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Contribution to the study of galls of *Ocotea puberula* (Rich.) Nees (Lauraceae): Antioxidant and biological properties of the alkaloid S-(+)–dicentrine

Contribuição ao estudo das galhas de *Ocotea puberula* (Rich.) Nees (Lauraceae): Propriedades antioxidantes e biológicas do alcaloide S-(+)-dicentrina

Contribución al studio de las agallas de *Ocotea puberula* (Rich.) Nees (Lauraceae): Propiedades antioxidantes y biológicas del alcalóide S-(+)-dicentrina

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Abstract

The *Ocotea puberula* (Rich.) Nees (Lauraceae) (popular name is canela-guaiacá) is rich in secondary metabolites, among them, the alkaloids. Dicentrine is an alkaloid from *O.puberula* and have pharmacological actions such as anti-inflammatory, analgesic, anxiolytic and antimalarial. The objective of this work was to investigate the antioxidant and biological potential of the alkaloid dicentrine isolated from the galls of *Ocotea puberula*. To evaluate the antioxidant potential, the techniques of the formation of the phosphomolybdenum complex, Prussian blue and the reduction of

DPPH• were used and the biological activity was evaluated using *Artemia salina*, sheep erythrocytes, *Aedes aegypti* larvae and two target species. The phosphomolybdenum method showed an antioxidant potential of 90.68% compared to rutin, 30.88% compared to BHT and 16.62% compared to ascorbic acid. By the DPPH• method it presented IC_{50} = 943.11 μg.mL^{-1}, indicating low antioxidant potential. When evaluating dicentrine in *Artemia salina*, a low toxic potential and a non-hemolytic effect were verified in sheep erythrocytes. In the larvicidal activity in *Aedes aegypti*, dicentrin showed LC_{50} = 23.62 μg.mL^{-1}. On *Lactuca sativa*, dicentrine did not influence germination, IVG and hypocotyl growth, but root growth was stimulated with 250 μg.mL^{-1} and inhibited with 500 μg.mL^{-1}. On *Allium cepa* dicentrine with 25 and 100 μg.mL^{-1} stimulated the germination percentage and the GVI. The growth of the *Allium cepa* root was not influenced, however, the coleoptile was stimulated with 25 μg.mL^{-1} and inhibited with 50 μg.mL^{-1}. After evaluating the results presented in the chosen tests, it was observed that dicentrine has a larvicidal and allelopathic potential.
Keywords: Dicentrine; *Aedes Aegypti*; Allelopathy; Antioxidant potential.

Resumo
A espécie *Ocotea puberula* (Rich.) Nees (Lauraceae) (nome popular é canela-guaiacá) é rica em metabólitos secundários dentre eles, os alcaloides. A dicentrina é um alcaloide de *O. puberula* e possui ações famacológicas tais como, anti-inflamatória, analgésica, ansiolítica e antimalárica. O objetivo trabalho foi investigar o potencial antioxidante e biológico do alcaloide dicentrina isolado das galhas de *Ocotea puberula*. Para avaliar o potencial antioxidante foram utilizadas as técnicas da formação do complexo fosfomolibdênio, azul da Prússia e a redução do DPPH• e a atividade biológica foi avaliada utilizando *Artemia salina*, eritrócitos de carneiro, larvas de *Aedes aegypti* e duas espécies alvo. Pelo método do fosfomolibdênio foi verificado um potencial antioxidante de 90,68% em comparação a rutina, 30,88% comparado ao BHT e 16,62% comparado ao ácido ascórbico. Pelo método DPPH• apresentou CI_{50} = 943,11 μg.mL^{-1}, indicando baixo potencial antioxidante. Ao avaliar a dicentrina em *Artemia salina* foi verificado baixo potencial tóxico e efeito não hemolítico em eritrócitos de carneiro. Na atividade larvicida em *Aedes aegypti* a dicentrina apresentou CL50 = 23,62 μg.mL^{-1}. Sobre *Lactuca sativa* a dicentrina não influenciou a germinação, o IVG e crescimento do hipocótilo, porém o crescimento da radícula foi estimulado com 250 μg.mL^{-1} e inibido com 500 μg.mL^{-1}. Sobre *Allium cepa* a dicentrina com 25 e 100 μg.mL^{-1} estimulou a porcentagem de germinação e o IVG. O crescimento da radícula de *Allium cepa* não foi influenciado, porém, o coleóptilo foi estimulado com 25 μg.mL^{-1} e inibido com 50 μg.mL^{-1}. Após avaliação dos resultados apresentados nos testes escolhidos foi observado que a dicentrina possui um potencial larvicida e alelopático.
Palavras-chave: Dicentrina; *Aedes Aegypti*; Alelopatia; Potencial antioxidante.

Resumen
La especie *Ocotea puberula* (Rich.) Nees (Lauraceae) (nombre popular es canela-guaiacá) es rica en metabolitos secundarios, entre ellos, los alcaloides. Dicéntrina es un alcaloide de *O. puberula* y tienen acciones farmacológicas como antiinflamatorias, analgésicas, ansiolíticas y antipalúdicas. El objetivo de este trabajo fue investigar el potencial antioxidante y biológico del alcaloide dicéntrina aislado de las agallas de *Ocotea puberula*. Para evaluar el potencial antioxidante se utilizaron las técnicas de formación del complejo de fosfomolibdeno, azul de Prusia y la reducción de DPPH• y se evaluó la actividad biológica utilizando *Artemia salina*, eritrocitos de oveja, larvas de *Aedes aegypti* y dos especies diana. El método del fosfomolibdeno mostró un potencial antioxidante de 90,68% en comparación con la rutina, 30,88% en comparación con BHT y 16,62% en comparación con el ácido ascórbico. Por el método DPPH• presentó IC_{50} = 943,11 μg.mL^{-1}, lo que indica un bajo potencial antioxidante. Al evaluar la presencia de dicéntrina en *Artemia salina*, se verificó un bajo potencial tóxico y un efecto no hemolítico en eritrocitos de ovino. En la actividad larvicida en *Aedes aegypti*, la dicéntrina mostró CL_{50} = 23,62 μg.mL^{-1}. En *Lactuca sativa*, la dicéntrina no influyó en la germinación, IVG ni en el crecimiento del hipocótilo, pero el crecimiento de las raíces se estimuló con 250 μg.mL^{-1} e inhibió con 500 μg.mL^{-1}. Sobre *Allium cepa* dicéntrina con 25 y 100 μg.mL^{-1} estimuló el porcentaje de germinación y la IVG. El crecimiento de la raíz de *Allium cepa* no fue influenciado, sin embargo, el coleoptilo fue estimulado con 25 μg.mL^{-1} e inhibido con 50 μg.mL^{-1}. Luego de evaluar los resultados presentados en las pruebas elegidas, se observó que la dicéntrina tiene potencial larvicida y alelopático.
Palabras clave: Dicéntrina; *Aedes Aegypti*; Alelopatía; Potencial antioxidante.

1. Introduction

Popularly known as canela-guaicá, canela-amarela, canela-parda ou canela-sebo, the *Ocotea puberula* (Rich.) Ness is often found in the Southern Plateau or Southern Brazilian Plateau (Brotto et al., 2013). It presents small and black colored fruits, however, in some specimens the occurrence of phenotypically different fruits from those described in the literature is observed (Souza & Moscheta, 2000).

According to Oliveira et al., (2006) galls may be related to biochemical changes in the plant, which in response to the attack by inducing organisms differ from the fruit's structures, leading to the modification and /or increase of certain metabolites. Among such changes in the plant species are hyperplasia and hypertrophy of the mesophile, and increased production of starches, flavones, flavonoids and flavonones, giving the galler protection and nutrition (Oliveira et al., 2006).

The phytochemical composition of *Ocotea puberula* (Rich.) Ness includes several classes of flavonoid compounds, tannins, steroids, triterpenes and mainly alkaloids (Araujo, 2000). Among the alkaloids of that species is dicentrine, an alkaloid of the aporphinoid type which has antinociceptive activity (Montrucchio et al., 2012). Studies have reported the cytotoxic activity of dicentrin in human leukemic cell lines (CCRF-CEM and HL-60), murine (L1210), human hepatoma lines (HuH-7 and MS-G2) esophageal carcinoma lines (HCE-6) (Stévigny et al., 2005; Hoet et al., 2004; Woo et al., 1999; Huang et al., 1998) in colon adenocarcinoma, hepatoma, leukemia, and squamous cell carcinoma cells (Lin et al., 2015).

Thus, this research aimed to research the antioxidant capacity and biological activity of the S-(+)- dicentrine of the modified fruits of *Ocotea puberula* (Rich.) Nees (Lauraceae).

2. Methodology

2.1 S-(+)-dicentrine obtention

The present work was carried out with the alkaloid dicentrin previously isolated from the galls and identified by Montrucchio et al., (2012) and assigned for the development and study of this research Access to the botanical material was permitted and licensed by Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) and was registered under number A0EB51A as required by the Brazilian legislation.

2.2 Antioxidant capacity

2.2.1 *Formation of the phosphomolybdenum complex method*

The sample (dicentrine) and standards (ascorbic acid, rutin and butylated hydroxytoluene (BHT)) were diluted in methanol at concentration of 200 μg.mL^{-1}. The methodology was described by Prieto et al., (1999). A 0.3 mL of each sample and standards was combined with 3 mL of reagent solution (28 mmol.L^{-1} of sodium phosphate, 4 mmol.L^{-1} of ammonium molybdate and sulfuric acid 0.6 mol.L^{-1}). The tubes were incubated at 95º C for 90 min and then cooled at room temperature. The absorbance of the solution was measured in spectrophotometer (UV-1601 PC Shimadzu®) at 695 nm. The antioxidant activity (AA%) was compared to ascorbic acid, rutin and BHT and, was evaluated by the formula (1), where A_{sample} is the absorbance of the test compound, A_{blank} is the absorbance of the white and $A_{\text{positive control}}$ is the absorbance of the positive control. The tests were performed in triplicate.

$$\textbf{(1)} \qquad AAR\,(\%) = \frac{Abs\ sample - Abs\ blank}{Abs\ positive\ control - Abs\ blank}$$

2.2.2 *Antioxidant potential by DPPH• (2,2-diphenyl-1-picrylhydrazyl-hydrate)*

The antioxidant potential of dicentrine was evaluated by reducing the DPPH• radical according to a modified methodology (Mensor et al., 2001; Salgueiro et al., 2014). The methanolic solution of DPPH• was used in the concentration of

0.03 mmol.L^{-1}. A stock solution (1mg.mL^{-1}) methanolic solution of dicentrine was prepared in concentrations ranging from 2 µg.mL^{-1} to 500 µg.mL^{-1}, in order to provide the best activity range. The tests were performed in microplates with 96-well round bottom in a 'U' shape, where 71 µL of the sample and 29µL of the DPPH• solution (0.3mM) were added. After thirty minutes of incubation in the dark, readings were performed on a Multiscan FC, Thermo Scientific® spectrophotometer at 518 nm. As standards, rutin, ascorbic acid and BHT were used. All tests were carried out in triplicate.

The ability of the extracts to reduce the radical was expressed in a percentage of inhibition calculated according to the formula (2).

$$\textbf{(2) \% DPPH radical scavenging effect} = \frac{100-(Abs_{amostra}-Abs_{branco})x100}{(Abs_{controle}-Abs_{branco})}$$

From the percentages of DPPH• inhibition, the IC_{50}% was calculated by linear regression, that is, the concentration required to exert 50% of the antioxidant potential.

2.2.3 *Reducing power antioxidant (Prussian blue) method*

The methodology was described by Yen & Chen (1995) and Jayanthi & Lalitha (2011). The sample (dicentrine) and standart (ascorbic acid) were diluted in water at concentration of 200 µg.mL^{-1} and they were transferred to 25mL test tubes, together with 0.2 mol.L^{-1} potassium phosphate buffer (pH 7.0) and a solution of potassium ferricyanide at 1.0%. The mixture was incubated at 45°C for 20 min, and then 1% trichloroacetic acid was added to the test tubes. Around 2.5 mL were transferred to 5.0 mL test tubes, and 1.5 mL of distilled H_2O, 1.0 mL of ethanol PA and 0.5 mL of $FeCl_3$ to 1.0% (*w/v*). The reading was measured in spectrophotometer (UV-1601 PC Shimadzu®) at 700 nm.

2.3 Evaluation of biological activities

2.3.1 *Brine shrimp (Artemia salina) lethality assay*

The assay was performed according to the methodology described by Meyer et al., (1982) with modifications. The eggs of *Artemia salina* (200 mg/400 mL) were placed in artificial seawater (30 g marine salts (Blue Treasure®) dissolved in 1000 mL distilled water) were placed in contact with the saline solution to hatch for 48 hours, aerated for one hour and exposed to constant lighting (20 W) and controlled temperature (27-30) ºC. As a positive control, quinidine sulfate (5, 50 and 500 µg.mL^{-1}) was used and as a negative control, methanol and saline solution. The test was carried out in triplicate.

Three concentrations of dicentrine (5, 50 and 500 µg.mL^{-1}) were used from a 5 mg. mL^{-1} solution in methanol, which were placed in flasks to evaporate for 12 hours at 37ºC. After 48 hours, ten *Artemia salina* nauplii were transferred to the flasks with dicentrine and controls. The volume was adjusted to 5 mL with saline and the flasks were incubated at (27-30) ºC for a period of 24 hours. Subsequently, the count of the alive and dead nauplii was performed, being considered alive those who presented any type of movement, when observed close to the light source.

2.3.2 Preliminary toxicicty against Aedes aegypti

The methodology applied was adapted from the World Health Organization (WHO) (1981a), WHO (1981b), WHO (2005) and Betim et al, 2019. The eggs of *Aedes aegypti* (Rockfeller strain provided by the Oswaldo Cruz Foundation – IOC/Fiocruz/Ibex/Entomologia/Laficav - Rio de Janeiro-RJ). The eggs were placed in mineral water and reared under laboratory conditions (27±3 °C, relative humidity of 80%, and incubated in a Bio-Oxygen Demand incubator) by feeding with fish feed (Aldon basic, MEP 200 complex) from hatching until the third stage of larval development.

Three concentrations (1000, 100 and 10μg.mL^{-1}) were tested in solution with 0.2% aqueous dimethyl sulfoxide (DMSO). In plastic cups, 5 mL of the solutions and 15 larvae were placed in triplicate. After 24 hours at room temperature, mortality was verified, considering larvae unable to reach the water surface when touched as dead. As a negative control, the aqueous solution of DMSO 0.2% was used.

2.3.3 In vitro hemolytic potential

The technique proposed by Banerjeeet al. (2008) adapted by Henneberg (2013). The sheep blood was washed with cold phosphate buffered saline (PBS) and centrifuged at 3000 rpm until a clear supernatant was obtained. With the resulting red blood meal, a 2% (m/v) solution in PBS was prepared, which was stored at a temperature of 4 °C. Dicentrine was diluted to 1000 μg.mL^{-1} in 10% methanol in PBS and then the concentrations of 100, 250, 500, 750 and 1000 μg.mL^{-1} were prepared. As positive controls, Triton 1% in PBS and potable water were used. As a negative control, PBS and 10% methanol in PBS were used. As white, concentrations and controls with PBS were used only.

The solutions (200 μL) were incubated with the erythrocyte solution (200 μL) and with PBS (200 μL) in 5 mL plastic tubes (Table 1) and at 37 °C for 3 hours. The tubes were kept open. Subsequently, the samples were centrifuged and 150 mL of the supernatant was transferred to a 96-well microplate for reading on a 540 nm UV spectrophotometer. The test was performed in triplicate.

Table 1. Quantities of solutions used in the verification of hemolysis.

Solutions
200 μL (dicentrine dilutions) + 200 μL sheep erythrocytes 2%
200 μL triton 1 % in PBS + 200 μL sheep erythrocytes 2%
200 μL potable water + 200 μL sheep erythrocytes 2%
200 μL PBS + 200 μL sheep erythrocytes 2 %
200 μL methanol 10% in PBS + 200 μL sheep erythrocytes 2 %
200 μL blank (sample and controls) + 200 μL PBS

Source: Authors.

The percentage of hemolysis was calculated using the formula **(3)**

$$(3)\ \%Hemólise = \frac{100-(Abs_{amostra}-Abs_{controlenegativo1}-Abs_{brancoamostra})}{(Abs_{controle\ positivo}-Abs_{branco\ controle\ positivo})} x100$$

2.3.4 Allelopathic test

The allelopathic activity of dicentrin was tested at concentrations of 250, 100, 50 and 25 μg.mL^{-1} on *Lactuca sativa* and *Allium cepa* according to Macias et al., (2000) and Dias et al., (2005) with modifications.

The dicentrin samples were prepared in chloroform and were placed (2 mL) on filter papers to evaporate 24 hours before the start of the test at a controlled temperature of 36 °C. Subsequently, the papers were transferred to plastic plates with a diameter of 6 cm.

In laminar flow, the papers were soaked with 2 mL of distilled water and twenty seeds of *Lactuca sativa* or *Allium cepa* were distributed. The plates were taken to a B.O.D incubator at 20 °C for 7 days for *Lactuca sativa* and 12 days for *Allium cepa*. Two triplicates were prepared for each concentration, evaluating germination and growth separately. Chloroform and water were used as controls, under the same conditions.

To assess germination, a daily reading was carried out, always at the same time, and the germinated seeds were removed from the plates. The germination percentage **(5)** and the germination speed index **(6)** were calculated.

$$(5)\ \%\ Germination = \frac{germination\ sample}{germination\ negative\ control} x100$$

$$(6) VGI = (\frac{n1}{1} + \frac{n2}{2} + \frac{nx}{x} + \cdots + \frac{nF}{F}) x100$$

Where: VGI = germination speed index; n1 = number of seeds germinated on day 1; n2 = number of seeds germinated on day 2; nx = number of seeds germinated on day x; nF = number of seeds germinated on the final day.

To assess growth, the plates were opened on the seventh and twelfth day (for *Lactuca sativa* and *Allium cepa* respectively). They were then measured with the aid of a ruler and tweezers, the radicle and hypocotyl, for *Lactuca sativa*, and radicle and coleoptile for *Allium cepa*.

2.4 Statistical analysis

For the antioxidant capacity tests, the statistical analysis was performed by the ANOVA test followed by the Tukey test, considering a significance level of 95% with the help of the Sisvar® software (Ferreira, 2014).

For the tests that evaluated the dicentrine activity on *Artemia salina* and *Aedes aegypti* results were submitted to the Probitos method, using the IBM SPSS® software Statistics version 22.0 (IBM Corporation, 2013, Armonk, NY, USA), which provided the values of CL_{50} and CL_{90} (lethal concentration for 50% and 90% of individuals) with 95% confidence intervals.

For the assessment of allelopathic activity, the results were analyzed statistically by the ANOVA test followed by the Scott-Knott test, with the aid of the Sisvar® program (Ferreira, 2014). The graphics were designed with the aid of the NUMBERS application for MAC version 6.2.1.

3. Results and Discussion

The result of it's an antioxidant capacity of dicentrine compared to DPPH•, phosphomolybdenum and Prussian blue methods are listed in Table 2.

Table 2. Antioxidant capacity from dicentrine.

Sample and controls	Phosphomolibdene method Average ± SD			DPPH method Average ± SD	Reducing Power (Prussian blue)
	RAA Ascorbic acid (%)	RAA BHT (%)	RAA Rutin (%)	IC_{50} (µg/mL)	AA (%)
Ascorbic acid	100,00 ± 0 [b]	-	-	5,94 ± 0,01 [a]	100,00 ± 0 [b]
BHT	-	100,00 ± 0 [b]	-	13,33 ±0,43 [a]	-
Rutin	-	-	100 ± 0 [a]	8,19± 0,19 [a]	-
Dicentrine	16,62 ± 4,04 [a]	30,88 ± 8,65 [a]	90,68 ± 24,67 [a]	943,11 ± 121,05 [b]	23,80 ± 3,84 [a]

Different letters in the same column indicate the statistical difference ($p < 0.05$) between dicentrine and controls. Statistical analysis using ANOVA followed by Tukey Test. RAA = relative antioxidant activity. SD= standard desviaton. BHT = butylated hydroxytoluene. IC = inibihition concentration 50%. Source: Authors.

In the phosphomolybdenum test, dicentrin had 90.68% of antioxidant activity related to rutin (Table 1), showing the antioxidant potential of dicentrin. However, in the trial using DPPH• dicentrine showed IC_{50} = 943.11 µg.mL^{-1} (Table 2), that is, low antioxidant potential compared to rutin.

Castilhos et al., (2007), from the ethanolic extract of *Rhodophiala bifida* (Amaryllidaceae family) extracted the alkaloid montanine and evaluated the antioxidant potential by the DPPH• method verifying AA activity = 36% in relation to

ascorbic acid. The moscamine alkaloid, isolated from *Croton echioides* (family Euphorbiaceae) demonstrates IC_{50} = 14.5% μg.mL^{-1} when evaluated by the DPPH• method (Novello et al., 2016). This technique was also used to evaluate alkaloids isolated from the species *Alseodaphne corneri* (family Lauraceae) (Zahari et al., 2016). The alkaloids (-)-gyrolidine (IC_{50} = 280.95), (+)-O-methyllimacusine (IC_{50} = 265.09 μg.mL^{-1}), (+)-2-norobaberine (IC_{50} = 254.95 μg.mL^{-1}), (+)-laurotetanine (IC_{50} = 131.72 μg.mL^{-1}) were tested (Zahari et al., 2016).

Souza (2008) tested the antioxidant potential by testing the reduction of the phosphomolybdenum complex of the alkaloids ulein and iombine isolated from *Himatanthus lancifolius* (Apocynaceae family) where the antioxidant potential was considered low in relation to ascorbic acid, with the ulein with 0.59% and the yombina 0.53% of antioxidant potential. From the species *Hammada scoparia* (family Amaranthaceae), the alkaloids carnegine and N-methylisosalsoline were tested by reducing the phosphomolibdenum complex and did not show measurable antioxidant potential in the test (Bouaziz et al., 2016).

The antioxidant properties of secondary metabolites can be attributed to the hydroxyl group that could donate electrons to free radicals, so the hydroxyl group that is present in alkaloids could be the reason why they have free radical scavenging activities (DPPH•) and that react stoichiometric form only with molecules that are good hydrogen donors (Pradines et al., 2002; Kedare; Singh, 2011). Among these molecules, those that have active hydroxyl groups, with ease to donate hydrogen, such as phenolic compounds and vitamins C and E. (Lu et al., 2010) stand out. Thus, it is expected that more polar molecules, because they have a greater number of compounds with available hydroxyls, will present the best results (Mensor et al., 2001). When analyzing the molecular structure of the dicentrine compound, it is observed that it does not have hydroxyls and this may justify the low antioxidant activity by the DPPH• method.

Dicentrine has been described with pharmacological (antinociceptive) activity (Montrucchio et al., 2012) and the literature suggests a correlation between anti-toxicity and anti-inflammatory properties, that is, substances with antioxidant potential reduce inflammation by decreasing free radicals that participate in the recruitment of cells to inflamed tissues (Thambi et al., 2009).

The results of *Artemia salina* and *Aedes aegypti* are listed in Table 3. In *Artemia salina*, dicentrin showed LC_{50} = 926.23 μg.mL^{-1} (Table 3) which can be considered according to Amarante et al. (2011) that considers low toxicity for LC_{50}> 500 μg.mL^{-1}, moderate for LC_{50} between 100 to 500 μg.mL^{-1} and very toxic for LC50 <100 μg.mL^{-1}.

Table 3. Concentration–mortality results of A*rtemia salina* and *Aedes aegypti* exposed to dicentrine.

Sample and control	Concentration (μg.mL^{-1})	Mortality (%)±SD	LC_{50} (μg.mL^{-1})	LC_{90} (μg.mL^{-1})
Artemia salina biossay				
Quinidine sulfate	5	0.00 ± 0.00	108.69	205.60
	50	6.67 ± 5.77		
	500	100.00 ± 0.00		
Dicentrine	5	0.00 ± 0.00	926.23	> 1000
	50	18.46 ± 10.11		
	500	38.33 ± 37.53		
Larvicide biossay with *Aedes aegypti*				
Dicentrine	10	26.67 ± 6.67	23.64	275.94
	100	88.89 ± 10.18		
	1000	93.33 ± 6.67		

CL_{50}= lethal concentration 50%. CL_{90}= lethal concentration 90%. SD= standard desviaton. Source: Authors.

In the species *Glechoma hederacea* (family Lamiaceae), Kumarasamy et al. (2003) isolated the alkaloids hederacin A and hederacin B and that were tested in *Artemia salina* and the alkaloids showed toxicity with LC_{50} of 3.2 and 14.0 μg.mL^{-1}, respectively. Another alkaloid that was tested for toxicity in *Artemia salina* was aspidospermin, isolated from the species *Geissospermum vellosii* (Apocynaceae) and showed activity with an LC_{50} of 232.63 μg.mL^{-1} (Dias, 2012).

Wangensteen et al. (2016) isolated the alkaloids dihydronitidine, isoarnotianamide from the bark of the stem of *Zanthoxylum heitzii* (family Rutaceae) and tested them for toxic potential in *Artemia salina*, with the two compounds presenting LC_{50} above 100 μg.mL^{-1}. It is noted that according to the classification by Meyer et al. (1982) the aforementioned alkaloids would be considered toxic and with the classification by Amarante et al. (2011) both would have moderate toxicity.

In the test on *Aedes aegypti* larvae, dicentrine showed LC_{50} = 23,62 μg.mL^{-1} (Table 2). With the presented result, the larvicidal potential is evidenced because according to Cheng et al., (2008), substances with LC_{50} values lower than 100 μg.mL^{-1} are considered good larvicidal agents. Still, the result on *Aedes aegypti* corroborates the result by Garcez et al. (2009), who used (+) - dicentrine isolated from the bark of the stem of *Ocotea velloziana* and this showed a larvicidal effect with LC_{50}= 30.2 μg.mL^{-1}. Chalom et al. (2019), isolated alkaloids from the aerial parts of *Stemona aphylla* (family Stemonaceae) and used them in larvicidal activity in *Aedes aegypti*. The alkaloid (2'S)-hydroxystemofoline showed LC_{50} and LC_{90} values of 3.91 and 7.14 μg.mL^{-1} and the stemopholine alkaloid showed LC_{50} and LC_{90} values of 4.35 and 7.60 μg.mL^{-1} (Chalom et al., 2019).

The control strategies of the main vector of dengue, *Aedes aegypti* are based on the use of chemical and biological products, integrated with environmental management programs (Luna et al., 2004). In Brazil, programs that aim to control *A. aegypti* mainly use chemical insecticides, with on organophosphates (temephos) and pyrethroids (cypermethrin) that require constant monitoring, as there is a strong correlation with resistance of these insecticides and the occurrence of mutations in the mosquito population (Luna et al., 2004).

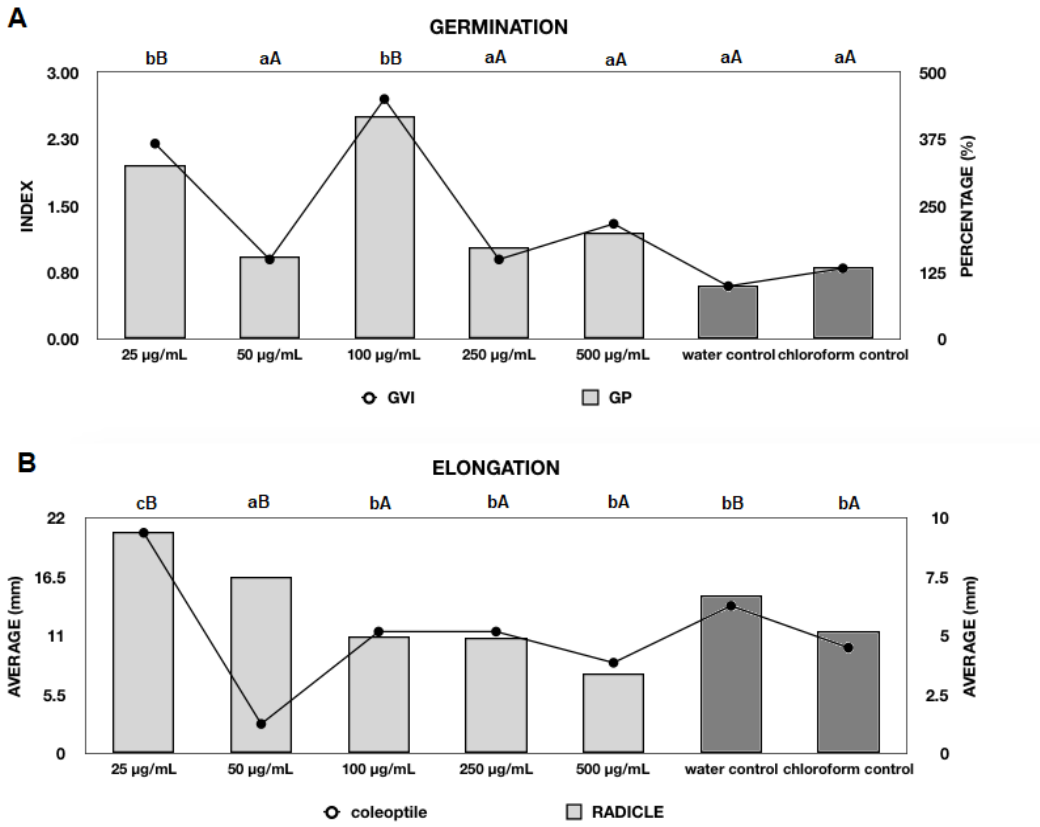
The advantage of natural insecticides is that they have less toxic bioactive compounds with a shorter half-life compared to synthetic compounds, resulting in less harmful residues in the environment and in the life cycles of humans and animals (Who 2012; Pavela 2016; Muangmoon et al., 2018, Betim et al., 2020).

The behavior of larvae treated with alkaloids, that is, the effects of their exposure generate, as a consequence, seizures, paralysis and death of the larvae (need not follow that order), and indicate that plant alkaloids are likely to have a toxic effect on the neuromuscular system of the larvae (Chaithong et al., 2006).

In the assay to verify the hemolytic potential *in vitro*, dicentrine was not able to cause hemolysis. In vitro hemolytic activity can be considered a good test of screening for toxicity of plant extracts and fractions since through evaluation of the mechanical stability of the mutton erythrocyte membrane we can characterize the damage that the compound causes (hemolysis) and correlate the toxicity of extracts or fractions with activity potential therapy (Murador & Deffune, 2007; Schulz et al., 2005).

On *Lactuca sativa* (Figure 1), dicentrine was not able to influence the germination and growth of the hypocotyl. However, when evaluating root growth, there was a stimulus with 250 μg.mL^{-1} and inhibition with 500 μg.mL^{-1}. Figure 2 shows that dicentrine at 25 μg.mL^{-1} and 100 μg.mL^{-1} stimulated the VGI and the germination percentage of *Allium cepa*. Root growth was not influenced by dicentrin, but coleoptile growth was stimulated by 25 μg.mL^{-1} and inhibited by 50 μg.mL^{-1}

Figure 1. Dicentrine allelopathic activity with *Lactuca sativa.*



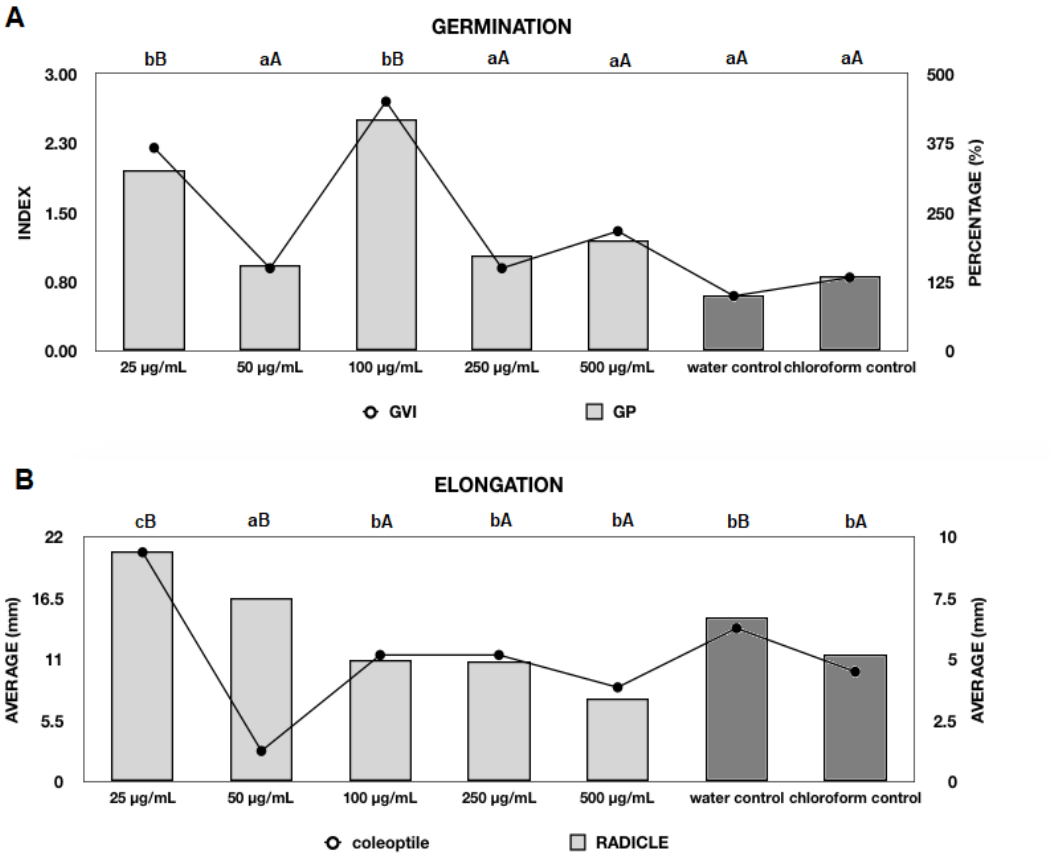
Statistical analysis using ANOVA followed by Scott-Knott Test. GVI: Germination Speed Index. GP: Germination Percentage.A = Different lowercase letter indicate the statistical difference ($p < 0.05$) between concentrations and controls for Germination Speed Index Different uppercase letters indicate the statistical difference ($p < 0.05$) between concentrations and Germination Percentage.B = Different lowercase letter indicate the statistical difference ($p < 0.05$) between concentrations and controls for coleoptile Different uppercase letters indicate the statistical difference ($p < 0.05$) between concentrations and controls for radicle. Source: Authors.

The results presented show the hormone effect both on the growth of *Lactuca sativa* and *Allium cepa*.

Generally the effects of allelochemicals tend to be more pronounced at higher concentrations, however, the allelopathic influence can escape this pattern since the observed effects result from the sum of a series of molecular changes, which justify that the results for allelopathy, obtained in the laboratory, they may not be repeated under natural conditions, due to the simultaneous occurrence of several biotic and abiotic factors that may mask this phenomenon (Maraschin-Silva & Aquila, 2006).

The allelopathic activity of the genus *Ocotea* has already been investigated. According to Carmo et al. (2007), in the species *Ocotea odorífera*, an allelopathic effect was observed, inhibiting the development of both the aerial part and the root system of the plants submitted to their extracts.

Figure 2. Dicentrine allelopathic activity with *Allium cepa.*



Statistical analysis using ANOVA followed by Scott-Knott Test. GVI: Germination Speed Index. GP: Germination Percentage.A = Different lowercase letter indicate the statistical difference ($p < 0.05$) between concentrations and controls for Germination Speed Index Different uppercase letters indicate the statistical difference ($p < 0.05$) between concentrations and Germination Percentage.B = Different lowercase letter indicate the statistical difference ($p < 0.05$) between concentrations and controls for coleoptile Different uppercase letters indicate the statistical difference ($p < 0.05$) between concentrations and controls for radicle. Source: Authors.

It is reported that genotoxic compounds have physical and chemical characteristics and properties capable of interacting with nucleic acids, which can lead to defects related to heredity through the mutations observed in germ cells. There are studies that corroborate the use of the *Allium cepa* test system as an important tool and biomarker in the monitoring of genotoxicity of extracts and infusions of medicinal plants and the results have indicated as main effects the increase and decrease of cell proliferation, establishing that many plants can present mutagenic and antimutagenic potential (Knoll et al. 2006). The analysis of the genotoxic action is evaluated by the reduction of the growth of the roots (Vicentini et al. 2001). Thus, it is noted that dicentrine, when increased in concentration, prevents the root growth of *Allium cepa*, and may indicate a potential genotoxic agent depending on the concentration used. To confirm the genotoxic potential, other tests should be used.

According to Imatomi et al. (2015), changes in the germination process may result from physiological processes in the seed, which are affected by phytotoxins, responsible for the suppression of enzymatic activities, or phytohormones, associated with the hydrolysis of the embryo's reserve materials at the beginning of development. According to Piña-Rodriguez et al. (2004), the VGI is used to validate seed vigor as its weakening leads to a progressive loss of productive capacity, with a reduction in germination uniformity.

4. Conclusion

Dicentrine showed antioxidant capacity in the phosphomolybdenum complex reduction assay, however using the DPPH method• it did not show potential. In the Artemia salina test, dicentrin had low toxic activity and did not have hemolytic potential. However, it showed a larvicidal potential on Aedes aegypti that can be considered as a natural larvicidal potential. On Lactuca sativa, dicentrine did not influence germination and the growth of the hypocotyl, but it did influence root growth. On Allium cepa, adicentrine influenced the germination and growth of the coleoptile. This study is a multidisciplinary and was indicated that the dicentrine metabolite has good potential for use, one of which is a larvicidal activity. Future studies with formulations containing dicentrine can be tested for new potentials of the molecule.

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