Acta Botanica Brasilica, 2025, 39: e20240246
doi: https://doi.org/10.1590/1677-941X-ABB-2024-0246

Original Article

Phytotoxic potential of leaf extracts from species occurring in two phytophysiognomies of the Atlantic Forest: a chemical and biological evaluation

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ABSTRACT

Some plants synthesize phytotoxic compounds that can inhibit the development of neighboring species. Phenolic compounds from some botanical families have been the focus of studies because of their effect on surrounding plants. This work aims to analyze the phytochemical profile of leaf methanolic extracts through the identification of major phenolic compounds, evaluation of their phytotoxicity, and their intraspecific variation. Leaves from *Calophyllum brasiliense*, *Psidium guineense,* and *Miconia cinnamomifolia* from the Ombrophilous Forest and Restinga phytophysiognomies were sampled. The phytotoxic activity was also tested against *Solanum lycopersicum*. The extract of *M. cinnamomifolia* presented the greatest chemical diversity and intraspecific variation. The *M. cinnamomifolia* forest extract presented potent inhibition of seed germination and root growth of the target species. Both *C. brasiliense* extracts affected the target species in higher concentrations. In the *P. guineense* Restinga extract, which had greater activity than the species' forest extract, 8-hydroxyluteolin-8-sulfate was identified. This is the first report of flavonoid sulphate for the Myrtaceae family, with reported biological activities and indicated for studies in bioprospection. Our study highlights the importance of conservation of Atlantic Forest species, due to the diversity of phenolic compounds and their phytotoxic effects, in response to distinct environmental conditions.

Keywords: chemical traits, Dense Ombrophilous Forest, intraspecific variation, phenolic compounds, Restinga

Received January 29, 2024; Accepted September 15, 2025
Editor-in-Chief: Thaís Elias Almeida; Associate Editor: Moemy Moraes

How to cite:
Amélia RP, Feitoza RBB, Oliveira RR, Gonçalves FMB, Da Cunha M, Lima HRP. 2025. Phytotoxic potential of leaf extracts from species occurring in two phytophysiognomies of the Atlantic Forest: a chemical and biological evaluation. Acta Botanica Brasilica 39: e20240246. doi: 10.1590/1677-941X-ABB-2024-0246

Introduction

Knowledge of the level of interaction among plant species belonging to a natural system is limited because the full potential of the special metabolites they produce in response to different environmental filters is unknown (Marsh *et al.*, 2019). The degree of interaction among species can be informed indirectly by floristic and phytosociological surveys since the plant communities studied are a result of interactions among species, e.g., competition and chemical signalization (Imatomi *et al.*, 2013a; Gillani *et al.*, 2024).

Plants synthesize a wide variety of secondary or special metabolites, whose diversity varies according to plant family and species (Kessler & Kalske, 2018). Such compounds play an essential role in the interaction of plants with their environment by triggering mechanisms that promote survival and adaptation (Verma & Shukla, 2015). Among the special metabolites, phenols and flavonoids stand out for their notable structural diversity (Lattanzio, 2013).

Phenolic compounds are widely distributed in bryophytes and mainly in vascular plants (Cheynier *et al.*, 2013). They act as signals in plant-microorganism interactions and in plant-plant relationships (Mousavi *et al.*, 2021), as well as in defense mechanisms against pathogens and herbivores. They are essential functional traits that attract pollinators and fruit and seed dispersers and contribute to responses to environmental filters (Cheynier *et al.*, 2013; Verma & Shukla, 2015). They are also considered a cause of ecological and economic problems, such as interfering with the regeneration of natural forests and annual crops due to soil sickness (Li *et al.*, 2010). Phytotoxic phenols tend to cause negative effects on other plants, such as delaying germination, hindering root and shoot growth (Cheynier *et al.*, 2013). Such compounds include flavonoids, hydroxycinnamic and benzoic acids, phenylpropanoids, coumarins, and tannins (Barral & Paradelo, 2011; Cheng & Cheng, 2015; Mousavi *et al.*, 2021).

The expression of special metabolites by individual plants of the same species in different locations may be affected by abiotic factors (Arnold *et al.*, 2019; Yang *et al.*, 2018), which may reflect differences in the production of phytotoxic compounds (Pilatti *et al.*, 2018). Abiotic factors such as light, temperature, and water influence, directly or indirectly, plant growth and metabolism, and the chemical diversity in plants is a result of their plasticity (Arnold *et al.*, 2019; Yang *et al.*, 2018).

Complex biomes, with multiple phytophysiognomies, present marked changes in plant chemical profiles due to differences in tree canopy and water availability (Cerqueira *et al.*, 2021). One example is the Brazilian Atlantic Forest, which comprises diverse phytophysiognomies that vary largely in vegetation structure, microclimate, and edaphic conditions (Pireda *et al.*, 2019). Coastal sandbanks, known as Restingas (RE), are characterized by high temperatures and irradiance, as well as lower availability of water and nutrients (Oliveira *et al.*, 2023). In contrast, the Dense Ombrophilous Forest (OF) is an Atlantic Forest phytophysiognomy characterized by higher water availability and low light irradiation due to the forest canopy (Pireda *et al.*, 2019; Borges *et al.*, 2022; Xavier *et al.*, 2023).

Thus, we hypothesize that individuals of the same species grown in different Atlantic Forest phytophysiognomies will present intraspecific variation at the chemical level, which may result in distinct phytotoxic activities of phenolic compounds from both studied areas. To test this hypothesis, species found in both OF and RE areas belonging to families with known occurrence of phenolic compounds were studied: Melastomataceae (Vasconcelos *et al.*, 2006; Sobrinho *et al.*, 2017; Bomfim *et al.*, 2021), Myrtaceae (Santos *et al.*, 2018; Macedo *et al.*, 2021; Imatomi *et al.*, 2013a; Imatomi et al., 2013b), and Calophyllaceae (García-Zebadúa *et*

al., 2014; Liu *et al.,* 2022; Zailan *et al.,* 2022). In addition, the studied families are characterized by their richness and abundance, as well as their high endemism in Brazilian forest formations (Lucas & Bünger, 2015; Cabral *et al.,* 2021; Wagner *et al.,* 2022; Goldenberg *et al.,* 2025).

As for the focus species, *Psidium guineense* Sw. (Myrtaceae) may present phenolic acids (caffeic, vanillic, and gallic acids), catechins, flavones, and isoflavonoids (Senanayake *et al.,* 2018). *Calophyllum brasiliense* Cambess. (Calophyllaceae) is reported as a producer of phenolic acids, flavonoids, xanthones, chromanones, and coumarins (García-Zebadúa *et al.,* 2014; Santos *et al.,* 2025). The chemical composition of *Miconia cinnamomifolia* (DC.) Naudin (Melastomataceae) is unknown until this date, although flavonoids may be found in other *Miconia* species (Bomfim *et al*., 2021).

Therefore, this study aimed to evaluate intraspecific variation between two distinct Atlantic Forest phytophysiognomies, with basis on the chemical composition of methanolic fractions of crude leaf extracts of individuals of *Calophyllum brasiliense* Cambess. (Calophyllaceae), *Psidium guineense* Sw. (Myrtaceae) and *Miconia cinnamomifolia* (DC.) Naudin (Melastomataceae). It also aimed to assess the phytotoxic potential of the extracts.

Materials and Methods

Plant Material

Leaves of *Psidium guineense*, *Miconia cinnamomifolia*, and *Calophyllum brasiliense* were sampled in April 2022, at approximately 9 a.m., from five individuals from each of the following two Atlantic Forest phytophysiognomies in the state of Rio de Janeiro, Brazil: Dense Ombrophilous Forest (OF), at Reserva Biológica de Poço das Antas (Rebio Poço das Antas), in the municipality of Silva Jardim (22º30' S – 22º33' S; 42º15' W – 42º19' W); and Restinga (RE) at Reserva Particular do Patrimônio Nacional Fazenda Caruara (RPPN Faz. Caruara), in the municipality of São João da Barra (21º75' S; 41º03' W). A voucher specimen of each species was deposited at the HUERJ Herbarium of the Universidade do Estado do Rio de Janeiro (Table 1).

During plant sampling, microclimatic data were collected using a digital thermohygrometer (TH01) and a radiometer (LI-250). The absolute mean values of the measured parameters revealed marked contrasts between the vegetation physiognomies (Table 1).

Table 1. Coordinates, microclimatic parameters, and herbarium voucher codes for the species in Dense Ombrophilous Forest (OF) and Restinga (RE) environments.

	Calophyllum brasiliense		***Miconia cinnamomifolia***		***Psidium guineense***	
	OF	**RE**	**OF**	**RE**	**OF**	**RE**
Geographic Coordinates	22º58'28'' S; 42º26'97" W	21º75'71''S; 41º03'56'' W	22º58'28" S; 42º26'97" W	21º75'71" S; 41º03'56'' W	22 º55'40" S; 42º29'57" W	21º75'71''S; 41º03'56''W
Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	245.56±70.33	1214.01±1.25*	153.9±44.19	1757.88±185.59*	99.72±45.97	1468.81±241.72*
Air Humidity (%)	75.58±3.4*	51.35±0.2	77.95±3.57*	48.17±4.5	74.73±2.42*	57.41±8.82
HUERJ Voucher	13597	13600	13598	13601	13599	13602

Legend: HUERJ: Herbarium of the Universidade do Estado do Rio de Janeiro. Irradiance and Air Humidity Values are displayed as mean ± standard deviation. * indicate significant differences between OF and RE after Welch's t test ($p < 0.05$).

Leaf Extracts

Leaves of *C. brasiliense, P. guineense,* and *M. cinnamomifolia* from each phytophysiognomy were pooled and dried in a forced air oven for three days at 40 °C and ground using a Marconi® MA330/1 mill. The powder obtained (12 g) was submitted to extraction with 60 mL of ethanol in an Ultronique® Q5.9/40 Eco-Sonics ultrasonic bath for 30 min at 60 °C and filtered using a Buchner funnel, to yield one crude extract per species.

Crude dry extracts of *P. guineense* and *M. cinnamomifolia* were dissolved in a minimum quantity of ethanol and subsequently added to microcrystalline cellulose (4.0 g). The solvent was eliminated by a Fisatom ® 802 rotary evaporator, using approximately 8 rpm rotation at 45 °C. Subsequently, the cellulose coated with the extract was deposited on a layer of cellulose (200 mg moistened with 3 mL of distilled water) that had been previously prepared in a Büchner funnel with a porous plate. The column was successively eluted in increasing order of polarity using 200 mL of hexane, chloroform, ethyl acetate, methanol, and water solvents. Individual fractions were collected, concentrated in a rotary evaporator, and lyophilized.

The *Calophyllum brasiliense* crude extract was initially subjected to the same chromatography procedure as the other plants; however, the extract obstructed the Büchner funnel with cellulose. The extract was subsequently subjected to a liquid-liquid fractionation in the separation funnel using the solvent system hexane/methanol/water (1/0.5/0.5 v/v). In this procedure, 2 g of the dry extract was solubilized in 50 mL of the low phase, and the solution was washed four times with 50 mL of the upper phase (UP). The UP was collected and reserved, and the lower phase (LP) was concentrated in a rotary evaporator and lyophilized. Following this, the LP fraction was subjected to a second liquid-liquid fractionation using the solvent system hexane/ethyl acetate/methanol/water (1/2/0.5/1 v/v). Next, the procedure outlined above was executed, and the new lower phase was collected and concentrated for drying. This second fraction was employed in the analysis to clarify the chemical compounds.

Phytochemical Analysis

High-performance liquid chromatography (HPLC) analysis was performed using solutions of the methanolic fractions of *M. cinnamomifolia* and *P. guineense*, and the LP precipitate of *C. brasiliense*, prepared at a concentration of 5 mg. mL^{-1} in a mixture of 5 % acetonitrile in ultrapure water in 2.0 ml Eppendorf microtubes. HPLC analysis was performed using a Hitachi LaChrom Elite® HPLC System chromatograph coupled to a diode array detector (DAD). A Luna Omega C-18 column (5 cm x 2 mm i.d.), 1.6 µm particle, and 100 A, equipped with a compatible 2.1 mm pre-column, was used. The column temperature was 40 °C, and the flow rate was 0.3 $mL.min^{-1}$ during 65 minutes of analysis. The mobile phase consisted of two solvents: (A) deionized water with 0.1 % formic acid; and (B) acetonitrile (HPLC grade) with 0.1 % formic acid (Tédia® and Sigma-Aldrich®). The solutions were injected into the HPLC system (5 µL, in triplicate). The chromatogram data showed absorption signal I at 254 nm, with detection of ellagic tannins at 280 nm, and signal I for UV absorption at 340 nm, referring to the B ring of flavonoids at 343 nm.

UPLC-DAD-MS analysis

UPLC-UV-ESI-MS/MS analysis was performed on the same chromatographic apparatus described in the previous section, coupled to a high-resolution mass spectrometer equipped with an electrospray ion source (ESI) and quadrupole time of flight mass analyzer (Q-TOF) (Bruker Daltonics, Bremen, Germany). Column effluent was introduced into the mass spectrometer at 0.25 $mL.min^{-1}$.

Nebulizer gas was high-purity nitrogen (N_2) produced online by a Peak Scientific NM32LA nitrogen generator. Analysis parameters were set using negative ionization mode with spectra acquired over a mass range from *m/z* 50 to 1400. The optimum ESI-MS parameters were capillary

voltage, +3.5 kV; drying gas temperature, 210°C; drying gas flow, 10.0 L/min; nebulizing gas pressure, 72.5 psi; collision RF, 200 Vpp; transfer time 120 µs; and pre-pulse storage, 3 µs. Moreover, automatic MS/MS experiments were performed, adjusting the collision-energy values as follows: *m/z* 500, 30 eV; above *m/z* 500, 50 eV; and using nitrogen as collision gas. The MS data were processed through Data Analysis 4.0 software (Bruker Daltonics, Bremen, Germany). External instrument calibration was performed using a Cole Palmer syringe pump (Vernon Hills, IL, USA) directly connected to the interface, eluting a sodium formate ($NaCHO_2$) solution cluster containing 5 mM sodium hydroxide (NaOH) and 0.2 % formic acid in water/isopropanol 1/1 (v/v).

The LC/MS system was controlled by HyStar 3.2 software (Bruker Daltonics, Bremen, Germany).

Phytotoxicity Bioassays

The methanolic fractions of the crude extracts from *C. brasiliense, M. cinnamomifolia,* and *P. guineense* (16 mg) were dissolved in 0.1 % dimethyl sulfoxide (DMSO, 5 µL per mL) and diluted in distilled water until reaching the concentrations of 2000, 1000, 500, 250, and 125 ppm. DMSO is widely used in phytotoxicity bioassays as it improves the dissolution of extract compounds (Lorenzo *et al.*, 2016). DMSO at 0.1 % does not affect the growth of target species, as reported by Castellano (2001). This bioassay adopted distilled water containing DMSO, as above, as a negative control. The positive control was the herbicide glyphosate (Roundup®), dissolved in DMSO and diluted as the plant extracts above (Macías *et al.,* 2000; Castellano, 2001).

The bioassays were performed in Petri dishes (Ø = 6 cm), each equipped with two layers of 80 g filter paper, with four replicates per treatment. Twenty seeds of *Solanum lycopersicum* L. cv. Santa Cruz Kada (Isla Sementes, Porto Alegre, Brazil), pre-disinfected with 1 % sodium hypochlorite, were placed directly on the filter paper of each dish along with 1 mL of the respective treatment. All dishes were labeled, sealed with Parafilm®, and placed in a germination chamber (Solab SL-224/210, Piracicaba, Brazil) set at 27 °C, under a 12/12 h photoperiod, for a period of seven days (Macías *et al.,* 2000; Silva *et al.,* 2017; Feitoza *et al.,* 2020).

During the bioassay, the number of germinated seeds was counted twice a day using a 12 h-interval between counts, until the end of the 7 days. The seeds were considered germinated with 2 mm of radicle protrusion. The percentage of Germination ($PG_{\%}$) was calculated as (1):

$$PG_{(\%)} = \frac{\sum X_i}{N} 100$$

where X_i is the number of germinated seeds and N is the total number of seeds, with the values expressed as percentages (Anjum & Bajwa, 2005).

To evaluate seed germination delay, the Germination Speed Index (GSI) was calculated as (2):

$$GSI = \sum \frac{G_i - G_{i-1}}{t_i}$$

where G is the number of germinated seeds at time t (in hours) during observation i (Anjum & Bajwa, 2005).

At day 7 of the bioassay, root length was measured (n=20, r=4) using a digital pachymeter, from the root-shoot transition zone to the root tip. Non-germinated seeds were counted as zeros in root length measures.

Statistical Analysis

Data were statistically analyzed using R 4.4.1v (R Core Team, 2024). Sample normality was evaluated by the Shapiro-Wilk test (p = 0.05). Non-normal data were compared using the Kruskal-Wallis test and Dunn's post-hoc test (p = 0.05). Bar graphs were produced in Excel® by calculating the percentage of the control, which is given by (3):

$$\%(Control) = \left(\frac{\bar{X}T - \bar{X}C}{\bar{X}C}\right) 100$$

where $\overline{X}T$ is the mean of measurements of each treatment, and $\overline{X}C$ is the mean of measurements of the negative control. Results are expressed as percentages. Negative values denote inhibition,

whereas positive values indicate stimulation (Feitoza *et al.,* 2018). Inhibition values were further submitted to Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis using Euclidean distance to evaluate similarities among extract effects.

Results

Phytochemical Analysis

The phenolic compounds profile of this species was obtained by UPLC-DAD-ESI-Q-TOF-MS analysis of the methanolic fractions of *Psidium guineense* and *Miconia cinnamomifolia,* and the *Calophyllum brasiliense* second low phase. Chromatograms with molecular absorption detection at wavelengths of 254 nm and 340 nm are shown in Figures S1-S6. The compounds are summarized in Table 2, which includes the compound name, molecular formula, MS/MS fragments, experimental mode m/z in negative mode [M-H]-, and retention time. The fragmentation profiles of all compounds were compared to those in the literature and databases, including Mass Bank and GNPS.

After analysis of the *C. brasiliense* leaf extract, three compounds were identified in the two phytophysiognomies: astilbin 3-O rhamnoside (5), quercetin hexoside (6), and quercetin rhamnoside (7). Two compounds were identified only in OF: gallic acid and dihydroxybenzoic acid hexoside (Table 2; Figure S1). The extract of *C. brasiliense* from RE indicated variation with the presence of protocatechuic acid (Table 2; Figure S2).

Some phenolic compounds of *P. guineense* leaf extracts were found common to both phytophysiognomies, namely quinic acid (1), galloyl hexoside (2), myricetin-3-glucoside (3), and myricetin 3-arabinoside (4) (Table 2; Figure S3 and S4). One compound was found only in RE, 8-hydroxyluteolin-8-sulfate (6) (Table 2; Figure S4).

Miconia cinnamomifolia exhibited the most diverse phenolic composition in both phytophysionomies. Gallic acid, quercetin rutinoside, and quercetin pentoside I were among the phenolic compounds identified in both phytophysiognomies. The following compounds were found only in OF: peduncalagin, casuarictin, tellimagrandin II, quercetin hexoside I, quercetin pentoside II, quercetin pentoside I, and quercetin rhamnoside (Figure S5). The following compounds were identified only in RE: vescalaginin (2), castalagin (4), methoxygalic acid (5), hydrolyzed tannin (6), isoorientin (8), ellagic acid (9), vitexin (11), rutin (12), and isovitexin (13) (Figure S6).

Phytotoxicity Analysis

The leaf extracts from *P. guineense*, *M. cinnamomifolia,* and *C. brasiliense* showed significant phytotoxic activity ($p < 0.05$) against *Solanum lycopersicum* (Fig. 1; Table S1). *Calophyllum brasiliense*, from both studied phytophysiognomies, demonstrated high phytotoxicity in particular, as evidenced by high inhibition (i.e., highly negative percentages of negative control) for percentage of germination (PG), Germination Speed Index (GSI), and root length for concentrations 500, 1000, and 2000 ppm. The OF and RE leaf extracts differed in effect at lower doses (125 and 250 ppm); the OF extract presented greater inhibition for PG and GSI than the RE extract, whereas the inverse was observed for root length. The OF extract and the herbicide glyphosate (positive control) had similar inhibition effects on PG and GSI at the lower concentrations (125 and 250 ppm). However, both OF and RE extracts at 2000 ppm presented greater inhibition of PG and GSI than glyphosate. In addition, the RE extract caused a greater decrease in root length than glyphosate at 125 and 250 ppm, and the OF extract caused greater inhibition than its positive control at 500 and 2000 ppm (Fig. 1).

In general, the effects of the leaf extract of *P. guineense* from OF differed significantly from the extract from RE and from glyphosate (Fig. 1; Table S1). The OF extracts exhibited the lowest phytotoxic effects on PG, GSI, and root length of 125 to 500 ppm, whereas no significant differences ($p > 0.05$) were found between OF and RE extracts above 1000 ppm. Moreover, the RE leaf extract

Table 2. List of compounds identified in the UPLC chromatograms at 254 nm and 340 nm of extracts of *Calophyllum brasiliense* Cambess., *Psidium guineense* Sw., and *Miconia cinnamomifolia* (DC.) Naudin. originating from two phytophysiognomies of the Atlantic Forest: Dense Ombrophilous Forest (OF) and Restinga (RE).

Species	Nº.	RT (mim)	Compound	Molecular formula	[M-H] m/z	Fragments MS/MS (m/z)	OF	RE	Ref.
Calophyllum brasiliense Cambess. (Callophylaceae)	1	1.5	NI		347.105	173, 137	X	X	–
	2	3.1	Gallic acid	$C_7H_6O_5$	169.0161	124	X	–	Pavlović *et al.* (2016)
	3	3.9	Protocatechuic acid	$C_7H_6O_4$	153.0223	109	–	X	Pavlović *et al.* (2016)
	4	4.5	Hexoside dihydroxybenzoic acid	$C_{13}H_{16}O_9$	315.0674	153, 109	X	–	Pavlović *et al.* (2016)
	5	25	Astibin 3-O rhamnoside	$C_{21}H_{22}O_{11}$	449.1203	125, 151, 179, 285	X	X	Zengin *et al.* (2019)
	6	26.4	Quercetin hexoside	$C_{21}H_{20}O_{12}$	463.4803	300	X	X	Yeo and Shahidi (2020)
	7	34.2	Quercetin rhamnoside	$C_{21}H_{20}O_{11}$	447.4386	300	X	X	Dos Santos *et al.* (2018)
Psidium guineense Sw. (Myrtaceae)	1	1.4	Quinic acid	$C_7H_{12}O_6$	191.0587	127, 191	X	X	da Silva and Oliveira (2018)
	2	2.6	Galloil hexosíde	$C_{13}H_{16}O_{10}$	331.0721	123, 125, 128, 165, 169, 211	X	X	Dos Santos *et al.* (2018)
	3	21.6	Myricetin-3-glucoside	$C_{21}H_{19}O_{13}$	479.5402	316	X	X	Dos Santos *et al.* (2018)
	4	23.6	Myricetin 3-arabinoside	$C_{20}H_{17}O_{12}$	449.0707	316	X	X	Dos Santos *et al.* (2018)
	5	26.1	NI		751.0883	301, 449	X	–	–
	6	26.1	8-hydroxyluteolin-8-sulfate	$C_{21}H_{20}O_{15}S$	380.9972	301, 151	–	X	Olatunde *et al.* (2021)
	7	26.9	Myricetin-rhamnoside	$C_{21}H_{19}O_{12}$	463.6016 463.0934	316	X	X	Dos Santos *et al.* (2018)
Miconia cinnamomifolia (DC.) Naudin. (Melastomataceae)	1	1,52	Gallic Acid	$C_7H_6O_5$	169.0132	124	X	X	Li *et al.* (2005)
	2	1,97	Vescalaginin	$C_{41}H_{26}O_{26}$	933.0574	933, 915, 871, 613, 589, 493, 467, 425, 301, 275, 249	–	X	Pereira *et al.* (2017)
	3	1,97	Peduncalagin	$C_{34}H_{24}O_{22}$	783.0642	783, 481, 301	X	–	Pereira *et al.* (2017)
	4	2,82	Castalagin	$C_{41}H_{26}O_{26}$	933.0602	933, 631, 589, 467, 425, 301	–	X	Pereira *et al.* (2017)
	5	4,48	Methoxygallic acid	$C_8H_8O_5$	183.029	155, 140, 127	–	X	Martins *et al.* (2023)
	6	9,09	Hydrolyzed tannin	NI	557.0177	513, 482, 363, 275, 249	–	X	–
	7	10,32	Casuarictin	$C_{41}H_{28}O_{26}$	935.0782	935, 783, 633, 481, 301, 275	X	–	Soares *et al.* (2019)
	8	17,64	Isoorientin	$C_{21}H_{20}O_{11}$	447.0792	357, 327, 285	–	X	Deng *et al.*, 2008
	9	19,90	Ellagic acid	$C_{14}H_6O_8$	300.9987	283, 273, 245, 229, 201	–	X	Abdulla *et al.* (2017)
	10	22,00	Tellimagrandin II	$C_{41}H_{30}O_{26}$	937.0907	767, 465, 301, 169,	X	-	Pannakal *et al.* (2023)
	11	20,44	Vitexin	$C_{21}H_{20}O_{10}$	431.0959	431, 341, 311, 283	–	X	Breiter *et al.* (2011)
	12	21,25	Rutin	$C_{27}H_{30}O_{16}$	609.143	609, 300	–	X	Manyelo *et al.* (2020)
	13	22,04	Isovitexin	$C_{21}H_{20}O_{10}$	431.0959	341, 323, 311, 283, 269	–	X	Breiter *et al.* (2011)
	14	22,62	Quercetin rutinoside	$C_{27}H_{30}O_{16}$	609.1438	609, 300	X	X	–
	15	23,84	Quercetin hexoside I	$C_{21}H_{20}O_{12}$	463.0883	463, 300	X	–	Breiter *et al.* (2011)
	16	26,72	Quercetin pentoside I	$C_{20}H_{18}O_{11}$	433.077	300	X	X	Sheng *et al.* (2021)
	17	27,71	Quercetin pentoside II	$C_{20}H_{18}O_{11}$	433.0774	300	X	–	Sheng *et al.* (2021)
	18	28,79	Quercetin pentoside I	$C_{20}H_{18}O_{11}$	433.0773	300	X	–	Sheng *et al.* (2021)
	19	30,48	Quercetin rhamnoside	$C_{21}H_{20}O_{11}$	447.0932	300	X	–	Sheng *et al.* (2021)

Legend: RT: Retention Time. NI: not identified. Ref.: References.

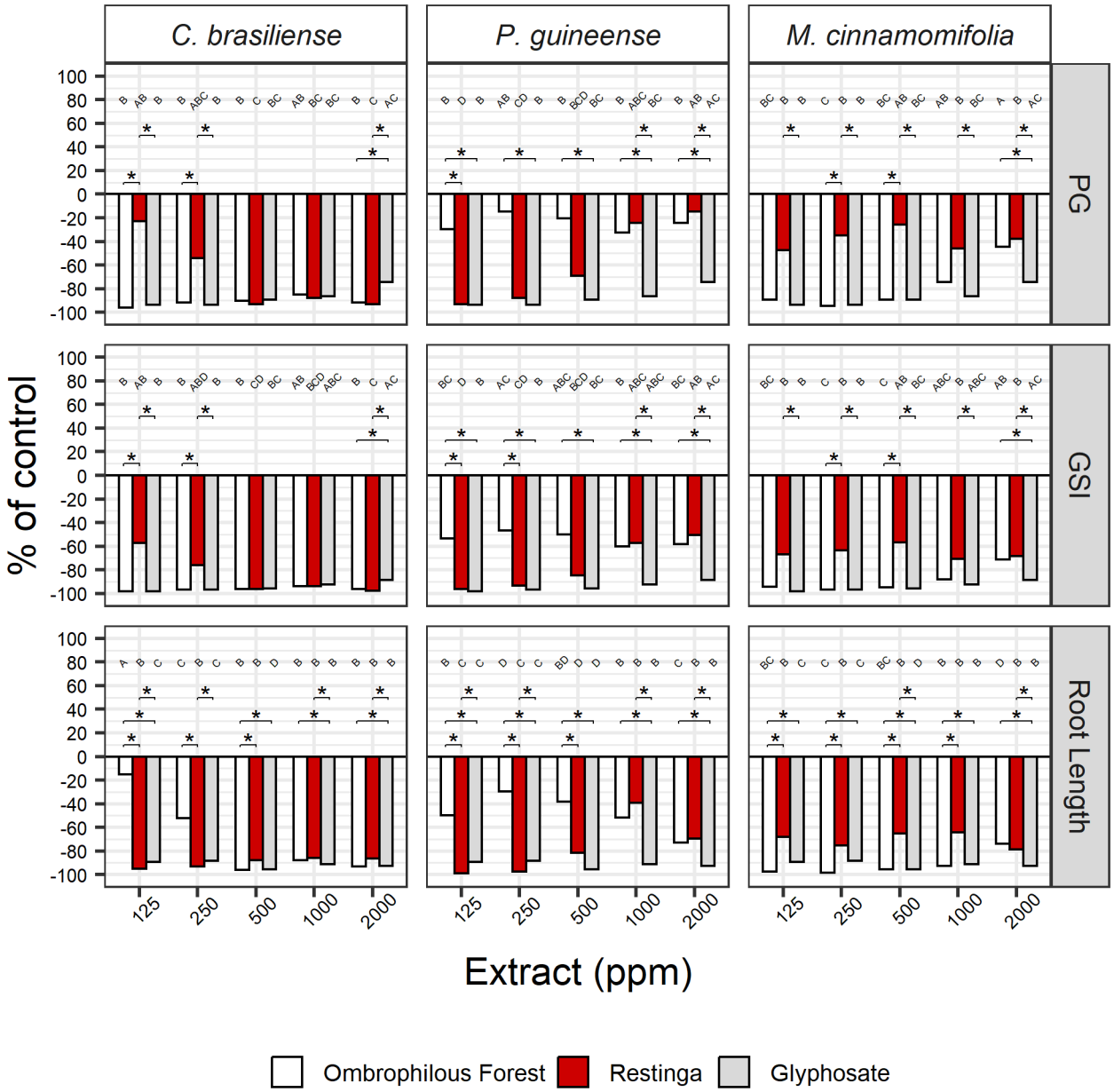

Figure 1. Effect of the methanolic partitions of *Miconia cinnamomifolia* and *Psidium guineense* and lower fraction of *Calophyllum brasiliense,* at concentrations of 2000, 1000, 500, 250, 125 ppm, on the Germination Percentage (PG), Germination Speed Index (GSI), and Root Length of *Solanum lycopersicum*. Bars are displayed as percentages of the negative control (distilled water). Negative percentage values indicate inhibition. Distinct uppercase letters indicate significant differences among concentrations after Kruskal-Wallis test, followed by the post-hoc Dunn test ($p < 0.05$), where the negative control (distilled water) represents "A". (*) indicates significant differences among Ombrophilous Forest and Restinga extracts and the herbicide Glyphosate, after Kruskal-Wallis test, followed by the post-hoc Dunn test ($p < 0.05$).

significantly decreased PG and GSI of 125 to 500 ppm, whereas root length was reduced in all concentrations in comparison to the negative control. The RE extract was less phytotoxic than glyphosate in most cases; however, this extract exhibited greater inhibition of root length than glyphosate at 125 and 250 ppm.

The OF leaf extract of *M. cinnamomifolia* significantly reduced PG and GSI of *S. lycopersicum* from 125 to 500 ppm, compared to the negative control (Fig. 1; Table S1). The RE extract generally exhibited the lowest inhibition values (i.e., less negative percentages of control), in comparison to the OF extract and glyphosate. When comparing

the two extracts, the effect of OF on PG and GSI was greater than that of the RE extract, at 250 and 500 ppm, and the same pattern was observed for root length at 125–1000 ppm. Moreover, the OF extract presented similar percentages of control to glyphosate for PG and GSI, and its inhibitory effect on root length was greater than that observed for the herbicide at 125, 250, and 1000 ppm.

The hierarchical UPGMA cluster analysis compared the similarities among the extracts of *C. brasiliense, P. guineense,* and *M. cinnamomifolia*, from both OF and RE, and the herbicide glyphosate, based on their effects on germination and initial development of tomato (Fig. 2). The analysis indicated the formation of two groups. Group A gathers the *M. cinnamomifolia* RE and the *P. guineense* OF extracts, based on lower inhibition percentages than the other treatments. Group B is the largest group and contains the remaining treatments. The *P. guineense* RE extract is the most distant treatment in Group B, due to its mild inhibitory effect at 1000 and 2000 ppm, whereas the lowest concentrations (125 and 250 ppm) were highly inhibitory. The *C. brasiliense* RE extract is separated from the other remaining treatments in Group B, since its 125 and 250 ppm concentrations caused lower inhibition in PG and GSI. Still inside Group B, the *M. cinnamomifolia* OF extract, the *C. brasiliense* OF extract, and the glyphosate herbicide were clustered together due to their high inhibition values at most concentrations. Since the *C. brasiliense* OF extract caused less inhibition of root length at 125 and 250 ppm (below -50 % of control), the *M. cinnamomifolia* OF extract and glyphosate were the most similar, as their phytotoxicity was high at all concentrations.

Discussion

The fractions of the analyzed extracts exhibited chemical profiles rich in phenolic compounds. All three species showcased hydrolysable tannins and flavonoids, but with some differences in flavonoid types. Flavonoids, phenolic compounds, and their degradation products play a significant role in phytotoxicity (Fiorentino *et al.,* 2008; Li *et al.,* 2010; Mousavi *et al.,* 2021; Weston & Mathesius, 2013). This is further evidenced by the inhibitory effect of their extracts on *S. lycopersicum* seed germination and root growth, regardless of their phytophysiognomy of origin. *Solanum lycopersicum* (tomato) is considered a suitable target species in phytotoxicity bioassays, due to its uniform germination and rapid growth (Macías *et al.,* 2000). Phytotoxicity studies suggest that phenolic compounds and flavonoids present inhibitory or stimulatory effects depending on the target species. Macías *et al.* (1997) found that methylflavonoids from *Helianthus annuus* L. inhibited tomato seed germination and growth, whereas they caused growth stimulation in *L. sativa* and *Lepidium sativum* L.

In this study, the *C. brasiliense* leaf extracts from both phytophysiognomies significantly inhibited *S. lycopersicum* germination and root growth. Above 500 ppm, those extracts caused similar effects as the glyphosate herbicide. Such direct interferences are indicative of the phytotoxic potential of *C. brasiliense* compounds to be explored in the development of bioherbicides. It is worth noting that gallic acid and dihydroxybenzoic acid hexoside were identified in the OF extract of *C. brasiliense*. The high inhibition in seed germination after the *C. brasiliense* OF extract can be attributed in part to the presence of these compounds. Pinho *et al.* (2017) investigated the phytotoxicity of 11 phenolic acids, including gallic acid, protocatechuic acid, and 4-hydroxybenzoic acid, and observed that the phytotoxicity of these acids increased with their hydrophobicity. On the other hand, protocatechuic acid was identified in the RE extract of the same species. Wang *et al.* (2013) observed that protocatechuic acid is a biodegradation product from (-)-epicatechin, and this process increased the inhibitory effect on *Lactuca sativa* L. seed germination and radicle growth. Among metabolites found in both OF and RE leaf extracts from this species, we detected quercetin glycosides and astibin 3-O-rhamnoside. Previous studies indicate that O-glycosyl ligands in flavonoids seem to be less phytotoxic than their aglycone counterparts (Martino *et al.,* 2012; Feitoza *et al.,* 2018).

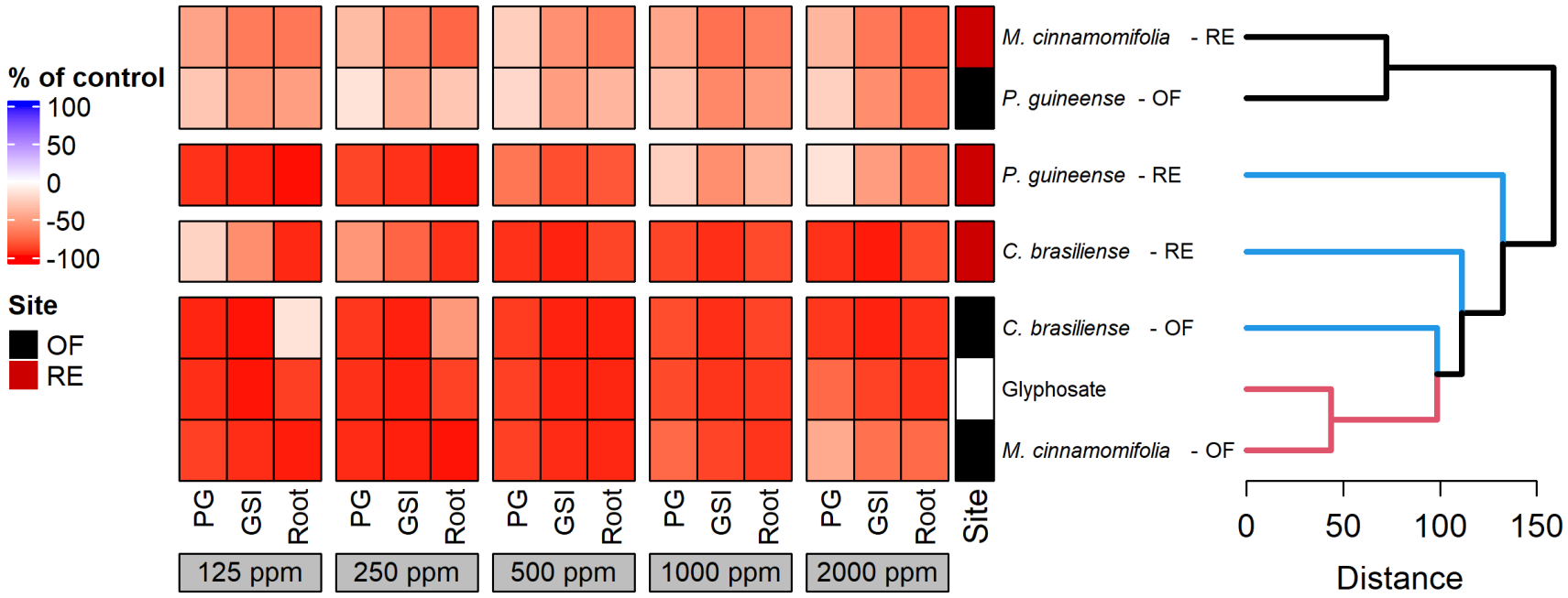

Figure 2. Hierarchical cluster analysis of the effects of the methanolic partitions of *Miconia cinnamomifolia* and *Psidium guineense* and lower fraction of *Calophyllum brasiliense,* at concentrations of 2000, 1000, 500, 250, 125 ppm, on the Germination Percentage (PG), Germination Speed Index (GSI), and Root Length (Root) of *Solanum lycopersicum*. The dendrogram was built using a Euclidean distance coefficient and the UPGMA (unweighted pair group method using arithmetic averages) method. Values used were percentage differences from the negative control (distilled water) and are displayed as a heatmap varying from 100 % inhibition (-100, red) to 100 % stimulation (100, blue).

Myrtaceae species have been the focus of studies on phytotoxicity (Imatomi *et al.,* 2013a;b). Extracts from *Myrcia tomentosa* Glaz. leaves, which were reported to produce 3-O-glycosylflavonols such as juglanin and avicularin, inhibited root length of *S. lycopersicum* seedlings (Imatomi *et al.,* 2013b). The literature highlights the presence of flavonoids in *Psidium*, including different types, such as isoflavones (Lapčík *et al.,* 2005), dihydrochalcone derivatives, flavonols (kaemperol, quercetin, and myricetin derivatives), dihydroflavonoids, and catechin (Beltrame *et al.,* 2021). The phytochemical analysis carried out on *P. guineense* leaves in the present study revealed the presence of flavonols, mainly myricetin, in samples from both phytophysiognomies. The bioassay performed here showed more pronounced phytotoxic action for the RE extract, which exhibited greater inhibition of root length than glyphosate at 125 and 250 ppm. Myricetin is considered phytotoxic (Einhellig *et al.,* 2004) and exhibits the ability to oxidize Fe^{2+} ions, impacting mitochondria and chloroplasts (Ximenez *et al.,* 2022). This disrupts homeostatic balance, inflicts damage to the photosynthetic apparatus, and triggers oxidative stress, culminating in seedling growth inhibition (Ximenez *et al.,* 2022).

Flavonoid sulphates are a large group of lesser-known compounds that may be found in marine and land plants, including various monocots and eudicots (Barron *et al.,* 1988). The 8-hydroxyluteolin-8-sulfate found in this study is the first report of flavonoid sulphate in Myrtaceae. Moreover, this metabolite was found only in *P. guineense* individuals in the Restinga environment. Among the functions associated with flavonoid sulphates in plants, other studies cite the relevance of such compounds in salt-tolerant plants, such as mangrove species, and in response to high reactive oxygen species (ROS) (Manurung *et al.,* 2021; Mohammed *et al.,* 2025). Supikova *et al.* (2022) suggest that, as seen in glycosylated flavonoids, sulfation of flavonoids is associated with compound storage in the vacuole, presenting less toxicity or biological activity.

Regarding luteolin, Beninger & Hall (2005) previously isolated and identified, from *Chrysanthemum morifolium* L. v. Ramat leaves, the luteolin 7-O-β-glucuronide. These authors demonstrated that the leaf extract of this species, at concentrations of 0.2 and 2.0 mM, significantly reduced the frond number and chlorophyll content of *Lemna gibba* L. The conclusion about the allelopathic activity of luteolin was likely attributed to the ortho-3′,4′-dihydroxy arrangement of the B-flavonoid ring in the compound. In our study, the 8-hydroxyluteolin-8-sulfate presents the aforementioned arrangement at the B-ring, as well as the sulfate group at the C-8 position. Perhaps this compound may exhibit phytotoxic effects as an isolated compound or synergistically with other compounds found. The results found in this previous study serve as a guide for future studies with the flavone identified in the RE extract of *P. guineense*.

Phytochemical studies of the genus *Miconia* have identified 148 metabolites, mostly flavonoids and phenolic acids, as well as terpenoids and steroids (da Silva *et al.,* 2022). Despite the extensive metabolite profile, allelopathic and phytotoxic studies are scarce for the genus (Gatti *et al.,* 2004; Isaza *et al.,* 2007; Santos *et al.,* 2015). The present study found *M. cinnamomifolia* to present the greatest chemical diversity and intraspecific variation in foliar extracts from both environments, including gallic and ellagic acid, tannins, flavones, and flavonols.

The OF extract of *M. cinnamomifolia* is the most promising treatment among all extracts, due to its diversity of phenolic compounds, its high phytotoxicity in all concentrations tested, and the high similarity of phytotoxic effect with the glyphosate herbicide. This inhibitory effect may be associated with the presence of flavonoids, mainly quercetin. The accumulation of quercetin in the meristematic zone of the root apex interferes with the transport of auxin to adjacent cells, causing changes in the hormonal gradient and, therefore, limiting cell proliferation and radicle growth (Cheynier *et al.,* 2013; Agati *et al.,* 2021; Singh *et al.,* 2021). This extract also contained pedunculagin and tellimagrandin II. These compounds demand close attention since they have been previously reported for their phytotoxic

potential, with impacts on the development of target species through interactions with proteins or metals (Barry *et al.,* 2001; Grundhöfer *et al.,* 2001).

The present bioassay with the RE extract of *M. cinnamomifolia* showed inhibitory effects on the root length of *S. lycopersicum* at all concentrations. Individuals of *M. cinnamomifolia* from RE exhibited a diverse array of phenolic compounds, with a predominance of hydrolyzed tannins, identified as vescalaginin and castalagin. Studies on the phytotoxic activity of these compounds are scarce. Our novel findings about *M. cinnamomifolia* phytochemical composition and phytotoxic potential highlight the importance of further elucidation of plant chemical diversity, plant chemical traits, and their potential biological activities.

The present bioassay analysis revealed significant inhibitory effects on germination potential, germination speed, and root growth after treatment with OF leaf extracts of *C. brasiliense* and *M. cinnamomifolia*. A similar negative impact on seedling development was observed following treatment with the RE leaf extract of *P. guineense*. Compounds such as quercetin (Fernández-Aparicio *et al.,* 2021), gallic acid (Patterson, 1981; Sodaeizadeh *et al.,* 2009), dihydroxybenzoic acid hexoside (Hussain *et al.,* 2020; Vieites-Álvarez *et al.,* 2023), luteolin and its derivatives (Beninger & Hall, 2005; Hussain *et al.,* 2020), which were found in all three of the studied species, have been cited in the literature as phytotoxic compounds on target plants. It is important to note that plant chemicals may interact with each other, either antagonizing their effect or presenting a synergic effect (Silva *et al.,* 2013; Feitoza *et al.,* 2018). In other words, mixtures of plant chemicals may present an increased or decreased phytotoxicity in comparison to their isolated effect. These unprecedented results may guide future experiments with isolated and/or combined compounds found in the studied species, are important guidelines for new ecological studies, and show the chemical potential produced by these species that allows their establishment in two different phytophysiognomies.

The results regarding abiotic factors in the two phytophysiognomies reiterate the same pattern of variation observed for the Restinga, characterized by high irradiance, and for the Ombrophilous Forest, where air humidity is higher (Pireda *et al.,* 2019; Oliveira *et al.,* 2023; Xavier *et al.,* 2023). Changes in abiotic factors, such as light and water availability, may be reflected in distinct chemical profiles (Arnold *et al.,* 2019; Yang *et al.,* 2018). Biosynthesis of plant phenolics occurs from the shikimate-phenylpropanoid pathway, and this biosynthetic route is affected by environmental stresses, including high light irradiation (Lattanzio, 2013). Phenylalanine ammonia-lyase (PAL) is a crucial enzyme of the phenylpropanoid pathway, and its activity is increased in response to biotic and abiotic factors, enhancing protection against UV radiation and ROS (Barros & Dixon, 2020).

In response to our hypothesis, only *M. cinnamomifolia* exhibited chemical intraspecific variation between the Atlantic Forest phytophysiognomies. The *M. cinnamomifolia* OF extract presented higher phytotoxicity, similar to the herbicide glyphosate. Moreover, *C. brasiliense* and *P. guineense* had little variation in the chemical profiles. While *C. brasiliense* extracts had similar phytotoxic effects at high extract concentrations, the *P. guineense* RE extract was more inhibitory in lower concentrations. We reported for the first time the occurrence of 8-hydroxyluteolin-8-sulfate in *P. guineense* RE extract. This uncommon flavonoid group, which is restricted to some plant families, is characterized by its biological activities, highlighting its potential in the development of new pharmaceuticals.

The most bioactive extracts in this study can be manipulated to produce bioherbicides through the isolation of promising compounds and their structural modification. Phenolic compounds, as the structures identified in this study, are generally associated with the response to UV radiation and radical scavenging ability, but our findings indicate a significant role of phenolic compounds in the mediation of plant-plant interaction processes in

natural plant communities. As the comprehension of chemical diversity in a plant community can be as important as knowing the taxonomic diversity, knowledge of the chemical profile can subsidize the selection of plant species for ecosystem restoration, preventing harmful effects to species diversity and associated components. Studies must be addressed to enhance the knowledge about the functionality of chemical traits and their biological activities.

Acknowledgements

We thank Herick Viana and Adilson Martins for collecting the species in the study areas. We thank Dr. Jose Fernando Andrade Baumgratz for taxonomic classification. We thank B. F. Ribeiro and Dr. Marcelo da Silva Mathias for technical work in the laboratory of LBCT/CBB/UENF and LCQ/CCT/UENF. We thank the Multiuser Center (CME-LBCT) for providing infrastructure and the Advanced Microscopy Unit (AMU-CENABIO) of the Federal University of Rio de Janeiro. This study is a part of the Ph.D. dissertation of R.P.A. at UERJ.

Supplementary Material

The following online material is available for this article:

Table S1: Effect of the methanolic partitions of *Miconia cinnamomifolia* and *Psidium guineense* and lower fraction of *Calophyllum brasiliense,* at concentrations of 2000, 1000, 500, 250, 125 ppm, on the Germination Percentage (PG), Germination Speed Index (GSI), and Root Length of *Solanum lycopersicum*. Values are displayed as mean ± standard deviation. Distinct uppercase letters indicate significant differences among concentrations after the Kruskal-Wallis test, followed by the post-hoc Dunn test ($p < 0.05$). Distinct lowercase letters indicate significant differences among Ombrophilous Forest and Restinga extracts and the herbicide Glyphosate, after the Kruskal-Wallis test followed by the post-hoc Dunn test ($p < 0.05$).

Figure S1: Chromatograms obtained for the methanolic fraction of *Calophyllum brasiliense* from the Dense Ombrophilous Forest at 254 nm (A) and 340 nm (B).

Figure S2: Chromatograms obtained for the methanolic fraction of *Calophyllum brasiliense* from the Restinga at 254 nm (A) and 340 nm (B).

Figure S3: Chromatograms obtained for the methanolic fraction of *Psidium guineense* Sw. from the Dense Ombrophilous Forest at 254 nm (A) and 340 nm (B).

Figure S4: Chromatograms obtained for the methanolic fraction of *Psidium guineense* Sw. from the Restinga at 254 nm (A) and 340 nm (B).

Figure S5: Chromatograms obtained for the methanolic fraction of *Miconia cinnamomifolia* (DC) Naudin. from the Dense Ombrophilous Forest at 254 nm (A) and 340 nm (B).

Figure S6: Chromatograms obtained for the methanolic fraction of *Miconia cinnamomifolia* (DC) Naudin. from the Restinga at 254 nm (A) and 340 nm (B).

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Authors' Contributions

Renata Pegoral Amélia and Rodrigo B. B. Feitoza developed the ideas, conceptualization, methodology, research, writing - original draft, writing - review, and editing. Rodrigo Rodrigues de Oliveira and Fernanda Manhães Braga Gonçalves contributed to the methodology, formal analysis, research, and writing – original draft. Maura Da Cunha and Helena R. P. Lima contributed to conceptualization, methodology, research, resources, writing – original draft, writing - review and editing, funding acquisition, supervision.

Conflict of Interest

All authors declare that there is no conflict of interest of a personal, scientific, commercial, political, or financial nature in the process of evaluation and publication of the aforementioned article.

Data Availability

Data and R scripts related to this article will be available upon request to the corresponding author.

Funding Information

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001; Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (308267/2021-4); and Fundação de Amparo à Pesquisa do Rio de Janeiro – FAPERJ (CNE – E26/203.995/2024; Temáticos – E-26/211.339/2021).