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ARTICLE

Allelopathic potential and chemical characterisation of ‘flor-de-papagaio’ bark (*Norantea guianensis*)

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Resumen

*Potencial alelopático de la corteza de la especie de ‘flor de loro’ (*Norantea guianensis*)*

El objetivo fue llevar a cabo la prospección fitoquímica de la cáscara de *Norantea guianensis* y evaluar su potencial alelopático en la germinación de semillas y el crecimiento inicial de plántulas de lechuga y tomate, utilizando extractos acuosos y etanólicos. Se identificaron compuestos fenólicos y sus derivados, flavonoides y taninos, así como cumarinas, antraquinonas, alcaloides y saponinas. Se produjo interferencia negativa en la germinación y el vigor, especialmente en las semillas de tomate, y los extractos afectaron negativamente el desarrollo de las plántulas. La especie *N. guianensis* tiene la capacidad de interferir en el desarrollo de otras plantas y por esta razón, su uso debe evaluarse cuidadosamente.

Palabras clave: Alelopatía; Aleloquímicos; Metabolitos secundarios.

Abstract

The purpose of thisstudy was to carry out phytochemical prospecting of Norantea guianensis bark and to evaluate its allelopathic potential in seed germination and the initial growth of lettuce and tomato seedlings using aqueous and ethanolic extracts. We identified phenolic compounds and derivatives, flavonoids, and tannins, as well as coumarins, anthraquinones, alkaloids, and saponins. Negative interference was observed in germination and vigour tests, mainly in tomato seeds, with extracts negatively affecting seedling development. The species *N. guianensis* has the ability to interfere in the development of other plants, and for this reason, its use should be carefully evaluated.

Key words: Allelopathy; Allelochemicals; Secondary metabolites.

Introduction

The species *Norantea guianensis* Aubl., Marcgraviaceae, popularly known as 'flor-de-papagaio' (Figure 1), is a tree or hemiepiphyte found in rupestrian fields and savannahs of the Cerrado and Amazon biome, in addition to other vegetation formations. Because it is easy to propagate, in addition to having lush flowers, it is suitable for landscape projects (Cunha *et al.* 2008).

In landscape projects, there is concern with the use of native species as ornamental plants taking into account the functionality of plant compositions, care with the bioclimatic conditions of each location, and respect for the geomorphological conditions found in nature in order to provide good plant development and to create comfortable microclimates for the user (Heiden *et al.* 2006). However, there is no concern on the part of the landscaping companies with the predominance of one species over the other in the same environment; although, Cardoso (2013) highlights the importance of studying species of Brazilian auctotone flora, used as ornamental plants, which have the ability to interfere with the establishment and/or development of other species.

The success of this ornamental species in landscape projects depends, among other factors, on its allelopathic effect among other species in the same environment. Certain species have chemical substances that, when released into the environment, will positively or negatively interfere in the development of other plants, a process called allelopathy, which has the function of reducing or eliminating the competition for resources through chemical substances; although, compounds that are toxic for one species may not be for others (Rice 1984, Fujii & Hiradate 2007).

Figura 1. Imagen de hojas y flores de *Norantea guianensis*. Fotografía: João Medeiros.

Figure 1. Image of leaves and flowers of *Norantea guianensis*. Photography: João Medeiros.

These chemical compounds, when released, such as by leaching from the aerial part (e.g. leaves) (Macias *et al.* 2003, Fujii & Hiradate 2007), can negatively interfere with the assimilation of nutrients, growth, photosynthesis, and respiration. For example, tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) (Macias *et al.* 2003, Ferreira 2004) by coumarins and alkaloids. To evaluate the allelopathic potential of a plant, the most common method There is a great diversity of allelochemicals that interfere in the germination and growth processes of plants, among which can be mentioned the phenolic compounds (flavonoids and tannins), terpenoids (saponins and triterpenes), heterosides is through studying the effects of the extracts of the species on seeds and/or seedlings of high-quality cultivated plants

There are no reports on the species *N. guianensis* and its behaviour when grown in gardens, in addition to an absence of studies citing tests for allelopathic activity of its bark, its chemical characteristics, and the class of secondary metabolites present. In this way, the main objective of this work was to test the hypothesis that this vegetal species has the capacity to negatively interfere with the growth of other vegetal species through allelopathy.

Materials and methods

Sample collection

The biological material (bark) of *N. guianensis* was collected manually in March 2017 in the morning with a machete from 10 matrices located in forests in the Taboco region, Corguinho municipality (19°49'S 54°50'W, 320 MASL), Mato Grosso do Sul, Brazil. The collected bark was stored in sterile polyethylene bags and taken to the Research Laboratory, Campo Grande, Mato Grosso do Sul.

Preparation of extracts

The bark was fragmented with pruning shears, dried in an oven (±27°C), and crushed in a mill (with 3 knives; 1,725 rpm; 20 mesh mesh). The material was stored in a glass bottle, sealed, labelled, and kept in the refrigerator until use. Aqueous (40 g of powder for 200 mL^{-1} of distilled water) and ethanolic (40 g of powder for 200 mL^{-1} of ethanol - absolute ethyl alcohol Merck® with

99.9% purity index) extracts were prepared at a 20% concentration (crude extract) following the methodology described by Oliveira *et al.* (2011) and used for phytochemical analysis and bioassays. The crude extract (20%) was diluted by adding distilled water to concentrations 2.5, 5, 10, 15, and 20%.

Phytochemical analysis

The collected plant material was dried in a circulating air oven at 40°C and finely ground in a wiley mill. Secondary metabolites were extracted from the powder (500 g) in an ultrasonic bath for 60 minutes. Phytochemical prospection was done through characterisation reactions to determine the class of secondary metabolites. All analyses were performed in triplicate, following an adapted methodology based on work by Matos (2009) and Simões *et al.* (2017).

For the assays, alterations in colour or precipitation were compared to the control following the method described by Fontoura *et al.* (2015). The observed intensities were classified into strongly positive (+++= 100%), moderately positive (++= 50%), weakly positive (+= 25%), and partially positive (±= 10%). A haze or partially changed colour and the absence of colour or precipitation were considered negative. The intensity of the colour or precipitation indicated an increased concentration of each class of secondary metabolite.

Aqueous and ethanolic extracts in concentrations of 2.5, 5, 10, 15, and 20% were used to quantify the total phenols (mg of gallic acid equivalents per g of extract - mg EAG/g^{-1}), determined by the Folin-Ciocalteu method, with gallic acid (10 to 350 mg mL^{-1}) as the standard ($y = 0.02x + 0.042$; $r^2=0.9999$) (Sousa *et al.* 2007). The total flavonoids content (equivalent mg of quercetin per g of extract -mg EQ/g^{-1}) was evaluated by the aluminium chloride method and as standard, quercetin (6 to 20 µg mL^{-1}) to construct the calibration curve ($y=0.042x+0.0081$; $r^2= 0.9999$) (Peixoto-Sobrinho *et al.* 2008). The extracts were also subjected to analyses of pH (pH DM-20, Digimed) and the average pH of the extracts was 4.2±0.2 (aqueous) and 5.6±0.4 (ethanolic). The osmotic potential was between - 0.042 Mpa (aqueous) and - 0.003 Mpa (ethanolic), determined using a formula proposed by Ayers & Westcot (1991). The results found for both parameters are considered adequate for the germination and seedling formation of the target species (Rice 1984, Pattnaik & Misra 1987, Ferreira & Aqüila 2000, Gatti *et al.* 2004).

Germination in a germination chamber

Five mililitres of the aqueous and ethanolic extracts, with concentrations of 2.5, 5, 10, 15, and 20% were placed on two sheets of germitest paper in Petri dishes (7 cm in diameter) sealed with film paper. Two controls were used: distilled water and ethanol (99.9%). In the control with ethanol, 5 ml of ethanol were placed on the Petri dishes and after their evaporation, the Petri dishes was moistened with 5 ml of distilled water. Four replicates were used with 25 lettuce seeds, cultivar “Maravilha Quatro Estações,” and 25 tomato seeds, cultivar “Santa Clara.” Observation of the seeds was done every 24 hours for 7 days, and seeds were considered germinated when there was a 2 mm protrusion of the primary root (morphological criterion of germination).

Growth in a germination chamber

Ten milliltres of the extracts were used, with concentrations of 2.5, 5, 10, 15, and 20%, and placed on two sheets of germitest paper in transparent plastic boxes (11 × 11 × 3.5 cm) sealed with film paper. Two controls were used: distilled water and ethanol (99.9%). In the control with ethanol, 10 ml of ethanol were placed on the plate and after their evaporation, the plate was moistened with 10 ml of distilled water. Each treatment consisted of 4 replications with 10 pre-germinated seeds with ±2 mm of primary root, and the evaluation was made after 10 days (no further moistening of the plates after sowing). The bioassays were maintained in germination chambers at a constant temperature of 20°C (lettuce) and 25°C (tomato) with a 12-hour photoperiod of white light (20 W fluorescent lamps). Measurement of the primary root was made from the seedling lap to the meristematic apex of the root system (mm) and the aerial part (mm), which is from the plant lap to the apical meristem apex, using a digital calliper.

Germination in a greenhouse

Emergency tests were performed using seeds from the 2 target species (4 replicates with 25 seeds) in expanded polystyrene (styrofoam) trays with 128 cells. Crushed bark was added to the substrate vermiculite at concentrations of 5% (950 g vermiculite + 50 g bark powder), 10% (900 g+100 g), 20% (800 g+200 g), and control (vermiculite only). The substrates were homogenised, moistened with distilled water, and placed in the trays. One seed of the target species was placed per cell and covered with a thin layer of substrate to evaluate germination and emergence speed index.

Growth in a greenhouse

The seedlings obtained in a greenhouse through 4 replications with 10 pre-germinated seeds with ±2 mm of primary root length, which were placed in the cells with the substrate in the same proportions as the germination experiment, were evaluated. Seedling growth was measured after 10 days of experimentation. Measurement of the primary root was made from the seedling lap to the meristemic apex of the root system (mm) and the aerial part (mm), which is from the plant lap to the apical meristem apex, using a digital calliper.

Evaluated parameters, experimental design, and statistical treatment

The germination and vigour percentage, indirectly measured by the mean germination time (MGT) in days, was evaluated, quantifying germination from a kinetic point of view (Labouriau & Agudo 1987) and the germination speed index (GSI) in addition to the emergency speed index (ESI) (Maguire 1962).

The experimental design was completely randomised, with four replications per treatment. The phytochemical evaluation was carried out with three repetitions for each concentration and the calculation of the means, accompanied by the standard deviation. The data of the evaluated characteristics were subjected to ANOVA, and when there was significance the means were compared using the Tukey test, with 5% probability, performed using the statistical program *Assistat*. There was the need for data transformation according to the Shapiro-Wilk tests for normality of ANOVA and Levene residues for homogeneity between variances, with untransformed data being presented, for easy understanding of the results.

Results

The phenolic compounds, tannins, coumarins, and alkaloids had a moderately positive intensity in both extracts, and the anthraquinones had a moderately positive intensity in the aqueous extract and a strong intensity in the ethanolic extract. Flavonoids and saponins had low intensity in both extracts (Table 1).

Regarding the content of phenolic and flavonoid compounds, the values of the aqueous and ethanolic extracts were statistically different. The highest values occurred in extracts 20% and the lowest in extracts 2.5%. The reduction in the concentration of phenolic compounds reached 23.2% and for flavonoids reached 73.2%. The aqueous extract was more efficient in extracting the compounds (Table 2).

The dilution process of the crude extract (20%) resulted in a reduction in the concentration of phenolic compounds and total flavonoids. The reduction was greater in the aqueous extract (22.8% for phenolic compounds and 72% for flavonoids) compared to the ethanolic extracts (12.9% for phenolic compounds and 50.7% for flavonoids).

Germination and growth

In the germination chamber, the germination of lettuce seeds was negatively affected in only the

Secondary metabolites	aqueous	ethanolic
Phenolic compounds	++	++
Tannins	++	++
Flavonoids	+	+
Coumarins	++	++
Anthraquinones	++	+++
Alkaloids	++	++
Saponins	+	+

Tabla 1. Metabolitos secundarios de extracto acuoso y etanólico, corteza de *Norantea guianensis*. Fuertemente positivo (+++= 100%), moderadamente positivo (++= 50%) e débilmente positivo (+= 25%).

Table 1. Secondary metabolites of aqueous and ethanolic extracts of *Norantea guianensis*. Strongly positive (+++= 100%), moderately positive (++= 50%), and weakly positive (+= 25%).

	Phenolic compounds		Flavonoids	
%	**aqueous extract**	**ethanolic extract**	**aqueous extract**	**ethanolic extract**
20	56.1±0.2 aA	48.9±0.3 aB	38.6±0.5 aA	21.3±0.3 aB
15	51.8±0.3 bA	46.8±0.1 bB	27.6±0.5 bA	18.1±0.1 bB
10	48.2±0.1 cA	46.4±0.9 bB	21.2±0.4 cA	16.1±0.2 cB
5	45.1±0.2 dA	44.1±0.4 cB	14.5±0.1dA	12.4±0.1 dB
2.5	43.3±0.5 eA	42.6±0.2 dA	10.8±0.2 eA	10.5±0.2 eA

Tabla 2. Contenido de compuestos fenólicos (mg EAG/g-1) y flavonoides totales (mg EQ/g-1) de los extractos acuosos y etanólicos de la corteza de *Norantea guianensis* en diferentes concentraciones. *Los promedios seguidos de la misma letra en la columna no difieren estadísticamente, prueba de Tukey ($p < 0.05$).

Table 2. Content of phenolic compounds (mg EAG/g-1) and total flavonoids (mg EQ/g-1), aqueous and ethanolic extracts of *Norantea guianensis*. *Means followed by the same lowercase letters in a column and capital letters on the lines do not differ significantly by the Tukey test ($p < 0.05$).

20% aqueous extract. The germination of tomato seeds suffered the effects of metabolites from the 5% aqueous and ethanolic extract (Table 3). Regarding the vigour of lettuce seeds, measured indirectly by GSI and MGT (Table 3), the aqueous extracts interfered negatively from the concentration of 5%; the ethanolic extracts from the concentration of 15%. Tomato seeds were affected by the concentration of 5% in both extracts.

The results indicated a greater effect of the 20% extracts on the germination of tomato seeds, reducing germination by 91% (ethanol extract) and 78% (aqueous extract). There is no reduction in the germination of lettuce seeds for any of the concentrations of the ethanolic extracts tested and in the case of the aqueous extracts only a 12% reduction in germination is observed in the presence of 20% aqueous extracts (Table 3).

At a concentration of 20%, the GSI of tomato seeds was also more affected, with a reduction between 87% (aqueous) and 92% (ethanolic), while lettuce was between 16% (ethanolic) and 46% (aqueous). Regarding the MGT, there was an increase in germination time of 108% (ethanol extract) and 21% (aqueous extract) for tomato seeds and of 31% (ethanol) and 70% (aqueous) for lettuce seeds (Table 3).

The seedling development results in a germination chamber (Table 4) demonstrated that all extracts had a negative influence on the development of roots and shoots, starting from concentrations of 5% in both extracts and in both types of seeds, the effect being more pronounced as the concentration of said extracts increases. In the greenhouse, there was also a significant reduction in seedling growth. The ethanolic and aqueous extracts, from the concentration of 2.5%, reduced the growth of the root system and the aerial part, lettuce and tomato seedlings, with the effect being more pronounced as the concentration of said extracts increases and the ethanolic extract leading to the death of tomato seedlings at a concentration of 20%. The results obtained in the greenhouse also demonstrated that the germination and emergence speed index of both target species were negatively affected at the 5% concentration, with seeds germinating less and taking longer to emerge (Table 5).

	Germination		GSI		MGT	
	ethanol.	aqueo.	ethanol.	aqueo.	ethanol.	aqueo.
Lettuce control	99 a	98 a	24 a	24.2 a	0.26 a	0.27 a
2.5%	99 a	98 a	24 a	22.7 ab	0.26 a	0.28 a
5%	99 a	100 a	24 a	21.9 b	0.26 a	0.33 b
10%	99 a	96 a	23.4 ab	20.3 b	0.28 a	0.35 bc
15%	98 a	96 a	21.3 b	16.6 c	0.32 b	0.39 c
20%	98 a	88 b	20.1 b	13.1 d	0.34 b	0.46 d
Tomato control	95 a	94 a	8.6 a	10.0 a	0.77 a	0.82 a
2.5%	91 a	94 a	8.9 a	8.7 a	0.91 a	0.80 a
5%	76 b	84 b	7.1 b	6.0 b	1.4 b	0.91 b
10%	24 c	65 c	3.1 c	4.4 c	1.4 b	0.99 c
15%	13 d	35 d	1.3 d	2.9 d	1.7 b	1.0 c
20%	9 d	22 e	0.7 e	1.3 e	1.6 b	0.99 c

Tabla 3. Germinación (%), índice de velocidad de germinación (GSI) y tiempo medio de germinación (MGT) de semillas de lechuga y tomate en extracto acuoso y etanólico, corteza de *Norantea guianensis*, cámara de germinación. *Los promedios seguidos de la misma letra en la columna no difieren estadísticamente, prueba de Tukey ($p<0.05$).

Table 3. Germination (%), germination speed index (GSI), and mean germination time (MGT) of lettuce and tomato seeds in aqueous and ethanolic extracts of *Norantea guianensis* in germination chamber. *Averages followed by the same letter in the column do not differ significantly by the Tukey test ($p<0.05$).

Discussion

In relation to phytochemical studies with the species *N. guianensis*, Saleh & Towers (1974) identified the presence of glyco-flavonoids in flowers, while Dressler (2004) indicated that terpenes, tannins, saponins, alkaloids, and phenolic compounds are common in this family. The analyses indicated that among the metabolites present in the aqueous and ethanolic extracts, phenolic compounds appear with medium intensity, which correspond to a group of allelochemicals that have

	Roots (mm)		Steams (mm)	
	ethanolic	aqueous	ethanolic	aqueous
Lettuce control	13.1 a	16.6 a	12.4 a	12.9 a
2.5%	5.4 b	12.7 b	10.1 b	11.6 b
5%	2.9 c	6.6 c	5.7 c	10.9 b
10%	2.3 c	4.9 c	3.6 d	8.2 c
15%	2.1 c	3.8 d	3.3 d	6.3 d
20%	1.4 d	2.3 e	1.1 e	4.2 e
Tomato control	68.4 a	65.3 a	33.2 a	37.7 a
2.5%	26.5 b	31.2 b	19.6 b	32.1 b
5%	13.8 c	24.5 b	17.3 b	29.5 b
10%	7.6 d	11.4 c	9.1 c	15.4 c
15%	0 e	6.6 d	1.9 d	17.1 c
20%	0 e	1.3 e	0 e	16.3 c

Tabla 4. Longitud promedio (mm) de raíces y tallos, plántulas de lechuga y tomate, extracto acuoso y etanólico, corteza de *Norantea guianensis*, en cámara de germinación. *Los promedios seguidos de la misma letra en la columna no difieren estadísticamente, prueba de Tukey ($p < 0.05$).

Table 4. Average length (mm) of the roots and stems of lettuce and tomato seedlings in aqueous and ethanolic extract of *Norantea guianensis* in germination chamber. *Averages followed by the same letter in the column do not differ significantly by the Tukey test ($p < 0.05$).

% *Norantea guianensis* powder in vermiculite	Germination		ESI		Seedlings	
	lettuce	tomato	lettuce	tomato	lettuce	tomato
Control	88 a	62 a	13.1 a	7.8 a	46.3 a	91.9 a
5	66 b	37 b	8.8 b	3.7 b	37.7 b	83.6 b
10	70 b	39 b	9.3 b	3.8 b	36.7 b	79.7 b
20	42 c	41 b	3.9 c	4.1 b	37 b	73.9 b

Tabla 5. Germinación (%), índice de velocidad de emergencia (ESI) y tamaño de las plántulas de lechuga y tomate (mm), sustrato de vermiculita con polvo de corteza de *Norantea guianensis* en diferentes proporciones (0, 5, 10 y 20%) en vivero. *Los promedios seguidos de la misma letra en la columna no difieren estadísticamente, prueba de Tukey (p<0.05).

Table 5. Germination (%), emergence speed index (ESI), and size of lettuce and tomato seedlings (mm) in substrate with *Norantea guianensis* powder in different proportions (0, 5, 10 and 20%) in greenhouse. *Averages followed by the same letter in the column do not differ significantly by the Tukey test (p<0.05).

a greater allelopathic effect. This class ranges from simple phenols and flavonoids to more complex structures such as tannins. Phenolic compounds are considered potent inhibitors of seed germination (Rice 1984), reducing the formation of lignin, which contributes to the reduction in root elongation, in addition to blocking mitochondrial breathing (Fujii & Hiradate 2007).

Tannins also occur at medium intensity and can be released under natural conditions since they are water soluble. However, there are few reports of the involvement of tannins in allelopathic activity, with Rice (1984) describing their involvement in the inhibition of the growth hormone gibberellin.

Other derivatives of phenolic compounds are flavonoids, which are found in low intensity. According to Rice (1984), they can inhibit seedling germination and growth, and they are also cited as allelochemicals responsible for causing negative effects, direct and indirect, in the cell division process. Because they have free hydroxyls, they are able to capture electrons and act as catalysts in the photochemical phase of photosynthesis and/or as regulators of ion channels involved in the oxidative phosphorylation of the process of obtaining photosynthetic energy (Piettá & Simonetti 1999). Their antioxidant activity explains the ability to regulate cell growth and inhibit germination and seedling growth, for example (Macias *et al.* 1997).

Coumarins, another important allelochemical found in medium intensity, according to Macias *et al.* (2003) and Willis (2007), act as potent inhibitors in germination and plant growth. For Abenalovi *et al.* (2006), this effect occurs due to their ability to block mitosis, decreasing water intake and oxygen consumption. Nazemi *et al.* (2018) describe that coumarin can reduce the amount and activity of Golgi body in lettuce, block mitosis in alfalfa and modify mitochondria structure in onion, demonstrating its strong allelopathic action.

Another class of secondary metabolites with an important role in allelopathic function are anthraquinones (Rice 1984, Simões *et al.* 2017), with medium and high intensities detected. Murakami *et al.* (2009), for example, reported allelopathic activity of anthraquinones in the germination and growth of *Lemna minor* L. Among anthraquinones, sorgoleone, found in sorghum (*Sorgum bicolor* (L.) Moench), is a potent allelochemical that inhibits the germination and growth of various plants, acting as an inhibitor of photosystem I (Gonzalez *et al.* 1998). The greater intensity of anthraquinones in the ethanolic extract could justify its more intense effect, in relation to the processes of germination and seedling formation. However, as already mentioned by Fujii & Hiradate (2007) and Souza Filho *et al.* (2010), the allelopathic potential can often be related to the joint action of phytochemicals (synergism). On the other hand, Luz *et al.* (2010), when evaluating the allelopathic activity of pairs of substances and isolated substances of *Acacia mangium* Willld. on seeds of *Mimosa pudica* L. and *Senna obtusifolia*, verified that, separately, the substances promoted effects superior to those effected by the substances in pairs.

Alkaloids, found in medium intensity, can suppress the germination and growth of other species in the environment, as reported by Fujii & Hiradate (2007) and Willis (2007). Classes like that of alkaloids are related to allelopathic action due to their toxicity, changing the permeability of the membrane of meristem cells, for example, leading to death (Fujii & Hiradate 2007, Mulac & Humpf 2011). Alves *et al.* (2003) report that glycosylated spirosolane alkaloids, such as solamargine and solasonine, can suppress seed germination and growth of other species, even in small concentrations.

The presence of saponins in low intensity, according to Rice (1984) and Macias *et al.* (2003), also indicates the allelopathic action of the extracts since they present action on the cell membrane, modifying its permeability and interfering in the germination and vigour of seeds, leading to damage to cell structures. Saponins are commonly cited as responsible for causing allelopathic effects, as they can be released under natural conditions, as they are water-soluble, reducing the respiratory rate, which inhibits plant germination and growth (Ferreira & Aqüila 2000, Maraschin-Silva & Áquila 2006), demonstrating its action potential.

Regarding the content of phenolic and flavonoid compounds, a lower concentration of compounds occurs as the dilution increases, which would be expected. The smallest reduction in the concentration of phenolic compounds demonstrates that they are less affected by the dilution process, with the leaching process having little impact on their solubility. Flavonoids, on the other hand, were more affected, which indicates that during the rainy season greater leaching in nature may occur and, consequently, more intense allelopathic action in the environment may occur. The aqueous extract was also more efficient in extracting both metabolites, a factor probably related to the greater polarity of the solvent water.

The action of phenolic compounds and derivatives (flavonoids and tannins), such as allelochemicals, has been described by several researchers. For example, Cipollini *et al.* (2008) attributed the allelopathic activity of the hydromethanolic extract of *Lonicera maackii* (Rupr) leaves on the germination of *Arabidopsis thaliana* (L.) seeds due to the presence of flavonoids, glycoflavonoids, and chlorogenic acid. These compounds can affect different physiological processes of plants, interfering in their development, for example, at the cellular level, influencing lipid metabolism and the biochemical mechanism of respiration, inhibiting glucose transport and cellulose synthesis, which negatively affects the seedling growth (Angelo & Jorge 2007).

Specifically, studies relating to allelopathy and the determination of phenolic and flavonoid compounds are scarce. Among these, Oliveira *et al.* (2014) indicated the allelopathic activity of aqueous and ethanolic extracts of *Palicourea rigida* Kunth leaf on the germination speed of lettuce and seedling development. The action of these compounds has also been reported by Rodrigues *et al.* (2010), who evaluated the effect of hydromethanolic extract and fractions of *Senna alata* (L.) leaves in concentrations of 50, 100, 150, and 200 mg L^{-1} on seed germination and seedling of three weeds from pastures (*Mimosa pudica*, *Senna obtusifolia*, and *S. alata*), attributing the allelopathic activity to the glycosylated flavonoids present in the fractions. Silva *et al.* (2013) found that the ethanolic extract and glycoflavonoids isolated from the green leaves of *Derris urucu* (Killi & Smith) Macbr. inhibited seed germination and *M. pudica* seedling development. These data show the ability of flavonoids to act in isolation or in complex mixtures to inhibit the tested seeds.

Regarding the germination process, the results showed that the extracts interfered with the germination of both species, with the tomato being more affected by the ethanolic extract. Although both extracts have equal qualitative compositions, the ethanolic extract, which used a solvent with less polarity, was more efficient in the extraction of certain compounds, which may have influenced seed germination and seedling growth.

Regarding the higher sensitivity of tomatoes (Solanaceae), according to Rizzi *et al.* (2016) this species is more sensitive to secondary metabolites than lettuce (Asteraceae), relating this factor to families with distinct biochemical and physiological processes. Pereira *et al.* (2018) also found similar results, with tomato seeds being more affected than lettuce when in the presence of *Anacardium humille* A.St.-Hil extracts. For Rizzi *et al.* (2016), evaluating how different species behave, in the face of the same allelochemicals, may allow studies aimed at the creation of bio-herbicides designed to combat unwanted species, affecting the others to a minimum.

It could be assumed that free anthraquinones, found in greater intensity in the ethanolic extract, were responsible for greatest action. However, it is not possible to make this statement because, in general, the allelopathic potential of plant extracts must be related to the joint action of phytochemicals (synergism). On the other hand, for certain species, allelochemicals can affect germination differently, being able to alter only the vigour of the seeds and/or the formation of seedlings (Fujii & Hiradate 2007, Souza Filho *et al.* 2010), as observed for lettuce.

Interference with germination and/or vigour may also be related to the concentrations used because, depending on this, the results may be different. Authors such as Capobiango *et al.* (2009)

have demonstrated that aqueous extracts in concentrations of 10% and 30% there were reductions in GSI, while aqueous extracts in concentrations 70, 90, and 100% did not show results. Evaluating the allelopathic effect of *Artemisia annua* L. on lettuce, Magiero *et al.* (2009) found that leaf aqueous extract in the concentration of 25% led to germination in a longer period of time than the control. Testing the aqueous and ethanolic extracts of *Vochysia divergens* Pohl, Oliveira *et al.* (2013) indicated an increase in the MGT of lettuce and tomato seeds from a concentration of 2.5%, demonstrating that the action patterns of the allelochemicals are different depending on the species studied.

Although there was a greater effect on tomato seeds, according to Ferreira (2004), germination may be less affected by allelochemicals. Authors such as Maraschin-Silva & Áquila (2006), working with *Erythroxylum argentinum* O.E. Schulz, *Luehea divaricata* Mart., *Myrsine guianensis* (Aubl.) Kuntze, and *Ocotea puberula* (Rich.) Nees (aqueous extracts), indicated that lettuce germination was slightly altered only in the extracts of *E. argentinum* and *L. divaricata*, confirming the statement by Ferreira (2004). Carmo *et al.* (2012), working with species considered to produce allelopathic substances (*Pinus elliottii* Engelm. and *Eucalyptus* spp.) in the field, did not find evidence of the allelopathic action of the mentioned species, indicating that the germination process can occur even in the presence of these metabolites.

For extracts of other species, such as *Casearia sylvestris* Sw., the germination test was only negatively influenced at the highest concentrations of 90% and 100%, while for *Joanesia princeps* Vell., the germination inhibition of lettuce seeds occurred from the extract in the concentration of 30% (Capobiango *et al.* 2009). Testing fresh leaf extracts of *Rheedia brasiliensis* (Mart.) Planch. & Triana in the germination of lettuce seeds, Oliveira *et al.* (2011) indicated that only at the concentration of 20% was there a negative effect on germination. On the other hand, Coelho *et al.* (2011), evaluating extract of juazeiro (*Ziziphus joazeiro* Mart.) seeds indicated that the effect occurred from the 75% concentration. Other authors, such as Oliveira *et al.* (2013), indicated that ethanolic and aqueous extracts of *Vochysia divergens*, with a concentration of 10%, influenced the germination of tomato and lettuce seeds. These results demonstrate different patterns depending on the species tested.

In the greenhouse, the germination and ESI of both target species were negatively affected, indicating the release of allelochemicals through leaching and their interference on the species. Evaluating the results in the germination chamber and greenhouse, it can be observed that in the chamber the effects of the extracts were more deleterious. In environments such as a greenhouse, where environmental conditions such as temperature, light intensity, the presence of ultraviolet and infrared radiation, and the development of microorganisms on the substrate, the effects can be different since these factors can affect the structure of allelochemicals, decreasing their action potential.

According to Almeida *et al.* (2008), when the presence of variable environmental conditions occurs, the metabolites can undergo oxidative processes, changing their mode of action and, often, reducing their effects. This situation was reported by Pereira *et al.* (2018), who evaluated *Anacardium humile* extracts and their action on lettuce, tomato, and fedegoso seeds (*Senna obtusifolia* (L.) Irwin & Barneby), with more intense action observed in a germination chamber.

The development of seedlings in a germination chamber indicated that the extracts negatively influenced the development of roots and shoots, with a significant reduction in all treatments. In the greenhouse, there was also a reduction in seedling growth, although on a smaller scale.

It can be observed that although the present compounds have negatively affected the entire seedling, the growth of the roots was more affected. Hagemann *et al.* (2010) described that the root system is the most sensitive to allelochemicals because, according to Fujii & Hiradate (2007), its rapid elongation depends on efficient cell division, which can be affected by the presence of these compounds. In addition, this structure tends to accumulate a greater amount of metabolites since it is in direct contact with the extract.

The increase in the concentration of the extracts caused a decrease in the growth of the plants, having the highest concentrations, the shortest lengths of seedlings, or causing their death, as observed in the tomato in the extract at a concentration of 20%. Results such as those found by Capobiango *et al.* (2009) demonstrate that in certain concentrations, such as 10% aqueous extract of *Joanesia princeps* leaves, the length of the roots decreased and in the 50% extract, growth does not occur. The authors also evaluated the aqueous extract of the leaves of *Casearia sylvestris* and observed that an increase in the concentration of the extracts led to less root growth.

Oliveira *et al.* (2013) found the same results with aqueous and ethanolic extracts of V*ochysia divergens*, demonstrating that at a concentration of 2.5% there was a significant reduction in the length of roots and stems of lettuce and tomato seedlings. This demonstrates that, depending on the species tested, the concentration of the extract from which a negative change in growth occurs is variable. These data corroborate the results found by this work, in which the extracts of *N. guianensis* caused reductions in seedling growth.

Conclusions

In laboratory and greenhouse conditions, extracts and dry powder added to vermiculite have allelochemical action, negatively interfering with the germination, especially of tomato seeds, and initial seedling growth of lettuce and tomato. These results demonstrate that the tested hypothesis is valid and that *Norantea guianensis* can negatively influence the development and survival of other plant species used in floristic compositions.

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