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Allelopathic potential of ethanolic extract and its fractions from leaves of *Geonoma schottiana Mart.*

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Abstract

Geonoma schottiana Martius (Arecaceae) is an understory palm species widely distributed in Brazil. The objective of this work was to determine the allelopathic potential of *Geonoma schottiana* leaf extract and its fractions of different polarities and evaluate *in vitro* and *in vivo* antioxidant activities. The germination of seeds of *Lactuca sativa* and *Panicum maximum* was tested using ethanolic leaf extract and its hexane, dichloromethane, ethyl acetate, and butanol fractions. In vitro antioxidant tests were performed through the DPPH, ABTS, FRAP, and molybdenum tests, whereas the SOD, CAT, and POX enzymatic tests were used to evaluate in vivo antioxidant activity. Chemical analyses were performed through phytochemical screening of the main groups of secondary metabolites by thin-layer chromatography and quantification of the total contents of phenolics, tannins, and flavonoids. The hexane and butanol fractions had allelopathic effects on *P. maximum* and low activity on *L. sativa*. The ethyl acetate fraction presented higher antioxidant activity in the DPPH test, whereas butanol presented higher antioxidant activity in the ABTS test. The most active fractions in terms of allelopathic effect interfered with the increases in the production of SOD and POX enzymes. The most polar fractions had higher contents of phenolic compounds. All analyzed chemical groups were found in the phytochemical screening, except the anthracene group.

Keywords: antioxidant, catalase, chemical analyses, peroxidase, prooxidants

Introduction

Studying allelopathic effects among plant species is one of the alternatives for identifying bioactive compounds from plant derivatives for controlling weeds in large crops. Allelopathy can affect the geographical distribution of a plant species and enabling the germination and survival of seedlings to allelopathy is an alternative management for maintaining plant populations. Allelochemicals are substances produced by plants that cause allelopathic effects on other plant species and can currently be used to meet the increasing need for replacing synthetic agricultural inputs with natural compounds, thus contributing to sustainable agricultural development and protection of natural resources (Schandry & Beaker, 2020). Studies have shown that the use of allelopathic properties of some plant species has resulted in positive effects for controlling weeds and lettuce growth, serving as indicators in bioassays to determine allelopathic potentials.

Plants of the family Arecaceae have significant economic importance, mainly for the food sector, including the genera *Bactris* (peach palm), *Cocos* (coconut palm), *Elaeis* (oil palm), and *Euterpe* (açai palm). Several studies have reported the benefits of some species of this family due to the presence of secondary metabolites with antioxidant activities, which assist in reducing free radicals within the cellular environment (Naskar et al., 2013). Free radicals can be any chemical species that exist independently with one or more unpaired electrons, which makes them highly reactive and able to attack biomolecules, including proteins, carbohydrates, lipids, DNA, and RNA. The formation of these compounds is determined by the loss or gain of electrons, presenting unpaired electrons in the atomic orbitals of different atoms. Several studies have shown that phenolic compounds found in Arecaceae species

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(William et al., 2018) can provide protective (antioxidant) action against damages caused by free radicals. The antioxidant activity of these compounds derives from their redox properties, which have an important function in neutralizing free radicals.

Geonoma schottiana Martius (Arecaceae), known in Brazil as aricanga, is an understory palm species widely distributed in the country, found in the Cerrado (Fontes, 2019) and Atlantic Forest (Braz et al., 2016) biomes. Ecological studies have indicated that its wide distribution is attributed to its high genetic variability (Loiseau et al., 2019), herbivory resistance (Santos Alves et al., 2018), and high germination potential (Braz et al., 2016). However, studies on the biological activities of this species are scarce. Thus, the objective of this study was to evaluate the allelopathic potential and in vitro and in vivo antioxidant activity of *G. schottiana* leaf ethanolic extract and their fractions of different polarities.

Material and Methods

Plant extract

Leaves were collected from five randomly selected individuals of *Geonoma schottiana* in the Aricanga Municipal Park, in Aracruz, Espirito Santo (ES), Brazil (19°49'18.04"S and 40°19'52.40"W). Exsiccate of the botanical material was deposited in the VIES Herbarium of the Federal University of Espirito Santo, Vitoria, ES, under the number 015065. The leaves were washed to remove dust and other residues, dried in an oven at 40 °C for 120 hours, and crushed in an industrial blender.

The leaves were macerated using commercial ethanol as a solvent. The material was filtered after 72 hours of extraction, separating the plant residue from the extract. The ethanolic extract obtained was concentrated in a rotary evaporator until complete ethanol evaporation. The recovered ethanol was then added back to the plant residue to continue the extraction process until the exhaustion of the material plant. The crude extract obtained was stored in a glass container under refrigeration at 8 °C. A portion of the ethanolic extract was resuspended in a mixture of water and ethanol (2:8 v v^{-1}) and successive liquid-liquid extractions were performed using solvents of increasing polarity to obtain the hexane, dichloromethane, ethyl acetate, and butanol fractions. The solvents were completely evaporated in a rotatory evaporator after fractionation.

Allelopathic potential

The allelopathic potential was evaluated through a germination bioassay using seeds of *Lactuca sativa* L. (lettuce) and *Panicum maximum* Jacq. (Guinea grass). The seeds were distributed onto Petri dishes moistened with 3.0 mL of the ethanolic extract and its hexane, dichloromethane, ethyl acetate, and butanol fractions at a concentration of 1.0 mg mL^{-1} each. A 1% dimethylsulfoxide solution (DMSO) was used as the experimental control. Each Petri dish contained 20 seeds, with five replicates (totaling 100 seeds per treatment); they were maintained in a BOD germination chamber at 20 °C (*L. sativa* seeds) and 25 °C (*P. maximum*) under constant light (MAPA, 2009).

Germination counts were carried out every 24 hours for seven days. Root and shoot lengths were measured on the seventh day. Seeds were considered germinated when the root length reached at least 50% of the seed length (Ferreira & Aquila, 2000). The following variables were evaluated to determine the allelopathic potential for germination and initial growth: germination index, germination speed index (Labouriau & Viladares, 1976), mean time for germination (Ferreira & Borguetti, 2004), allelopathic index (Alves et al., 2004), root length, shoot length, root fresh weight, and shoot fresh weight (Borella & Pastorini, 2010). The fresh weight was obtained by weighing 10 randomly selected samples from the seedlings that had their root and epicotyl lengths evaluated. The shoot was separated from the root and weighed separately. Weighing was performed using a precision analytical balance and the results were expressed as grams per root and grams per shoot.

In vivo evaluation of pro-oxidant and antioxidant activity

Malondialdehyde (MDA)

The oxidation level and cell damage were determined by measuring the MDA content, which is the final product of lipid peroxidation (Morales &Munné-Bosch, 2019). Samples of 300 mg of plant tissue were macerated using liquid nitrogen in 2.0 mL of 0.1% trichloroacetic acid (w v^{-1}). The homogenate was centrifuged at 12,000 × g at 4 °C for 15 minutes and the supernatant was collected. Subsequently, aliquots of 1.0 mL were collected from the supernatant and added to 1.0 mL of a 0.5% (w/v) thiobarbituric acid solution prepared in 20% trichloroacetic acid (w v^{-1}). The samples were incubated at 90 °C for 35 minutes and rapidly cooled on ice. Readings were performed at 532 nm and 600 nm. The MDA content was calculated using the molar extinction coefficient of 155 mM cm^{-1} based on the formula: MDA content (ηM) = $[(A_{532} - A_{600}) / 1.56] \times 105$.

Enzymatic activity of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD)

Sample preparation for assessing CAT, POX, and SOD enzymatic activities

The activity of the antioxidant enzymes SOD, POX, and CAT was evaluated using samples obtained from 300 mg of *P. maximum* seeds and roots subjected to pre-germination treatments for 18 hours in the hexane fraction and for 7 days in the butanol fraction, both at a concentration of 1.0 mg mL^{-1}. The material was homogenized with 0.1 M potassium phosphate buffer (pH 6.8), 0.1 mM EDTA-Na_2, and 1% polyvinylpolypyrrolidone (w v^{-1}). Extractions were performed using liquid nitrogen and the homogenate was centrifuged at 1,2000 × g at 4 °C for 15 minutes. The supernatant was used to assess the SOD, CAT, and POX activities. SOD activity was assessed according to Del-Long et al. (1993) by conducting the reaction at 25 °C in a 15-W lamp chamber for 6 minutes, followed by reading at 560 nm. POX activity was assessed according to Kar &Mishra (1976) by conducting the reaction at room temperature for 2 minutes, followed by reading at 420 nm. CAT activity was assessed according to Anderson et al. (1995) by conducting the reaction at room temperature for 2 minutes with subsequent readings at 240 nm. Three replicates with duplicates were performed in a completely randomized design.

Evaluation of in vitro antioxidant activity of extracts and fractions

DPPH assay

The antioxidant activity of the extracts was analyzed through the DPPH free radical capture method (Rufino et al., 2007) with adaptations. The samples were prepared using 1mL of a 0.1mM DPPH solution and 3.0 mL of ethanolic extract and its fractions at a concentration of 1.0 mg mL^{-1}. Each solution was prepared by diluting the sample in its respective extraction solvent. The samples remained to react for 30 minutes at room temperature and in the dark until the absorbance readings. The standard curve was determined using 4 mL of DPPH solution at concentrations of 10µM, 20µM, 30µM, 40µM, 50µM, and 60µM, with dilutions made in methanol. The study was conducted in triplicates and the readings were taken in glass cuvettes. Absorbance readings were carried out using a spectrophotometer (Biospectro SP-220) at a wavelength of 515 nm. Trolox at concentrations of 100µM, 500µM, 1000µM, 1500µM, and 2000µM was used as the standard antioxidant, and the results were expressed as antioxidant capacity equivalent to µM Trolox g^{-1} of extract, i.e., TEAC (Trolox equivalent antioxidant capacity).

ABTS assay

The antioxidant activity of the ethanolic extract and their fractions was carried out through the capture of $ABTS^{\bullet+}$ radical cations (Sánchez-González et al., 2005). The ABTS radical solution was prepared by reacting 7 mM ABTS with 140 mM potassium persulfate. The $ABTS^{\bullet+}$ radical solution was kept in the dark at room temperature for 16 hours for complete reaction and stabilization of the radical. Then, it was diluted in ethanol to obtain an absorbance of 0.7 ± 0.05 at 734 nm. The calibration curve of the Trolox standard was prepared at concentrations of 100µM, 500µM, 1000µM, 1500µM, and 2000µM. The concentration used to evaluate the antioxidant capacity of the ethanolic extract and its fractions was 1.0 mg mL^{-1}. A 30µL aliquot of each standard solution was transferred to test tubes in a dark environment and 3.0 mL of the $ABTS^{\bullet+}$ radical solution was added. Absorbances were measured at 734 nm after 6 minutes of reaction, using ethanol as the blank. The same procedure was carried out for the solutions of the ethanolic extract and its fractions. Trolox was used as the antioxidant standard, and the results were expressed as TEAC.

Ferric ion reducing antioxidant power (FRAP)

The FRAP test was conducted according to Sánchez-González et al. (2005). According to this assay, antioxidants added to the reaction medium reduce the Fe^{3+} TPTZ complex to form a blue Fe^{2+}-TPTZ complex, resulting in an increased absorbance. The FRAP of the ethanolic extract and its fractions was assessed by mixing 900 µL of FRAP reagent (freshly prepared and preheated at 37 °C) with 90 µL of distilled water and 30µL of the ethanolic extract and its fractions at a concentration of 1.0 mg mL^{-1}, as well as the standard. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$, and 25 mL of 0.3 mM acetate buffer at pH 3.6. Readings were taken at 595 nm every 15 seconds, using a spectrophotometer (Beckman DU-640; Beckman Instruments Inc., Fullerton, USA) coupled to a thermostatic cell holder. The temperature was maintained at 37 °C. Readings at 30 minutes were selected to calculate the FRAP values. The standard curve was generated by replacing the sample aliquot with a 10µL aliquot of Trolox at concentrations of 0.5µM, 0.75µM, 1.0µM, 1.25µM, 1.5µM, 1.75µM, and 2.0µM. The antioxidant results were expressed as equivalent µM Trolox g^{-1} of extract.

Phosphomolybdenum

The total antioxidant capacity (TAC) of the

ethanolic extract and its hexane, dichloromethane, ethyl acetate, and butanol fractions were evaluated following the phosphomolybdenum method (Parimelazhagan, 2016). A 300µL aliquot of the extract and each fraction at a concentration of 1.0 mg mL^{-1} was added to 3 mL of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture solution was subject to incubation in a water bath at 95 °C for 90 minutes. The absorbance was measured at 695 nm using a UV/VIS spectrophotometer. TAC was expressed as ascorbic acid equivalent (µg AAE mg^{-1}).

Phytochemical profile
Thin-layer chromatography (TLC)

TLC analysis was performed according to the methodology of Wagner & Bladt (1996). The stationary phase consisted of using silica gel 60 chromatography plates (ALUGRAM® Xtra SIL G/UV_{254}, 10 × 10 cm), with 0.5 cm application bands for the samples and a mobile phase elution of 8.0 cm bands. A 10µL aliquot of samples was added using a micropipette (Hamilton 705SN). Chemical groups, chromatographic conditions, and developing agents for each group are shown in (**Table 1**).

Table 1. Thin-layer chromatography (TLC), elution systems, and developing reagents for chemical groups

Chemical groups	Elution system	Reagent
Alkaloids	Toluene: Ethyl acetate: diethylamine (70:20:10, v v^{-1})	Dragendorff
Derivatives from anthracene	Ethyl acetate: methanol: water (1000:13:5:10, v v^{-1})	10% KOH in ethanolic extract
Coumarins	Toluene: Ethyl Ether: (1:1 saturated with 10% acetic acid, v v^{-1})	10% KOH in ethanolic extract
Lignans	Chloroform: methanol: water (70:30:4, v v^{-1})	Sulfuric vanillin
Mono and diterpenes	Toluene: ethyl acetate (93:7, v v^{-1})	Sulfuric vanillin
Naphthoquinones	Toluene: formic acid (99:1, v v^{-1})	10% KOH in ethanolic extract
Triterpenes and steroids	Toluene: chloroform: ethanol (40:40:10, v v^{-1})	Liebermann-Burchard

Determination of total phenolic, tannin, and flavonoid contents

Total phenolics were quantified using the Folin-Ciocalteau method (Sánchez-Rangel et al., 2013). Samples of the ethanolic extract and its fractions were prepared to obtain a final concentration of 1.0 mg mL^{-1} each, using the extraction solvents for solution preparation. An aliquot of 0.2 mL was withdrawn from each sample, and 0.5 mL of 10% (v/v) Folin-Ciocalteau solution, 1.0 mL of 7.5% (w/v) sodium carbonate solution, and 8.4 mL of water were added, in the dark. The absorbance was measured at 760 nm in a a spectrophotometer after 30 minutes.

Total tannins were quantified using the casein precipitation method (Seigler et al., 1986). A volume of 1.0 g of powdered casein was added to an Erlenmeyer flask, flowed by the addition of 6.0 mL aliquots of the samples, dilution in 12 mL of water, and constant agitation for three hours under room temperature (25 °C). A control sample was prepared similarly using only water and casein. Subsequently, the samples were filtered through filter paper, and the volume of the resulting filtrate was brought to 25 mL in a volumetric flask. These solutions were subjected to residual phenol testing using the Folin-Ciocalteu method. The total tannins corresponded to the difference between the value found in the total phenolics reading and the residual phenolics. The same procedure was used for the standard solutions of tannic acid at the following concentrations: 100µg mL^{-1}, 50µg mL^{-1}, 25µg mL^{-1}, 12.5µg mL^{-1}, 6.25µg mL^{-1}, 3.12µg mL^{-1}, 1.56µg mL^{-1}, and 0.78µg mL^{-1}. The determinations were performed in triplicate, and the results of total phenolics and total tannins were expressed as tannic acid equivalent (µg TAE mg^{-1} of the sample).

The content of total flavonoids in the ethanolic leaf extract and its polar fractions was quantified using the colorimetric method with aluminum chloride (Djeridane et al., 2006). A volume of 1.5 mL of 2% $AlCl_3$ solution in ethanol was mixed with 1.5 mL of each sample solution. The absorbance was measured at 415 nm after a 10-minute incubation at room temperature using a UV/VIS spectrophotometer (Thermo, Waltham, MA, USA). Ethanol was used as a blank to replace the sample. Quercetin was used as a standard at concentrations of 100µg mL^{-1}, 50µg mL^{-1}, 25µg mL^{-1}, 12.5µg mL^{-1}, 6.25µg mL^{-1}, 3.12µg mL^{-1}, 1.56µg mL^{-1}, and 0.78µg mL^{-1} for the calibration curve, and the results were expressed as quercetin equivalent per milligram of the sample (µg QE mg^{-1}).

Experimental Design and Statistical Analysis

The experiments were conducted in a completely randomized design. The data obtained for the ethanolic leaf extract and its fractions were subjected to analysis of variance (ANOVA) and the means were compared

using Tukey's test ($p \leq 0.05$ and 0.01). Enzymatic activity and lipid peroxidation data were subjected to the T-test at 5% significance level.

Results and Discussion

Allelopathic potential

The data found for the allelopathic potential of the ethanolic extract and its fractions from leaves of *Geonoma schottiana* are shown in (**Table 2**). The ethanolic extract and its hexane and butanol fractions significantly inhibited the germination index and germination speed index of *Panicum maximum*; the hexane and butanol fractions presented an allelopathy index close to 50%. All treatments affected the root and shoot growth and biomass accumulation of *P. maximum*.

Regarding *Lactuca sativa*, the ethanolic extract and its ethyl acetate fraction hindered root and shoot growth and biomass accumulation, however, without damaging the germination process; only the acetate fraction decreased the germination speed index and increased the meantime for germination compared to the control. High allelopathy indexes were not found for this species.

Table 2. Germination index (GI), germination speed index (GSI), mean time for germination (MTG; in days), allelopathy index (AI), root length (RL; cm), shoot length (SL; cm), root fresh weight (RFW; g) and shoot fresh weight (SFW; g) of *Lactuca sativa L.* and *Panicum maximum* Jacq subjected to leaf extracts of Geonoma schottiana Mart at concentration of 1.0 mg mL^{-1}

Test plant	Extracts	GI	GSI	MTG	AI	RL	SL	RFW	SFW
Lactuca sativa	Control	92^{abc}	21.13^{bc}	1.24^{b}	0.00^{b}	5.11^{a}	0.82^{a}	0.0339^{b}	0.0612^{a}
	Ethanolic	97^{ab}	23.91^{a}	1.04^{c}	-5.85^{c}	3.10^{b}	0.59^{d}	0.0200^{d}	0.0425^{b}
	Hexane	94^{ab}	23.25^{ab}	1.02^{c}	-2.54^{bc}	4.75^{a}	0.78^{b}	0.0346^{b}	0.0608^{a}
	Dichloromethane	81^{c}	19.87^{c}	1.03^{c}	11.76^{a}	4.70^{a}	0.68^{c}	0.0260^{c}	0.0290^{d}
	Ethyl acetate	87^{bc}	16.31^{d}	1.53^{a}	5.49^{a}	3.35^{b}	0.44^{e}	0.0167^{e}	0.0379^{c}
	Butanol	100^{a}	24.83^{a}	1.02^{c}	-8.99^{c}	5.01^{a}	0.80^{ab}	0.0452^{a}	0.0613^{a}
Panicum maximum	Distilled water	85^{a}	7.26^{a}	3.10^{d}	0.00^{b}	4.70^{a}	2.19^{a}	0.0797^{a}	0.0610^{a}
	Ethanolic	74^{b}	5.03^{b}	3.83^{bc}	12.26^{b}	1.72^{c}	1.55^{c}	0.0343^{c}	0.0492^{b}
	Hexane	43^{d}	2.88^{c}	3.87^{abc}	49.19^{a}	2.51^{b}	1.09^{d}	0.0187^{d}	0.0133^{e}
	Dichloromethane	79^{ab}	5.27^{b}	4.19^{a}	8.98^{b}	1.89^{c}	1.86^{b}	0.0475^{b}	0.0402^{c}
	Ethyl acetate	83^{ab}	5.45^{b}	4.10^{ab}	4.37^{b}	2.05^{c}	1.77^{b}	0.0561^{ab}	0.0372^{c}
	Butanol	54^{c}	3.76^{c}	3.72^{c}	52.62^{a}	0.82^{d}	1.52^{c}	0.0102^{e}	0.0287^{d}

In Vivo Pro-Oxidant Activity of Malondialdehyde (MDA)

The results from the analysis of lipid peroxidation indicated that the exposure of *P. maximum* seeds to the hexane and butanol fractions—the most toxic for germination of this species—resulted in a significant increase in malonaldehyde production compared to the control (**Figure 1**).

In vitro and in vivo of antioxidant activity of the plant extract and its fractions

In vitro antioxidant activity

The antioxidant activity of the ethanolic extract and its hexane, dichloromethane, ethyl acetate, and butanol fractions obtained through the DPPH, ABTS, FRAP, and phosphomolybdenum tests are shown in (**Table 3**). The results obtained using the TEAC (Trolox equivalent antioxidant capacity) method presented a higher capacity of DPPH redox for the ethyl acetate (271.91µM Trolox g^{-1}) and dichloromethane (245.09µM Trolox g^{-1}) fractions. The ethanolic extract and its fractions presented antioxidant activity by inhibiting the ABTS$^{\bullet+}$ radical, mainly the butanol fraction (197.47µM Trolox g^{-1}). The dichloromethane fraction presented the highest antioxidant activity in the FRAP test (10.97µM Trolox g^{-1}). The hexane fraction (89.22µg AAE mg^{-1}) presented higher antioxidant activity in the phosphomolybdenum test compared to the ethanolic extract and the other fractions.

In vivo antioxidant activity

The analysis of enzymatic activity was carried out using the hexane and butanol fractions, which showed the most promising results in the allelopathic potential and in vitro antioxidant activity tests for *P. maximum*. These fractions increased the antioxidant activity of all evaluated enzymes. The SOD and POX enzymes stood out by presenting the highest increases in antioxidant activity, regardless of the extractor or plant organ analyzed (**Figure 2**).

Chemical analyses and phytochemical profile

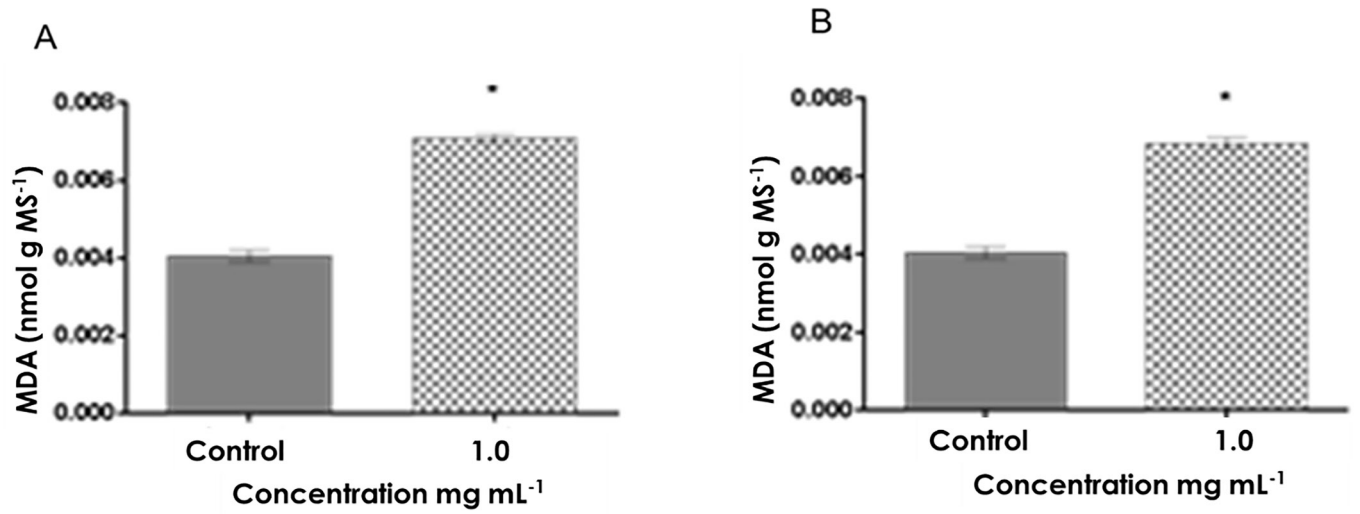

Figure 1. Graphical representation of lipid peroxidation in seeds of *Panicum maximum* subjected to treatments with hexane (A) and butanol (B) fractions (concentration of 1.0 mg mL^{-1}) of ethanolic extract from leaves of *Geonoma schottiana*, compared to the control. * = significant difference by the T-test at 5% significance level.

Table 3. Antioxidant activity in leaf ethanolic extract of *Geonoma schottiana* and its fractions (concentration of 1.0 mg mL^{-1}) obtained through DPPH, ABTS, FRAP (µM Trolox g^{-1}), and phosphomolybdenum (µM AAE g^{-1}) tests

Extract and its fractions	DPPH	ABTS	FRAP	Phosphomolybdenum
Ethanolic extract	227.48 ± 3.409 ^{b}	152.81 ± 2.857 ^{b}	6.30 ± 0.174 ^{d}	59.99 ± 1.229 ^{b}
Hexane	141.74 ± 1.056 ^{c}	163.84 ± 1.549 ^{b}	7.54 ± 0.037 ^{c}	89.22 ± 1.082 ^{a}
Dichloromethane	245.09 ± 2.752 ^{ab}	158.60 ± 1.194 ^{b}	10.97 ± 0.072 ^{a}	87.73 ± 1.036 ^{a}
Ethyl Acetate	271.91 ± 4.558 ^{a}	164.58 ±1.437 ^{b}	7.87 ± 0.023 ^{bc}	41.54 ± 1.009 ^{c}
Butanol	232.15±0.8145 ^{b}	197.47 ± 1.002 a	7.93 ± 0.100 ^{b}	49.47 ± 1.047 ^{bc}

Means followed by the same letter in the column are not statistically different from each other by Tukey's test at 5% significance level.

The total phenolic, tannin, and flavonoid contents found in the ethanolic extract and its fractions are shown in (**Table 4**). The butanol fraction exhibited the highest total contents of phenolics (344.62µg TAE mg^{-1}), tannins (311.11µg TAE mg^{-1}), and flavonoids (8.71µg QE mg^{-1}). These contents decreased as the polarity of the fractions decreased.

Thin-layer chromatography

The analyses by thin-layer chromatography (TLC) presented the main chemical groups found in the ethanolic extract and its fractions (Table 8). The ethanolic extract presented all analyzed groups, except the naphthoquinone group, which also was not found in the other fractions. The hexane fraction presented the mono and sesquiterpene, steroid, and lignin groups. The dichloromethane and ethyl acetate fractions presented the same groups of metabolites, except for the flavonoid group which was found in the ethyl acetate fraction. The mono and sesquiterpene, flavonoid, lignin, and alkaloid groups were found in the butanol fraction (**Table 5**).

The analysis of allelopathic activity of ethanolic extract and its fractions from leaves of *G. schottiana* on *P. maximum* showed that all treatments negatively affected the evaluated growth variables; the hexane and butanol fractions presented the most significant results, including seed germination damage and high allelopathy indexes (~50%). However, this result could not be confirmed in the analysis of the extract's effects on *L. sativa*, as no treatment had a toxic effect on the germination process and no increases in allelopathy index were found, despite the decreases in plant growth and biomass accumulation caused by the ethanolic extract and the ethyl acetate fraction. Allelopathy indexes equal to or higher than 50% denote a strong allelopathic effect, which was found only for the hexane and butanol fractions on *P. maximum*.

Assessing allelopathic effects on test seeds, such as *L.* sativa, and on seeds of weed species is important when a product does not negatively affect the test plant while affecting the target plants. The results found in the present work showed that mainly the hexane and butanol fractions had allelopathic effects on *P. maximum* and no evident damage to *L. sativa*. Considering the processes of obtaining plant extracts, the solvent used is one of the main determining factors for the capacity of extracting chemical components present in plants (Fioresi et al.,

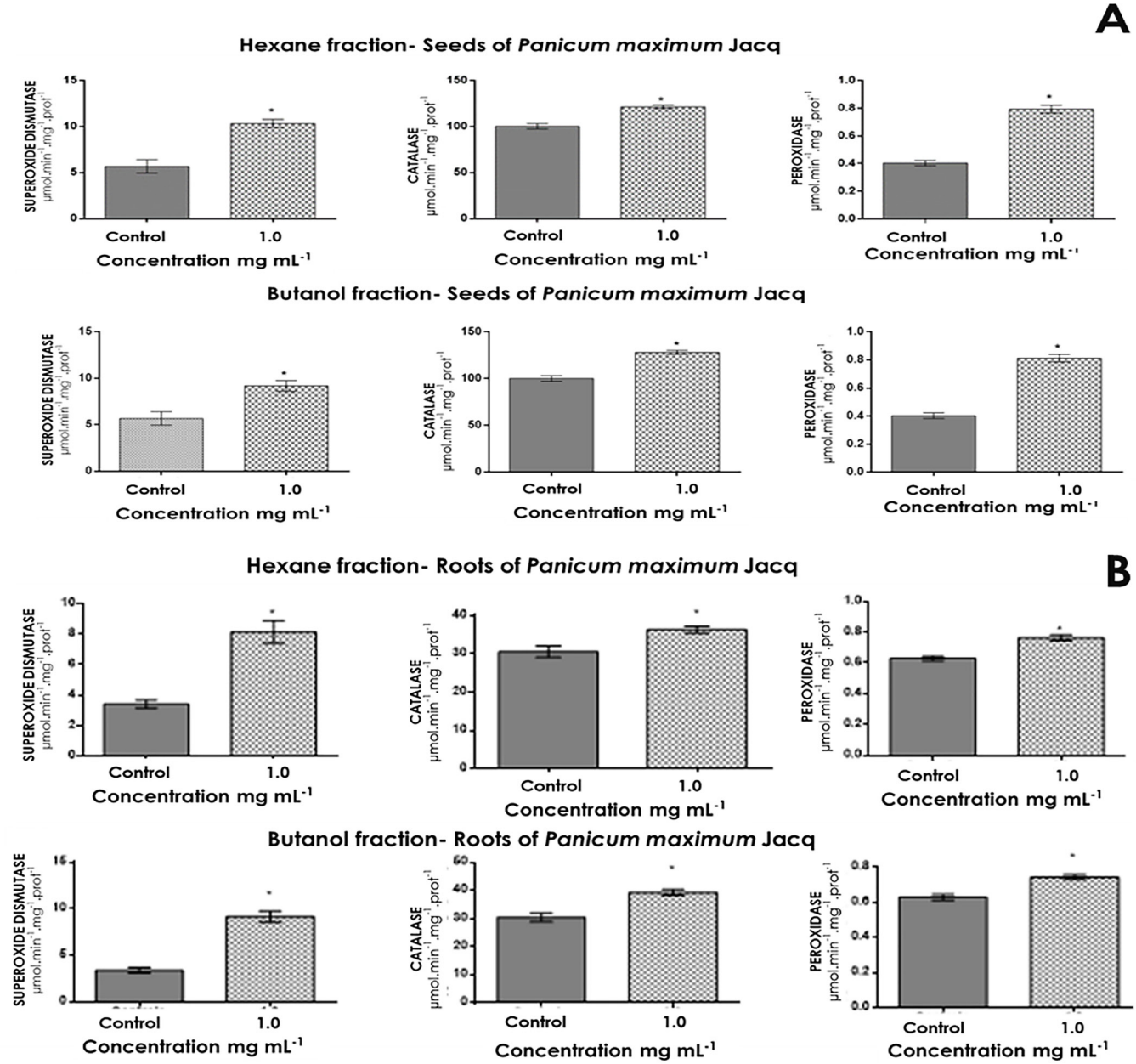

Figure 2. SOD, CAT, and POX enzymatic activity in seeds (A) and roots (B) of Panicum maximum to treatments with hexane (A) and butanol (B) fractions (concentration of 1.0 mg mL-1) of ethanolic extract from leaves of Geonoma schottiana, compared to the control. * = significant difference by the T-test at 5% significance level.

Table 4. Total contents of phenolics, tannins, and flavonoids in ethanolic extract and its fractions from leaves of *Geonoma schottiana*

Extract and fractions	Total phenolics (µg TAE mg^{-1})	Total tannins (µg TAE mg^{-1})	Total flavonoids (µg QE mg^{-1})
Ethanolic extract	209.44 ± 2.158^{ab}	109.25 ± 2.002 ^{c}	6.62 ± 0.023 ^{b}
Hexane	65.02 ± 1.835 ^{c}	35.18 ± 0.763 ^{d}	0.39 ± 0.009 ^{d}
Dichloromethane	164.44 ± 2.098^{b}	154.81 ± 1.677 ^{b}	0.39 ± 0.005 ^{d}
Ethyl acetate	265.02 ± 1.984^{ab}	183.33 ± 1.076 ^{b}	2.56 ± 0.014 ^{c}
Butanol	344.62 ± 2.580 ^{a}	311.11 ± 2.390 ^{a}	8.71 ± 0.023 ^{a}

The means followed by the same letter in the column are not statistically different from each other by the Tukey's test at 5% significance level.

2021). The use of non-selective solvents, such as ethanol, and selective solvents of different polarities, such as hexane, dichloromethane, ethyl acetate, and butanol, results in the concentration of secondary metabolites in fractions with similar polarities to those of the solvents.

The hexane and butanol fractions affected all the variables evaluated for allelopathic effect on seeds of *P. maximum*, which may denote the presence of chemical groups with polar and non-polar characteristics for the desired biological effect. Steroids, terpenes, flavonoids,

Table 5. Results of phytochemical screening by thin-layer chromatography (TLC) in ethanolic extract and its fractions from leaves *of Geonoma schottiana*

Chemical groups	Samples				
	Ethanolic	Hexane	Dichloromethane	Ethyl acetate	Butanol
Mono and sesquiterpenes	(+)	(+)	(-)	(-)	(+)
Steroids	(+)	(+)	(+)	(+)	(-)
Flavonoids	(+)	(-)	(-)	(+)	(+)
Coumarins	(+)	(-)	(+)	(+)	(-)
Lignins	(+)	(+)	(-)	(-)	(+)
Alkaloids	(+)	(-)	(+)	(+)	(+)
Anthracene	(+)	(-)	(+)	(+)	(-)
Naphthoquinones	(-)	(-)	(-)	(-)	(-)

and alkaloids stand out among the compounds found in these fractions, as they have shown effects on the germination of several species, including *P. maximum* (Chamoun et al., 2021). Furthermore, Lima et al. (2011) evaluated some of the same extractors used in the present study and plants of the genus *Euterpe* (family Arecaceae) and found similar results, reinforcing the allelopathic potential of species of this family on the germination of *P. maximum*.

Eventually, germination may exhibit low sensitivity to the action of secondary metabolites, which can more severely affect the initial stage of plant development. The damage to this development stage may result in the emergence of abnormal seedlings, as contact with allelochemicals causes physiological changes that inhibit metabolic activities, leading to disturbances in cell membranes and consequently affecting plant development, as found in the present study for *P. maximum*, which presented a delay in root and shoot growth and in biomass accumulation.

The antioxidant activity of *G. schottiana* extract and its fractions are mainly due to the presence of phenolic compounds, as found by phytochemical screening using thin-layer chromatography. The butanol fraction presented higher total phenolic, tannin, and flavonoid contents, followed by the ethyl acetate fraction. Phenolic compounds are usually found in these fractions, which may explain their antioxidant potential against DPPH and ABTS.

Information on the antioxidant activity of *G. schottiana* leaf extracts assessed through DPPH, ABTS, FRAP, and phosphomolybdenum tests, as well as their allelopathic effects, are not found in the literature. However, species of the genus *Euterpe* present diversity of phenolic compounds and high antioxidant activity (AL Nasser & Mellor, 2022). Considering the antioxidant test with phosphomolybdenum, more non-polar fractions exhibit higher activity when compared to more polar fractions. This test is based on the reduction of molybdenum with the formation of a green complex in the acid medium; it is mainly applied to plant extracts containing vitamin E, which is usually found in more non-polar fractions in fractionated extraction processes (Joshi et al., 2021).

Allelopathic effects on germination and initial growth stage can be correlated with changes in the activities of several plant enzymes. The enzymatic activity tests for catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) were applied as parameters to analyze the allelopathic effects of the *G. Schottiana* extract on seeds and roots of *P. maximum*. These enzymes are essential for plant physiology because they metabolize reactive oxygen species (ROS), which are chemical compounds that naturally occur, however, when present in excess, they can cause several damages to the organism.

A significant increase in the activity of these three antioxidants enzymes was found in the present study, both in seeds and roots of *P. maximum*, denoting increases in ROS and oxidative stress due to the application of the butanol and hexane fractions of the extract. This increase was higher than 100% compared to the control in some cases and may be associated with increases in MDA levels in the plant, indicating increases in lipid peroxidation of membranes caused by free radicals. Oxidative stress is an imbalance between the production of pro-oxidants and antioxidants and can occur in different forms; but lipid peroxidation estimated by the production of MDA (the final product of the oxidation process) is widely considered one of the most reliable indicators of oxidative stress in plants and damage to the metabolism caused by allelochemicals (Pinto et al., 2016).

SOD removes the superoxide radical anion (O^{2-}) from the intracellular environment by catalyzing its dismutation into hydrogen peroxide (H_2O_2) and oxygen (O_2). However, H_2O_2 is still harmful to the plant and needs to be metabolized, which is done by CAT and POX by reacting with H_2O_2 and resulting in water and O_2, which are non-toxic byproducts. Additionally, POX may be associated with plant growth and elongation, as hydroxyl radical (OH^-) is formed from H_2O_2 during the metabolic

pathway and is an important catalyst agent for lignin biosynthesis (Nascimento et al., 2020). These effects may indicate an allelopathic characteristic for *G. schottiana* leaf extracts with a consequent increased oxidative stress in target plants with damage to germination and development processes.

Conclusion

The hexane and butanol fractions of leaf extract of *Geonoma schottiana* Mart. presented higher allelopathic potential on the germination and initial development of *Panicum maximum* (monocotyledon) with low effects on *Lactuca sativa* (dicotyledon).

The ethyl acetate and butanol fractions presented higher antioxidant activity in the DPPH, ABTS, and FRAP tests, which was associated with the presence of phenolic compounds. The hexane and butanol fractions had effects on *P. maximum* through the activity of oxidative enzymes, mainly superoxide dismutase, and peroxidase.

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