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Phenolic Composition and Allelopathy of *Libidibia ferrea* Mart. ex Tul. in Weeds

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Abstract

Considering the need to produce effective bioherbicides to control weeds and thus reduce the contamination of environments through the use of agrochemicals in control of these plants, the scientific community has been studying the allelopathic activity of several species of Caatinga, once studies indicate that some species of this biome presentind to have activity allelopathic about other plants. On this, the present study aimed to evaluate the allelopathic potential and phenolic composition of extracts of *Libidibia ferrea* Mart. ex Tul. on seed germination and seedling development of *Calotropis procera* (Aiton) WT Aiton. and *Cenchrus echinatus* L. For the allelopathy test, leaf, bark and root extracts, both hot (100 °C) and cold (25 °C), were used, followed by a control group (distilled H_2O). Phytochemical prospecting was performed by High Performance Liquid Chromatography (HPLC). The results showed that the hot *L. ferrea* bark extract has allelopathic activity on *C. procera* and *C. echinatus*, which was observed in all parameters analyzed. The phytochemical results showed that *L. ferrea* extracts present several phenolic compounds which are possibly responsible for the results observed against the two weed species studied, with standing out Gallic acid, Catechin, Caffeic acid, Ellagic acid, and Quercetin. It is therefore necessary to isolate these compounds in view of a potential future use for *L. ferrea* extracts in the production of a bioherbicide.

Keywords: bioherbicide, high performance liquid chromatography, phenolic compounds, synthetic herbicides

1. Introduction

Currently the indiscriminate use of agrotoxic has been a cause of great concern in several countries, since the sintect herbicides are one of the methods most used in weeds control, which has caused environmental consequences such as poluition of soil, water, air and food (Santos & Silva, 2014). However, an alternative to the use of synthetic herbicides is the use of products derived from the secondary metabolism of plants known as allelochemicals. These compounds are produced by the plant's defense mechanism and are released into the environment through volatilization, root exudation, leaching and residue decomposition (Silva, Medeiros Filho, Duarte, & Moreira, 2014). The allelochemicals can have a positive or negative effect on the germination and development of other plants, whiching can be used in search for new sources of bioherbicides (Ferreira & Aquila, 2000). The production of these substances can be influenced by several environmental factors, such as temperature, light intensity, water and nutrient availability, soil texture and the presence of microorganisms (Scognamiglio, D'Abrosca, Esposito, & Fiorentino, 2015).

Research in the allelopathy field permits the search for plant allelochemicals, which can be used to control invasive species and weeds in agriculture, and thus reduce or even eliminate environmental contamination for herbicides sintetic, in the way there by preserving natural resources to ensure the supply of quality food. Currently, chemical substances with allelopathic activity have been studied in order to be used in bioherbicide

formulation or to be modified, in order to increase their biological activity (Formagio, Masetto, Baldivia, Vieira, & Zarate, 2010).

Allelopathic effects are mediated by chemical substances belonging to different secondary compound groups, such as terpenoids, steroids, alkaloids, tannins, phenols, coumarins, flavonoids, glycosides, cyanogenics, derived from benzoic acid, fatty acids and quinones, among others (Bulegon et al., 2015). These can occur in different plant parts including, leaves, flowers, roots, rhizomes, stems and seeds (Borella, Martinazzo, & Aumonde, 2011).

Unlike synthetic herbicides, herbicides of vegetable origin have low ecotoxicological properties, as they present low toxicity to humans and animals, rapid degradation, reduced environmental impact and they do not present cumulative properties along the food chain (Isman, 2006; Benelli, Flamini, Canale, Cioni, & Conti, 2012). Because there is a need for effective bioherbicides for the control of weeds, the scientific community has been studying the allelopathic activity of several Caatinga species, these studies have shown the great allelopathic potential that species of this ecosystem possess (Centenaro et al., 2009; Coelho, Maia, Oliveira, & Diógenes, 2011; Rêgo Junior et al., 2011; Silveira, Maia, & Coelho, 2012).

Fabaceae is a greatly diverse monophyletic taxon with roughly 18 thousand species distributed across 630 genera, being therefore the third largest family (Judd et al., 2009; Lewis, Schrire, Mackinder, & Lock, 2005). *Libidibia ferrea* Mart. Ex Tul. is one species from this family, formerly belonging to the *Caesalpinia* genus, popularly known as "jucá" or "Pau-ferro". This species is endemic to the North and Northeast (Alagoas, Bahia, Ceará, Paraíba and Pernambuco), especially in geographic areas dominated by the Caatinga ecosystem, a dry Tropical Forest from Northeast Brazil (Ferreira & Soares, 2015). As this species possesses allelopathic activity against *Lactuca sativa* L. seeds (Oliveira, Coelho, Maia, & Diogenes, 2012), hypothesis that it also possesses allelopathic activity against weed seeds exist.

Among the species of weeds, two stand out: *Calotropis procera* (Aiton) WT Aiton. known as "papai-noel" or "ciumeira" belonging to Apocynaceae, native to South-West Asia and Africa where it occurs in subspecies and common in the Northeast region of Brazil behaving as a natural environment invader (Costa, Medeiros, Alves, & Medeiros, 2009); and *Cenchrus echinatus* Linn. (Poaceae), popularly known as carrapicho, that is widespread in almost all cropping regions of Brazil. It has been found infesting various commercial crop fields, such as peanut, citrus, bean, cassava, soybean, sugarcane, mint, onion and tomatoe, as well as in pastures (Silva et al., 2015). The aforementioned species are considered important invasive plants (weeds) and their control is exercised through synthetic herbicide means, which have caused numerous environmental and human health damages.

Therefore, the authors of this study evaluated the allelopathic potential and phenolic composition of *L. ferrea* extracts on *C. procera* and *C. echinatus* seed germination and seedling development.

2. Methodology

2.1 Botanic Material

The botanical material was collected from the city of Potengi, in the state of Ceará, Brazil, in June 2016, under coordinates 07°05′.99″ S; 040°00′.07″ W, with an altitude of 613 m. For botanical identification, fertile material was collected, which was herborized, assembled in an exsiccate and deposited in the Caririense Dárdano de Andrade-Lima Herbarium - HCDAL/URCA with voucher number 12,781.

2.2 Extract Preparation

Libidibia ferrea leaves, barks and roots were collected for the extracts. Two 50 g portions of each aforementioned plant organs were used. The two 50 g portions were packaged separately into six beakers. Each plant part received a hot treatment (500 mL distilled water at 100 °C) and a cold treatment (500 mL distilled water at 25 °C), which lasted 30 minutes. After this period, each beaker content was ground in an industrial blender for one minute. The extract was then filtered using a perfex® cloth to retain all fibrous material and the filtrates were subsequently subjected to 3000 rpm centrifugation for 10 min.

Physical-chemical Characterization

Two physicochemical parameters of the extracts were measured: osmolarity and pH. When the extracts behaved as acids (< 6.5) or bases (> 7.5), these were adjusted to neutral values close to 7 with the aid of 0.1 mol/L KOH and 5% HCl, as recommended by Macias, Galindo and Molinillo (2000).

For the osmolarity, the extract's osmotic potentials were measured using the molar concentration from 2.5 mL of each extract with an osmometer (Model PZL-1000). The measurements were obtained in mOsm/kg and converted to osmotic pressure (MPa) using the equation below (Larcher, 2004):

$$\pi = - W \times 0.00832 \times Tabs \quad (1)$$

Where, π is osmotic Pressure in MPa; W is osmotic Potential in Osm/kg; Tabs is absolute temperature, expressed in Kelvin degrees.

2.3 Seeding and Procedures

The experimental design was completely randomized with seven treatments: hot leaf extract (EQF 100 °C), cold leaf extract (EFF 25 °C), hot bark extract (EQC 100 °C), cold bark extract (EFC 25 °C), hot root extract (EQR 100 °C), cold root extract (EFR 25 °C) and istilled water (control treatment) with four 25 seed replicates from *C. procera* and *C. echinatus* seeds each. In each petri dish, 3 mL of the crude extract were applied to two germitest paper sheets which had been previously autoclaved. The appropriately sterilized petri dishes were maintained in a BOD type chamber at 25 °C temperature and a light/dark cycle of 12 hours for 7 days for *C. procera* and 5 days for *C. echinatus*. The germinated seeds were counted every 24 hours, considering seed germination as those with a primary root length equal to or greater than 2 mm.

2.4 Variables Analyzed

2.4.1 Number of Germinated Seeds (NGS)

The number of germinated seeds was calculated by the following formula:

$$NGS = (N/Nt) \times 100 \quad (2)$$

Where, N refers to the number of germinated seeds; Nt refers to the total number of seeds sown.

2.4.2 Germination Velocity Index (GVI)

Germination Velocity Index (GVI) was calculated according to Maguire (1962) using the formula:

$$GVI = G1N1 + G2/N2 + ... + Gn/Nn \quad (3)$$

Where, G1, G2, Gn is number of germinated seeds computed in the first, second and last count; N1, N2, Nn is number of days of sowing to the first, second and last count.

2.4.3 Seedling Development

In order to analyze whether the extracts presented any developmental allelopathic activity, the caulicle and radicle lengths were measured. Caulicular measurements (distance in mm from the hypocotyl to the apex) and radicular measurement (distance in mm from the hypocotyl to the meristematic apex) of the seedlings were made using a ruler duly adjusted in mm.

2.5 Chemicals, Apparatus and General Procedures

All chemical were of analytical grade where methanol, formic acid, acetic acid, gallic acid, ellagic acid and caffeic acid were purchased from Merck (Darmstadt, Germany)., whereas luteolin, quercetin, catechin and epicatechin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

2.6 High Efficiency Liquid Chromatography with Diode Array Detector (HPLC-DAD)

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} columns (4.6 mm × 250 mm) packed with 5µm diameter particles. The mobile phase with a 0.6 mL/min flow rate consisted of a mixture of solvents: A (water/acetic acid, 98:2% v/v) and B (methanol/water/formic acid, 70:28:2% v/v) using the following linear eluting gradient: 0-3 min: 0% B in A; 3-25 min: 30% B in A; 25-43 min: 50% B in A; 43-55 min: 60% B in A; 55-60 min: 80% B in A; 60-65 min: 50% B in A; and 65-69 min: 0% B in A; following the method described by Boligon et al. (2015) with some modifications. *Libidibia ferrea* extracts (10 mg/mL) and mobile phase were filtered through 0.45 µm membrane filters (Millipore) and degassed by ultrasonic bath prior to use. Standard reference stock solutions were prepared at a concentration range of 0.030-0.250 mg/ml. Quantifications were carried out by peak integration using the external standard method, at 254 nm for gallic and ellagic acids; 280 nm for catechin and epicatechin; 327 nm for caffeic acid; and 366 nm for luteolin and quercetin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). All chromatography procedures were carried out at ambient temperature and in triplicates.

2.7 Statistical Analysis

Statistical analyzes were performed using the GraphPad Prism 6 software. A two-way analysis of variance (two-way ANOVA) and mean comparison using Tukey's test at 5% probability were performed for the

germination tests. As for seedling length, a one-way ANOVA followed by the Tukey's test at 5% probability was performed. Differences between HPLC groups were assessed by an analysis of variance model and Tukey's test. The level of significance for the analyzes was set to $p < 0.05$. These analyzes were performed by using the free R software version 3.1.1 (R Core Team, 2014).

3. Results

3.1 Physical-Chemical Analysis

According to Table 1, we can observe that the pH values obtained for the *L. ferrea* extracts varied between 4.75 and 5.49, considering the extracts with certain acidity. Therefore, the extracts were adjusted in order to reach values between 6.5 and 7.5, considered ideal for allelopathy tests according to Macias et al. (2000). For the osmotic potential values, the extracts did not present much solute; therefore, they would not cause false-positive results due to a high concentration of solutes.

Table 1. Physical-chemical characteristics of the *L. ferrea* extracts used in the bioassays to evaluate their allelopathic potential on *C. procera* and *C. echinatus* seeds

Treatments	EQC	EFC	EQF	EFF	EQR	EFR
Measured pH	4.75	4.92	4.98	5.15	5.49	5.46
Adjusted pH	6.55	6.55	6.96	6.96	6.72	6.53
Osmolarity (Mpa)	-0.109	-0.079	-0.145	-0.084	-0.040	-0.029

Note. EQC: Hot Peel Extract; EFC: Cold Peel Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract.

3.2 Chemical Composition of the Extracts

The HPLC profile of *L. ferrea* extracts was also acquired, analysis is shown in Figures 1A-1F. The samples contains other minor compounds in addition to gallic acid (retention time-t_R = 12.35 min, peak 1), catechin (t_R = 17.49 min, peak 2), caffeic acid (t_R = 25.11 min, peak 3), ellagic acid (t_R = 34.08 min, peak 4), epicatechin (t_R = 37.62 min, peak 5), quercetin (t_R = 50.13 min, peak 6) and luteolin (t_R = 61.94 min, peak 7).

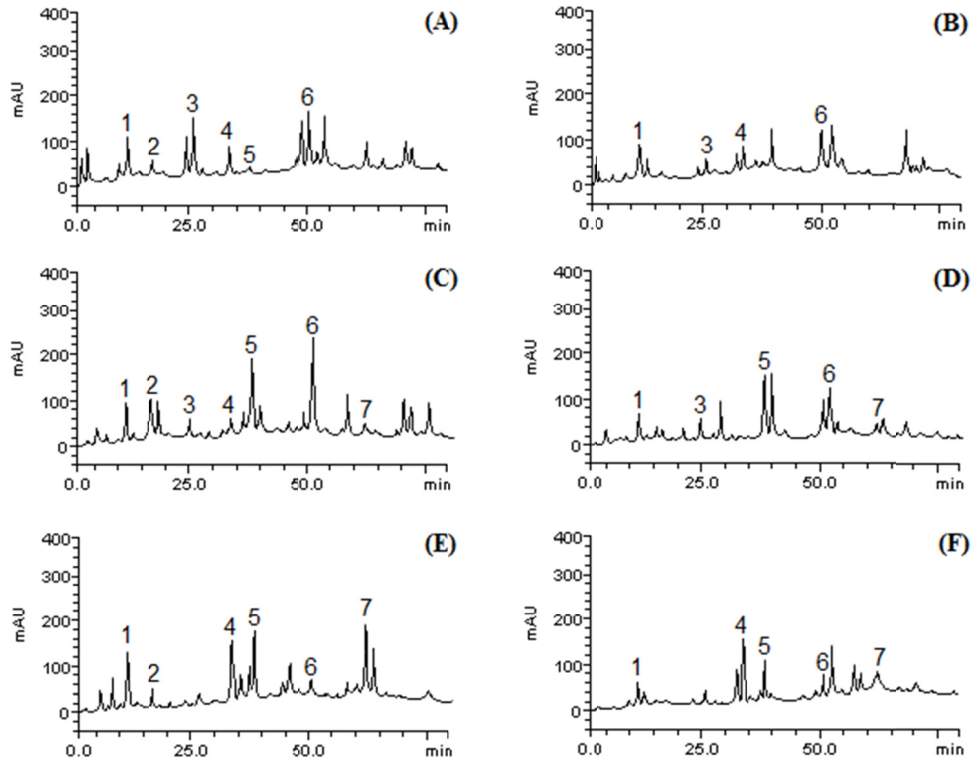

Figure 1. Representative high performance liquid chromatography profile from *L. ferrea* extracts

Note. A: Chromatographic profile of the hot bark extract. B: Chromatographic profile of the cold bark extract. C: Chromatographic profile of the hot leaf extract. D: Chromatographic profile of the cold leaf extract. E: Chromatographic profile of the hot root extract. F: Chromatographic profile of the cold root extract.

The HPLC analysis of the *L. ferrea* extracts showed variations in phenolic composition, with the compounds that presented themselves as a main constituent being Quercetin in the hot bark extract (5.01 mg/g), the cold bark extract (2.71 mg/g) and the hot leaf extract (6.01 mg/g); Epicatechin in the cold leaf extract (4.93 mg/g); Luteolin in the hot root extract (5.19 mg/g); Ellagic acid in the cold root extract (4.97 mg/g) (Table 2).

Table 2. Polyphenolic compound concentrations analyzed by HPLC-DAD from *L. ferrea* extracts

Compounds	EQC	EFC	EQF	EFF	EQR	EFR
	---------- mg/g ----------					
Gallic acid	2.79±0.01 a	2.65±0.04 a	2.49±0.01 a	1.27±0.03 a	4.38±0.01 a	1.10±0.05 a
Catechin	0.98±0.03 b	-	2.54±0.03 a	-	1.03±0.01 b	-
Caffeic acid	4.81±0.04 c	0.94±0.05 b	1.13±0.07 b	1.16±0.05 a	-	-
Ellagic acid	2.06±0.01 d	1.39±0.02 c	1.09±0.02 b	-	4.57±0.03 c	4.97±0.03 b
Epicatechin	0.15±0.01 e	-	5.36±0.01 c	4.93±0.01 b	5.11±0.01 d	2.25±0.01 c
Quercetin	5.01±0.04 c	2.71±0.01 a	6.01±0.03 d	3.05±0.02 c	1.06±0.07 b	1.07±0.01 a
Luteolin	-	-	1.03±0.01 b	0.46±0.04 d	5.19±0.02 d	1.54±0.02 d

Note. Results are expressed as mean ± standard deviation (SD) from three determinations. The means followed by different letters differ using Tukey's test with $p < 0.05$.

3.3 Number of Germinated Seeds

According to the data represented in Figure 2, the hot extracts from the different *L. ferrea* (leaves, barks and roots) organs presented allelopathic activity, inhibiting *C. procera* seed germination with significant differences in relation to the control group.

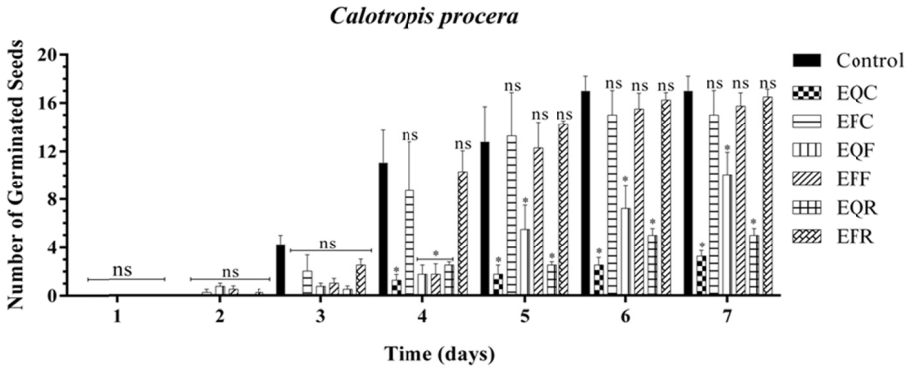

Figure 2. Allelopathic effect of *L. ferrea* extracts against the number of germinated *C. procera* seeds

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect $P < 0.05$. ns: corresponding to the allelopathic effect no significant comparing to control group.

With regards to the germination of *C. echinatus* seeds, these had their germination inhibited by bark extracts, and by the hot *L. ferrea* leaf extract (Figure 3).

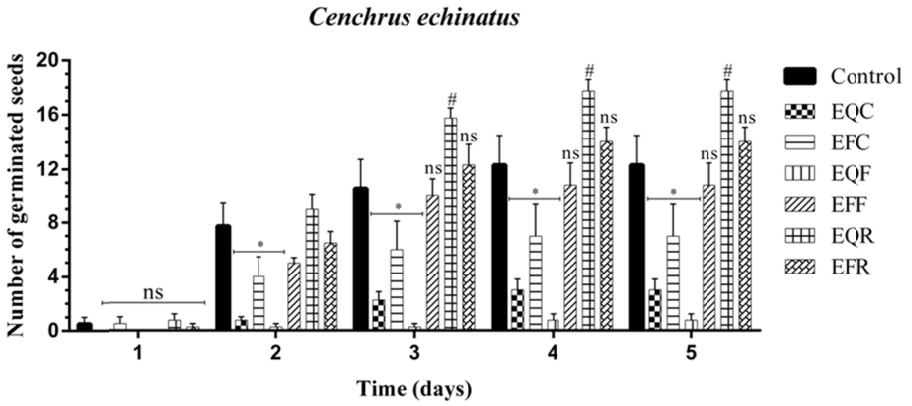

Figure 3. Allelopathic effect of *L. ferrea* extracts against the number of germinated *C. echinatus* seeds

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect and # to the positive allelopathic effect $P < 0.05$. ns: corresponding to the allelopathic effect no significant comparing to control group.

3.4 Germination Velocity Index (GVI)

Table 3. Germination Velocity Index of *C. procera* and *C. echinatus* under the effect of hot and cold *L. ferrea* extracts from leaves, barks and roots

Treatament	Control	EQF	EFF	EQC	EFC	EQR	EFR
C. procera	4.11±0.64a	1.43±0.74a	3.28±0.47a	0.54±0.37b	3.18±1.82a	2.1±2.09a	2.37±1.96a
C. echinatus	5.56±0.38ae	0.25±0.28b	4.35±0.96c	1.06±0.44bd	3.16±2.41acd	7.62±1.07e	5.72±1.14ace

Note. Letters in the same line differ statistically using Tukey's test at 5%.

Regarding the germination velocity index (GVI) of *C. procera* seeds, the hot *L. ferrea* bark extract reduced the germination speed of *C. procera* seeds differing significantly from the control group (Table 3).

For the germination velocity values of *C. echinatus* seeds, the hot leaf extract, cold leaf extract and the hot *L. ferrea* bark extract caused a delay in the germination velocity of *C. procera* seeds, which were significant in relation to the control group (Table 3).

3.5 Shoot Length

With regards to the shoot length of *C. procera* seedlings, the hot *L. ferrea* leaf and bark extracts showed a decrease in *C. procera* length which was significant when compared to the control (Figure 4).

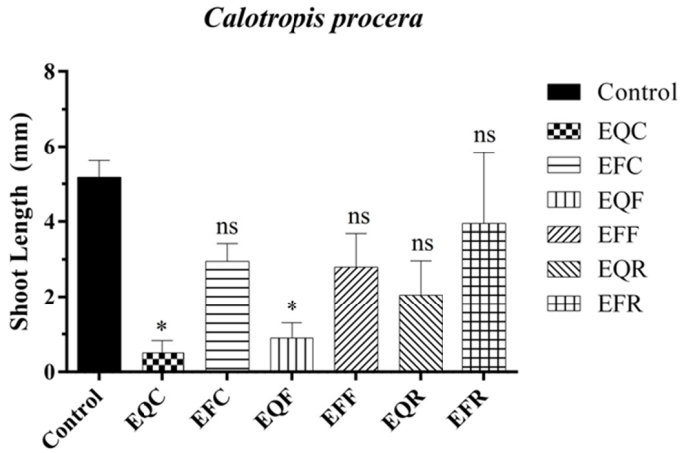

Figure 4. Allelopathic effect of *L. ferrea* extracts on *C. procera* shoot growth

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect. ns: corresponding to the allelopathic effect no significant comparing to control group.

As shown in Figure 5, we can see that the length of the shoot of *C. echinatus* seedlings was affected by the hot extract of the barks and leaves of *L. ferrea*, differing significantly from the control group.

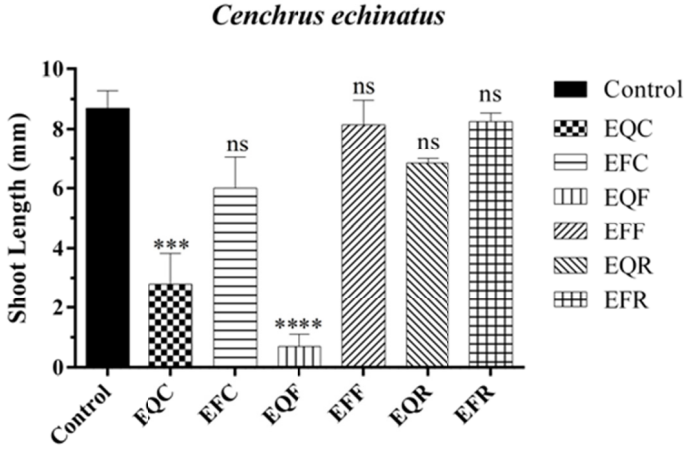

Figure 5. Allelopathic effect of *L. ferrea* extracts on *C. echinatus* shoot growth

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect. ns: corresponding to the allelopathic effect no significant comparing to control group.

3.6 Root Length

In relation to *C. porcera* seedling root length, analysis of the obtained data showed that there was a significant difference between the different *L. ferrea* extracts, with the hot bark extract having a negative influence on root

growth, while the cold leaf extract promoted an increase in its length, however this increase was not significant when compared to the control (Figure 6).

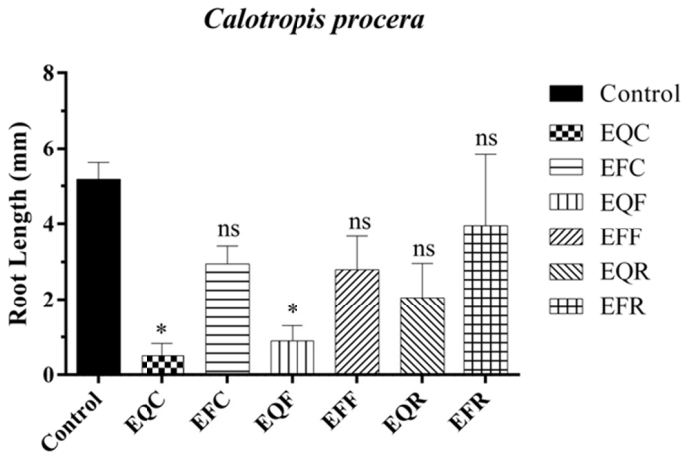

Figure 6. Allelopathic effect of *L. ferrea* extracts on *C. procera* root growth

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect. ns: corresponding to the allelopathic effect no significant comparing to control group.

According to the data shown in Figure 7, the root length of *C. echinatus* seedlings were affected by the hot leaf and bark extracts from the donor species, differing significantly from the control group.

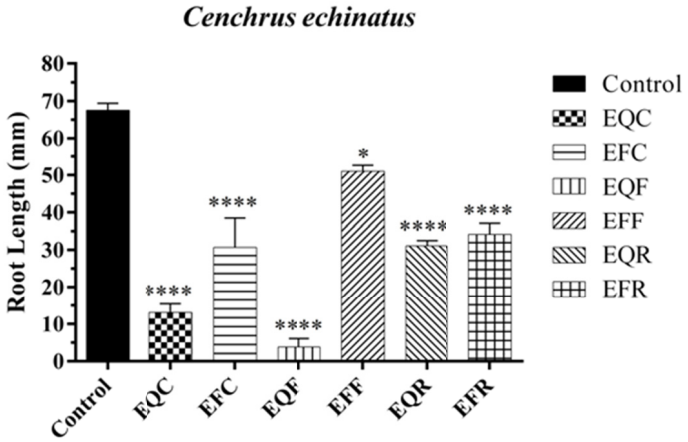

Figure 7. Allelopathic effect of *L. ferrea* extracts on *C. echinatus* root growth

Note. EQC: Hot Bark Extract; EFC: Cold Bark Extract; EQF: Hot Leaf Extract; EFF: Cold Leaf Extract; EQR: Hot Root Extract; EFR: Cold Root Extract. * Corresponds to the negative allelopathic effect.

4. Discussion

There is currently a series of studies highlighting the allelopathic activity of Caatinga species on the germination process of other species (Centenaro et al., 2009; Coelho, Maia, Oliveira, & Diógenes, 2011; Rêgo Junior et al., 2011; Silveira, Maia, & Coelho, 2012), even though this process is considered less sensitive to the action of these allelochemicals (Ferreira & Áquila, 2000). Oliveira et al. (2012) found that extracts from different *L. ferrea* organs negatively influenced seed germination and lettuce seedling development. Thus, the present study brings forward important results for the allelopathic activity of this species' extracts against the two tested weeds.

With regards to the pH and osmolarity of the extracts under study, the control of these variables is extremely important for research in allelopathy since substances such as sugars, amino acids and organic acids may

influence the pH and may be osmotically active, in which case this activity may mask the allelopathic effect, as well as lead to false positive results (Borella & Pastorini, 2010). The available literature information on how the pH effects seed germination and seedling development basically refers to species from temperate regions. However, these data demonstrate that both germination and seedling development are negatively affected under extremely acid or extremely alkaline conditions (Rosa, Fortes, Mauli, & Marques, 2011).

As for the osmotic potential, this should not exceed the -0.2 value (Gatti, Perez, & Lima, 2004), since they can exhibit certain solutes which can modify the water property, leading to an osmotic pressure in the solution different from zero, if they are not within the standard (Silveira et al., 2012). In allelopathy studies, osmolarity verification of the extracts is of extreme importance since a high osmotic potential may be reflected in seed germination time, delaying germination velocity, thus masking the allelopathic effect (Ferreira & Áquila, 2000).

Regarding the phytochemistry of the *L. ferrea* extracts, caffeic acid, a simple phenylpropanoide present in its bark and leaf extracts, is reported in the literature as an allelochemical compound that inhibits both germination and the development of other plants (Barkosky, Einhellig, & Butler, 2000). In addition, Loffredo, Monaci, and Senesi (2005) report that caffeic acid has an allelopathic effect and inhibits seed germination and tomato growth. Thus, it is likely that this compound, alone or in combination with other compounds, inhibited the germination of the recipient species. This study corroborates with that by Araújo et al. (2014), in which *L. ferrea* polyphenols were quantified, and the presence of gallic acid and catechin in the plant bark is confirmed. It is noteworthy that in the cold *L. ferrea* bark extract the presence of catechin was not verified, which may be justified by the preparation of the extract.

Results similar to the present study were also found by Oliveira et al. (2012), which indicated that gallic acid and ellagic acid present in *L. ferrea* leaf and bark extracts are considered possible allelopathic components on the development of alface seedlings, where the appearance of abnormal seedlings and a reduction in shoot and root length in the presence of these compounds were observed. Moreover, gallic acid is mentioned in the literature as an important germination reducer of a large number of plants (Fiorenza et al., 2016). Souza Filho et al. (2006) also show that this compound has allelopathic potential over the germination of *Mimosa pudica* L. seeds, with significant germination effects demonstrating up to 71% inhibition.

For the germination assays, this study showed that *L. ferrea* leaf, bark and root extracts present allelopathic activity over *C. procera* and *C. echinatus* seeds. Similar results were observed in the study by Oliveira et al. (2012), in which the effect of different *L. ferrea* organ extracts on *Lactuca sativa* germination were analyzed, and it was verified that the leaf, bark mature pod extracts presented allelopathic activity on seed germination of the recipient species.

In the aforementioned study, in which extraction methods, extract concentration and interaction were analyzed, significant differences were observed for these variables with all analyzed characteristics, demonstrating a higher allelopathic effect when the seeds were exposed to the extract obtained by infusion (at 100 °C), thus corroborating with the results obtained in the present study.

These results are probably due to a higher availability of the allelochemicals in the solution when infusion extraction is applied since *L. ferrea* presents a high tannin content (Frasson, Bittencourt, & Heinzmann, 2003), which is more readily solubilized in hot water (Trugillo, Mori, Lima, & Cardoso, 2003). It is important to emphasize the observed differences between infusion and cold extraction, where considering the results of the present study, extract preparation with hot water is widely used with the purpose of obtaining greater extraction especially of plant substances which are less soluble (Ferreira & Áquila, 2000).

For Ferreira and Aquila (2000), changes in germination patterns may be the result of effects over membrane permeability, transcription and DNA translation, secondary messenger function; respiration, sequestration of oxygen (phenols), conformation of enzymes and receptors, or the combination of these factors. Regarding the Germination Velocity Index, results similar to the present study were found by Oliveira et al. (2012) in a research carried out with hot *L. ferrea* bark extracts, which reduced the germination speed of *L. sativa* seeds.

In relation to the root system, Conti and Franco, (2011), point out that plant roots are more sensitive to the action of allelopathic substances since their elongation depends on cell divisions which, once inhibited, affect their normal development. Thus, it is very important to evaluate seedling normality, as allelochemicals may lead to the formation of abnormal seedlings, where root necrosis is one of the most common phenomena (Ferreira & Áquila, 2000). This is an important ecological aspect, since, with development inhibition of the root system, there can be a reduction in the plant's competitive pressure, thus favoring the growth of neighboring species allowing these to establish dominant aspects (Formagio et al., 2010).

It is also worth mentioning that during the development phase of the plant, the roots are in direct contact with the soil solution and may absorb allelopathic substances which are being released in sufficient quantities and which can cause germination, radicular growth and aerial part growth inhibition (Oliveira, Pinto, Araújo, Nunes & Brito, 2015). These results are important as the sharp root reduction may affect the competitive capacity and productivity of the plant, where the reduction of the aerial part may diminish the plant's capacity to compete for light (Cândido et al., 2010).

5. Conclusion

The *L. ferrea* extracts have a high allelopathic effect on the two tested weeds. The hot *L. ferrea* bark extract showed allelopathic activity over *C. procera* and *C. echinatus* seeds, which was observed at all analyzed parameters. The hot leaf extract inhibited seed germination of the two tested species, moreover, it caused a decrease in shoot length in their seedlings, in addition to reducing germination velocity and causing a decrease in *C. echinatus* root length. In the phytochemical analysis, *L. ferrea* extracts presented chemical compounds with allelopathic activity, with standing out Gallic acid, Catechin, Caffeic acid, Ellagic acid, and Quercetin, which show a significant effect on the studied weeds. The results highlight the need to increase research where these compounds are isolated with the future aim of utilizing *L. ferrea* extracts for the production of a bioherbicide.

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