

DEREPLICATION OF ISOQUINOLINE ALKALOIDS FROM *Duguetia quitarensis* (Benth.) AND EVALUATION OF PHYTOTOXIC ACTIVITY

DESREPLICACIÓN DE ALCALOIDES ISOQUINOLÍNICOS DE *Duguetia quitarensis* (Benth.) Y EVALUACIÓN DE LA ACTIVIDAD FITOTÓXICA

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ABSTRACT

Duguetia quitarensis (Benth.) is a species distributed across the Amazon region, from Venezuela to Bolivia. This study aimed to characterize the chemical profile of the methanolic extract of *D. quitarensis* through dereplication by direct infusion and to evaluate its phytotoxic potential against *Allium cepa* L. and *Lactuca sativa* L. Ten isoquinoline alkaloids were annotated, including aporphine-, benzyl-tetrahydroisoquinoline-, 7-hydroxyaporphine-, and oxoaporphine-type compounds. The methanolic extract exhibited phytotoxic effects of varying intensity, as indicated by seedling growth inhibition and germination assays. Root elongation was more sensitive than hypocotyl growth, particularly in *A. cepa*. The presence of multiple isoquinoline alkaloids suggests their contribution to the observed phytotoxicity, possibly through interference in cellular processes such as respiration and DNA synthesis. These findings expand the chemical knowledge of *D. quitarensis* and indicate its potential as a natural source of allelopathic or bioherbicide compounds.

Keywords: *Duguetia quitarensis*; annonaceae; alkaloids; dereplication; phytotoxic activity.

RESUMEN

Duguetia quitarensis (Benth.) es una especie distribuida en la región amazónica, desde Venezuela hasta Bolivia. Este estudio tuvo como objetivo, caracterizar el perfil químico del extracto metanólico de *D. quitarensis*, mediante desreplicación por infusión directa, y evaluar su potencial fitotóxico frente a *Allium cepa* L. y *Lactuca sativa* L. Se anotaron diez alcaloides isoquinolínicos, incluidos compuestos de los tipos aporfina, bencil-tetrahydroisoquinolina, 7-hidroxiaporfina y oxoaporfina. El extracto metanólico mostró efectos fitotóxicos de diferente intensidad, evidenciados por los ensayos de inhibición del crecimiento de plántulas y de germinación. El alargamiento radicular fue más sensible que el crecimiento del hipocótilo, especialmente en *A. cepa*. La presencia de múltiples alcaloides isoquinolínicos sugiere su contribución a la fitotoxicidad observada, posiblemente mediante interferencia en procesos celulares, como la respiración y la síntesis de ADN. Estos hallazgos amplían el conocimiento químico de *D. quitarensis*, e indican su potencial como fuente natural de compuestos alelopáticos o bioherbicidas.

Palabras clave: *Duguetia quitarensis*; annonaceae; alcaloides; desreplicación; actividad fitotóxica.

INTRODUCTION

In plant communities, plants can interact in positive, negative, or neutral ways, with it being more common for neighboring plants to interact negatively, such that the emergence and/or growth of one or both is inhibited. These interactions between neighboring organisms are referred to as interference.

Allelopathy is an example of such interference, caused by chemical substances produced by certain organisms, which affect other community components in the environment.⁽¹⁾

This interference is not competition, as it does not involve a dispute for limited resources like light, water, and nutrients, but rather it is a toxic effect of substances produced by other plants. Allelopathy can be an ecological interaction strategy, as through this mechanism, one plant can interfere with the growth of another, recognized as an important ecological mechanism that influences plant dominance, succession, the formation of plant communities, and climax vegetation, as well as crop management.

The allelopathic compounds released by a plant can affect growth, impair normal development, and inhibit seed germination of other plant species.⁽²⁾

Phytotoxicity is one of the main problems faced by farmers, manifesting as crop invasion by weeds. In cereals and vegetables, losses due to the presence of weeds can reach 100 %, while in some forest and fruit species, the losses are smaller. Thus, when the economic damage threshold is exceeded, it is necessary to use control practices, including chemical control through herbicides, which have numerous disadvantages such as environmental impact and harmful effects to human health.⁽³⁾ Natural compounds represent a great opportunity for the discovery of new herbicides, safer for the environment, called bioherbicides, which are based on structures derived from plants and other living organisms. It is estimated that there are 24 million organic compounds with great allelopathic potential.⁽⁴⁾

Chemical compounds (allelochemicals) can be produced in all plant tissues and differ between species. Compounds with allelopathic activity can be found in various classes of natural substances such as phenols, alkaloids, glycosides, flavonoids, terpenoids, coumarins, and tannins, among others. Allelopathic compounds vary according to the species and have different effects on target species.⁽⁵⁾

The Annonaceae family consists of 2 106 species and more than 130 genera. It is concentrated in the tropics, with about 900 species being neotropical, 450 being

afrotropical, and the rest being indomalayan. Annonaceae play an important ecological role in terms of species diversity, especially in tropical forest ecosystems. In Brazil, the family has confirmed occurrence in all states, with 380 species described and distributed in 32 genera. The Amazon biome comprises 268 species, the Atlantic Forest 98 species, and the Cerrado 52 species.⁽⁶⁾

The genus *Duguetia* A. St.-Hill includes 94 species, of which 89 occur in Neotropical regions and 4 in Africa. In Brazil, the genus is one of the most represented in Brazilian flora. The species *D. quitarensis* (synonyms: *Aberemoa quitarensis* Benth. REFr., *Duguetia ibonensis* Rusby, and *Duguetia tessmannii* REFr.) is one of the most common species of the genus and is distributed in the Amazon region from Venezuela to Bolivia. Economically, its wood is used in the construction sector.⁽⁷⁾

From a biological perspective, Annonaceae species have shown a wide spectrum of biological activities, such as antiarthritic, anticancer, anticonvulsant, antidiabetic, antiplasmodial, hepatoprotective and bilirubin-reducing, insecticidal, gastroprotective, molluscicidal, and wound-healing activities.⁽⁸⁾

A preliminary screening of crude extracts from *D. quitarensis*⁽⁷⁾ revealed that the methanolic extract was the most abundant in alkaloids, which was also confirmed by thin-layer chromatography. Thus, we proposed to investigate the presence of these alkaloids in the crude methanolic extract of this species, not yet described in the literature. For this, the dereplication technique by direct infusion was used. The phytotoxic potential (*in vitro*) of the crude methanolic extract of *D. quitarensis* was evaluated based on the percentage of stimulation or inhibition in the length of seedlings (root and hypocotyl) and the percentage of inhibition of germination in *Allium cepa* L. (onion) and *Lactuca sativa* L. (lettuce).

For this study, dereplication analysis was employed, a technique that gained popularity in the 1990s as a crucial step in the screening of crude extracts to identify already known bioactive compounds and avoid the re-isolation of non-novel substances. Mass spectrometry (MS) has been widely used in the dereplication of natural matrices due to its versatility, speed, and high sensitivity, which allows the use of smaller sample quantities.⁽⁹⁾ A preliminary screening of the crude extracts of *Duguetia quitarensis* revealed that the methanolic extract is the most abundant in alkaloids, as confirmed by thin-layer chromatography. Therefore, the present study aimed to investigate the alkaloid composition of the crude methanolic extract

of *D. quitarensis*, which has not yet been described for this species, using dereplication by direct infusion (ESI-MS/MS), and to evaluate its in vitro phytotoxic potential on seedling growth (root and hypocotyl) and the germination of *Allium cepa* L. and *Lactuca sativa* L.

MATERIALS AND METHODS

Plant Materials

The species *D. quitarensis* (Benth.) was collected in June 2018 on the banks of the Rio Jaci Paraná (10° 17' 59.87" S, 64° 14' 40.31" W) in the city of Porto Velho, Rondônia, and identified by botanist Eduarda A. Dias. A specimen of the plant material was deposited in the Rondonian Herbarium of the Federal University of Rondônia and registered under the number RON 648.

Extraction and Analysis by Direct Infusion (ESI-IT-MS/MS)

Leaves and small stems of *D. quitarensis* (200 g) extracted from a single plant specimen were crushed and then subjected to exhaustive extraction by maceration at room temperature. Initially, hexane (Synth brand) was used to remove apolar substances that are not soluble in water or ethanol, such as lipids or oils, so that ethanol could subsequently extract more polar compounds; the resulting extract was discarded. Afterwards, methanol (500 mL, Synth brand) was used. After filtration and removal of the solvents by evaporation at reduced pressure and drying, the crude extracts of hexane (5.4 g) and methanol (8.5 g) were obtained.

Exploratory analyses of the methanolic extract were performed by electrospray ionization mass spectrometry using a TSQ Quantum Access equipment (Thermo Scientific, San Jose, CA, USA) with ion-trap analyzer (ESI-IT-MS/MS), in positive mode, to infer the chemical composition of this species. The methanolic extract of *D. quitarensis* was resuspended in methanol (HPLC grade) generating stock solutions (1 mg. mL⁻¹). Then, the solutions were diluted to 5 µg.mL⁻¹ and analyzed by direct infusion in the mass spectrometer.

Seeds Used in Phytotoxicity Assays

To verify the phytotoxic effect of the methanolic extract from the leaves and small stems of *D. quitarensis*, seeds from the *A. cepa* (*Amaryllidaceae*), a monocotyledon and the dicotyledon from *L. sativa*

(*Asteraceae*) were used, known as standard target species STS according to Macías.⁽¹⁰⁾

Phytotoxicity Assay

A total of 10 mL of methanolic extract at concentrations of 0.156 25, 0.325, 0.625, 1.25, 2.5 and 5.0 mg/mL were added to Gerbox germination boxes (11 cm x 11 cm x 3.5 cm) containing two sheets of filter paper. The solvent was evaporated for 24 hours at room temperature and protected from light, and then 10 mL of distilled water and 20 seeds, commercially obtained, were added (three boxes, n = 60). Distilled water was used as a negative control. The Gerbox germination boxes with the seeds were conditioned in a germination chamber with a photoperiod model (SL-224/300, 300 L, Solab) at 25 °C with a photoperiod of 12 hours for 120 hours for *A. cepa* and *L. sativa*.⁽¹¹⁾

The evaluated parameters were the percentage of inhibition in seedling growth and germination. The lengths of the seedlings were measured with the help of a professional digital caliper with an LCD screen, model MTX, 150 mm. The level of activity was expressed as a percentage of inhibition,⁽¹²⁾ according to equation (1):

$$\% \text{ Inhibition} = \left(\frac{XT - XC}{XC} \right) \cdot 100 \quad (1)$$

where

XT: mean elongation of the treatments.

XC: mean elongation of the control.

The percentages of seed germination inhibition were calculated based on the difference from the control. For the root and shoot, the distribution used was the measurements of each variable in each germination box, known as the distribution of means. Positive values were considered stimulation, and negative values were considered inhibition.⁽¹²⁾

Preliminary Phytochemical Analysis

The qualitative phytochemical analysis of the crude methanolic extract of *D. quitarensis*⁽⁷⁾ using standard procedures,⁽¹³⁾ was carried out to determine the main classes of secondary metabolites (phenols, tannins, flavonoids, alkaloids, steroids, and triterpenoids).

Statistical Analysis

The experimental design was completely randomized and carried out in three replications to verify the

percentage of stimulation and inhibition of seedling length (root and hypocotyl) and the germination inhibition percentage. The means followed a normal distribution by the Shapiro-Wilk test ($p > 0.05$) and were then subjected to analysis of variance (ANOVA). For the analysis of the germination inhibition percentage, treatments were compared to the control, and when treatment effects showed significant differences, the means were compared by Dunnett's test ($p < 0.05$). The means for each treatment of seedling length were compared using Tukey's test ($p < 0.05$). Normality and variance analyses were performed using the GraphPad Prism 8.0.1 (244) program.

RESULTS AND DISCUSSION

The identification of the major alkaloids present in the crude methanolic extract of *D. quitarensis* was carried out through MS/MS fragmentation analysis and comparisons with data described in the literature. Ten alkaloids were annotated, including four aporphine-type: nornuciferine (1), nuciferine (2), corytuberine (3), nandigerine (4); two benzyl tetrahydroisoquinoline-type: N-methylcoclaurine (5), armepavine (6); one 7-hydroxyaporphine type: guatterine (7) and its oxide (8); and two oxoaporphine alkaloids: lysicamine (9) and liriodenine (10), as shown in (Table 1). The structures of the identified compounds are presented in (Figure 1).

The presence of isoquinoline alkaloids in species of the Annonaceae family is widely recognized in the field of natural products. However, the occurrence of this class of compounds is not exclusive to Annonaceae, as isoquinoline alkaloids are broadly distributed across several plant families, including Amaryllidaceae, Berberidaceae, Chenopodiaceae, Dioncophyllaceae, Lauraceae, Rubiaceae, and Siparunaceae, among others.⁽¹⁴⁾ In order to expand the chemical knowledge of another species belonging to the Annonaceae family, the composition of the crude methanolic extract of *Duguetia quitarensis*, a species not yet described in the literature, was investigated. Dereplication analysis was employed, a methodology established as a fundamental step in the screening of crude extracts to identify known bioactive compounds and avoid unnecessary re-isolation of previously characterized substances. Mass spectrometry (MS) was applied in this process due to its versatility, speed, and high sensitivity, which enable the analysis of minimal sample quantities.⁽⁹⁾

In the total ion analysis of the crude methanolic extract from the leaves and small branches of *D. quitarensis*, in positive mode, various ions were observed, as the electrospray ionization (ESI) mass spectra for many alkaloids show abundant $[M+H]^+$ ions, due to the strong basicity of secondary amine groups.⁽¹⁵⁾ However, we focused our attention on ions in the range of m/z 200-400, which correspond to the molecular mass of various isoquinoline alkaloids

Table 1- Precursor ion and key fragments observed in the ESI-IT-MS/MS experiment

Compound	Name	Molecular Formula	$[M+H]^+$	MS ^a
1	Nornuciferine	C ₁₈ H ₁₉ NO ₂	282	265, 250, 235, 207
2	Nuciferine	C ₁₉ H ₂₁ NO ₂	296	265, 250, 234
3	Corytuberine	C ₁₉ H ₂₁ NO ₄	328	297, 265
4	Nandigerine	C ₁₈ H ₁₇ NO ₄	312	295, 265, 237
5	N-methylcoclaurine	C ₁₈ H ₂₁ NO ₃	300	269, 237, 209, 192, 107
6	Armepavine	C ₁₉ H ₂₃ NO ₃	314	283, 299, 206, 107
7	Guatterine	C ₁₉ H ₁₉ NO ₄	326	308, 295, 265, 247
8	Guatterine N-oxide	C ₁₉ H ₁₉ NO ₅	342	324, 295, 265
9	Lysicamine	C ₁₈ H ₁₃ NO ₃	292	277, 260, 248, 220
10	Liriodenine	C ₁₇ H ₉ NO ₃	276	248, 220, 218

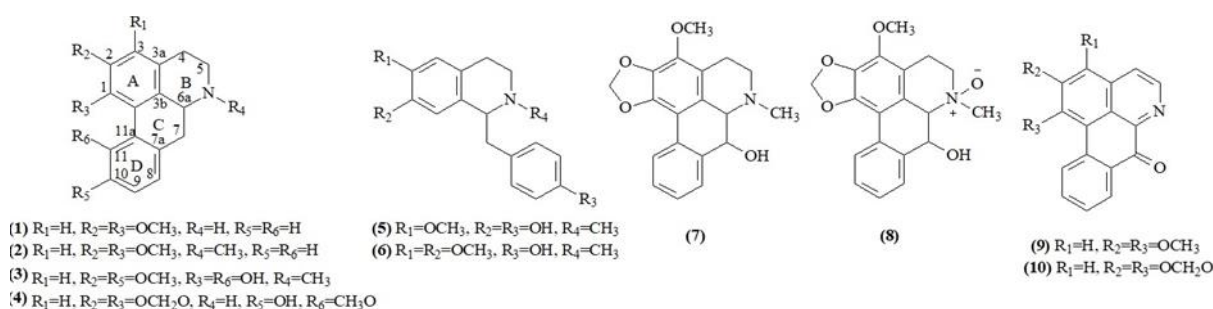


Fig. 1- Chemical structure of the compounds 1-10

found in Annonaceae. Within this range, several ions with even m/z were observed, suggesting the presence of alkaloids. The proposed fragmentations of compounds (**1-10**) are presented in supplementary material. An ion at m/z 282 was observed, where the ionic fragments obtained by MS^n were characterized through key fragmentations. Following a typical pattern for this class of alkaloids, the initial loss of the amine group ($282 \rightarrow 265$) occurred, followed by subsequent losses indicating two pathways: (a) the loss of a CH_3 group ($265 \rightarrow 250$) and (b) the loss of the methoxyl group ($265 \rightarrow 234$). The analysis of these fragmentations and comparisons with data described in the literature⁽¹⁶⁾ allowed the annotation of this compound as nornuciferine (**1**).

This class of alkaloids also possesses methyl groups attached to the nitrogen, and in this case, the initial loss will be equivalent to 31 Daltons (Da). This was observed for the ion at m/z 296 with the initial loss of the methylamine group ($296 \rightarrow 265$) and subsequent losses suggesting (a) the loss of methyl ($265 \rightarrow 250$) and (b) the loss of the methoxyl group ($265 \rightarrow 234$). The comparison of these values with those described in the literature^(16,17) led to the annotation of this compound as nuciferine (**2**).

For the ion m/z 328 ($[M+H]^+$), an initial loss of the methylamine group ($328 \rightarrow 297$) was observed, followed by a subsequent loss of methanol ($297 \rightarrow 265$). Unlike the ions at m/z 282 and m/z 296, which showed MS^3 losses related to methoxyl groups, the MS^3 loss for the ion at m/z 328 occurred through the methanol molecule. This difference occurs when different vicinal groups are attached to ring A, in this case, OH adjacent to CH_3O .⁽¹⁸⁾ The analysis of the fragmentations and comparison with data described in the literature⁽¹⁹⁻²¹⁾ suggest compatibility with the alkaloid corytuberine (**3**).

The ion m/z 312 initially lost the amine group ($312 \rightarrow 295$), followed by subsequent losses of the methylenedioxy group ($295 \rightarrow 265$) and CO ($265 \rightarrow 237$). Through the proposed fragmentation and comparison with data from the literature,⁽²⁰⁾ it was possible to suggest that this compound is nandigerine (**4**).

The ions at m/z 300 and m/z 314 showed initial losses of the methylamine group ($300 \rightarrow 269$) and ($314 \rightarrow 283$). For the ion at m/z 300, subsequent losses of methanol ($269 \rightarrow 237$) were observed, suggesting the presence of CH_3O adjacent to the OH group on ring A, and CO ($237 \rightarrow 209$). Through the analysis of the general fragmentation pattern for benzyl tetrahydroisoquinoline alkaloids (Fig. 2), we observed

the presence of two ions at m/z 107 and m/z 192, generated through McLafferty rearrangement involving the proton from nitrogen and the aromatic ring of the benzyl substituent.⁽¹⁸⁾

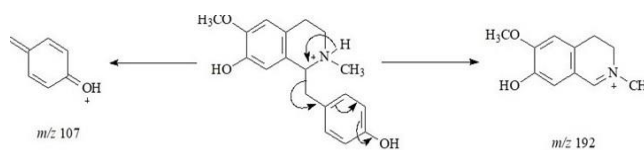


Fig. 2- General pathway for the fragmentation of benzyl-tetrahydroisoquinoline-type alkaloids

This type of rearrangement was also observed for the ion at m/z 314, generating ions at m/z 107 and m/z 206. Based on these fragmentation patterns and comparisons with data from the literature,^(17,22) we found that the ions at m/z 300 and m/z 314 are consistent with the benzyl tetrahydroisoquinoline alkaloids *N*-methylcoclaurine (**5**) and armepavine (**6**), respectively.

For the ion m/z 326, an initial loss of methylamine ($326 \rightarrow 295$) and a subsequent loss of the methylenedioxy group ($295 \rightarrow 265$) were observed. However, the base peak at m/z 308 corresponds to the loss of a water molecule, suggesting it is a 7-hydroxy-aporphine-type alkaloid. The fragmentation analysis allowed us to suggest that the compound is guatterine (**7**).

For the ion at m/z 342, the initial loss of the methylhydroxylamine group ($342 \rightarrow 295$) and a subsequent loss of the methylenedioxy group ($295 \rightarrow 265$) were observed. The ion at m/z 324 indicates the loss of a water molecule, suggesting it is another 7-hydroxy-aporphine-type alkaloid. However, upon analyzing the initial loss of the methylhydroxylamine group and the loss of the water molecule, we observed that it was an oxidation product of guatterine. Thus, when compared with the data described in the literature⁽²³⁾ for guatterine oxide, we found the results to be consistent. Therefore, we suggest that the compound at m/z 324 is *N*-oxide of guatterine (**8**).

The total ion spectrum also presented two ions at m/z 292 and m/z 276, which refer to oxoaporphine-type isoquinoline alkaloids. Unlike most classes of isoquinoline alkaloids that have the heterocyclic nitrogen atom as the main basic group and consequently, as the site of protonation, oxoaporphine alkaloids have more than one plausible site. In Electrospray Ionization in positive mode (ESI (+)) experiments, the proton can be attached to the nitrogen of the heterocyclic imine or the ketone portion. Density Functional Theory (DFT)

calculations showed that although there are two protonation sites for oxoaporphine alkaloids, protonation is slightly preferred at the imine group of the heterocyclic ring. These results indicate that structures with protonated nitrogen can distribute the charge throughout the molecule, generating a more stable cation.⁽²⁴⁾

The ion at m/z 292 underwent an initial loss of a methyl group ($292 \rightarrow 277$), and protonation occurred at the imine nitrogen of the heterocyclic ring. This fragmentation behavior indicates that peripheral groups are more susceptible to cleavage than the remote loss of a CO charge. Studies on electronic energies demonstrate that the C-1 position is more stable than the C-2 position, and this stability may result from the formation of intramolecular hydrogen bonds with the hydrogen attached to C-11, which stabilizes the resulting ion.⁽²⁴⁾ Based on the fragmentation pattern of the ion at m/z 276 and comparison with data reported in the literature,⁽⁶⁾ the compound was identified as lycicamine.⁽⁹⁾

According to da Silva,⁽²⁴⁾ the ion at m/z 276 presents a single peripheral group, in this case, a methylenedioxy bridge that undergoes an initial loss of 28 Da relative to the loss of CO ($276 \rightarrow 248$). The delocalization of π -electrons through rings A and B allowed the rupture of the methylenedioxy bridge followed by the loss of CO. However, our proposal is different, as we suggest that the initial loss of CO occurs from the ketone group ($276 \rightarrow 248$) and subsequently the rupture of the methylenedioxy bridge ($248 \rightarrow 218$). The analysis of fragmentations and comparisons with data described in the literature^(16,24) allowed us to annotated this compound as liriodenine (**10**).

The evaluation of the phytotoxic effect of the crude methanolic extract of *D. quitarensis* on the percentage of inhibition on seedling growth inhibition (root and hypocotyl) of *A. cepa* and *L. sativa* is presented in [Figures 3](#) and [4](#), respectively, and the germination inhibition percentage of *A. cepa* and *L. sativa* is presented in [Figure 5](#).

Means were compared using Tukey's test ($p < 0.05$). Different letters indicate significant differences. Positive values represent stimulation and negative values indicate inhibition. A = roots; B = hypocotyls.

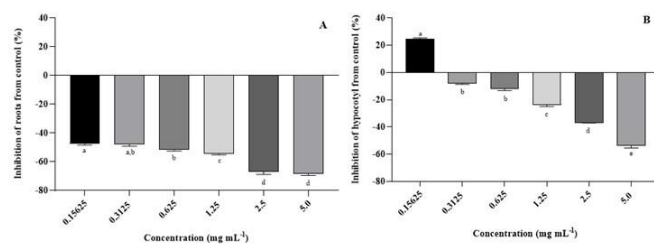


Fig. 3- Percentage of inhibition of *A. cepa* roots and hypocotyls in response to different concentrations of the crude methanolic extract of *D. quitarensis*

Means were compared by Tukey's test ($p < 0.05$); different letters indicate significant differences. Positive values indicate stimulation, and negative values indicate inhibition. C = Roots; D = Hypocotyls.

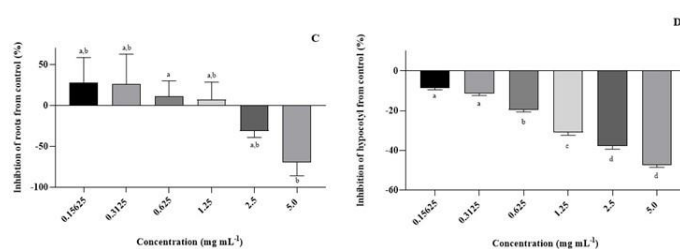


Fig. 4- Percentage of inhibition of *L. sativa* (lettuce) roots and hypocotyls in response to the crude methanolic extract of *D. quitarensis* at different concentrations

Means were compared to the control using Dunnett's test ($p < 0.05$); different letters indicate significant differences. E = *A. cepa*; F = *L. sativa*.

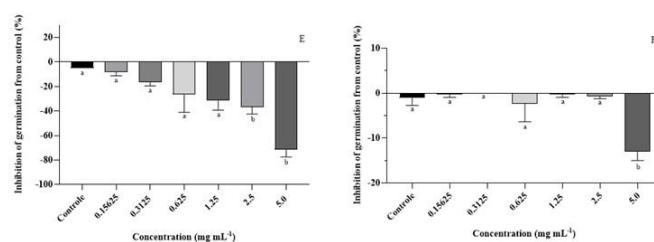


Fig. 5- Percentage of germination inhibition of *A. cepa* (onion) and *L. sativa* (lettuce) in response to the crude methanolic extract of *D. quitarensis* at different concentrations

The assays on the phytotoxicity of the crude methanolic extract of *D. quitarensis* on *A. cepa* seedlings ([Fig. 3](#)) showed inhibition in the percentage of root length growth at all tested concentrations, and for the hypocotyl, there was stimulation at the concentration of $0.15625 \text{ mg mL}^{-1}$ (24.48 %), and gradual inhibition at the other concentrations. The

results obtained for *L. sativa* (Fig. 4) showed less sensitivity to the methanolic extract of *D. quitarensis* when evaluating the percentage of root growth inhibition. Initially, stimulation was observed at concentrations of 0.15625 to 1.25 mg mL⁻¹ and inhibition at concentrations of 2.5 and 5.0 mg mL⁻¹. However, unlike what was observed for *A. cepa*, the hypocotyls of *L. sativa* were sensitive to the methanolic extract of *D. quitarensis* at all tested concentrations.

Root growth is generally suggested as a better indicator of the phytotoxicity of plant extracts, and this trend of greater sensitivity has been widely reported in the literature. This event likely occurs because the roots are in direct contact with the extracts and are therefore exposed to higher doses of chemical compounds.⁽¹¹⁾ The presence of these compounds, known as allelopathic compounds, can induce the appearance of abnormal seedlings, with radicle necrosis being one of the most common symptoms.⁽²⁵⁾

The presence of the identified alkaloids in the crude methanolic extract of *D. quitarensis* and the knowledge of their phytotoxic potential suggests that they are responsible for the observed activity, as the

CONCLUSIONS

The phytochemical and biological analyses revealed, for the first time, that the methanolic extract of *D. quitarensis* exhibits different degrees of phytotoxicity on the growth of seedlings and the germination of *A. cepa* and *L. sativa*. This is the first experimental evidence of the phytotoxic activity of this species, suggesting that the identified alkaloids are directly related to the observed effects. Therefore, this work expands the knowledge of the biological properties of *D. quitarensis* and contributes to understanding the ecological role of isoquinoline alkaloids in plant-plant interactions. In addition to providing novel data on the alkaloid composition of *D. quitarensis*, this study highlights the potential of the species as a natural source of allelopathic compounds. The integration between direct infusion dereplication and phytotoxicity assays demonstrates an efficient approach for the prospection and characterization of secondary metabolites, reinforcing the scientific and ecological importance of this Amazonian species.

mechanisms of action of alkaloids with phytotoxic potential indicate an attack on more than one plant target at the same time. It is known that the range of targets includes key enzymatic functions, photosynthesis, respiration, transcription, protein synthesis, membrane stability, signal transduction, electron transport, and replication. Thus, a variety of alkaloids bind to DNA or DNA/RNA processing enzymes, or intercalate with them, and can disrupt replication or transcription. These types of molecular processes manifest in plant bioassay results, such as the impact of colchicine, harmaline, papaverine, and salsoline on the root growth of *Lepidium sativum*, where solutions of 0.01 % of these alkaloids reduced radicle length to about 50 % or less compared to the control.⁽²⁶⁾

Regarding the percentage of *A. cepa* seed germination inhibition gradual inhibition was observed with increasing concentration. *Lactuca sativa* seeds were again less sensitive than the *A. cepa* seeds, with their peak inhibition at a concentration of 5.0 mg mL⁻¹ (13.0 %) (Fig. 5). Ferreira and Aquila⁽²⁵⁾ report that germination is less sensitive to allelochemicals than seedling growth, which may have resulted in the low inhibition percentage of *L. sativa* germination.

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INTEREST CONFLICT

The authors declare that there are no conflicts of interest relevant to the content of this article.

AUTHOR'S CONTRIBUTION

The research work described in this manuscript involved the collaboration of all authors as specified:

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