

## Absence of cytotoxic and genotoxic effects of the aqueous extract of *Stryphnodendron adstringens* (Barbatimão) bark using *Allium cepa* test

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### ABSTRACT

Barbatimão [*Stryphnodendron adstringens* (Mart.) Coville] is a plant of Mimosaceae family found in several regions of Brazil. Its bark is intensively used in folk medicine, mainly for its healing and antimicrobial actions. Medicinal plants could be dangerous to human health because some of them may have many toxicity products. The aim study was to investigate the cytotoxic and genotoxic effects of aqueous extract from the stem bark of barbatimão through *Allium cepa* test. The *A. cepa* seeds were watered with two concentrations (50 and 100 mg.mL<sup>-1</sup>) of barbatimão extract, a negative control (distilled water) and positive control (MNU solution 0.125 mg.L<sup>-1</sup>). It were analyzed 4000 cells per treatment and observed the number of cells in each phase of mitosis and changes in the cell cycle. Statistical analysis of the data was performed through analysis of variance (one-way ANOVA), with a probability level <0.05. About the cytotoxic effects, the four groups did not differ in the germination rate (p>0.05) but mean values of root length, weight and mitotic index were significantly lower in the positive control group (p<0.05). Regarding genotoxic effects, there were no significant differences between the barbatimão extract treatments and the negative control (p>0.05). At the positive control, however, there was a significant increase in micronucleus frequency (p=0.0042) and mitotic cycle anomalies (p=0.0076). It was concluded that the barbatimão extract did not present cytotoxic or genotoxic effects.

**Keywords:** medicinal plants; folk medicine; toxicity; infusion; ethnobotany.

### Ausência de efeitos citotóxicos e genotóxicos do extrato aquoso da casca de *Stryphnodendron adstringens* (Barbatimão) pelo teste de *Allium cepa*

### RESUMO

Barbatimão [*Stryphnodendron adstringens* (Mart.) Coville] é uma planta da família Mimosaceae encontrada em várias regiões do Brasil. Sua casca é intensamente usada na medicina popular, principalmente por suas ações de cicatrização e antimicrobianas. O uso de plantas medicinais pode ser perigoso à saúde humana devido eventualmente possuírem produtos tóxicos. O objetivo deste estudo foi investigar os efeitos citotóxicos e genotóxicos do extrato aquoso da casca do caule do Barbatimão através do teste *Allium cepa*. As sementes de *A. cepa* foram irrigadas com duas concentrações (50 e 100 mg.mL<sup>-1</sup>) de extrato de barbatimão, além de um controle negativo (água destilada) e um controle positivo (solução MNU 0,125 mg.L<sup>-1</sup>). Foram analisadas 4000 células por tratamento e observado o número de células em cada fase de mitose e alterações no ciclo celular. A análise estatística dos dados foi realizada através de análise de variância (ANOVA: um critério), com um nível de probabilidade <0,05. Em relação aos efeitos citotóxicos, os quatro grupos não diferiram na taxa de germinação (p>0,05), mas os valores médios do comprimento da raiz, dos pesos e do índice mitótico foram significativamente menores no grupo controle positivo (p<0,05). Quanto aos efeitos genotóxicos, não houve diferenças significativas entre os tratamentos do extrato de barbatimão em comparação com o controle negativo (p>0,05). No controle positivo, no entanto, houve um aumento significativo na frequência do micronúcleo (p=0,0042) e anomalias do ciclo mitótico (p=0,0076). Concluiu-se que o extrato de barbatimão não apresentou efeitos citotóxicos ou genotóxicos.

**Palavras-chaves:** plantas medicinais, medicina popular, toxicidade, infusão, etnobotânica.

### Introduction

Numerous natural products have been identified from the ethnopharmacological knowledge of some plants and phytotherapy are often the starting point for many research (ROCHA, 2013). Medicinal plants correspond to the oldest "weapons" used by man in the treatment of many diseases, that is, the use of plants in the prevention and/or cure of diseases is a habit that has always existed in the culture of humanity (MORAES; SANTANA, 2001). Currently, about 70% of the worldwide population uses medicinal plants in primary health care (FRESCURA et al., 2012).

Medicinal plants normally have their use generalized because many people understand that everything natural is

neither toxic nor harmful to health (CUNHA et al., 2012). It is an erroneous concept because there are a great variety of medicinal plants that endowed with high toxicity besides other detrimental properties to the human organism (CUNHA et al., 2012).

The Barbatimão (*Stryphnodendron adstringens*) is a plant of the Mimosaceae family common in the cerrado, with a wide geographical distribution, occurring in several states, from Pará crossing the Central Brazilian Plateau, to the north of Paraná (VASCONCELOS et al., 2004). Its bark is intensively used in folk medicine mainly for its cicatrizing and antimicrobial actions (FELFILI et al., 1999; PEREIRA et al., 2013). As this plant is present in the National List of

Medicinal Plants of Interest to the Brazilian National Health System (Rénisus), knowledge about its toxic effects has great importance to the community (Ministério da Saúde, 2009).

In onion bioassays (*Allium cepa*), after exposure in a certain period, it is possible to evaluate both the cytotoxic effects by the root growth reduction or mitotic index decrease and the genotoxic effects by the micronucleus analysis or cell division abnormalities (mitotic cycle changes) (FISKESJÖ; LEVAN, 1994; CHANDRA et al., 2005). Thus, the study described in this article used the *A. cepa* test system to investigate the cytotoxic and genotoxic effects of the aqueous extract from the stem bark of *Stryphnodendron adstringens* (Mart.) Coville like as used in the popular knowledge.

## Material and Methods

A completely randomized experimental design with two replicates was adopted, each experimental parcel using four Petri dishes. Intending to reproduce popular usage the bark was acquired in a local store of natural products located in the city of Belem (Pará, Brazil) where the herbs came from farmers from the amazon. Following one of the forms of popular knowledge preparation in Amazon Region, was obtained a concentration of 100 mg.mL<sup>-1</sup>. To prepare the barbatimão aqueous extract (infusion), pieces of the tree bark (25g) was immersed 30 minutes in distilled water (250mL) at room temperature (25°C); the extract was filtered and diluted to 50 mg.mL<sup>-1</sup> concentration and then stored under refrigeration (6°C-10°C) until the use in the bioassay.

The seeds were distributed in petri dishes (40 in each) lined with filter paper in two replicates. The groups were identified as: Positive Control (PC), watered with N-methyl-N-nitrosourea (MNU) solution at 0.125 mg.L<sup>-1</sup>; Negative control (NC), watered with distilled water; Treatment T1, treated with the aqueous extract at 50 mg.mL<sup>-1</sup>; Treatment T2, treated with the aqueous extract at 100 mg.mL<sup>-1</sup>, all seeds were watered daily. Some radicles from each group were collected and fixed in Carnoy solution (methanol: acetic acid = 3:1, v/v) until the slide preparation by the crushing technique (GUERRA; SOUZA, 2002). The slides were examined under an optical microscope and the cells observed with a 100x magnification.

Two thousand cells for each treatment were analyzed in both replicates, observing the cell numbers of interphase and the cell cycle phases (prophase, metaphase, anaphase, and telophase). The mitotic index (MI) was obtained by the following equation: MI = (m/T) x 100, where m = number of cells in mitosis and T = total cells analyzed (PIRES et al., 2001). The same slides were used to count the cell cycle changes: micronuclei (interphase abnormality), anaphase bridge, telophase bridge, vagrant chromosome and irregular metaphase (mitotic cycle abnormalities). (PINHO et al., 2010; COSTA et al., 2016). Statistical analysis was conducted through the Analysis of Variance (One-way ANOVA), with a level of probability < 0.05, through the statistical software BioEstat 5.0 (AYRES et al., 2007).

## Results and Discussion

Results for the germination rate, length, and rootlets weight are summarized in Table 1. Germination rate did not differ statistically (p>0.05) between the four groups, but the

radicles length and weight averages were significantly lower in the positive control.

**Table 1.** Effects of *Stryphnodendron adstringens* bark extract on germination rate, length, and weight of *Allium cepa* radicles.

Groups	Germination rate (%)	Length (cm)	Weight (mg)
Positive control (PC)	75.00 <sup>a</sup>	0.715 ± 0.37 <sup>b</sup>	7.205 ± 1.50 <sup>d</sup>
Negative control (NC)	71.25 <sup>a</sup>	2.755 ± 1.34 <sup>c</sup>	15.685 ± 6.50 <sup>e</sup>
Treatment (T1)	77.50 <sup>a</sup>	2.420 ± 1.12 <sup>c</sup>	13.675 ± 3.49 <sup>e</sup>
Treatment (T2)	71.25 <sup>a</sup>	3.225 ± 1.35 <sup>c</sup>	16.340 ± 5.22 <sup>e</sup>

Equal letters in the same column indicate absence of statistical difference between the groups at the level of p<0.05 (ANOVA).

Mitotic index analysis is an expressive parameter of cytotoxic effects from secondary metabolites, as it reveals alterations in cellular proliferation (CHAROENYING et al., 2010). Table 2 presents details of the mitotic index analysis in *A. cepa*, showing that treatments with the barbatimão bark extract did not significantly alter the cell cycle, although this occurred in the positive control.

**Table 2.** Frequencies of cell cycle phases (mean ± standard deviation) and Mitotic index (MI) in *Allium cepa* root cells exposed to the *Stryphnodendron adstringens* bark extract.

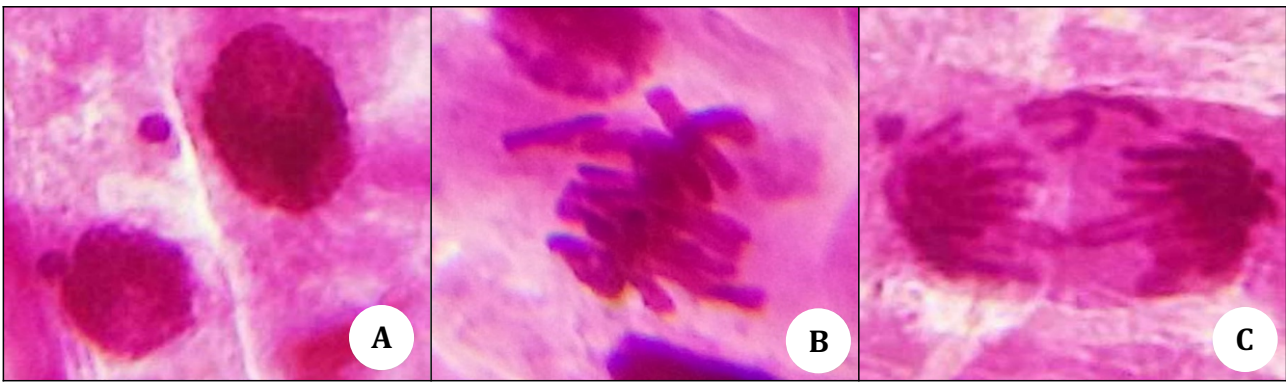
	Positive control	Negative control	Treatment (T1)	Treatment (T2)
Interphase	1970.5±6.5	1946.0±10.0	1920.0±1.0	1954.5±5.5
Prophase	12±2.0	28.5±0.5	38.0±4.0	22.5±0.5
Metaphase	5±2.0	5.0±4.0	15.0±2.0	9.5±0.5
Anaphase	3±1.0	4.0±0.0	4.5±1.5	5.0±1.0
Telophase	9.5±5.5	16.5±6.5	22.5±8.5	8.5±4.5
MI (%)	1.46 <sup>a</sup>	2.70 <sup>b</sup>	4.00 <sup>b</sup>	2.75 <sup>b</sup>
Total cells	4000	4000	4000	4000

Equal letters in the same line indicate absence of statistical difference between the groups at the level of p<0.05 (ANOVA).

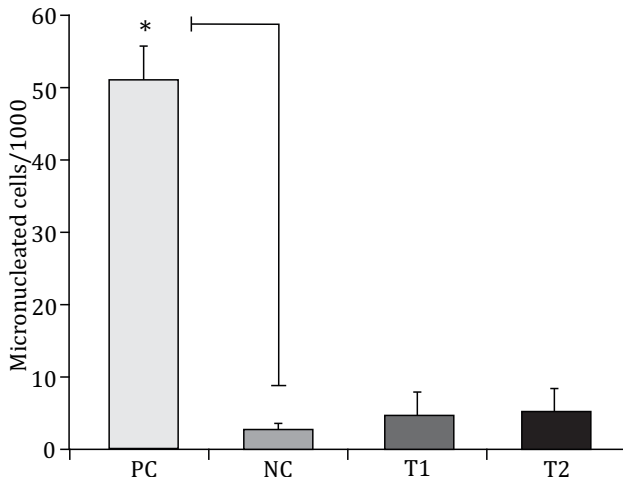
Plant extracts effects on the mitotic index in *A. cepa* root meristem vary according to the species tested. Iganci et al. (2006) concluded that aqueous extracts of *Plectranthus barbatus*, *P.amboinicus* and *Vernonia condensata* significantly increased mitotic index. However, Fachinetto et al. (2007) tested the toxicity of *Achyrocline satureioides* (marcela plant) and found that the mitotic index of the *A. cepa* cells decreased significantly in relation to the control. Jorge et al. (2009) obtained similar results in *Tuja occidentalis* (tuaia ou arbor vitae) and Giustina and Vieira (2010) in *Cinnamomum zeylanicum* (cinnamon).

On the other hand, Ribeiro et al. (2012) studied aqueous extracts phytotoxicity of barbatimão leaves in a bioassay with lettuce (*Lactuca sativa*). The extract presented higher phytotoxicity in view of the greater inhibition on germination and mitotic index. These results are due to the extract chemical composition, presenting high contents of total phenolics and flavonoids. This extract composition was probably due to its mode of preparation, infusion in distilled water at 90 °C for 30 minutes. For this reason, the results may have been different from ours, since in the present study the extract was prepared at room temperature, around 25 °C. In addition to that, the number and types of chemicals from different parts of the plant may be different (GLASENAPP, 2007).

Regarding the genotoxic effects, cell cycle abnormalities and micronucleus (Fig. 1 and Graph. 1), there were no significant differences between treatments with barbatimão extract and negative control (p > 0.05). In the positive control, however, there was a significant increase in the frequency of mitotic cycle abnormalities (p = 0.0076) and micronuclei (p = 0.0042).



**Figure 1.** Examples of cell cycle abnormalities found in the *Allium cepa* root meristem in the positive control: (A) cells with micronuclei; (B) metaphase with chromosomal loss; (C) anaphase bridge with chromosomal loss.



**Graphic 1.** Average numbers of micronucleated cells in the onion radicles meristem. Aqueous extract from Barbatimão bark does not induce genotoxicity in the *Allium cepa* assay. PC = positive control; NC = negative control; T1 = treatment with *S. adstringens* bark extract at 50 mg.mL<sup>-1</sup>; T2 = treatment with *S. adstringens* bark extract at 100 mg.mL<sup>-1</sup>. \* Statistical difference compared to negative control ( $p < 0.05$ ).

The micronuclei result from chromosomal fragments (clastogenesis) or whole chromosomes that, due to the anaphase incomplete migration, were excluded from the main nucleus (aneugenesis) (FENECH, 2000). Some onion cells exhibited fragments loss or whole chromosomes, which persisted into telophase, probably resulting in micronuclei.

Chiavegatto et al. (2017) and Pastori et al. (2015) evaluated genotoxic effects of plants in the root meristem of *A. cepa* and also reported the induction of delayed chromosomes during anaphase involving mitotic spindle damage and consequent production of micronuclei. Studies with mice also revealed genotoxic effects when a frequency increase of micronucleated polychromatic erythrocytes (MNPCE) was observed by using a 30 mg.mL<sup>-1</sup> aqueous extract concentration (CHAVES et al., 2017).

On the other hand, mice and *Artemia salina* larvae treated with an aqueous fraction of the stem bark did not present genotoxic effect. The mice treated with the highest concentration of 2250 mg.kg<sup>-1</sup> did not have a significant difference of micronucleated polychromatic erythrocytes (MNPCE) when compared to the control. Moreover, the extract fraction was shown to be antimutagenic, reducing the damage caused by cyclophosphamide. While *A. salina* larvae treated with 1000 mg.L<sup>-1</sup> had no inhibition of 50% of the population (COSTA et al., 2010).

Sousa et al. (2003) do not find mutations and recombination in somatic cells or chromosome damage in *Drosophila melanogaster* germ cells when treated with stem bark extracts. These diverse results in regarding barbatimão toxicity can be due to the different forms of extracts preparation, as well as the enormous amount of

different models studied. A good outline study using the same form of extract preparation and using it in several tests could be a more efficient way to determine the toxic effects that this plant could have.

### Conclusion

Nevertheless our results suggest, that the aqueous extract of barbatimão bark is not clastogenic, aneugenic and not even cytotoxic. Remembering, that toxicity of extracts obtained with other solvents, or from other parts of the plant, cannot be ruled out. In conclusion, the barbatimão bark solution did not present cytotoxic and genotoxic effects against the *A. cepa* test system.

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