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Effect of the peel extracts from two *Campomanesia* (Myrtaceae) species on *Allium cepa* L. (Amaryllidaceae)

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ABSTRACT

Species from the genus *Campomanesia* have been studied due to their biological activity and low toxicity. However, studies on the fruit peel of these species are scarce. In this context, the effects of ethanol extract from the fruit peel of *Campomanesia sessiliflora* (O. Berg) Mattos (CS) and *Campomanesia guazumifolia* (Cambess) O. Berg (CG) were evaluated concerning the effect on germination and meristem cells of roots of *Allium cepa*. The CG ethanolic extract showed flavonoid compounds higher than CS ethanolic extract. The CS extract showed no antioxidant activity. CS showed greater antiproliferative activity with reduced root size in *A. cepa* seeds. The two extracts did not induce mutagenicity and cell death at the studied concentration of 1 mg mL^{-1}.

Keywords: Germination, Allelopathy; Ethanolic extract.

Efeito dos extratos de cascas dos frutos de duas espécies de *Campomanesia* (Myrtaceae) em *Allium cepa L.* (Amaryllidaceae)

RESUMO

As espécies do gênero *Campomanesia* têm sido estudadas devido às suas atividades biológicas e baixa toxicidade. No entanto, existem poucos estudos com as casca dos frutos dessas espécies vegetais. Nesse contexto, foram avaliados os efeitos dos extratos etanólicos das cascas dos frutos de *Campomanesia sessiliflora* (O. Berg) Mattos (CS) e *Campomanesia guazumifolia* (Cambess) O. Berg (CG) em relação ao efeito na germinação e células do meristema das raízes de *Allium cepa*. O extrato etanólico de CG apresentou níveis de flavonóides maiores que o extrato etanólico de CS. O extrato de CS apresentou maior atividade antiproliferativa, com redução do tamanho da raiz nas sementes de *A. cepa*. Os dois extratos não induziram mutagenicidade e morte celular na concentração estudada de 1 mg mL^{-1}.

Palavras-chave: Germinação; Alelopatia; Extrato etanólico

1. Introduction

The Cerrado is a Brazilian biome that presents rich biodiversity, being the most diversified tropical savanna globally, with a great variety of native fruits with potential for sustainable use (Nascimento et al., 2020). However, it is common that the fruits are not used in their entirety, generating a residue rich in nutrients and with biotechnological potential, which is why industries have been exploring these by-products, with the stalks and husks (Antunes et al., 2018; Oliveira and Pandolfi, 2020).

In this context, fruit peels can generate extra income when exploited as by-products, this possibility has been studied for several species due to the presence of secondary metabolites and other compounds with biological activity (Rafiq et al., 2016; Vu et al., 2016; Vu et al. al., 2018; Freitas et al., 2020). Anticona et al. (2021) have already optimized the obtainment of bioactive compounds with antioxidant activity from the mandarin peel (*Citrus reticulata*), and Liang et al. (2021) analyzed the bioactive properties of the peel of 15 kiwi cultivars.

Among the fruits of the Cerrado used by the population, there are members of the genus *Campomanesia*, generally known as "gabiroba", "guabiroba", "gabiroba-do-campo", or "guavira" (Lima et al., 2017; Duarte et al., 2021). Several species of this genus are used for medicinal purposes and fresh human food (Duarte et al., 2020; Catelan et al., 2021). Fruits of this genus can also be consumed as juice, ice cream, and liqueur (Scalon et al., 2012).

Campomanesia guazumifolia (Cambess.) O. Berg is one of these species, traditionally known as "sete-capotes", "sete-capas", "capoteira", "sete-cascas" (Legrand and Klein, 1978). The essential oil from the leaves of *C. guazumifolia* has antioxidant and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Santos et al., 2017). Also, anti-inflammatory activity has already been identified in the infusion of *C. guazumifolia* leaves in rats (Catelan et al., 2018a). The ethanol extract has a photoprotective property (Catelan et al., 2019). Santos et al. (2020) optimized the obtainment of extracts rich in phenolic compounds and antioxidant activity from the leaves of *C. guazumifolia*. In addition, Catelan et al. (2018a) identified that the infusion of *C. guazumifolia* leaves does not present toxicity in mice.

On the other hand, *Campomanesia sessiliflora* (O. Berg) Mattos is popularly known as "guabiroba-verde" and can be found in the Brazilian states of Minas Gerais and Mato Grosso do Sul, in the Southeast and Midwest regions of the country, respectively (Kataoka and Cardoso, 2013). Catelan et al. (2019) identified the presence of phenolic compounds, flavonoids, and *in vitro* photoprotective activity of ethanol extracts from *C. sessiliflora* leaves. Kataoka and Cardoso (2013) identified phenolic compounds and flavonoids in the extracts obtained with ethanol:water (80:20 v/v) and determined the antioxidant potential.

In the peels of fruits of the *Campomanesia* genus, antidiarrheal activity for the methanol extract of the peels of *Campomanesia adamantium* (Cambess) O. Berg was identified *in vitro* analysis. Also, inhibition of COX-1 and COX-2 was found (Lescano et al., 2016). Essential oil from the fruit peel of this same species also showed pain inhibition and anti-inflammatory effect in rats (Viscardi et al., 2017). Silva et al. (2018) isolated a dimethylchalcone in peels of *C. adamantium* fruits with antiproliferative activity in rat melanoma (B16-F10). Compounds have already been identified from the peels of *C. adamantium* fruits (Viscardi et al., 2017; Silva et al., 2018) and *Campomanesia lineatifolia* Ruiz & Pav. (Osorio et al., 2006).

However, there are still no studies exploring the fruit peels from the species *C. guazumifolia* and *C. sessiliflora* and the safety of their use. In this sense, it is essential to ensure that the plants consumed do not present health risks, so it is relevant to analyze the effect of these plants in biological models to verify the effect on the cell cycle (Tedesco and Laughinghouse IV, 2012).

Plants are considered good biological models to indicate mutagenic action and micronuclei formation, and *Allium cepa* L. (onion) is considered one of the best biological models for preliminary genotoxicity analysis due to the reliability of the results and easy obtainment (Sposito et al., 2019; Furini et al., 2020). The mutagenic effect can be identified by interruptions in metaphase; however, the best region for this analysis is the root meristematic tissue since the cells do not differentiate and accelerate cell growth (Lopes and Sousa, 2019). The fact that *A. cepa* cells have large chromosomes in a reduced number also facilitates the detection of proliferation and mitotic spindle disorders (Silva et al., 2019).

Castro et al. (2020) identified that the extract from the infusion of *C. sessiliflora* leaves does not induce cell death or alters the mitotic index at a concentration of 0.5 mg mL^{-1} in *A. cepa* and presents antiproliferative activity. Infusion and essential oil of *Campomanesia xanthocarpa* (Mart.) O. Berg (Pastori et al., 2013) and the ethanol extract of the leaves of *Campomanesia pubescens* (Mart. Ex DC.) O. Berg have also been evaluated in the biological model of *A. cepa* (Catelan et al., 2018b), but are still no studies on mutagenicity and antiproliferative activity of *C. sessiliflora* and *C. guazumifolia* fruit peels. In this sense, this study aimed to evaluate the effect of ethanol extracts of the fruit peels of *C. sessiliflora* and *C. guazumifolia* on *A. cepa* cells.

2. Material and Methods

The fruits of *C. sessiliflora* (CS) and *C. guazumifolia* (CG) were collected in Dourados and Amambai, Mato Grosso do Sul, Brazil, respectively, in the fruiting period (November 2017). The species were sent for botanical identification, and exsiccates were deposited in the Herbarium of the Federal University of Grande Dourados (UFGD), Dourados-MS, Brazil, under numbers 4746 for CG and 2193 for CS.

The peels were manually separated from the fruit pulp, then crushed and frozen until the beginning of the experiments. We used the proportion of 30 g of thawed crushed peel for each 100 mL of 95% ethanol in 2 L containers for both species. After seven days, the sample was filtered through cotton, and the procedure was repeated twice. Finally, the solvent was evaporated using a rotary evaporator (Tecnal TE-210, Tecnal, Brazil) at a temperature of 40 °C.

The extracts were solubilized at 1 mg mL^{-1} in distilled water. The blank was distilled water. Analyzes took place in triplicate. The determination of flavonoids followed the method used by Djeridane et al. (2006); 1000 µL of $AlCl_3.6H_2O$ 2% in methanol were added for each 1000 µL of each sample, with 15 minutes of reaction. The reading was performed in a spectrophotometer at a wavelength of 430 nm.

To calculate the flavonoid concentration, an analytical curve was drawn using rutin as a standard at concentrations from 1 to 50 µg mL^{-1}. The linear regression of the analytical curve had an R^2 of 0.9990. It resulted in Equation 1, which was used to obtain the flavonoid concentration (C_f) through the sample absorbance (Abs 430 nm) after the chemical reaction.

$$C_f = \frac{Abs_{430\ nm} - 0.0017}{0.0103} \quad (1)$$

Ethanol extracts of fruit peels of *C. guazumifolia* and *C. sessiliflora* were prepared in distilled water at a single concentration of 1.0 mg mL^{-1}. The methodology was similar to those used by Castro et al. (2020) and Santos et al. (2021). Analyzes took place in duplicate. The analysis was performed with 30 seeds of *A. cepa* (Isla brand) free of pesticides, using distilled water as a negative control (NC) and trifluralin at a concentration of 0.84 µg mL^{-1} as a positive control (PC). For this purpose, the samples were submitted to 3 mL of the samples and were incubated in B.O.D. for 96 hours at 23 ± 3ºC.

The size of the roots (SR) was evaluated with the aid of a digital caliper (Digmess, model 100170, Brazil). The seeds germinated in the sample (SG) were counted in each replicate to calculate the germination index (GI), relating to the number of seeds germinated in the negative control (SGC) through equation 2.

$$GI = \frac{100 \times SG}{SGC} \quad (2)$$

The dye fixation process in the roots after the germination step was carried out as described by Castro et al. (2020). The slides were prepared with the meristems of *A. cepa* roots exposed to the samples. The evaluation was performed under a microscope (Eclipse E200, Nikon, USA) at 400x magnification, with ten slides per sample and evaluating 500 cells per slide.

The calculations of the analyzed parameters were performed as described by Franscisco et al. (2018). To calculate the mitotic index (MI), the number of cells in cell division (CD) was analyzed concerning the total number of cells analyzed (CA), using equation 3. The number of dead cells (DC) was also counted to calculate the cell death index (CDI) through equation 4.

$$MI = \frac{100 \times CD}{CA} \quad (3)$$

$$CDI = \frac{100 \times CD}{CA} \quad (4)$$

The CA was calculated by counting. The mutagenicity index (MTI) was estimated by counting the cells that present micronuclei (CMi) using equation 5.

$$MTI = \frac{100 \times CMi}{CA} \quad (5)$$

Statistical analysis of *A. cepa* was performed using the R software 13.6.1. Kruskal-Wallis test and Dunn test with a significance level of 0.05 were applied.

3. Results and Discussion

The ethanol extract of the fruit peels of *C. guazumifolia* presented higher levels of flavonoids (49.06 ± 0.40 mg RE g^{-1} ± SD) than *C. sessiliflora* (4.67 ± 0.22 mg RE g^{-1} ± SD). Phenolic compounds have several beneficial properties for human health, being secondary metabolites, much studied in medicinal plants due to this versatility (Tungmunnithum et al., 2018). Flavonoids associated with various biological activities are among these compounds (Santos and Rodrigues, 2017).

The extract of the fruit peels of *C. guazumifolia* and *C. sessiliflora* showed flavonoid contents lower than those reported in the literature for other extracts of the same species (Table 1). However, there are no studies of the chemical composition of these for both species. Flavonoids may be associated with antiproliferative activity, and CG showed greater potential. In this context, the *A. cepa* model can be useful for analyzing this biological activity (Hister et al., 2017). The germination test showed a significant difference in the germination index and root size concerning the negative ($p < 0.05$) and positive ($p < 0.05$) controls for the two

extracts evaluated. Root size (SR) and mitotic index (MI) were smaller for *C. sessiliflora*, indicating a more pronounced antiproliferative activity for *C. guazumifolia* (Table 2).

Changes in SR and MI are associated with the allelopathic effect (Pires and Oliveira, 2011). Allelopathy is the ability of a plant to interfere with the germination and development of other plants through the release of chemical compounds present in different parts of the plant (bark, leaves, flowers, fruits, roots, and among others), and these compounds can be from different classes, such as phenolic compounds, terpenes, alkaloids, and others (Silva, 2019).

The ethanol extract from the fruit peels of *C. sessiliflora* showed higher antiproliferative activity than *C. guazumifolia*; however, it did not present the highest levels of flavonoids, indicating that this species has other classes of compounds that provide this biological activity. However, the presence of flavonoids in *C. guazumifolia* can be used for biotechnological purposes (Rafiq et al., 2016; Vu et al., 2016; Freitas et al., 2020). The GI reduction for this species was not high; however, the MI reduction was significant, and the SR reduction was superior to the positive control, indicating a great potential of the extract from *C. sessiliflora* fruit peels to inhibit root growth.

The *A. cepa* model provides a sign of whether the extract has the potential to induce cell death and mutagenicity, helping to verify the risk of consuming a plant extract for pharmacognostic purposes (Bagatini et al., 2007). Both extracts showed the absence of cell death (CDI) and mutagenicity index (MTI) at a concentration of 1 mg mL^{-1}, similarly to the negative control, with distilled water. The mitotic index showed a significant difference in the germination index and root size concerning the negative ($p < 0.05$) and positive ($p < 0.05$) controls for the two extracts evaluated (Table 2).

Both extracts reduced MI, although the extract from *C. sessiliflora* fruit peels showed a greater reduction. Reducing this parameter is one requirement for anticancer drugs (Hister et al., 2017), and the absence of CDI and MTI in the concentration studied is positive for this purpose. The extract obtained by infusion of *C. sessiliflora* leaves also showed an absence of CDI and MTI at concentrations lower than 0.5 mg mL^{-1} (Castro et al., 2020), indicating that the extract of *C. sessiliflora* fruit peels can be used at concentrations higher than the infusion of leaves. However, it is necessary to study other biological models to ensure safety in its use.

Other species of the *Campomasia* genus have already been evaluated in the biological model of *A. cepa*. Pastori et al. (2013) identified that the infusion of *C. xanthocarpa* leaves has antiproliferative activity and genotoxicity. In contrast, in the study by Catelan et al. (2018b), ethanol extract of *C. pubescens* leaves shows cytotoxicity and genotoxicity.

The infusion of *C. guazumifolia* leaves has already been evaluated in rodents to verify its toxicity, with no clinical signs of acute toxicity (Catelan et al., 2018a). The absence of CDI and MTI at a concentration of 1 mg mL^{-1} for the extract from fruit peels of *C. guazumifolia* (Table 2) associated with flavonoids indicates that this extract has pharmacological potential with low toxicological potential.

Table 1. Flavonoid contents from different extracts of the *C. guazumifolia* (CG) and *C. sessiliflora* (CS) reported in literature.

Species	Samples	Flavonoids	References
CS	Infusion of the leaves	47.58[a]	Castro et al. (2020)
CS	Ethanol extract of the leaves	299.79[a]	Catelan et al. (2019)
CS	Infusion of the leaves	6.40 – 11.11[b]	Kataoka and Cardoso (2013)
CS	Ethanol-water extract of the leaves	17.09 – 35.60[b]	Kataoka and Cardoso (2013)
CG	Ethanol extract of the leaves	312.73[a]	Catelan et al. (2019)

NA = Not analyzed; GAE = Gallic acid equivalent; RE = equivalent rutin; QE = Quercetin equivalent; [a](mg RE g^{-1}); [b](µg QE mL^{-1}). Source: The authors.

Table 2. Germination index and average root size, mitotic index, mutagenicity index and cell death index of the ethanol extracts of the fruit peels of C. *guazumifolia* (CG) and *C. sessiliflora* (CS).

Samples	RGI ± SD (%)	SR ± SD (nm)	MI ± SD (%)	MTI ± SD (%)	CDI ± SD (%)
CG	92.01 ± 4.74	4.01 ± 0.47	2.7 ± 0.2	-	-
CS	80.68 ± 10.87	2.59 ± 1.17	1.2 ± 0.1	-	-
NC	100	4.97 ± 1.82	5.3 ± 0.2	-	-
PC	50.21 ± 0.10	2.85 ± 0.89	0.4 ± 0.1	3.5 ± 0.2	2.5 ± 0.1

RGI = Relative germination index; SR = Size of roots; MI = mitotic index; MTI = Mutagenicity index; CDI = Cell death index; NC = Negative control (distilled water); PC = Positive Control (Trifluralin 0.84 µg mL^{-1}); SD = standard deviation. Source: The authors.

4. Conclusions

The ethanol extract from the fruit peels of *C. guazumifolia* has higher content flavonoids; however, the ethanol extract from the fruit peels of *C. sessiliflora* showed a greater antiproliferative activity, with potential for use as a root inhibitor. Both extracts showed an absence of mutagenicity and cell death at the studied concentration of 1 mg mL^{-1}.

Both samples show the absence of mutagenicity and cell death in the *A. cepa* model at a concentration of 1 mg mL^{-1}. The ethanol extract of *C. sessiliflora* fruit peels has natural herbicide potential. The two extracts presented promising results for using this residue from the fruits of these species.

Authors' Contribution

Bruno Araújo Silva contributed to the execution of the experiment, data collection and writing of the manuscript. Thiago Luis Aguayo de Castro contributed to execution of the experiment, analysis and interpretation of results and writing of the manuscript. Lucilene Finoto Viana contributed to execution of the experiment and analysis and interpretation of results.

Maria do Socorro Mascarenhas Santos contributed to the analysis and interpretation of results and writing of the manuscript and final correction of the manuscript. Claudia Andrea Lima Cardoso contributed interpretation of results and writing of the manuscript and final correction of the manuscript.

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