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Original Article

Phytotoxicity and cytogenetic action mechanism of leaf extracts of *Psidium cattleyanum* Sabine in plant bioassays

Fitotoxicidade e mecanismo de ação citogenético de extratos foliares de *Psidium cattleyanum* Sabine em bioensaios vegetais

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Abstract

The search for more environmental friendly herbicides, aiming at the control of agricultural pests, combinated with less harmfulness to human health and the environment has grown. An alternative used by researchers is the application of products of secondary plant metabolism, which are investigated due to their potential bioactivities. Thus, species belonging to the Myrtaceae family are potential in these studies, since this family is recognized for having high biological activity. A species belonging to this genus is *Psidium cattleyanum*, which has a medicinal effect and its fruits are used in human food. Thus, the objective of this research was to evaluate and compare the phyto-cyto-genotoxicity of aqueous and ethanolic leaf extracts of the specie *P. cattleyanum*, from plant bioassays, as well as to identify the main classes of compounds present in the extracts. For this, the extracts were prepared, characterized and biological tests were carried out by evaluating, in seeds and seedlings of lettuce and sorghum, the variables: percentage of germination, germination speed index, root growth and aerial growth; and in meristematic lettuce cells the variables: mitotic phases, mitotic index, nuclear alterations and chromosomal alterations. Flavones, flavonones, flavonols, flavononols, flavonoids, alkaloids, resins, xanthones and anthraquinone glycoside were characterized in the ethanolic extract. Both evaluated extracts, in the highest concentration, inhibited the initial plant development. All treatments caused alterations in the mitotic phases and inhibited mitotic index. In addition, the treatments promoted an increase in nuclear and chromosomal alterations. The mechanism of action presented was aneugenic, clastogenic and determined in epigenetic alterations. The ethanolic extract was more cytotoxic, since it had a more expressive effect at a lower concentration. Despite the cytotoxicity of the extracts under study, they promoted alterations at lower levels than the glyphosate positive control.

Keywords: aqueous extract, citotoxicity, ethanolic extract, genotoxicity, *Lactuca sativa*.

Resumo

A busca por herbicidas mais amigáveis ao meio ambiente, visando o controle de pragas agrícolas, aliado a uma menor nocividade à saúde humana e ao meio ambiente tem crescido. Uma alternativa utilizada pelos pesquisadores é a aplicação de produtos do metabolismo secundário de plantas, que são investigados em virtude do seu potencial bioativo. Assim, espécies pertencentes à família Myrtaceae são potenciais nestes estudos, uma vez que esta família é reconhecida por possuir alta atividade biológica. Uma espécie pertencente a este gênero é *Psidium cattleyanum*, que possui efeito medicinal e seus frutos são utilizados na alimentação humana. Assim, o objetivo desta pesquisa foi avaliar e comparar a fitocitogenotoxicidade de extratos foliares aquosos e etanólicos da espécie *P. cattleyanum*, a partir de bioensaios vegetais, bem como identificar as principais classes de compostos presentes nos extratos. Para isso, os extratos foram preparados e caracterizados e foram realizados testes biológicos avaliando, em sementes e plântulas de alface e sorgo, as variáveis: porcentagem de germinação, índice de velocidade de germinação, crescimento radicular e crescimento aéreo; em células meristemáticas de alface foram avaliadas as variáveis: fases mitóticas, índice mitótico, alterações nucleares e alterações cromossômicas. Flavonas, flavononas, flavonóis, flavononóis, flavonóides, alcalóides, resinas, xantonas e glicosídeo de antraquinona foram caracterizados no extrato etanólico. Ambos os extratos avaliados, na maior concentração, inibiram o desenvolvimento inicial da planta. Todos os tratamentos causaram alterações nas fases mitóticas e inibiram o índice mitótico. Além disso, os tratamentos promoveram aumento de alterações nucleares e cromossômicas. O mecanismo de ação apresentado foi aneugênico, clastogênico e epigenético. O extrato etanólico foi mais citotóxico, pois teve efeito mais expressivo em menor concentração. Apesar da citotoxicidade dos extratos em estudo, eles promoveram alterações em níveis inferiores ao controle positivo glifosato.

Palavras-chave: extrato aquoso, citotoxicidade, extrato etanólico, genotoxicidade, *Lactuca sativa*.

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1. Introduction

Most of the known organic compounds can be found in nature, and plants are the collaborators in the formation and supply of these molecules. The production is through the natural synthesis of phytochemicals in the plant, which are commonly known as primary and secondary metabolites. This classification is carried out considering the role played by each compound in the plant (Mohan et al., 2019; Almeida et al., 2021).

The molecules and substances necessary for plant growth, such as chlorophyll, sugars, proteins, amino acids, lipids, among others, are considered primary metabolites. The compounds intended to defend the individual's biotic and abiotic stresses, such as essential oils, alkaloids, tannins, flavonoids, saponins, terpenoids, phenolic compounds, among others, are the secondary metabolites (Mohan et al., 2019; Oszmiański et al., 2020). These phytochemicals products of secondary metabolism have biological activities, being studied due to this potential (Alves et al., 2018; Oszmiański et al., 2020).

Studies have been developed seeking to prove the effects of the different uses and applications of plant materials. In this way, plant chemical compounds have been applied in various activities of human interest, such as in the synthesis of agrochemicals, cosmetics, medicines and condiments (Braga et al., 2019; Alves et al., 2018; Oszmiański et al., 2020).

Different ways of extracting and obtaining plant products are described (Alothman et al., 2009; Ferreira-Dias et al., 2003; Ozer et al., 2016; Saklani et al., 2017), in order to optimize your applications. One of the ways uses water as a solvent, obtaining aqueous extracts as a product. This type of extract is the most friendly to the environment, in addition, water is the most accessible and inexpensive solvent, making it very applicable to the extraction of bioactive vegetable compounds (Vuong et al., 2013). This type of extraction determines the greater obtaining and release of hydrophilic compounds (Kubiliene et al., 2018). Another way is ethanol extraction, which allows greater access to lipophilic substances, such as phenolic acids, aromatic acids, flavonoids and terpenes (Kubiliene et al., 2018). Thus, research that evaluates the bioactivity of the products of the different methods of obtaining plant metabolites is relevant and can provide illuminating results related to the different applications of these compounds.

Moreover, the understanding of bioactivity, as well as the differential effect of aqueous and ethanolic extract is important for the establishment of cultures, the study of possible bio-chemicals, in addition to allowing knowledge about the allelopathic activities of organisms.

To investigate the bioactivity of natural products against plant development, bioassays with model plants are used (Alves et al., 2018), for elucidating the different levels of toxicity of the test agent and indicating potential agrochemicals (Pinheiro et al., 2015). In addition, they feature fast response and are low cost (Alves et al., 2018). The species *Lactuca sativa* L. (lettuce) and *Sorghum bicolor* (L.) Moench are applied as a model in bioassays, as they are easily found agricultural supply stores and have a large amount of small-sized seeds (Aragão et al., 2015), in addition to germinating within 24 hours (Alves et al., 2018).

The Myrtaceae family agroup taxon with high biological activity. Among the species of this family with potential phytochemical content is the araça *Psidium cattleyanum* Sabine (Vinholes et al., 2018), which presents fruits used in human food and has bioactive compounds previously proven in studies that investigated its medicinal activity (Pereira et al., 2018; McCook-Russell et al., 2012; Vinholes et al., 2018).

Given the above, the objective of the present study was to evaluate and compare the phyto-cyto-genotoxicity of aqueous and ethanolic leaf extracts of the species *P. cattleyanum*, from bioassays with model plants, as well as to identify the main classes of compounds present in the extracts.

2. Material e Methods

2.1. Material vegetal

Young leaves were collected from adult individuals of *Psidium cattleyanum* at a height of 1.30 m, in the month of February (summer), in the morning period, at the experimental field of the Center of Agricultural Sciences and Engineering (CCAE) (altitude 254 m, coordinates 20°45' 41°31') at the Federal University of Espírito Santo (UFES), and used as test agents. The voucher was collected, dried, and deposited at the MBML-Herbario (48718). The number of SISGEN authorization is AGF0DB3.

Seeds of two species were adopted as plant models (Alves et al., 2018):

a) *Lactuca sativa* L. 'Crespa Grand Rapids' (Isla Pak) (eudicot), with germination rate of 97%, purity of 100% and within the validity period indicated by the supplier;
b) *Sorghum bicolor* L. Moench 'AL Precioso' (BR Seeds) (monocot), with germination rate of 87%, purity of 99.7% and within the validity period indicated by the supplier.

2.2. Extract preparation

To obtain the test extracts, the collected leaves were dried in forced air circulation oven at 60°C for 72 h and subsequently ground in a blender.

a) Aqueous extract

30 g of the dried leaf powder were weighed and 300 mL of distilled water at 100°C were added. After 10 minutes, the infusion was filtered, yielding the extract at the concentration of 100 mg mL^{-1} (Almeida et al., 2006; Prichoa et al., 2013), from which dilutions were made to obtain the concentrations of 50, 25 and 12.5 mg mL^{-1}.

b) Ethanolic extract

10 g of the dried leaf powder were weighed and 100 mL of 70% ethanol were added, being kept on a shaker for three days. Subsequently, the solution was filtered and placed in a rotary evaporator, yielding a concentrate of 500 mg mL^{-1} (concentration not tested). From this solution, dilutions were made to obtain the tested concentrations of 100, 50, 25 and 12.5 mg mL^{-1} (the same as tested with the aqueous extract).

2.3. Chemical characterization of aqueous and ethanolic extracts

The phytochemical screening to determine the main classes of secondary metabolites present in aqueous and ethanolic leaf extracts were performed as described in the literature for phenols, hydrolyzable tannins, condensed tannins, anthocyanins, anthocyanidins, leucoanthocyanidins, flavones, flavonols, flavononols, flavanones, xanthones, chalcones, aurones, catechins, steroids, triterpenoids, saponins, strong fixed acids, resins and alkaloids (Matos, 2009); for anthraquinone glycosides (Joshi et al., 2013); and for cardiac glycosides (Ayoola et al., 2008).

2.4. Phytotoxicity assay

The experiment was established following the method of direct treatment application in completely randomized design using five repetitions per treatment, with 25 seeds per repetition [22]. Distilled water was used as negative control (C-) and the commercially available herbicide glyphosate (0.1%) as positive control (C+) (Pinheiro et al., 2015). The following variables were analyzed (Alves et al., 2018; Silveira et al., 2017):

a) Germination percentage (GP) – number of germinated seeds after 48 h of exposure to the treatments, calculated by the ratio between the number of germinated seeds times 100 divided by the total number of exposed seeds per repetition.
b) Germination speed index (GSI) – number of germinated seeds counted every 8 h during the first 48 h of exposure to the treatments, calculated by the following formula (Equation 1):

$$\left(N\,1\,*1\right) + \left(N\,2 - N\,1\right)\,*1/2 + \left(N\,3 - N\,2\right)\,*1/3 + \ldots \left(N\,y - \left(N\,y-1\right)\right)\,*1/y \quad (1)$$

Where: Ny refers to the number of seeds germinated within a given period; y: represents the total number of time intervals (Maguire, 1962).

c) Root growth (RG) – measured (in mm) with the aid of a digital caliper after 48 h of exposure to the treatments.
d) Aerial growth (AG) – measured (in mm) after 120 h of exposure to the treatments with the aid of a digital caliper.

2.5. Cyto-genotoxicity assay

To assess cyto-genotoxicity, tip of roots of lettuce were fixed in an methanol: acetic acid fixative (3:1/vv^{-1}) after 48h of exposure to treatments (Alves et al., 2021; Dutra et al., 2020), and then they were stored at -20ºC. Two fastener changes were made; the first after ten minutes of fixation and the second after 24 hours. The roots remained fixed for at least 24 hours, until the end of the last fixation step.

The roots were washed three times, for ten minutes each, in distilled water and hydrolyzed in 5N HCl at 25ºC for 18 minutes. For each slide, semi-permanent and prepared by the crushing technique, two root meristems were used, which were cut, stained with 2% acetic orcein for 15 minutes and sealed with colorless enamel. For each treatment, five slides were prepared and 1000 cells were evaluated per slide, totaling 5000 cells per treatment. The following variables were evaluated:

a) Mitotic index (MI) - refers to the number of cells that are dividing, calculated by the ratio between the number of cells in division and the total number of cells observed (Fiskesjö, 1985).
b) Chromosomal alterations (CA) - refers to the changes observed at the chromosomal level, calculated by the ratio between the number of cells with CA and the total number of cells observed (Fiskesjö, 1985).
c) Nuclear alterations (NA) - refers to the changes observed at the nuclear level, calculated by the ratio between the number of cells with NA and the total number of cells observed (Fiskesjö, 1985).

The CA and NA were assessed separately according to their categories and their frequencies were measured individually (Pinheiro et al., 2015).

a) The CA are – c-metaphase, adherence, bridge, lost chromosome, chromosome not oriented, fragmentation, polyploidization, multipolarity, the frequency of each alteration being calculated individually by the ratio of the number of cells with each CA to the total number of cells in division.
b) The NA are – micronucleus and condensed nucleus the frequency of each alteration being calculated individually by the ratio of the number of cells with each NA to the total number of cells observed.

2.6. Statistical analysis

The values obtained in each observation were tabulated. The means were obtained by analysis of variance and submitted to the Tukey test ($p<0.05$) using the statistical program Genes and the graphics were plotted in the program R, version 3.3.2 (R Development Core Team, 2020; Cruz, 2013). Regression analysis was used to assess the mitotic index (MI). The polynomial regression models were adjusted according to the significance of ANOVA F and the quality of the models was assessed by the coefficient of determination (R^2). The analysis were performed using the R computational environment (R Development Core Team, 2020).

3. Results and Discussion

3.1. Chemical characterization of the aqueous and ethanolic extracts

Leucoanthocyanidins, catechins, anthocyanins, anthocyanidins, aurones, chalcones, condensed tannins and triterpenoids were not observed in any of the samples (Table 1). This observation corroborates another investigation that in which neither the aqueous or the ethanolic extract of *P. cattleyanum* presented anthocyanins, anthocyanidins, aurones and chalcones, confirming this finding for the specie (Gavilla and Muro, 2018).

Strong fixed acids, steroids, simple phenols, saponins, hydrolyzable tannins, cardiac glycosides and terpenoids were identified in both evaluated extracts (Table 1). Saponins – previously described in the composition of

Table 1. Classes of secondary metabolites found in the aqueous and ethanolic extracts of *Psidium cattleyanum*. The signals (+) and (-) respectively indicate the presence or absence of the chemical classes in the analyzed plant material.

Chemical class	Aqueous extract	Ethanolic extract
Strong fixed acids	+	+
Alkaloids	-	+
Catechins	-	-
Steroids	+	+
Simple phenols	+	+
Flavonoids	-	+
Anthocyanins	-	-
Anthocyanidins	-	-
Aurones	-	-
Chalcones	-	-
Flavones	-	+
Flavanones		+
Flavonols	-	+
Flavononols	-	+
Leucoanthocyanidins	-	-
Resins	-	+
Saponins	+	+
Condensed tannins	-	-
Hydrolyzable tannins	+	+
Xanthones	-	+
Anthraquinone glycosides	-	+
Cardiac glycosides	+	+
Triterpenoids	-	-
Terpenoids	+	+

aqueous and ethanolic extracts of *P. cattleyanum* – show insecticide activity, causing destruction of hemolymph components, leading to alterations in the coagulation, leakage, and ultimately death of the insect (Tello, 2014).

Alkaloids, resins, xanthones and anthraquinone glycosides were identified only in the ethanolic extract (Table 1). Alkaloids, previously described in the composition of the ethanolic extract of *P. cattleyanum*, but not in the aqueous one (Gavilla and Muro, 2018), have been described as insecticides owing to their detrimental effect on the nervous system of most insects, acting in the ganglion-cerebral disorientation, culminating in alteration of the insects' perception (Tello, 2014).

Also flavones, flavonones, flavonols, flavonnnols and flavonoids were only identified in the ethanolic extract of *P. cattleyanum* (Table 1). Different types of quercetin in the chromatographic profile of *P. cattleyanum* leaves further demonstrated (Wang et al., 2017). Thus, there are possibly different types of quercetin among the occurring flavonoids, which are related to different biological activities (Díaz-de-Cerio et al., 2017).

Considering the above, several compound classes with biological activity already described in the literature were observed in the studied extracts, demonstrating their potential in investigations of novel biological activities.

3.2. Phytotoxicity assay

The lettuce seeds treated with ethanolic extract at the concentrations of 100, 50 and 25 mg mL^{-1} did not germinate, being completely inhibited (Figure 1a).

Allelochemical compounds exert direct and indirect action on the plant metabolism (Maraschin-Silva and Aquila, 2006). The production of metabolites is dependent on the environmental conditions, such as nutrient availability, soil biota, chemical characteristics of the soil, interaction between the different populations, among others. Changes in these conditions that are caused by the presence of the allelochemical are considered as indirect action. In turn, the direct action comprises alterations at the level of cells and plant metabolism, including changes in physiological processes (e.g. respiration, photosynthesis) and in cell functioning (e.g. membrane permeability), among others (Reigosa et al., 1999; Rice, 1984; Rizvi et al., 1992).

In this way, when such effects of phytotoxic activity are compared to the phytochemical screening, it is noted that the treatment with aqueous extract was that allowed germination of lettuce seeds (Figure 1a). This extract did not present flavonoid compounds, such as flavones, flavonols and flavononols (Table 1). Since flavonoids are allelochemicals known to promote inhibition of plant development, by direct and indirect action (Carvalho et al., 2019), it can be concluded that the observed result is related to the absence/presence of these chemical constituents in the extracts.

The variable GP remained similar among all treatments in the sorghum model (Figure 1b), whereas in the lettuce model it was significantly inhibited by all treatments compared to C- (Figure 1a). However, greater effectiveness of the ethanolic extract is observed in lettuce compared to the aqueous extract, as total inhibition of GP occurred at its three highest concentrations. This greater effectiveness of the ethanolic vs. aqueous extract may be associated to the presence of alkaloids only in the first (Table 1). This metabolite class has been described to promote rupture of the cell membrane (Santos et al., 2019), allowing leakage of electrolytes, thus inhibiting the development of seeds/plantlets in a direct way.

It is important to highlight that C+ reduced the GP in lettuce by approximately 20% when compared to C-, with the reductions caused by the extracts being superior to that promoted by C+ (Figure 1a). Studying the allelopathic effect of the aqueous extract of *P. cattleyanum*, other study also reported inhibition of the germination of lettuce seeds treated at the concentration of 75 g L^{-1}, associating this inhibition to the presence of allelopathic compounds, which act by interfering with membrane permeability, cell division and enzyme activation (Hister et al., 2016).

The variable GSI in the sorghum model did not present significant difference between the treatments, including C+ (Figure 1d). However, the lettuce model displayed several significant alterations (inductions and inhibitions), also in

relation to C+ (Figure 1c), demonstrating that the extracts are more effective in lettuce than in sorghum. Alterations in the GSI evince the occurrence of changes in the metabolic processes related to germination (Maraschin-Silva and Aquila, 2006). In addition, highlighted that allelochemicals selectively inhibit and alter the growth or development patterns of the plants (Merino et al., 2018). Thus, such effect can be related to (1) the different responses of the seeds in the germination process of monocots and eudicots, and (2) the greater sensitivity of lettuce to the metabolites produced by *Psidium*. Studying the phytotoxicity of essential oils from different species of this genus, also reported greater sensitivity of lettuce in comparison to sorghum (Vasconcelos et al., 2019).

For the variable RG, significant inhibition was observed in both plant models (Figure 2). Comparing both test agents, the ethanolic extract was more efficient in lettuce (comparing the highest concentration), whereas the aqueous extract was more efficient in sorghum (Figure 2). Some authors have already reported differential response for monocots and eudicots, relating similar results with the physiology of the plants (Alves et al., 2018; Pinheiro et al., 2015; Vasconcelos et al., 2019).

For the lettuce model, inhibition of AG was observed in the treatment with ethanolic extract only at the concentration of 12.5 mg mL^{-1}, in 35.4% of the plantlets, compared to C- (Figure 2c). The concentrations of aqueous

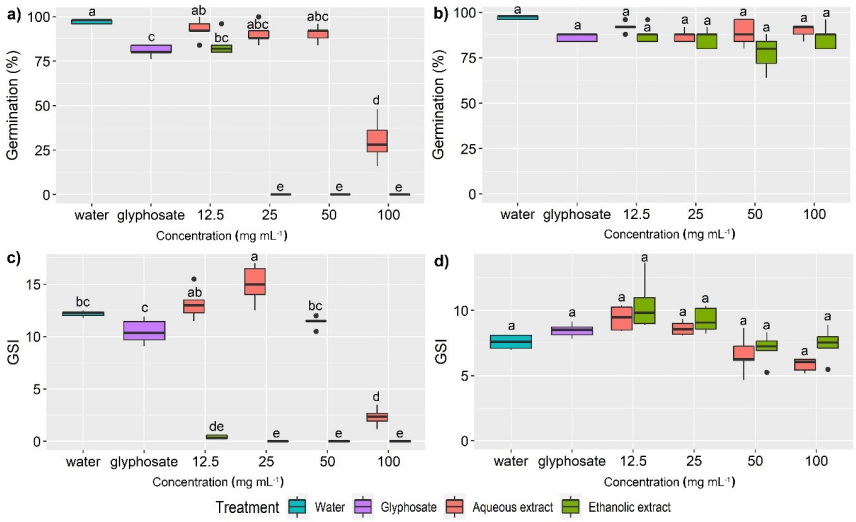

Figure 1. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the germination percentage of (a) *Lactuca sativa* and (b) *Sorghum bicolor* and germination speed index (GSI) of (c) *L. sativa* and (d) *S. bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

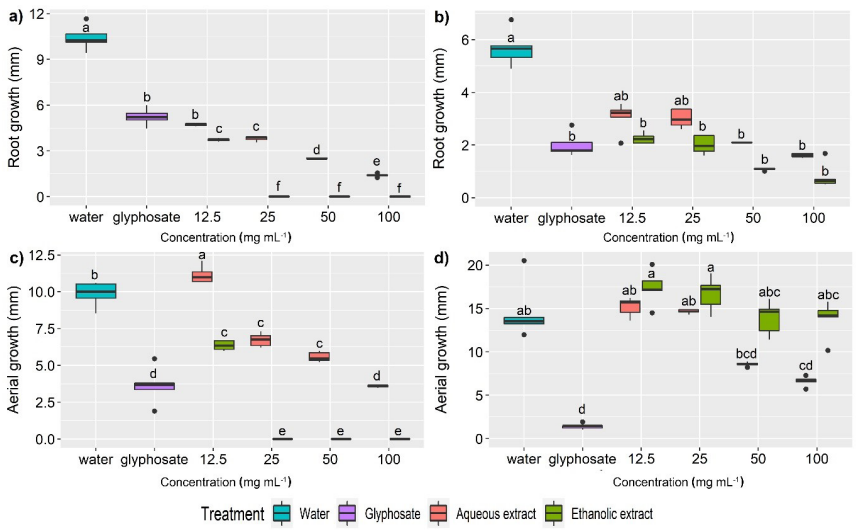

Figure 2. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the root growth of (a) *Lactuca sativa* and (b) *Sorghum bicolor* and aerial growth of (c) *L. sativa* and (d) *S. bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

extract that presented inhibitory effect were 25, 50 and 100 mg mL^{-1}, reaching 31.6%, 43.4% and 63.4% of the plantlets, respectively, in comparison to C- (Figure 2c). Several factors are determining for RG and AG, including nutritional and cellular conditions. In order for the plantlets to grow, it is necessary that cell multiplication and/or elongation occur. Moreover, the initial development is dependent on the formation of the cambium and xylem, which occurs according to the availability and distribution of nutrients in the plantlets (Merino et al., 2018).

The AG of sorghum was inhibited by the aqueous extract at the concentration of 100 mg mL^{-1} in 54.8% of the plantlets, compared to C- (Figure 2d). The evaluation of germination parameters and of the initial growth of the plantlets is considerably elucidative regarding the toxicity of compounds, since these are critical stages of the plant development and subject to high error rates, owing to the seeds presenting lower tolerance to different conditions imposed by the environment (Merino et al., 2018).

Overall, higher toxicity of the evaluated extracts was observed for the variable RG than for AG (Figure 2). The greater sensitivity of RG was also described by Cândido et al. (2010). These authors highlighted that, when comparing the action of phytotoxic agents in roots and aerial parts of plantlets, the effects are more prominent in the roots, as they remain in direct contact with the allelochemical, which increases the possibility of toxic agents influencing the development of this region.

Considering all variables of the assay, it was demonstrated that the ethanolic extract was the most toxic agent for lettuce, whereas the aqueous extract was the most efficient in the sorghum model.

3.3. Cyto-genotoxicity assay

The three largest concentrations (100, 50 and 25 mg mL^{-1}) of ethanolic extract completely inhibited the emission of roots in the model plant (lettuce). Thus, they could not be evaluated for cytotoxic parameters.

The mitotic index (MI) of all evaluated treatments, suffered a significant reduction, less than 50%, when compared to the C- (Figure 3). According Fiskesjö (1985), an effectively cytotoxic agent has MI inhibition greater than 50%, as is the case with the C+ used in the study, which showed a 73.7% reduction when compared to C- (Figure 3).

The most inducing treatment for chromosomal alterations (CA) was C+, although all other treatments promoted more CA than C- (Figure 3), showing that the treatments are less toxic to chromosomes/DNA than glyphosate. This result is important, since, it is sought, compounds with high biological activity and that promote lesser impacts to the environment (Pinheiro et al., 2015; Aragão et al., 2015; Alves et al., 2018; Aragão et al., 2017).

The treatments with aqueous extract did not promote an increase in nuclear alterations (NA), with the opposite being observed with the ethanolic extract (Figure 3). NA are those that alter the cell nucleus of the cell metabolically or morphologically or are also related to the appearance of DNA in a compartmentalized way, as if new nuclei were being formed, as is the case of micronuclei (Alves et al., 2018; Aragão et al., 2015; Santos et al., 2019; Andrade-Vieira et al., 2011). These changes reflect "mistakes" that are occurring during the split. In some cases, such as micronuclei (MNC), the goal is to reestablish the DNA content inside the nucleus; in others, as in the formation of condensed nuclei (Figure 4b), the damage is large enough to activate the cell death process, the latter being considered cytological evidence of the occurrence of cell death (Andrade-Vieira et al., 2011; Costa et al., 2017).

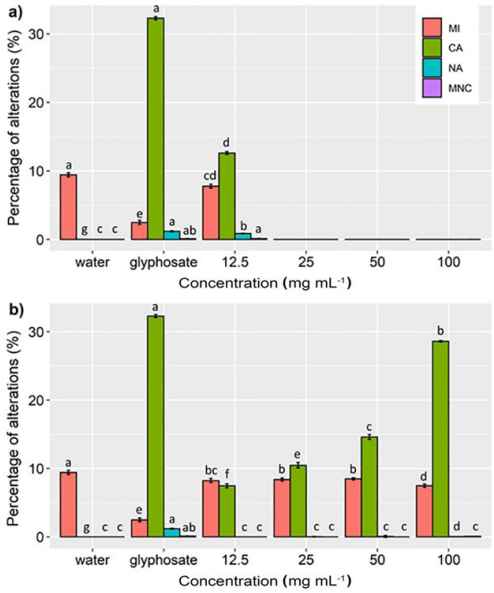

Figure 3. Percentage of observed alterations in the cell cycle of meristematic cells from the tip of the *Lactuca sativa* root exposed to (a) ethanolic extract and (b) aqueous extract from *Psidium cattleyanum* leaves. The small letters above the bars indicate significant differences between the treatments by the Tukey test ($p<0.05$). MI = mitotic index; CA = chromosomal alterations; NA = nuclear alterations, MNC = micronuclei.

The types of chromosomal alterations observed in plant bioassays allow classify the cellular action mechanism of the evaluated test agent. Thus, test agents that promote chromosomal changes resulting from changes in the formation of the mitotic spindle are considered aneugenic (Fernandes et al., 2009; Pinheiro et al., 2015; Alves et al., 2018), since they will change the chromosomal number of the daughter cells, but they will not affect their DNA sequences. Whereas, test agents that determine chromosomal changes that alter the DNA sequence of daughter cells, such as bridges and chromosomal fragments, are considered clastogenic (Bernardes et al., 2015; Vasconcelos et al., 2019). Test agents that cause chromosomal alterations that modify chromosomal signaling, such as adherence, are considered as epigenetic action mechanisms because it is triggered by changes in the phosphorylation pattern of serine 10 in histone 3 (Freitas et al., 2016; Alves et al., 2018). It is important to note that test agents can have more than one action mechanism simultaneously.

Both NA MNC (Figure 4a) and CA bridge (Figure 4e) showed an increase in treatments: ethanolic extract and aqueous extract (100 mg mL^{-1}) (Figures 3 and 5).

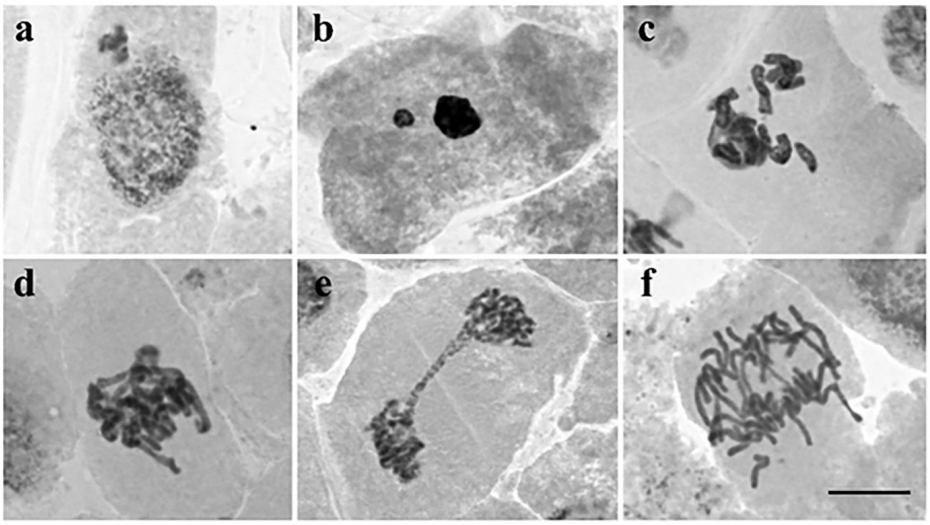

Figure 4. Alterations observed in meristematic cells of *Lactuca sativa* treated with aqueous and ethanolic extracts of *Psidium cattleyanum*. Where it is illustrated: (a) micronucleus; (b) condensed nucleus; (c) c-metaphase; (d) adherent; (e) bridge in telophase; (f) anaphase bridge with lost chromosome. Bar = 10µm.

The increase in these alterations are related, since the chromosome bridge is associated with the break-fusion-break cycle. In this case, telomeres are lost by fragmentation leaving cohesive ends of the chromosomes exposed. Thus, ends of different chromosomes connect, and at the time of chromosomal segregation, the formation / visualization of chromosomal bridges occurs. These linked chromosomes, which are being pulled to opposite poles, undergo "traction" by depolymerizing the microtubules, so that a new break occurs giving continuity to the break-fusion-break cycle (Silveira et al., 2017; Santos et al., 2019; Costa et al., 2020). These fragments formed in the cycle are organized in MNC, after the end of the division, to be exported from inside the cells (Leme and Marin-Morales, 2009; Andrade-Vieira et al., 2011; Silveira et al., 2017), resulting in the formation of MNC.

The frequency of c-metaphases (Figure 4c) increased in all treatments evaluated when compared to C- (Figure 5). This alteration derives from the total dysfunction of the mitotic spindle, showing that microtubule polymerization is not occurring, consequently, the chromosomes are not organized in the cell's equatorial plane (Silveira et al., 2017; Santos et al., 2019; Costa et al., 2020).

The lost chromosomes (Figure 4f) were observed, significantly, in the cells treated with the evaluated concentration of the ethanolic extract and in the concentrations of 25 and 100 mg mL^{-1} of the aqueous extract (Figure 5). The lost chromosomes, as well as the c-metaphases, refer to the bad organization of the spindle. However, the lost chromosomes are due to a partial change, while the c-metaphases of a total change in the formation of spindle fibers (Fernandes et al., 2009; Santos et al., 2019).

Chromosomal adherence (Figure 4d) was observed, significantly, in the cells treated with all evaluated extracts (Figure 5). This alteration refers to a series of changes that occur. This alteration indicates changes in the functioning

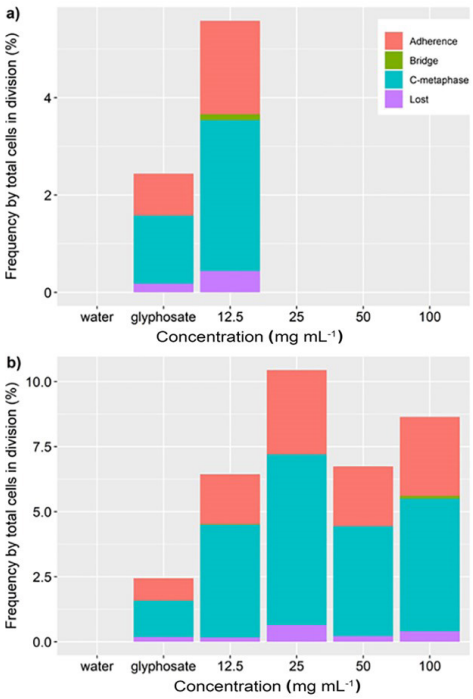

Figure 5. Distribution of observed chromosomal alterations in the cell cycle of meristematic cells from the tip of the *Lactuca sativa* root exposed to (a) ethanolic extract and (b) aqueous extract of *Psidium cattleyanum*.

of the mitotic machinery, alteration in the chromosomal constitution, as well as alteration in the phosphorylation of amino acids that constitute the chromosomes, thus modifying its signaling (Freitas et al., 2016; Silveira et al., 2017; Alves et al., 2018; Santos et al., 2019).

The observed CAs elucidated the action mechanism of the treatments. Thus, all treatments were aneugenic, as well as having an epigenetic effect, acting on mitotic machinery and chromosomal signaling. In addition, ethanolic extract in the investigated concentration and aqueous extract in the concentration of 100 mg mL^{-1} were clastogenic, altering the DNA sequence of the cells.

The knowledge of biological activity in the cell cycle, as well as the cellular action mechanism of plant extracts is important for directing studies and understanding relational dynamics between organisms. In addition, it helps to elucidate possible adverse health effects, assisting in the indication and restriction of the daily use of plants, whether for nutrition, body care and / or as a herbal medicine.

4. Conclusion

Elucidating the differential effects of extracts according to the applied extraction method and the target organism is important for the varied applications of natural products. This work contributed in the context of showing that the choice of extraction method should be in accordance with the aimed purpose; accordingly, the ethanolic extract favors the extraction of flavones, flavonones, flavonols, flavonnnols and flavonoids, alkaloids, resins, xanthones and anthraquinone glycosides.

With regard to phytotoxic potential, the ethanolic extract was more effective in lettuce, whereas the aqueous extract was more effective in inhibiting the sorghum model. Thus, differential toxicity was observed between the two used extraction methods and for the both model species.

The evaluated extracts were shown to be cytotoxic, with mechanisms of aneugenic action and promoters of epigenetic alterations, against the lettuce cell cycle. The ethanolic extract and the higher concentration of the aqueous extract promoted a significant increase in nuclear alterations, when compared to water. In addition, these treatments proved to be clastogenic. These results demonstrate the bioactivity of these extracts, as well as their toxic potential for biological applications.

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