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Phytotoxic and Enzymatic Study of *Philodendron meridionale* on seeds of *Lactuca sativa* L.

Estudo fitotóxico e enzimático de *Philodendron meridionale* em sementes de *Lactuca sativa* L.

Estudio fitotóxico y enzimático de *Philodendron meridionale* en semillas de *Lactuca sativa* L.

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Abstract

In an effort to identify novel biopesticides, the present study aimed to assess the effects of *Philodendron meridionale* (Buturi & Sakur) stem and leaf ketonic and ethanolic extracts (SKE, SEE, LKE, and LEE, respectively) on the germination, growth, root respiration, and enzymatic activities of *Lactuca sativa* L. seeds, and to measure the

associated saponins, phenolics, and flavonoids that may possess herbicidal, pharmaceutical, or pesticidal activities. The extracts were obtained using a modified Soxhlet apparatus and methanolic extracts of 0.1, 0.25, 0.5, 0.75 and 1.0 $mg.mL^{-1}$ were tested, with water and methanol as a control. The presence of saponins and the concentration of phenolic compounds were determined. Allelopathic activity was evaluated in tests of germination, growth, cellular respiration and enzymatic activity. The presence of saponins and the concentration of phenolic compounds equivalent to 225.12 for LKE, 240.45 for LEE, 193.28 for SKE, and 265.14 for SEE ($mg \cdot g^{-1}$.gallic acid), and flavonoids 52.74 for LKE, 54.31 for LEE, 72.74 for SKE, and 67.21 for SEE ($mg.g^{-1}$.quercetin) were determined. The allelopathy of the *P. meridionale* extracts against *L. sativa* was confirmed through negative effects on *L. sativa* seed germination, radical growth and morphology, dry mass, and the concentrations of α-amylase (EC 3.2.1.1), ascorbate peroxidase (EC 1.11.1.11), catalase (EC 1.11.1.6), and polyphenol oxidase (EC 1.10.3.1). It was likely that the allelopathic action of the *P. meridionale* extracts was related to its effects on the membrane permeability and oxidative stress of the treated *L. sativa* seeds. The *P. meridionale* extracts contained saponins, calcium oxalate crystals, and flavonoids, including phenolic compounds, which are known allelochemicals with herbicidal activities.
Keywords*: Araceae;* Enzymatic activity; Allelopathic; Phenolic compounds; Saponins.

Resumo

Em um esforço para identificar novos biopesticidas, o presente estudo teve como objetivo avaliar os efeitos dos extratos cetônicos e etanólicos de caule e folha de *Philodendron meridionale* (Buturi & Sakur) (SKE, SEE, LKE e LEE, respectivamente) na germinação, crescimento, respiração radicular e atividades enzimáticas de sementes de *Lactuca sativa* L., e medir o índice de saponinas, fenólicos e flavonóides associados que podem possuir atividades herbicidas, farmacêuticas ou pesticidas. Os extratos foram obtidos em aparelho Soxhlet modificado. Foram testados os extratos metanólicos de 0,1; 0,25; 0,5; 0,75 e 1,0 $mg.mL^{-1}$, tendo água e metanol como controle. Foram determinadas a presença de saponinas e a concentração de compostos fenólicos. A atividade alelopática foi avaliada em testes de germinação, crescimento, respiração celular e atividade enzimática. A presença de saponinas e a concentração de compostos fenólicos equivalente a 225,12 para LKE, 240,45 para LEE, 193,28 para SKE e 265,14 para SEE ($mg.g^{-1}$.ácido gálico) e flavonóides 52,74 para LKE, 54,31 para LEE, 72,74 para SKE e 67,21 para SEE ($mg.g^{-1}$.quercetina). A alelopatia dos extratos de *P. meridionale* contra *L. sativa* foi confirmada por meio de efeitos negativos na germinação de sementes de *L. sativa*, crescimento e morfologia do radical, massa seca e concentrações de α-amilase (EC 3.2.1.1), ascorbato peroxidase (EC 1.11.1.11), catalase (EC 1.11.1.6) e polifenol oxidase (EC 1.10.3.1). É provável que a ação alelopática dos extratos de *P. meridionale* esteja relacionada aos seus efeitos na permeabilidade da membrana e no estresse oxidativo das sementes tratadas de *L. sativa*. Os extratos de *P. meridionale* continham saponinas, cristais de oxalato de cálcio e flavonóides, incluindo compostos fenólicos, que são conhecidos aleloquímicos com atividades herbicidas.
Palavras-chave*: Araceae;* Atividade enzimática; Alelopatia; Compostos fenólicos; Saponinas.

Resumen

En un esfuerzo por identificar nuevos bioplaguicidas, el presente estudio tuvo como objetivo evaluar los efectos de los extractos cetónicos y etanólicos de tallo y hoja de *Philodendron meridionale* (Buturi & Sakur) (SKE, SEE, LKE y LEE, respectivamente) sobre la germinación, el crecimiento y la raíz. respiración y actividades enzimáticas de las semillas de *Lactuca sativa* L., y medir las saponinas, fenólicos y flavonoides asociados que pueden poseer actividades herbicidas, farmacéuticas o pesticidas. Los extractos se obtuvieron utilizando un aparato Soxhlet modificado y se ensayaron extractos metanólicos de 0,1, 0,25, 0,5, 0,75 y 1,0 $mg.mL^{-1}$, con agua y metanol como control. Se determinó la presencia de saponinas y la concentración de compuestos fenólicos. La actividad alelopática se evaluó en pruebas de germinación, crecimiento, respiración celular y actividad enzimática. La presencia de saponinas y la concentración de compuestos fenólicos equivalentes a 225,12 para LKE, 240,45 para LEE, 193,28 para SKE y 265,14 para SEE ($mg.g^{-1}$.ácido gálico), y flavonoides 52,74 para LKE, 54,31 para LEE, 72,74 para SKE y 67,21 para SEE ($mg.g^{-1}$.quercetina). La alelopatía de los extractos de *P. meridionale* contra *L. sativa* se confirmó através de los efectos negativos sobre la germinación de semillas de *L. sativa*, el crecimiento y morfología de radicales, la masa seca y las concentraciones de α-amilasa (EC 3.2.1.1), ascorbato peroxidasa (EC 1.11.1.11), catalasa (EC 1.11.1.6) y polifenol oxidasa (EC 1.10.3.1). Es probable que la acción alelopática de los extractos de *P. meridionale* estuviera relacionada con sus efectos sobre la permeabilidad de la membrana y el estrés oxidativo de las semillas de *L. sativa* tratadas. Los extractos de *P. meridionale* contenían saponinas, cristales de oxalato de calcio y flavonoides, incluidos compuestos fenólicos, que son aleloquímicos conocidos con actividad herbicida.
Palabras clave: *Araceae;* Actividad enzimática; Alelopatía; Compuestos fenólicos; Saponinas.

1. Introduction

Knowledge about the mechanisms of action caused by secondary metabolites, physical-chemical characterization and biological tests of botanical products facilitated the development and industrial use of products, including medicines, cosmetics, foods, insecticides and herbicides, generated from of native species (Pino et al., 2013).

Philodendron meridionale (Buturi & Sakur) is a recently described and non-threatened Brazilian endemic, found in the states of Paraná and Santa Catarina (Buturi et al., 2014). The species is a hemiepiphyte and, so far, has not been the focus of any investigative research. However, other species of *Philodendron* have been reported to contain substances with biological activities, including anti-hemorrhagic (Moura et al., 2015), cytotoxic (Hassanein et al., 2011; Ghareeb et al., 2015), insecticide (Santiago et al., 2014) and larvicidal (Alliance et al., 2017).

Allelopathy is the influence, beneficial or harmful, of one species of plant on another (Zimdahl, 2018) and occurs when plants release substances (ie, allelochemicals), usually secondary metabolites, which affect the growth and survival of the surrounding vegetation (Bogatek & Gniazdowska, 2007). Substances can work by affecting plant respiration, photosynthesis, enzymatic activity, water relations, stomatal opening, phytohormone levels, mineral availability, cell growth and the structure and permeability of cell membranes and walls (Rezende et al., 2003).

The resistance or tolerance of secondary metabolites that serve as allelochemicals is becoming more and more defined and is found in more sensitive species than others, such as *Lactuca sativa L*. (lettuce) (Ferreira & Aquila, 2000; Reigosa et al., 2013). This is the most common plant used as a target species among hydrophytes, due to the short time required for germination (24 to 48 h) and growth (Elakovich, 1999, Gatto et al., 2020).

The aim of the present study was to evaluate the effects of ketone and ethanolic extracts of *P. meridionale* stems and leaves on germination, growth, root respiration and enzymatic activities of *L. sativa* seeds and to analyze saponins, phenolics and associated flavonoids that may have herbicides, pharmaceutical products or pesticides.

2. Material and Methods

2.1 Collection of botanical material

Philodendron meridionale specimens were collected at the Botanical Garden Campus of Federal University of Paraná, Brazil (25°26′58″ S, 49°37′12″ W – 906 m altitude). The herbarium specimens were deposited in the collection of the Botanical Museum of Curitiba, under registration number 390207, and were identified by a *Philodendron* specialist, Dr. Mônica Cássia Sakuragui.

Access to the botanical material was permitted and licensed by Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) and was registered under number A0EB51A as required by the Brazilian legislation.

2.2 Preparation of ketonic na ethanolic extracts

To prepare leaf ketonic extracts (LKE) and stem ketonic extracts (SKE), *P. meridionale* leaves and stems were separately dried, crushed in cutting and hammer mills, and extracted using a Soxhlet apparatus with propanone. The remaining residues were extracted using 99.9% ethanol in a modified Soxhlet apparatus (Carvalho et al., 2009) to obtain leaf ethanolic extracts (LEE) and stem ethanolic extracts (SEE). For each extraction procedure, the extracts were filtered, using a Büchner funnel, concentrated, and then refrigerated.

2.3 Measurement of total phenolic contents (TPC)

All extracts were diluted in methanol (1.0 $mg.mL^{-1}$), and total phenolic contents of the solutions were measured using Folin-Ciocalteu reagent, as described by Slinkard and Singleton (1977). Total phenolic content was calculated using a calibration curve for gallic acid ($y = 0.0392x – 0.057$, $R^2 = 0.9967$) and was expressed in mg equivalents of gallic acid per g crude extract.

2.4 Measurement of flavonoid contents

Flavonoid contents were measured using the guidelines of Chang et al. (2002) and expressed in mg equivalents of quercetin per g crude extract ($y = 0.0313x - 0.016$, $R^2 = 0.9997$).

2.5 Saponin screening

Three assay tubes, each with equal volumes of aqueous extract (0.02 g of all extracts were diluted in 5 mL of water, in triplicate), were shaken vigorously for 5 min and then allowed to rest for 30 min. The presence of saponins was then confirmed by the persistence of >1 cm foam in the tube (Reginatto, 2017).

2.6 Preparation of the allelopathic test

Six milliliters of the methanolic solution (LKE, SKE, LEE, and SEE) of various concentrations (0.1, 0.25, 0.5, 0.75, and 1.0 $mg.mL^{-1}$) were added to boxes (i.e., gerbox) that contained two sheets of Whatmann® filter paper (1.0) that had been autoclaved at 120°C for 20 min. For preparation, the extracts were diluted and kept at 30°C for 24 h to allow evaporation of the solvent. They were then resuspended using the autoclaved filter paper pre-moistened with 6 mL distilled water. A similar procedure was also performed using methanol (solvent control) and water (negative control). The experiment was conducted in quadruplicate, and the gerbox with *L. sativa* seeds were incubated in a germination chamber (Bio-Oxygen Demand incubator), with a relative humidity of ~80% and constant temperature of 25°C (Brasil, 2009; Krause et al., 2015; Merino et al., 2018; Gatto et al., 2020).

2.6.1 *Germination experiment*

Germination of the seeds was checked over a period of 7 days, and germinated seeds were removed from the gerbox. Seeds were considered to have germinated when the radical appeared to protrude through the tegument, and germination was measured in terms of rate (speed and percentage; (Laboriau, 1983) and germination velocity index (GVI; Maguire, 1962). The GVI was calculated for each replicate, according to the following equation: GVI: G1/N1 + G2/N2 + Gn/Nn, where GVI represents the germination speed index.G1, G2, ..., Gn represent the number of seeds that germinated on each day, and N1, N2, ..., Nn represent the number of days from sowing until the seventh day, using the sum of the germinated seed quantity ratio and the day of germination (Krause et al., 2015; Merino et al., 2018).

The natural germination rate (percentage) of the *L. sativa* seeds was assessed in a parallel test, in which one hundred *L. sativa* seeds (≤ 25 per gerbox) were treated with water.

2.6.2 *Growth experiment*

On the last day of the germination test (i.e., 7th day), the hypocotyl and radical lengths were measured using a pachymeter, and to measure dry mass, the seedlings were dried in an oven at 60°C until reaching a constant weight (Macias et al., 2000; Merino et al., 2018)

2.6.3 *Root respiration experiment*

Root respiration capacity was measured according to Steponkus and Lanphear (1967); Krause et al. (2015) and Merino et al. (2018). Briefly, 1.0 cm pieces were cut from the ends of 10 roots and transferred into test tubes with 5 mL 0.6% (m/v) triphenyl tetrazolium hydrochloride and 1 mL sodium phosphate (mono and dibasic) buffer (0.05 $mol.L^{-1}$ pH 7.0). The tubes were incubated at room temperature for 2 h and then transferred to a water bath (30 °C). After 15 h, the solutions were drained, the roots were rinsed with distilled water, 7 mL 95% (v/v) ethanol was added, and the tubes were heated in a boiling

water bath (~100 °C) for 15 min. After cooling to room temperature, 10 mL 95% (v/v) ethanol was added to each tube, and the absorbance of each tube at 530 nm was measured, using 95% (v/v) ethanol as a blank.

2.6.4 ***Enzyme activity experiment***

Methanolic extracts of 1.0 mg.mL^{-1} concentration were prepared at the end of the determined germination period and growth of the seedlings exposed to *P. meridionale* extracts. The seedlings were triturated using liquid nitrogen and then frozen (-20°C). Subsequently, 10 mL potassium phosphate buffer (0.2 mol.L^{-1}, pH 7.0) was added, and the samples were centrifuged (2500 rpm for 20 min at 4°C). The resulting supernatants were kept refrigerated (4°C) and used as enzymatic extracts (Putter, 1974) for the following assays.

2.6.4.1 ***Catalase (CAT; EC 1.11.1.6) activity***

Catalase activity was measured according to Aebi (1984). Briefly, 100 µL enzyme extract was combined with 3 mL aqueous hydrogen peroxide (H_2O_2, 12.5 mmol.L^{-1}) at 30 °C, and CAT activity was measured as the reduction in absorbance at 240 nm, which occurred as consequence of H_2O_2 consumption, using the molar extinction coefficient ($\varepsilon = 3.6\ 10^1$ mol.L^{-1}·cm^{-1}) (Anderson et al., 1995).

2.6.4.2. ***α-Amylase (EC 3.2.1.1) activity***

The α-Amylase activity was measured according to AOAC International (1965). Briefly, 400 µL enzyme extract was incubated in a water bath (70 °C for 20 min), combined with 0.5 mL acetate buffer (1.0 mol.L^{-1} pH 7.0) and 1.0 mL starch solution (1%), and then incubated at 30 °C. After 5 min, 0.5 mL lugol solution (50%) and 1 mL distilled water were added, and absorbance was measured at 620 nm using a spectrophotometer. The negative control contained 3 ml acetate buffer (1.0 mol.L^{-1}, pH 7.0), 3 mL starch solution (1%), and 1 mL lugol solution (50%), and the blank solution contained 2 mL starch solution (1%) and 6 mL acetate buffer (1.0 mol.L^{-1},pH 7.0).

2.6.4.3 ***Polyphenol oxidase (PPO; EC 1.10.3.1) activity***

To measure PPO activity, 1 mL enzyme extract was combined with 1 mL potassium phosphate buffer (0.05 mol.L^{-1} pH 6.0) and 0.5 mL catechol (0.5 mol.L^{-1}; (Merck Brasil, Rio de Janeiro, Brasil). The mixture was incubated at 30 °C for 30 min, and then transferred to an ice bath. Thereafter, 0.5 ml of perchloric acid (1.4%) was added, and the mixture was allowed stand for 10 min. As control (white), a mixture of 2 mL potassium phosphate buffer (0.05 mol.L^{-1}, pH 6.0) and 0.5 ml catechol (0.1 mol.L^{-1}; Duangmal & Apenten, 1999) was used. Absorbance was measured at 395 nm using a spectrophotometer.

2.6.4.4 ***Ascorbate peroxidase (APX; EC 1.11.1.11) activity***

Ascorbate peroxidase activity was measured by monitoring the oxidation rate of ascorbate at 290 nm ($\varepsilon = 2.80 \cdot 10^3$ mol.L^{-1}·cm^{-1}), as described by Amako et al. (1994). Briefly, 500 µl of enzyme extract was combined with 3 ml potassium phosphate buffer (50 mmol.L^{-1}, pH 7.0) that contained 1 mmol.L^{-1} H_2O_2, 1 mM ascorbic acid, and 1 mmol.L^{-1} ethylenediamine tetraacetic acid. For calibration, as white, 6 ml of the solution without enzymatic extract was used.

2.7 Analysis and Statistics

The analyses were conducted in triplicate and the readings were completed in spectrophotometer UV/Vis SHIMADZU-1601.

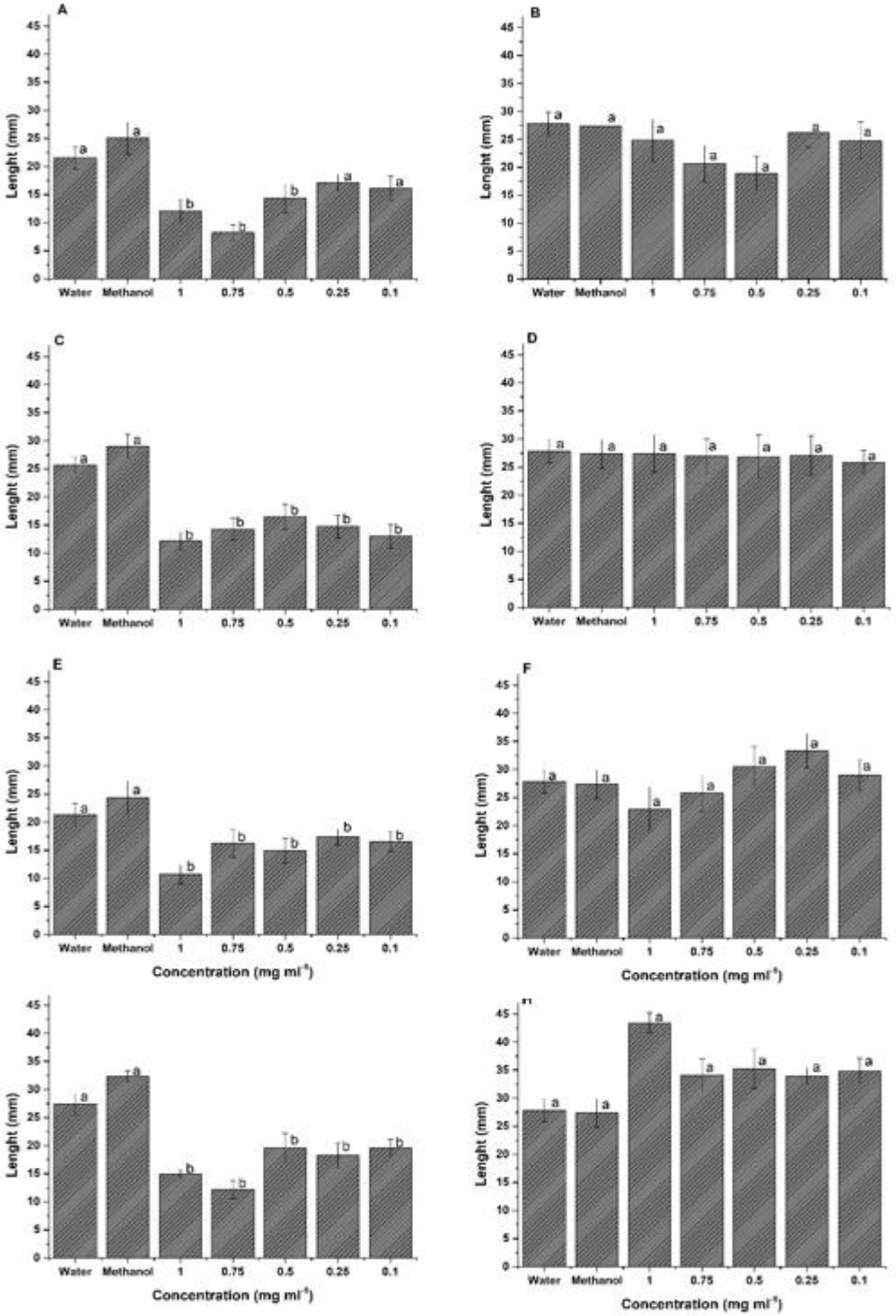
For the germination and growth bioassays, the experimental delineation was completely randomized, with six treatments (solvent control, negative control, and four extracts) and four replicates per treatment, and a total of twenty *L. sativa* seeds were used for each treatment. The germination and growth data were subject to variance analysis (ANOVA), using Origin 9.0 (OriginLab, Northampton, MA), and the means of each variable were compared using the Tukey test, at $p = 0.05$.

3. Results and Discussion

Most of the control *L. sativa* seeds germinated within 24 h, whereas most of the seeds treated with high extract concentrations (0.5, 0.75, and 1.0 $mg \cdot mL^{-1}$) germinated between 24 and 48 h; the effect of the extracts increased as the treatment concentration increased. The treatment had no effect on GVI value.

The extract treatments inhibited root growth, regardless of concentration, but did not affect hypocotyl growth (Figure 1). Interference with the primary root growth of treated plants, as observed in this study, is one of the main indicators used for the study of extracts with allelopathic potential (Souza-Filho et al., 1997).

Figure 1. Effects of ketonic and ethanolic *Philodendron meridionale* extracts on the growth of *Lactuca sativa* roots and hypocotyls**.**

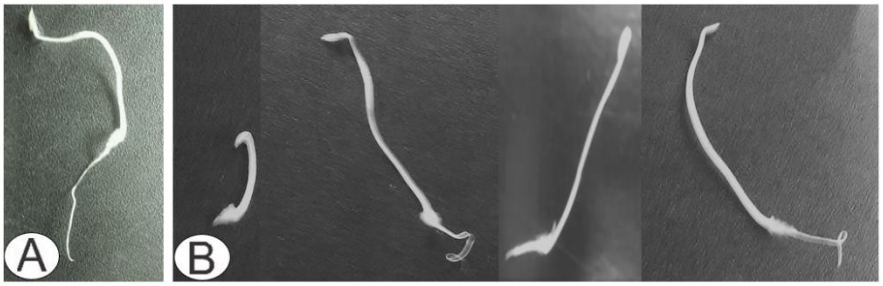


Being (A) LKE about root; (B) LKE about hypocotyls; (C) LEE about root; (D) LEE about hypocotyls; (E) SKE about root; (F) SKE about hypocotyls; (G) SEE about root; (H) SEE about hypocotyls. LKE: ketonic leaf extract; LEE: ethanolic leaf extract; SKE: ketonic stem extract; SEE: ethanolic stem extract; Negative Control: water; Solvent Control: methanol. Bars with the same letters within each plot are not significantly different at the 0.05 level (ANOVA).
Source: Authors (2020).

The extract treatments also affected seedling morphology, for example, causing roots to become atrophied and disproportionate (Figure 2). When compared with the roots of the control seedlings, the roots of the extract-treated seedlings were also thicker, and either hairier or hairless.

Several researchers have described effects that are similar to those observed in the present study and suggested that roots are damaged more than hypocotyls, mostly likely as a result of the direct contact of roots with the treatment solutions (Cruz-Ortega et al. 1998; Chung et al. 2001; Aumonde et al. 2012; Sousa et al. 2020; Torquato et al. 2020, Silva et al. 2020). Munns and Tester (2008) reported that root growth can be restricted by water deficit stress and ionic toxicity, both of which can cause metabolic and physiological damage. However, Marenco and Lopes (2005) noted that, although shoot length was unaffected, the damage of plants from water deficit could be discounted, as there was evidence that the extracts had toxic effects on plant growth factors.

Figure 2. Changes in the morphological aspect of radicles of *L sativa*, under action of *P. meridionale* extracts.



Being: A*: L sativa* seedling with normal development by negative control action; B: Seedlings of *L sativa* with development altered by the action of extracts of *P. meridionale*.
Source: Authors (2020).

Extract treatment also increased the concentrations α-amylase, APX, CAT, and PPO (Table 1) and, regardless of concentration, reduced the dry mass of the *L. sativa* seedlings, although not by more than 43% (Table 1).

Table 1. Effect of ketonic and ethanolic *Philodendron meridionale* extracts on the enzymatic activity, respiration, and dry mass of *Lactuca sativa* seeds.

	APX	CAT	PPO	α-Amylase	Respiration	Dry Mass (%)
LKE	25.44 ± 0.86*	0.38 ± 0.01*	20.5 ± 0.34*	44.48 ± 0.33*	0.040 ± 0.12	81.13*
LEE	24.36 ± 0.13*	0.34 ± 0.00*	20.1 ± 0.41*	54.03 ± 1.07*	0.042 ± 0.34	80.92*
SKE	33.89 ± 0.28*	0.28 ± 0.03*	20.34 ± 1.83*	46.25 ± 1.20*	0.053 ± 0.11	84.11*
SEE	29.94 ± 0.29*	0.30 ± 0.01*	19.09 ± 1.60*	52.59 ± 1.63*	0.062 ± 0.21	83.02*
Negative Control	5.15 ± 0.13	0.06 ± 0.01	6.60 ± 0.07	27.21 ± 0.88	0.050 ± 0.24	96.41
Solvent Control	5.88 ± 0.17	0.06 ± 0.01	6.73 ± 0.10	28.81 ± 2.84	0.060 ± 0.42	96.39

LKE: ketonic leaf extract; LEE: ethanolic leaf extract; SKE: ketonic stem extract; SEE: ethanolic stem extract; Negative Control: water; Solvent Control: methanol; APX: ascorbate peroxidase; CAT: catalase; PPO: polyphenol oxidase. Values followed by asterisks (*) are significantly different from other values in that column at the 0.05 level (ANOVA).
Source: Authors (2020).

Hoffmann et al. (2007) also reported that plant extracts reduced seedling mass. An alternative explanation is that the seedlings accumulated less water as a result of the action of the extracts, which would thereby inhibit growth and potentially alter cell membrane permeability. These processes also reflect the increased production and accumulation of free-radicals, which could indirectly affect the translocation and allocation of assimilates by affecting enzyme activities (Aumonde et al., 2013).

In the present study, the observed increases in CAT, APX, and PPO activities suggested that the plant defense systems were activated, perhaps in response to allelopathy, and that this caused oxidative stress in the treated seedlings. Meanwhile, the elevated α-amylase activity indicated that the plant energy system was weakened. According to Almeida et al. (2008), these factors result from changes in seedling growth.

CAT and APX are the two most important contributors to H_2O_2 detoxification (Bhatt & Tripathi, 2011). Furthermore, cell wall-bound APX catalyzes the polymerization of phenols to form the lignins that function as mechanical barriers to prevent the entry of substances (Kirkby & Römheld, 2007; Viecelli et al., 2010). It is likely that the contribution of PPO to disease resistance is related to the oxidization of phenolic and quinone compounds; like polymers, these can form protein complexes that act as physical barriers against the penetration of pathogens (Campos et al., 2004). The elevated levels of PPO observed in the present study suggested that phenolic compounds might act as allelochemicals. The elevated α-amylase activity observed in the present study could be attributed to defense-related increases in cellular energy expenditure.

Allelochemicals can also alter the cellular respiration of plants that are exposed to botanical extracts (Carmo et al., 2007). In the present study, we observed alterations in the membranes of treated *L. sativa* seeds, especially in their permeability. However, the *P. meridionale* extracts did not affect seedling respiration (Table 1).

In the present study, phenolic and flavonoid compounds were dosed in extracts of *P. meridionale*; the concentration of phenolic compounds were equivalent to $mg.g^{-1}$ of gallic acid — 225.12 for LKE, 240.45 for LEE, 193.28 for SKE, and 265.14

for SEE. The flavonoid contents of the *P. meridionale* LKE, LEE, SKE, and SEE were 52.74, 54.31, 72.74, and 67.21 $mg.g^{-1}$ quercetin, respectively. The observation of persistent froth also suggested the presence of saponins in the extracts.

Saponins, tannins, and flavonoids are among the allelochemicals that are often considered to have direct or indirect effects and can be used under natural conditions owing to their hydrosolubility (Rice 1984; Ferreira & Aquila, 2000). Saponins may exert phytotoxicity through their effects on membrane lipids or through effects on specific enzymes (Duke & Oliva, 2005). Einhellig (2005); Maraschin-Silva and Aquila (2006) reported that flavonoids and phenolic acids altered the permeability of cell membranes.

Lucchesi and Oliveira (1988) and Hoffmann et al., (2007) noted that crystals, such as calcium oxalate raphides, and natural polymers, such as tannins, lignins, and resins, can also have allelopathic effects. Lignins, resins, druses and raphides of calcium oxalate have been reported in *P. meridionale* (Swiech et al., 2016) and the present study confirmed the presence of saponins and quantified phenolic compounds, including flavonoids. These substances might be responsible for the observed effects, either through isolated actions or a synergic mechanism.

The phytotoxicity of many products probably results from a general disturbance to the cell growth of treated plants, rather than from any specific mechanism (Einhellig, 1994; Borella & Pastorini, 2009). Furthermore, as allelochemicals belong to a variety of chemical classes, it is unlikely that they will share modes of action. As suggested by Reigosa et al., (1999), this might also explain the paucity of described dose-dependent relationships, as in the present study.

4. Conclusions

In the present study, we found that extracts of *Philodendron meridionale* affect the germination rate, enzymatic processes and morphology of *Lactuca sativa* seeds. These findings demonstrate effects on membrane permeability and oxidative stress. The combined action and potential synergy of the metabolites found in the extracts of *P. meridionale* make the species suitable for use in cultures (mainly organic) and the metabolic actions characterized in this model can be correlated in drug therapies.

Further studies are needed in planting houses and in the field to observe if the *P. meridionale* extracts induce allelopathic action, given that climatic conditions and plant-soil-microorganism interactions can interfere with growth and germination conditions.

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References

Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzimology*. 105**,** 121–126.

Alliance, D., Santos, C. B. R., Barbosa, L. M. C., Gomes, J. S., Lobato, C. C., Viana, J. C., Ramos, R., Ferreira, R. M. A. & Souto, R. N. P. (2017). Chemical study, predictions "In Silico"and larvicide activity of the essential oil of root *Philodendron deflexum* Poepp. *J Comput Theor Nanosci*. 14(7), p3330-3337.

Almeida, G. D., Zucoloto, M., Zetun, M. C., Coelho, I. & Sobreir, F. M.(2008). Oxidative stress in vegetable cells mediated by allelochemicals. *Revista Facultad Nacional de Agronomía Medellín*. 61, 4237–4247.

Amako, K., Chen, G. & Asada, K. (1994). Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant and Cell Physiology*. 35, 497–504.

Anderson, M. D., Prasad, T. K. & Stewart, C. R. (1995). Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotylus of maize seedlings. *Plant Physiology*. 109, 1247–1257.

AOAC - Association of Official Agricultural Chemists. (1965). *Official methods of analysis*., (10th ed.), Editorial Board, Washington.

Aumonde, T. Z., Martinazzo, E. G., Borella, J., Pedó, T., Amarant,e L., Villela, F. A. & Moraes, D. M. (2012). Physiological alterations in seeds and the antioxidative metabolism of lettuce seedlings exposed to the activity of leaf extracts of *Zantedeschia aethiopica* Spreng. *Interciência*. 37, 845–851.

Aumonde, T. Z., Martinazzo, E. G., Borella, J., Pedó, T., Amarante, L., Villela, F. A. & Moraes, D. M. (2013). Physiological responses of seeds and seedlings submitted to the extract of *Philodendron bipinnatifidum. Semina–Ciências Agrárias.* 34, 3181–3192.

Bhatt, I. & Tripathi, B. N. (2011). Plant peroxiredoxins: catalytic mechanisms, functional significance and future perspectives. *Biotechnology Advances*. 29, 850–859.

Bogatek, R., & Gniazdowska, A. (2007). ROS and phytohormones in plant-plant allelopathic interaction. *Plant Signal Behav*. 2(4), 317-318.

Borella, J. & Pastorini, L. H. (2009). The allelopathic influence of *Phytolacca dióica* L. in the germination and initial growth of tomatoes and picão-preto. *Revista Biotemas*. 22, 67–75.

Brasil. (2009). Ministry of Agriculture, Livestock and Food Supply. Rules for the analysis of seeds. Mapa/ACS, Brasília.

Buturi, C. V., Temponi, L. G. & Sakuragui, C. M. (2014). A new species of *Philodendron* Schott (Araceae) in Paraná, Brazil. *Phytotaxa*. 174, 144-148.

Campos, A. D., Ferreira, A. G., Hampe, M. M. V., Antunes, I. F., Brancão, N., Silveira, E. P., Osório, V. A., & Augustin, E. (2004). Peroxidase and polyphenol oxidase activity in the resistance of beans to anthracnose. *Revista Agropecuária Brasileira*. 19, 637–643.

Carmo, F. M.S., Borges, E. E. L. & Takaki, M. (2007). Alelopatia de extratos aquosos de canela-sassafrás (*Ocotea odorifera* (Vell.) Rohwer). *Acta Bot Bras.* 21, 697- 705.

Carvalho, J. L. S., Cunico, M. M., Dias, J. F. G., Miguel, M. M. & Miguel, O. G. (2009).

Termoestabilidade de processos extrativos de *Nasturtium officinale* R. Br., brassicaceae por sistema Soxhlet modificado. *Quím. Nova*. 32(4), 1031-1035.

Chang, C. C., Yang, M. H., Wen, H. M. & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*.10, 178–182.

Chung, I. M., Ahn, L. K. & Yun, S. J. (2001). Assesment of allelopathic potential of barnyard grass (*Echinochloa crus-galli*) on rice (*Oriza sativa* L.) cultivars. *Crop Protec*. 20, 921-928.

Cruz-Ortega, R., Anaya, A. L., Hernández-Bautista, B. E. & Laguna-Hernández, G. (1998). Effects of allelochemical stress produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. *J Chem Ecol*. 24(12), 2039-2057.

Duangmal, K., & Apenten, R. K. O. (1999). A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanun tuberosum* var. romano). *Food Chem.* 64, 351-359.

Duke, S. O. & Oliva, A. (2005). Mode of Action of Phytotoxic Terpenoids. In: Macias, F. A., Galindo, J. C. G. & Molinillo, J. M. G. *Allelopathy: chemistry and mode of action of allelochemicals*. Taylor & Francis e-Library, 374p.

Einhellig, F. A. (1994). Mechanism of action of allelochemicals in allelopathy. *Allelopathy*. 9, 96-116. https://doi.org/10.1021/bk-1995- 0582.ch007

Einhellig, F. A. (2005). Mode of Allelochemical Action of Phenolic Compounds. In: Macias, F. A., Galindo, J. C. G. & Molinillo, J. M. G. (Eds.). *Allelopathy: chemistry and mode of action of allelochemicals*. Taylor & Francis e-Library, 392p.

Elakovich, S. D. (1999). *Biossays applied to allelopathic herbaceous vascular hydrophytes*. In: Inderjit, Dakshini, K. M. M. & Foy, C. L. (eds) Principles and practices in plant ecology. CRC Press. Boca Raton: 45–56.

Ferreira, A. G. & Aquila, M. E. A. (2000). Allelopathy: an emerging area of ecophysiology. *Revista Brasileira de Fisiologia Vegetal*. 12, 175–204.

Gatto, L. J., Fabri, N. T., Souza, A. M., Fonseca, N. S. T., Furusho, A. S., Miguel, O. G., Dias, J. F. G., Zanin, S. M. W. & Miguel, M. D. (2020). Chemical composition, phytotoxic potential, biological activities and antioxidant properties of *Myrcia hatschbachii* D. Legrand essential oil. *Brazilian Journal of Pharmaceutical Sciences*, 56, e18402.

Ghareeb, M. A., Refahy, L. A., Saad, A. M., El-Shazely, M. A., Mohamed, A. S. & Osman, N. S. (2015). Cytotoxic screening of three egyptian plants using brine shrimp lethality test. *Int J Pharm Pharm Sci*. 7(9), 507-509.

Hassanein, H. I., El-Ahwany, E. G., Salah, F. M., Hammam, O. A., Refai, L. & Hamed, M. (2011). Extracts of five medicinal herbs induced cytotoxicity in both hepatoma and myeloma cell lines. *J Cancer Sci Ther*. 3(10), 239-243.

Hoffmann, C. E. F., Neves, L. A. S., Bastos, C. F. & Wallau, G. L. (2007). Allelopathic activity of *Nerium oleander* L. and *Dieffenbachia picta* Schott in seeds of *Lactuca sativa* L. and *Bidens pilosa* L. *Revista de Ciências Agroveterinária*. 6,11–21.

Kirkby, E. A. & Römheld, V. (2007). Micronutrientes na fisiologia de plantas: funções, absorção e mobilidade. *Encart Inf Agr*. 118, 1-24.

Krause, M. S., Duarte, A. F. S., Merino, F. J. Z., Paula, C. S., Miguel, M. D., & Miguel, O. G. (2016). Phytotoxic Analysis of Extract of Leaves of *Solanum megalochiton* Mart. Solanaceae on *Lactuca sativa* L. and *Allium cepa* L. *International Journal of Sciences*. 5(11), 36-42.

Lucchesi, A. A., & Oliveira, R. F. (1988). Inhibitory effects in germination, induced by the cabbage extract (*Brassica oleracea* L. var. acephala DC.). *Annals of the Higher School of Agriculture Luiz de Queiroz*. 45, 167–178.

Macias, F. A., Castellano, D., & Molinillo, J. M. G. (2000). Search for a standart phytotoxic bioassay for allelochemicals. Selection of standard target species. *J Agric Food Chem.*, 48(6), 2512-2521.

Maraschin-Silva, F., & Aquila M. E. A. (2006). Allelopathic potential of native species in the germination and early stages of growth of *Lactuca sativa* L. (Asteraceae). *Acta Botânica Brasileira*. 20, 61–69.

Marenco, R. A., & Lopes, N. F. (2005). *Plant physiology: photosynthesis, respiration, hydric relations and mineral nutrition*. Boca Raton: UFV, Viçosa.

Merino, F. J. Z., Ribas, D. F., Silva, C. B., Duarte, A. F. S., Paula, C. S., Oliveira, M., Dias, J. F. G., Miguel, M. D. & Miguel, O. G. (2018). A study of the phytotoxic effects of the aerial parts of *Senecio westermanii* Dusén (Asteraceae) on *Lactuca sativa* L. and *Allium cepa* L. seeds. *Brazilian Journal of Pharmaceutical Sciences*, 54, 1-10.

Moura, V. M., Sousa, L. F., Santos, M. C., Raposo, J. D. A., Lima, A. E., Oliveira, R. B., Silva, M. N. & Mourão, R. H. V. (2015). Plants used to treat snakebites in Santarém, western Pará, Brazil: An assessment of their effectiveness in inhibiting hemorrhagic activity induced by *Bothrops jararaca* venom. *J. Ethnopharmacol*. 161, 224-232.

Munns R., & Tester M. (2008). Mechanism of salinity tolerance. *Annual Review of Plant Biology*. 59, 651–681.

Pino, O., Sánchez, Y. & Rojas, M. M. (2013). Plant secondary metabolites as an alternative in pest management. I: Background, research approaches and trends. *Rev. Protección Veg*. 28(2), 81-94.

Putter, J. (1974). Peroxidases. In: Bergmeyer, H. U. (Ed.) *Methods of enzymatic analysis*, 2nd Edn. Verlag Chemie, Weinheim, Germany. 685–690.

Reginatto, F. H. (2017). Introdução à análise fitoquímica. In: Simões, C. M. O., Schenkel, E. P., Gosmann, G., Mello, J. C. P. D., Mentz, L. A. & Petrovick, P. R. *Farmacognosia da Planta ao Medicamento*. Porto Alegre: Artmed, 69-83.

Reigosa, M. J., Sánchez-Moreiras, A., & González, L. (1999). An ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences*. 18, 577–608.

Reigosa, M., Gomes, A. S., Ferreira, A. G. & Borghetti, F. (2013). Allelopathic research in Brazil. *Acta Botanica Brasilica*, *27*(4), 629-646.

Rezende, C. P., Pinto, J. C., Evangelista, A. R. & Santos, E. I. P. A. (2003). *Alelopatia e suas interações na formação e manejo de pastagens*. Bol Agr, Universidade Federal de Lavras, MG. 54, 1-55.

Rice, E. L. (1984). *Allelopathy*. (2nd ed.), Orlando: Academic, 422p.

Santiago, J. A., Cardoso, M. G., Figueiredo, A. C. S., Moraes, J. C., Assis, F. A., Teixeira, M. L., Santiago, W. D., Sales, T. A., Camargo, K. C., & Nelson, D. L. (2014). Chemical characterization and application of the essential oils from *Chenopodium ambrosioides* and *Philodendron bipinnatifidum* in the control of *Diabrotica speciosa* (Coleoptera: Chrysomelidae). *American Journal of Plant Sciences*. 5, 3994-4002.

Silva, J. N., Silva, M. A. D., Rocha, A. K. P., Alves, R. M., Silva, E. F., Leal, L. S. G. & Moura, D. P. (2020). Allelopathy of *Piptadenia moniliformis* and *Capparis hastata* on the vigor of *Mimosa hostilis* seeds. *Research, Society and Development*, . 9(8), e472985527.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*. 28, 49–55.

Sousa, J. D., Leite, T. R., Linhares, K. V., Sousa, J. D., Bezerra, J. W. A., Santos, M. A. F., Torquatto, H. I. S., Boligon, A. A., Bezerra, J. S., Campos, N. B., & Silva, M. A. P. (2020). Chromatographic profile and allelopathic potential of the essential oil of *Acritopappus confertus* (Gardner) R. M. King & H . Rob. (Asteraceae). *Research, Society and Development*. 9(12), e1991210450.

Souza-Filho A. P. S., Rodrigues, L. R. A. & Rodrigues, T. J. D. (1997). Effects of the allelopathic potential of three leguminous plants on three invasive species of grasslands. *Revista brasileira de agricultura*. 32, 165–170.

Steponkus, P. L. & Lanphear, F.O. (1967). Refinement of the triphenyl tetrazolium chloride method of determining cold injury. *J Plant Physiol*. 42, 1423-1426.

Swiech, J. N. D., Bobek, V. B., Folquitto, D. G., Silva, R. Z., Budel, J. M., Farago, P. V., Miguel, M. D. & Miguel, O. G. (2016). Morpho-anatomy of the vegetative organs of *Philodendron meridionale* Buturi & Sakur. *Latin American Journal of Pharmacy*. 10, 2142–2148.

Torquato, I. H. S., Costa, N. C., Pereira, K. S., Campos, N. B., Oliveira, A. A., Generino, M. E. M., Bezerra, J. W. A., Santos, M. A. F., Sousa, J. D., Boligon, A. A., & Silva, M. A. P. (2020). Composição polifenólica e potencial alelopático de *Senna cearensis* Afr. Fern. (Fabaceae). *Research, Society and Development*. 9(8), e577986207.

Viecelli, C. A., Stangarlin, J. R., Kuhn, O. J., & Schwan-Estrada, K. R. F. (2010). Induction of resistance in beans to angular leaf spots by extracts of mycelium of *Pycnoporus sanguineus. Summa Phytopatholica*. 36, 73–80.

Zimdahl, R. L. (2018). *Chapter 9 – Allelopathy*. Fundamentals of Weed Science (Fifth Edition), 253-270. https://doi.org/10.1016/B978-0-12-811143-7.00009-3.