were

then washed again in distilled water with removal of the meristematic region and stained with acetic orcein 2%. The analysis of these blades was performed under a 40X magnification optical microscope, taking into account the phase of the cell cycle in which the cells were present, such as interphase, prophase, metaphase, anaphase and telophase. To calculate the mitotic index was considered the sum of the number of cells in prophase, metaphase, anaphase and telophase, divided by the total number of cells observed (Sehgal et al. 2006; Vieira et al. 2009). The result is presented as a percentage.

2.5. Statistical analysis

The data related to the mitotic index were submitted to the Chi-square test ($\chi 2$) with the aid of the statistical program BioEstat 5.3 (Ayres et al. 2007).

3. Results

The results found for the controls and treatments evaluated in relation to the mitotic index (MI) are presented in Table 2. The cells observed for the counting and evaluation of the treatments were in interphase and cell division (Figure 1). The negative control (T1) presented higher MI (6.12%), differing significantly from all other treatments evaluated. The positive control (T2) also differed statistically from the negative control, showing lower MI, thus confirming its antiproliferative action. Results differed significantly between treatments and controls (Table 2). In the comparison between the negative control (T1) (MI= 6.12%) and the positive control (T2) (MI= 4.55%), there was a significant difference and decreased IM, which indicates inhibition of cell division.

Positive control (T2) differed significantly from treatments with bark extract (T5 and T6) and root extract (T7 and T8), and the extracts further inhibited cell division in relation to glyphosate. Comparing the treatments of aqueous extracts of leaves at both concentrations (T3 and T4) with glyphosate treatment (T2), there was the same behavior, as the MI did not differ statistically, showing a decrease in cell division. Treatments with aqueous extracts of dried bark were significantly different. The extract with higher concentration (T6) strongly inhibited cell division, being the lowest mitotic index observed (2.8%). In relation to treatments with dried root extracts, the two differed statistically from each other, but unlike what occurred with treatments with bark extracts, it was the treatment with the lowest concentration (T7) that most reduced the MI (2.95 %).

4. Discussion

All treatments differed significantly from the negative water control (T1) demonstrated by the decrease in MI values. This means that there has been a reduction in cell division of the meristematic cells of *Allium cepa*. This decrease indicates antiproliferative activity of aqueous extracts from the leaves, bark and roots of cabreúva at both concentrations used. The observed cells of the onion bulb root that were submitted to the lowest concentrations of extracts prepared by infusion (T3) and decoction (T5) obtained a cellular stimulus, resulting in the increase of the IM compared to those with higher concentrations (T4 and T6). This means that the higher concentrations of leaf and bark extracts caused the reduction of MI. In contrast, the aqueous extract from dried roots, at higher concentration (T8), increased the MI compared to the lower concentration treatment (T7). Therefore, aqueous extracts of dried leaves and bark show antiproliferative capacity, especially at high concentrations.

By studying *Luehea divaricata* in two populations and at concentrations of 6 g L^{-1} and 30 g L^{-1}, Frescura et al. (2012) also observed this same behavior regarding cell proliferation. The higher concentration led to a decrease in MI values, concluding that there was an increase in antiproliferative capacity with increasing concentration. Coelho's research (2013), analyzing two populations of *Echinodorus grandiflorus* at concentrations of 6 g L^{-1} and 24 g L^{-1}, also found increased antiproliferative activity at the highest concentration, except for a commercial extract treatment. In this study, regarding the aqueous extracts of leaves, there was no significant difference between the concentrations studied (Table 2). Similarly, Kuhn (2015), when analyzing the *Peltodon longipes* species, observed that leaf extracts at different concentrations (5 g L^{-1} and 15 g L^{-1}) did not differ significantly. Other tree species such as aroeira (*Myracrodruon urundeuva*) (Trentin et al. 2013), graviola (*Annona muricata*), ipê-roxo (*Handroanthus impetiginosus*) (Melo et al. 2010), angico-branco (*Anadenanthera colubrina*) (Lima et al. 2014) and pau-ferro (*Libidibia ferrea*) (Guerra et al. 2017) also have antiproliferative activity found in their extracts from various plant parts, such as bark and leaves. Some species even belong to the same family as *Myrocarpus*, reaffirming the results obtained in this study.

Myrocarpus frondosus bark extracts had an MI of 3.67% at a concentration of 2.5 g, while the highest concentration had an MI of 2.8%, reducing cell proliferation when the concentration of the extract increased. There was a significant difference between the

two concentrations (Table 2). However, other authors, such as Frescura et al. (2012), studying the species *Luehea divaricata*, did not observe significant differences between bark extracts at concentrations 32 g L^{-1} and 160 g L^{-1}. Considering the results observed for the root decoctions at different concentrations, there is a difference between the concentrations, because the inhibition of cell division was smaller as the extract concentration increased (Table 2). Studying aqueous extracts of *Lavandula angustifolia* roots at a concentration of 0.29 g in 250 mL of distilled water, Freitas et al. (2016) found antiproliferative potential compared to controls. However, Rodrigues et al. (2017), analyzing the same species in higher concentration (3.75 grams in 200 mL of distilled water) observed proliferative potential. The increased concentrations of aqueous extracts of *Lavandula angustifolia* roots induced an increase in the proliferative capacity of the species, a behavior also observed in this study with *Myrocarpus frondosus* roots.

When studying the roots of *Myrocarpus frondosus in vivo* and *in vitro*, Bottamedi et al. (2018) found antioxidant and anti-inflammatory activity which were attributed to the presence of flavonoids and phenolics. In addition, the analyzes performed on the essential oil of *Myrocarpus frondosus* leaves showed the presence of α-thujene, α-pinene, sabinene, β-pinene, mircene, p-cymene, limonene, β-bourbonene, β-caryophyllene, D-germacrene, bicyclogermacrene, spatulenol and globulolem, with predominantly β-pinene and bicyclogermacrene (Cabrera et al. 2012; Santi et al. 2017). In other studies with *Echinodorus grandiflorus* (Coelho, 2013), *Baccharis trimera*, *Baccharis articulata* (Fachinetto and Tedesco, 2009), *Caesalpinia echinata* (Bastos et al. 2011) and *Myracrodruon urundeuva* (Romano et al. 2013) were also found substances like flavonoids and tannins. These chemical components were attributed to the antiproliferative capacity presented by the researched plants mentioned above. Flavonoids exert a broad spectrum of health-beneficial biological activities, including the antiproliferative effect on cancer cells (Gibellini et al. 2011; Tsai et al. 2016), reaffirming the results obtained with the species *Myrocarpus frondosus*.

5. Conclusion

The aqueous extracts of leaves, bark and roots of *Myrocapus frondosus* at concentrations of 2.5 and 5.0 grams have antiproliferative effect on the cell division of *Allium cepa* meristematic cells.

Aqueous extracts from the 5.0 gram *Myrocapus frondosus* bark exhibit high antiproliferative capacity.

The antiproliferative activity found in the species *Myrocapus frondosus* may be associated with the presence of flavonoid and phenolic compounds in the plant tissue composition of the species.

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Conflict of interest statement

The authors declare no conflict of interest.

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Tables

Table 1. Treatments evaluated in the *Allium cepa* test.

Treatments
T1. Distilled water - Negative control.
T2. Glyphosate 2% - Positive control.
T3. Infusion of 2.5 g of dried leaves.
T4. Infusion of 5.0 g of dried leaves.
T5. Decoction of 2.5 g of dried bark.
T6. Decoction of 5.0 g of dried bark.
T7. Decoction of 2.5 g of dried roots.
T8. Decoction of 5.0 g of dried roots.

Table 2. Interphase, cell division and mitotic index in *Allium cepa* cells.

Treatments	Cells analyzed	Interphase cells	Dividing cells	MI (%)
T1. Distilled water	4000	3.755	245	6.12 a*
T2. Glyphosate 2%	4000	3.818	182	4.55 b
T3. 2.5 g de DL	4000	3.811	189	4.72 b
T4. 5.0 g de DL	4000	3.816	184	4.60 b
T5. 2.5 g de DB	4000	3.853	147	3.67 c
T6. 5.0 g de DB	4000	3.888	112	2.80 d
T7. 2.5 g de DR	4000	3.882	118	2.95 d
T8. 5.0 g de DR	4000	3.858	142	3.55 c
Total	32000	30681	1319	-

*Averages followed by the same letter in the column do not differ from each other by the $\chi 2$ test at a 5% error probability level. DL= dried leaf; DB= dried bark; DR= dried root; MI= mitotic index.

Figures

Figure 1. Cell cycle phases of *Allium cepa* with interphase cell and division cells. Interphase (A). Prophase (B). Metaphase (C). Anaphase (D). Telophase (E). Scale 10 μm.

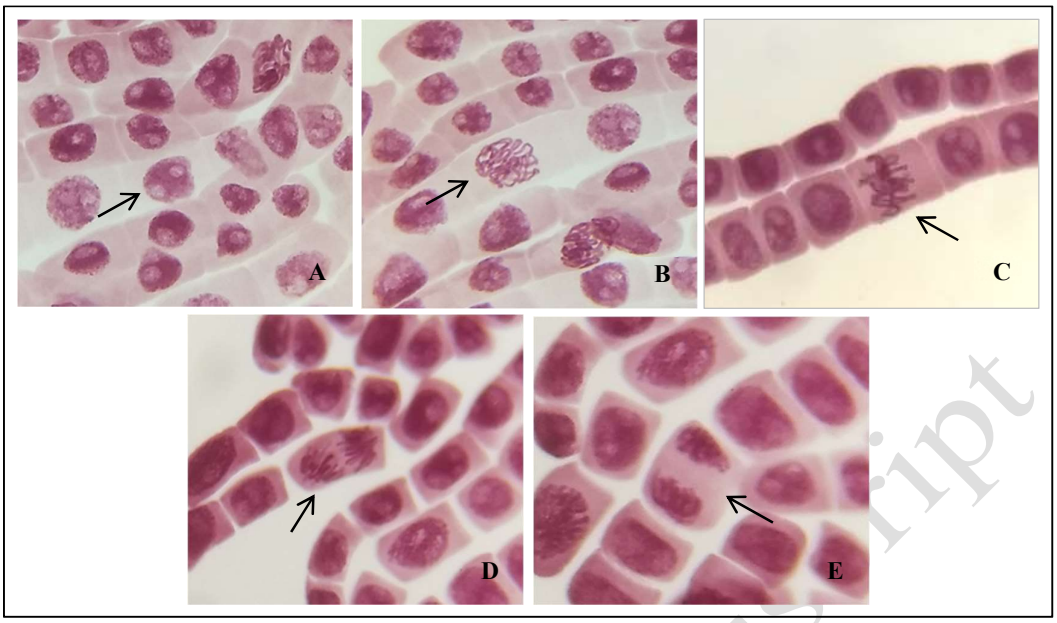

Figure 1