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Toxicogenetic potential of *Mimosa pigra* (Fabaceae) infusion in *Allium cepa* meristematic cells

M.S. Araújo[1], S.P. Santos[2], B.A. Barros-Filho[3], M.M.O. Lima[4] and A.S. Leite[4*]

[1] Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, MG, Brasil
[2] Departamento de Fitotecnia, Universidade Federal do Piauí, Teresina, PI, Brasil
[3] Departamento de Química, Instituto Federal do Piauí, Parnaíba, PI, Brasil
[4] Departamento de Biologia, Instituto Federal do Piauí, Floriano, PI, Brasil

Corresponding author: A.S. Leite
E-mail: aracellileite@ifpi.edu.br

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ABSTRACT. *Mimosa pigra* is a plant commonly used for medicinal purposes in the treatment of several diseases. However, studies regarding its toxicological properties are scarce. We evaluated the toxic, cytotoxic, genotoxic and mutagenic activities of leaf and stem infusions of *M. pigra* collected in the region of Delta do Parnaíba, Piauí, Brazil, through an *in vivo Allium cepa* test. Three concentrations of leaf and stem treatments were used (leaf treatment 1 and stem treatment 1 = 0.771 g/L; leaf treatment 2 and stem treatment 2 = 1.542 g/L; leaf treatment 3 and stem treatment 3 = 3.084 g/L), in addition to the negative controls (water) and positive controls (copper sulfate - 1.2 mg/mL - *A. cepa* test). All the treatments were toxic, causing significant root growth inhibition. Leaf treatment 1, leaf treatment 3 and stem treatment 1 showed significant inhibition of the mitotic index. Leaf treatment 2, stem treatment 1 and stem treatment 3 showed significant genotoxic activity based on the frequency of chromosomal aberrations. The tested concentrations did not provoke significant mutagenic activity

when compared to the negative control. We suggest that further studies with other biological systems are needed to evaluate the safetyness of *M. pigra* infusions for therapeutic purposes.

Key words: *Mimosa pigra*; Cytotoxic; Genotoxic; Mutagenic

INTRODUCTION

The use of medicinal plants for therapeutic purposes is an ancient tradition that is still widely practiced (Bermejo-Villanueva and Fornari, 2017; Mishra et al., 2018). According to the World Health Organization (WHO), more than 60% of the world population and about 80% of developing countries use plants as a medicine to treat human diseases (WHO, 2013; Sharifi-Rad et al., 2018). As a result of this practice, the scientific community has been performing toxicological studies to verify the real effectiveness of these natural resources, in order to promote their safe use (Melo et al., 2017).

Due to the climatic and geomorphological conditions of Brazil, the country has been considered a natural collection of great importance to the planet, presenting wide variability of biomes (Sousa et al., 2012). The Fabaceae family is the third largest plant family in the world due to its wide geographical distribution, being found throughout the country. The subfamilies Mimosoidae and Caesalpinioideae are commonly found in tropical and subtropical regions (Forzza et al., 2010).

Mimosa pigra is popularly known in Brazil as calumbi d'água, giquiri, malissa-de-boi and other names (Silva, 2004; Souza, 2012). The species has its origin center located in tropical America and it is also found in several tropical and subtropical regions of the world (Paynter, 2005). In Brazil, *M. pigra* is distributed in many regions of the country, with reports of its medicinal use by the population (Mota, 1997; Okonkwo et al., 2016; Oliveira et al., 2016). The medicinal use is for treatment of headaches, diarrhea, cold, and heart problems, due to the plant's antimicrobial activity (Grosvenor and Supriono, 1995; Rosado-Vallado et al., 2000), and to the plant's antioxidant and anti-inflammatory activities (Rakotomalala et al., 2013).

The municipality of Ilha Grande is the largest fluvial-marine island in Delta do Parnaíba River, located between the States of Piauí and Maranhão. It has the appearance of a floodable and non-floodable sandbank nucleated by tree species (Santos-Filho et al., 2015). In a floristic study performed at the region of Delta do Parnaíba, it was observed that 10% of the population was composed of representatives of the genus *Mimosa*. The species *M. pigra* was found frequently in humid and swampy environments (Guzzi, 2012).

The use of plants as bioindicators is widely practiced in toxicological studies (Biruk et al., 2017; Bortolotto et al., 2017; Ghosh et al., 2017; Abdelsalam et al., 2018; Mercado and Caleño, 2020). Among the widely used plants, *A. cepa* was one of the most reported in the literature (Leite et al., 2015; Silva and Monteiro, 2017; Verma and Srivastava, 2018). It is an important bioindicator of *in vitro* cytogenotoxicity, widely indicated for the assessment of environmental risks (Garcia et al., 2017; Mercado and Caleño, 2020), and data can be extrapolated to animal models (Mohammed et al., 2015; Pathiratne et al., 2015; Rodríguez et al., 2015; Haq et al., 2017; Rahman et al., 2017; Datta et al., 2018; Verma and Srivastava, 2018; Maity et al., 2020), due to its high sensitivity in detecting genotoxic and mutagenic activities (Leme and Marin-Morales, 2009; Khan et al., 2019).

It is possible to evaluate some macroscopic parameters using the *A. cepa* test, such as toxicity, cytotoxicity, genotoxicity and mutagenicity (Leme and Marin-Morales, 2009; Mercado and Caleño, 2020). Toxic activity is assessed when root growth is inhibited (Leite et al., 2015; Bolonhesi and Lopes, 2018), while cytotoxic activity is verified when mitotic index is inhibited (Mercado and Caleño, 2020; Mercado and Bayona, 2020). Genotoxicity is assessed through the frequency of chromosomal aberrations (Khan et al., 2020), while mutagenicity is evaluated through the micronucleus frequency (Silva et al., 2013). Thus, the *A. cepa* system is a key *in vivo* model in the evaluation of natural and synthetic substances, capable of identifying damage at the cellular level (Leite et al., 2015).

As little research has been conducted towards the evaluation of *M. pigra* biological activities, an understanding about the therapeutic properties of this plant is necessary. Therefore, we evaluated the toxicity, cytotoxicity, genotoxicity and mutagenicity of the *M. pigra* plant infusion in an *A. cepa* assay.

MATERIAL AND METHODS

Plant material

The species *M. pigra* was collected in the region of Delta do Parnaíba, Piauí, Brazil (latitude 02º54'17" S, longitude 41º46'36" W, and altitude 5 m). After collection, the plant material (a wild specimen) was taken to the Chemistry Laboratory of the Federal Institute of Piauí (IFPI), where the leaves and stems were separated. The plant material was dried at room temperature until reaching constant mass and subsequently crushed.

Preparation of plant extract

To prepare the extract, dried and crushed leaves (35 g) and stems (72.4 g) of *M. pigra* were extracted three times with ethanol at room temperature. After extraction, the organic solvent was removed on a rotary evaporator (802 - Fisatom model) under reduced pressure and at temperatures ranging from 40º to 50ºC to obtain the ethanolic extract of leaves (4.37 g; 12.48% yield) and the ethanolic extract of stems of *M. pigra* (3.08 g; 4.25% yield).

$$Yi = (M_{extract} / M_{dry\ matter}) \times 100\% \quad \text{(Eq. 1)}$$

In which:
Yi = Total extract yield (%);
$M_{extract}$ = Extract mass (g);
$M_{dry\ matter}$ = Dry leaf mass (g).

Preparation of Infusions

For preparation of infusions, three concentrations of leaves and stems of *M. pigra* were used (leaf treatment 1 and stem treatment 1 = 0.771 g/L; leaf treatment 2 and stem treatment 2 = 1.542 g/L; leaf treatment 3 and stem treatment 3 = 3.084 g/L), measured according to the recommended dose of industrialized tea sachets. Similar concentrations

have been used in the preparation of teas, which may vary depending on the manufacturer (Bagatini et al., 2009; Nishiyama et al., 2010). The infusions were prepared with drinking water, in order to accurately simulate the conditions performed by people who use the tea. The volume used was 200 mL of water at 100°C, which was poured over the extracts. The infusion time established was 10 min for both leaves and stems, since higher infusion periods are not indicated for preparation of teas (Infante et al., 2010; Koch et al., 2012).

Allium cepa Test

The test followed the Fiskesjö protocol (1985), with adaptations proposed by Leite et al. (2015). Small, healthy and uniformly sized onions purchased at the Floriano (PI) market were used. The bulbs were cleaned and placed under running water for 15 min and prepared to germinate, with the bottom immersed in the test solutions. Dechlorinated water was used as a negative control, while copper sulfate (1.2 mg/mL) was used as a positive control. The Dechlorinated water was obtained through an aerating sprayer with the aid of an activated carbon filter, used for a period of 24 h on an open surface. This negative control is used because it does not cause damage to cells, unlike copper sulfate, which has a cytotoxic effect (Leite et al., 2015). The dead outer scale leaves of the bulbs were removed without damaging the primary roots to promote the growth of new roots. The onions were placed in contact with each dose of treatments. For each treatment, five onion bulbs were used. After 48 h of exposure in a dark environment at room temperature, the roots were measured with the aid of a ruler. Subsequently, the onion roots of each control were cut and placed in Carnoy solution and stored in 70% ethanol solution.

The roots were washed three times with distilled water for five minutes and subsequently placed in 1N HCl solution for 11 min. After being washed with distillated water, the roots were kept in dark flasks containing Schiff's reagent for two hours and then washed to remove excess dye. For slide preparation, a meristematic root region of approximately 1 mm was isolated, using forceps and scalpel, and placed on a slide. A drop of 2% acetic carmine was added to the sample, using squashing technique, and a coverslip was placed above it. The material was taken to the microscope (1000) for analysis of cytotoxicity, genotoxicity and mutagenicity. The parameters observed were mitotic index, frequency of chromosomal aberrations in anaphase and telophase and *M. pigra* frequency of Micronuclei in 1000 cells. Mitotic index, frequency of cells with chromosomal aberrations and Micronuclei were obtained based on equation 2, 3 and 4, respectively.

$$\text{Mitotic index} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \text{x}100 \quad \text{(Eq. 2)}$$

$$\%\ \text{Chromosomal aberrations} = \frac{\text{Number of chromosomal aberrations}}{\text{Total number of cells}} \text{x}100 \quad \text{(Eq. 3)}$$

$$\%\ \text{Micronuclei} = \frac{\text{Total number of micronuclei}}{\text{Total number of cells in interphase}} \text{x}100 \quad \text{(Eq. 4)}$$

Statistical analysis

The results were presented as mean ± standard deviation (SD). The data were analyzed through Analysis of Variance (ANOVA) followed by the Tukey test, using the

GraphPad Prism 7.0 software (Intuitive Software for Science, San Diego, C.A.) and considering the probability $P < 0.05$ as the confidence level.

RESULTS AND DISCUSSION

The tested concentrations of *M. pigra* leaf and stem infusion showed toxic effects when compared to the negative control (leaf treatment 1 $P < 0.001$; leaf treatment 2, leaf treatment 3, stem treatment 1, stem treatment 2 and stem treatment 3 $P < 0.0001$), promoting the reduction of root growth. Leaf treatment 1, leaf treatment 3 and stem treatment 1 showed significant differences ($P < 0.05$) for inhibition of mitotic index in *A. cepa* roots, reflecting the presence of cytotoxic effects. Significant values ($P < 0.05$) were also found for chromosomal aberrations measured in leaf treatment 2, stem treatment 1 and stem treatment 3. No significant mutagenic effects ($P > 0.05$) were found for the tested concentrations of *M. pigra* leaf and stem infusion (Table 1).

Table 1. Toxic, cytotoxic, genotoxic and mutagenic effects of *Mimosa pigra* leaf and stem infusions observed by root growth, inhibition of mitotic index, frequency of chromosomal aberrations and frequency of micronuclei in *Allium cepa.*

Treatments	Toxicity (Root length (cm))	Mitotic index (Cells in division/1000)	Frequency of Chromosomal Aberrations (%)	Frequency of Micronuclei (%)
NC[a]	1.3 ± 0.5	54.8 ± 13.6	0.9 ± 0.3	1.5 ± 0.7
PC[b]	0.1 ± 0.08***	11.3 ± 4.8***	2.8 ± 0.4***	6.0 ± 1.4
Leaf treatment 1 (0.771g/L)	0.7 ± 0.5**	09.1 ± 4.7*	0.4 ± 0.2	4.0 ± 0.0
Leaf treatment 2 (1.542g/L)	0.3 ± 0.2***	24.7 ± 13.7	0.06 ± 0.08*	2.0 ± 0.0
Leaf treatment 3 (3.084g/L)	0.6 ± 0.3***	04.0 ± 3.7*	0.0 ± 0.0	6.0 ± 5.6
Stem treatment 1 (0.771g/L)	0.3 ± 0.2***	12.3 ± 5.7*	0.02 ± 0.04*	3.5 ± 3.5
Stem treatment 2 (1.542g/L)	0.3 ± 0.2***	21.9 ± 16.6	0.0 ± 0.0	1.5 ± 0.7
Stem treatment 3 (3.084g/L)	0.3 ± 0.2***	20.8 ± 17.0	0.10 ± 0.12*	8.5 ± 6.3

Values are expressed as mean ± standard deviation (SD); [a]NC (Negative Control) = Dechlorinated Water; [b]PC (Positive Control) = Copper Sulfate (1.2 mg/L); * = $P < 0.05$; ** = $P < 0.001$; *** = $P < 0.0001$ ANOVA and Tukey test.

Reduction of the mitotic index is an important indicator of the cytotoxic action suffered by the cell, directly reflecting on the toxicity of compounds tested in biological systems. This toxicity can cause interferences in the replication of DNA and protein synthesis (Tkalec et al., 2009). Potentially phytotoxic substances in certain plants has been investigated, even allowing the extraction of these compounds for application against weeds in agriculture (Dayan et al., 2012). When evaluating the crude methanolic extract of *M. pigra* for fungicidal action, De Morais et al. (2017) identified flavonoid heterosides and aglycones as possible substances responsible for the antifungal activity. In addition, the authors reported that toxicogenetic studies for this specimen are still scarce.

In a similar study, Koodkaew et al. (2018) identified that the extract of *M. pigra* had phytotoxic effect, causing inhibition of the growth of meristematic cells of lettuce (*Lactuca sativa*) and snap pod (*Ruellia tuberosa*). Such inhibition was proportional to the increase in extract concentrations. The reduction in root length may be related to changes in the cell cycle, which can also be proven by the mitotic index and the analysis of chromosomal aberrations (Pittol et al., 2016).

Cytotoxicity can be evaluated by observing mitotic index inhibition, which can be assessed by the significant increase in frequency of cells with chromosomal aberrations. This index is considered an efficient parameter to estimate the frequency of cells in cell division (Fernandes et al., 2007; Leme and Marin-Morales, 2009). It was observed that all mitotic indices were lower than those of the negative control. According to Hoshina (2002) and to Caritá and Marin-Morales (2008), this result indicates that the growth and development of the exposed organism has been affected by the tested compound. Suppression of mitotic index may occur probably due to blocking of G1 or G2 phases, or to suppression of DNA synthesis, preventing cells from starting mitosis (Feretti et al., 2008; Griffiths et al., 2016). In addition, it can inhibit microtubule formation, nucleoprotein synthesis and it can reduce levels of Adenosina triphosphate (ATP), necessary for achromatic spindle elongation, chromosomal movement and microtubule dynamics (Majewska et al., 2003).

In a similar study, toxicogenetic effects of organic green tea and fennel tea infusions in three concentrations were evaluated using the *A. cepa* test. A significant inhibition of growth and root mitotic index ($P < 0.001$) was found in relation to the negative control (Reis et al., 2012). Sumitha and Thoppil (2016) obtained similar results when assessing the cytotoxic potential of polar and nonpolar extracts in *Hyptis suaveolens* and *Leucas indica*, using the *A. cepa* test. They observed a significant reduction in the mitotic index compared to the negative control, in addition to an increase in cells with chromosomal aberrations, when the extract concentration increased.

This study showed genotoxic activity of the tested *M. pigra* concentrations. The presence of chromosomal aberrations during the cell division process is a direct indication of DNA damage, which could not be easily repaired by the cell (Jolanta and Jolanta, 2005). This macroscopic parameter is considered a reliable indication of mutagenic activity (Mohandas and Grant, 1972). There is evidence of correlation between chromosomal damage and toxic effects of extracts on different plants (Sumitha and Thoppil, 2016), whose results may be correlated with results obtained in mammals (Nilüfer et al., 2008).

The formation of micronuclei is an excellent indicator of mutagenicity, chemical genotoxicity and aneugenic effects (Al-Sabti and Metcalfe, 1995; Khanna et al., 2016; Fatma et al., 2018). The absence of micronuclei based on the three concentrations of stem and leaves is indicative that there were no significant chromosomal alterations.

Species of the *Mimosa* genus have demonstrated cytotoxic activities in some tumor cell lines from colon (HCT-116), ovary (OVCAR-8) and glioblastoma (SF-295) by the MTT test in *M. caesalpiniifolia*. Deoxiflavone found in *M. diplotricha* has also shown a cytotoxic effect (Lin et al., 2011). *M. paraibana* also exhibited cytotoxic activity through the *Artemia salina* test (Nunes et al., 2008). Apoptosis can be initiated with cell cycle activation (Marks-Honczalik et al., 1998), cytokine production (Neurath et al., 1998), infectious agents (Shibata et al., 1999), or as an adverse response to medications (Fiorucci et al., 1999). However, it has also been reported that *Mimosa* species constituents have antioxidant activity (Nunes et al., 2008; Rakotomalala et al., 2013).

FINAL CONSIDERATIONS

The concentrations tested for *M. pigra* leaf and stem infusion showed toxic effects in all controls. When evaluating cytotoxic effects, a significant inhibition of the mitotic

index was observed in leaf treatment 1, leaf treatment 3 and stem treatment 1 when compared to the negative control. Furthermore, leaf treatment 2, stem treatment 1 and stem treatment 3 showed significant genotoxic activity due to their frequency of chromosomal aberrations.

Therefore, it is necessary to make an evaluation through other genotoxicity biomarkers and an identification of the plant compounds that may have this effect. The conduction of more detailed studies is required in order to achieve a less harmful concentration, using other biological tests to effectively assess the applicability of the infusion of *M. pigra*, obtained in the Delta do Parnaiba region for therapeutic purposes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdelsalam NR, Abdel-Megeed A, Ali HM, Salem MZM, et al. (2018). Genotoxicity effects of silver nanoparticles on wheat (*Triticum aestivum* L.) root tip cells. *Ecotoxicol. Environ. Saf.* 155: 76-85.

Al-Sabti K and Metcalfe CD (1995). Fish micronuclei for assessing genotoxicity in water. *Mutat. Res.* 343: 121-135.

Bagatini MD, Fachinetto JM, Silva ACF, Tedesco SB, et al. (2009). Cytotoxic effects of infusions (tea) of *Solidago microglossa* DC. (Asteraceae) on the cell cycle of *Allium cepa*. *Rev. Bras. Farmacogn*. 19: 632-636.

Bermejo-Villanueva D and Fornari T (2017). Recent advances in the processing of green tea biomolecules using ethyl lactate. A review. *Trends Food Sci. Technol.* 62: 1-12.

Biruk LN, Moretton J, Abrizio A, Weigandt C, et al. (2017). Toxicity and genotoxicity assessment in sediments from the Matanza Riachuelo river basin (Argentina) under the influence of heavy metals and organic contaminants. *Ecotoxicol. Environ. Saf.* 135: 302-311.

Bolonhesi IBTM and Lopes DD (2018). Analysis of toxicity from the effluent generated in a furniture industry spray booth using the species *Lactuca sativa* and *Allium cepa*. *Rev. Ambient. Água*. 13: 2246.

Bortolotto T, Da Silva J, Sant'Ana AC, Tomazi KO, et al. (2017). Evaluation of toxic and genotoxic potential of a wet gas scrubber effluent obtained from wooden-based biomass furnaces: a case study in the red ceramic industry in southern Brazil. *Ecotoxicol. Environ. Saf.* 143: 259-265.

Caritá KV and Marin-Morales JE (2008). Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*. 72: 722-725.

Datta S, Singh J, Singh J, Singh S, et al. (2018). Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test. *Sustain. Environ. Res.* 28: 171-178.

Dayan FE, Owens DK and Duke SO (2012). Rationale for a natural products approach to herbicide discovery. *Pest Manag. Sci.* 68: 519-528.

De Morais CB, Scopel M, Pedrazza GPR, et al. (2017). Anti-dermatophyte activity of Leguminosae plants from Southern Brazil with emphasis on *Mimosa pigra* (Leguminosae). *J. Mycol. Med*. 27: 530-538.

Fatma F, Verma S and Kamal A (2018). Monitoring of morphotoxic, cytotoxic and genotoxic potential of mancozeb using *Allium* assay. *Chemosphere*. 195: 864-870.

Feretti D, Zerbini I, Ceretti E, Villarini H, et al. (2008). Evaluation of chlorite and chlorate genotoxicity using plant bioassays and in vitro DNA damage tests. *Water Res.* 42: 4075-408.

Fernandes TCC, Mazzeo DEC and Marin-Morales MA (2007). Mecanismo de formação de micronúcleos em células poliploidizadas de *Allium cepa* expostas ao herbicida trifluralina. *Pestic Biochem Physiol*. 88: 252-259.

Fiorucci S, Antonelli E, Santucci G, Morelli Ó, et al. (1999). Gastrointestinal safety of nitric oxide-derived aspirin is related to inhibition of ICE-like cysteine proteases in rats. *Gastroenterology*. 116: 1089-1106.

Fiskesjö G (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas*. 102: 99-112.

Forzza RC, Leitman PM, Costa A, Carvalho Jr. AA, et al. (2010). Catálogo de plantas e fungos do Brasil. 2 ed. Instituto de Pesquisas Jardim Botânico, Rio de Janeiro.

Garcia C, Souza R, Souza C, Christofoletti C, et al. (2017). Toxicity of two effluents from agricultural activity: comparing the genotoxicity of sugar cane and orange vinasse. *Ecotoxicol. Environ. Saf.* 142: 216-221.

Ghosh P, Thakurb I and Kaushik A (2017). Bioassays for toxicological risk assessment of landfill leachate: a review. *Ecotoxicol. Environ. Saf.* 141: 259-270.

Griffiths AJF, Susan RW, Carroll SB, Doebley J, et al. (2016). Introdução à genética. 11 ed. Guanabara Koogan, Rio de Janeiro.

Grosvenor AC and Supriono AG (1995). Medicinal plants from Riau province, Sumatra, Indonesia. Part 2: Antibacterial e Antifungical activity. *Ethnopharmacol*. 45: 97-111

Guzzi A (2012). Biodiversidade do Delta do Parnaíba: litoral piauiense. Edufpi, Parnaíba.

Haq I, Kumar S, Raj A, Lohani M, et al. (2017). Genotoxicity assessment of pulp and paper mill effluent before and after bacterial degradation using *Allium cepa* test. *Chemosphere*. 169: 642-650.

Hoshina MM (2002). Evaluation of a possible contamination of the waters of the Claro River municipality of Rio Claro, part of the Corumbatai River basin, with the mutagenicity tests using *Allium cepa*. State University of Sao Paulo, Rio Claro, SP

Infante R, Rubio P, Contador, L, Moreno V, et al. (2010). Effect of drying process on lemon verbena (*Lippia citrodora kunth*) aroma and infusion sensory quality. *Food Sci. Technol.* 45: 75-80.

Jolanta M. and Jolanta J (2005). Plant Genotoxicity: A Molecular Cytogenetic Approach in Plant Bioassays. *Arh. Hig. Rada Toksikol*. 56: 177-184.

Khan IS, Ali MN, Hamid R and Ganie SA (2020). Genotoxic effect of two commonly used food dyes metanil yellow and carmoisine using *Allium cepa* L. as indicator. *Toxicol. Rep*. 7: 370-375.

Khan S, Anas M and Malik A (2019). Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays. *Toxicol. Rep*. 6: 193-201.

Khanna N, Rauh LA, Lachiewicz MP, Horowitz IR, et al. (2016). Margins for cervical and vulvar cancer. *J. Surg. Oncol.* 113: 304-309.

Koch AS, Mullera M, Joubert E, Rijst MVD, et al. (2012). Sensory characterization of rooibos tea and the development of a rooibos wheel and lexicon. *Food Res. Inter*. 46: 217-228.

Koodkaew I, Senaphan C, Sengseang N, Suwanwong S, et al. (2018). Characterization of phytochemical profile and phytotoxic activity of *Mimosa pigra* L. *Agric. Nat. Res*. 52: 162-168.

Leite AS, Dantas AF, Oliveira GL, Gomes Júnior AL, et al. (2015). Evaluation of toxic, cytotoxic, mutagenic, and antimutagenic activities of natural and technical cashew nut shell liquids using the *Allium cepa* and *Artemia salina* bioassays. *Biomed Res. Int*. 2015: 1-16.

Leme DM and Marin-Morales MA (2009). *Allium cepa* test in environmental monitoring: a review on its application. *Mutat. Res*. 682: 71-81.

Lin LC, Chiou CT and Cheng JJ (2011). 5-Deoxyflavones with cytotoxic activity from *Mimosa diplotricha*. *J. Nat. Prod.* 74: 2001-2004.

Maity S, Chatterjee A, Guchhait R, De S, et al. (2020). Cytogenotoxic potential of a hazardous material, polystyrene microparticles on *Allium cepa* L. *J. Hazard. Mater*. 385: 121-560.

Majewska A, Wolska E, Śliwińska E, Furmanowa M, et al. (2003). Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhodiola rosea* roots. *Caryologia*. 56: 337-351.

Marks-Honczalik J, Chu SC and Moss J (1998). Cytokine-mediated Transcriptional Induction of the Human Inducible Nitric Oxide Synthase Gene Requires Both Activator Protein 1 and Nuclear Factor kappaB-binding Sites. *J. Biol. Chem.* 273: 22201-22208.

Melo CR, Lira AB, Alves MF, Lima CMBL, et al. (2017). O uso de plantas medicinais para doenças parasitárias. *Rev. Acta Bras.* 1: 28-32.

Mercado SAS and Bayona HAM (2020). Evaluation of the cytotoxic potential of sodium hypochlorite using meristematic root cells of *Lens culinaris*. *Med. Sci. Total Environ*. 701: 134992.

Mercado SAS and Caleño JDQ (2020). Cytotoxic evaluation of glyphosate, using *Allium cepa* L. as bioindicator. *Sci Total Environ*. 700: 134452.

Mishra AP, Sharifi-Rad M, Shariati MA, Mabkhot YN, et al. (2018). Bioactive compounds and health benefits of edible Rumex species-A review. *Cell. Mol. Biol.* 64: 27-34.

Mohammed KP, Aarey A, Tamkeen S, Jahan, P, et al. (2015). Forskolin: genotoxicity assessment in *Allium cepa*. *Mutat. Res*. 777: 29-32.

Mohandas T and Grant WF (1972). Cytogenetic effect of 2, 4-D and amitrol in relation to nuclear volume and DNA content in some higher plants. *Can. J. Genet. Cytol*. 14: 773-778.

Mota CN (1997). Os filhos de Jurema na floresta dos espíritos: ritual e cura entre dois grupos indígenas do norteste brasileiro. Edufal, Maceió.

Neurath MF, Becker C and Barbulescu K (1998). Role of NF-kappa B in immune and inflammatory responses in the gut. *Gut*. 43: 856-860.

Nishiyama MF, Costa MAF, Costa AM, Souza CGM, et al. (2010). Chá verde brasileiro (*Camellia sinensis* var assamica): efeitos do tempo de infusão, acondicionamento da erva e forma de preparo sobre a eficiência de extração dos bioativos e sobre a estabilidade da bebida. *Ciênc. Tecnol. Aliment*. 30: 191-196.

Nilüfer A, Serap C, Senay S, Dilek Y, et al. (2008). Evaluation of clastogenicity of 4,6-Dinitro-o-cresol (DNOC) in *Allium* root tip test. *J. Biol. Environ. Sci.* 2: 59-63.

Nunes XP, Mesquita RF, Silva DA, Lira DP, et al. (2008). Constituintes químicos, avaliação das atividades citotóxica e antioxidante de *Mimosa paraibana* Barneby (Mimosaceae). *Rev. Bras. Farmacogn.* 18: 718-723.

Okonkwo CJ, Njoku OU, Okonkwo TJN, Afieroho OE, et al. (2016). Two new acylated flavonol glycosides from *Mimosa pigra* L. leaves sub-family Mimosoideae. *Future J. Pharm. Sci*. 2: 71-75.

Oliveira MS, Silva EO, Ferreira AWC and Guarçoni EAE (2016). Conhecimento e uso tradicional das espécies madeireiras e medicinais utilizadas no município de Aldeias Altas, Maranhão, Brasil. *Enciclop. Biosf.* 13: 1160-1173.

Pathiratne A, Hemachandra CK and De Silva N (2015). Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. *Environ. Monit. Assess.* 187: 1-12.

Paynter Q (2005). Evaluating the impact of a biological control agent Carmenta mimosa on the woody wetland weed *Mimosa pigra* in Australia. *J. Appl. Ecol.* 42: 1054-1062.

Pittol M, Tomacheski D, Simões DN, Ribeiro VF, et al. (2017). Macroscopic effects of silver nanoparticles and titanium dioxide on edible plant growth. *Environ. Nanotechnol. Mon. Manage*. 8: 127-133.

Rahman MM, Rahman MF and Nasirujjaman K (2017). A study on genotoxicity of textile dyeing industry effluents from Rajshahi, Bangladesh, by the *Allium cepa* test. *J. Chemical Ecol.* 33: 434-446.

Rakotomalala G, Agard C, Tonnerre P, Tesse A, et al. (2013). Extract from *Mimosa priga* attenuates chronic experimental pulmonar hypertension. *J. Ethnopharmacol.* 148: 106-116.

Reis TA, Luz EB, Araújo TB, Rocha LSS, et al. (2012). Análises tóxica, citotóxica e genotóxica de Infusões de *Pimpinella anisum* através do teste *Allium cepa*. In: VII Congresso de Pesquisa e Inovação da Rede Norte Nordeste de Educação Tecnológica, Palmas.

Rodríguez YA, Christofoletti CA, Pedro J, Bueno OC, et al. (2015). *Allium cepa* and *Tradescantia pallida* bioassays to evaluate effects of the insecticide imidacloprid. *Chemosphere*. 120: 438-442.

Rosado-Vallado M, Brito-Loeza W, Mena-Rejón GJ, Quintero-Marmol E, et al. (2000). Antimicrobial activity of Fabaceae species used in Yucatan traditional medicine. *Fitoterapia*. 71: 570-573.

Santos-Filho F, Almeida Jr. E, Lima P, Soares C, et al. (2015). Checklist of the flora of the restingas of Piauí State, Northeast Brazil. *Check List*. 11: 1598-1608.

Sharifi-Rad M, Roberts TH, Matthews KR, Bezerra CF, et al. (2018). Etnobotânica do gênero Taraxacum - fitoquímicos e atividade antimicrobiana. *Phytother Res.* 32: 2131-2145.

Shibata J, Goto H, Arisawa T, Niwa Y, et al. (1999). Regulation of tumor necrosis factor (TNF)-induced apoptosis by soluble TNF-receptors in *Helicobacter pylori* infections. *Gut.* 45: 24-31.

Silva GH and Monteiro RTR (2017). Toxicity assessment of silica nanoparticles on *Allium cepa*. *Ecotoxicol. Environ. Contam.* 12: 25-31.

Silva MF (2004). Nomes populares das Leguminosas do Brasil. Edua, Manaus.

Silva VA, Gonçalves GF, Pereira MSV, Gomes IF, et al. (2013). Assessment of mutagenic, antimutagenic and genotoxicity effects of *Mimosa tenuiflora*. *Rev. Bras. Farmacogn*. 23: 329-334

Sousa RS, Hanazaki N, Lopes JB, Barros RFM, et al. (2012). Are Gender and Age Important in Understanding the Distribution of Local Botanical Knowledge in Fishing Communities of the Parnaíba Delta Environmental Protection Area?. *Ethnobot. Res. Appl*. 10: 551-559.

Souza LAG (2012). Guia da biodiversidade de Fabaceae do Alto Rio Negro. Fronteiras, Manaus.

Sumitha KV and Thoppil JE (2016). Genotoxicity assessment of two common curing weeds: *Hyptis suaveolens* (L.) Poir. and *Leucas indica* (L.) R. Br. *Cytotechnology*. 68: 1513-1527.

Tkalec M, Malarić K, Pavlica M, Pevalek-Kozlina B, et al. (2009). Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of *Allium cepa* L. *Mutat. Res*. 672: 76-81.

Verma S and Srivastava A (2018). Morphotoxicity and cytogenotoxicity of pendimethalin in the test plant *Allium cepa* L. A biomarker based study. *Chemosphere*. 206: 248-254.

WHO (World Health Organization) (2013). WHO strategy on Traditional Medicine 2014-2023. WHO, EUA.