Original Article

ALLELOPATHY AND INFLUENCE OF LEAVES OF *Palicourea rigida* (Rubiaceae) ON SEED GERMINATION AND SEEDLING FORMATION IN LETTUCE

ALELOPATIA E A INFLUÊNCIA DE FOLHAS DE Palicourea rigida (Rubiaceae) NA GERMINAÇÃO DE SEMENTES E NA FORMAÇÃO DE PLÂNTULAS DE ALFACE

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ABSTRACT: *Palicourea rigida* is a shrub found in South America. So far, no studies have been forthcoming with regard to the species' allelopathic potential. Therefore, the current work seeks to verify the phytochemistry and potential allelopathic effect of different leaf extract concentrations of *P. rigida* on the germination of lettuce seeds and seedling growth. *P. rigida* leaf samples were collected from the Campo Grande region in Mato Grosso do Sul state, Brazil. From these samples, extracts were prepared in 20% ethanol and aqueous and submitted to phytochemical analysis. Bioassays were carried out in laboratory with aqueous and ethanolic extracts in concentrations of 0% (control), 1, 2.5, 5, 10 and 20%, with completely randomized experimental design, analyzing the percentage and germination time, besides seedling growth. Chemical prospecting identified the presence of phenolic compounds, tannins, flavonoids, cathechins, coumarins, steroids, triterpenes, alkaloids and saponins, with the foaming rate (*Afrosymmetric*) of 1250, considered high. The rate of germination from lettuce seeds was little affected by the different concentrations of extracts. However, the speed of germination was adversely affected in almost all concentrations. The development of primary roots and aerial part was notably impeded in 5, 10 and 20% (aqueous extracts) and 10 and 20% (ethanolic extracts).

KEY WORDS: Allelochemical. Allelopathic potential. 'Dourandinha'. Foaming rate.

INTRODUCTION

Studies about the germination and growth of different plant species are common, but less studied are the relationships involving allelopathy, which consists of important ecological processes that influence the composition, structure and dynamism in primary and secondary plants in native and cultivated vegetation (SCRIVANTI et al., 2003; WILLIS, 2010).

Allelopathy is the capacity in plants to produce chemical substances that, when transferred to the environment, are able to influence the development of adjacent vegetation; these substances can be released from various plant parts, mainly from the stem, leaves and roots. They can interfere directly or indirectly in the metabolism of other organisms (RICE, 1984; FUJII; HIRADATE, 2007), causing, among other actions, modifications in the function of the membrane used for the absorption of nutrients and water, as well as modifications within the activity of photosynthesis and respiration, as a result of the presence of chemical substances, such as phenols, terpenes, alkaloids, polyacetylenes, etc (RICE, 1984; WILLIS, 2010).

Allelochemicals found in various plant species can be used to create synthetic agro-chemicals that make agricultural production more sustainable and less aggressive ecologically. Many allelopathic substances offer great potential in terms of the bio-control of weeds and can even directly interfere with one or more specific species, thereby conditioning agricultural production. Indeed, their effects can inhibit or reduce germination, diminishing in strength or even killing the seedlings, as well as causing the yellowing of leaves and the atrophy of roots (ZENG et al., 2010; WILLIS, 2010).

Zeng et al. (2010) suggested that allelopathic substances could be used directly in the formulation of bio-herbicides, thus making allelopathy a rational and reversible method of controlling invasive plants, as opposed to using chemical herbicides. Grisi et al. (2012), working with *Sapindus saponaria* L. indicates this new tendency, with less aggressive products for weed control.

Given the vast Brazilian territory, with its enormous floral diversity, studies about the effectiveness of allelopathy in native species are important, with particular emphasis being placed on their application in a regional context. Amongst

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native species which have received little attention in terms of their allelopathic potential is *Palicourea rigida* Kunth, a species commonly known as 'douradão', 'douradinha' or 'gritadeira', which belongs to the Rubiaceae family and occurs throughout South America, in tropical regions (STEYERMARK, 1974).

The 'douradão' is used as a local source of medicine in regions of Brazilian Cerrado (savannah), used for treatment of inflammation (BOLZANI et al., 1992). Its chemical analysis indicates the presence of triterpenes (BOLZANI et al., 1992), iridoids, alkaloids (LOPES et al., 2004) and flavonoids (ROSA et al., 2010).

Due to the fact that studies have shown the use of *P. rigida* leaves in traditional medicine and presence of secondary metabolites in the genus, the present study evaluated the presence of secondary metabolites in leaves and verified its allelopathic potential.

MATERIAL AND METHODS

Collection plant material

Palicourea rigida leaves were taken from different specimens from areas in the municipality of Campo Grande, Mato Grosso do Sul, Brazil, in July 2011. The site is a savannah area, regionally called 'Cerrado". The Geographical coordinates are: 20°26'25.9 S and 054°32'28.2 W, altitude, 676 meters. The collected materials were placed in polythene sacks and transported in damp chambers. One of the branches was herborized (classified) and the excicate deposited to the herbarium (herbar) at the 'Laboratory of Plant Morphology', Campo Grande, Mato Grosso do Sul, Brazil, where it was registered (RG: 4400) and incorporated into the existing collection.

Preparation of extracts

Extraction from leaves of *P. rigida* was carried out at the 'Research Laboratory [Bio-diversity and Environmental Systems]' at the Anhanguera-Uniderp University. The plant material was used to prepare a water and ethanol-based solution (40 g of plant material to 400 mL of commercial ethanol (92^{o}) or water – extract 20%) by turbulizer (BRANDT et al., 2009).

Extracts were submitted to ultrasound bath for 60 minutes and allowed to stand in the dark for 24 hours in a cold room at 16 °C, filtered through a funnel lined with filter paper and cotton. The extracts were collected in a Becker, and the crude extracts (20%) were diluted with ethanol or water to obtain the following concentrations (v/v): 10, 5, 2.5 and 1%, with adapted methodology of Oliveira et al. (2011).

Phytochemical screening was carried out by humidification, using the aqueous (20%) and ethanolic (20%) extracts, according to colorimetric testing and/or the chemical precipitation methods (MATOS, 2009), with qualitative character of the following chemical groups, investigated: reducing sugars (Benedict reaction), phenolic compounds (precipitation reaction with ferric chloride), naphthoquinone (acid/base reaction), characterization of flavonoids (cyanidin reaction and sulfuric acid), tannins (reaction with iron salts, protein precipitation), coumarins (observation under ultraviolet light), triterpenes and steroids (Liebermann-Burchard reaction), identification of cardiotonic glycosides (Baljet test and test Kedde) and characterization of saponins (Lieberman-Buchard reaction and the rate of foam), which when positive were scored, according to their concentration, as strongly (+++), moderately (++), weakly (+) and more or less positive (+/-).

The results were compared and contrasted, observing any alteration in colour or precipitation (COSTA, 2001). Confirmation of the class of secondary metabolites was carried out by chromatographic analysis by thin layer chromatography and revealing specifically: vanillin/ethanol-H_2SO_4 1.0% for terpenes, Dragendorff reagent for alkaloids, Ferric chloride 1.0% for phenolic compounds and a solution of boric acid, oxalic acid and methanol (1:1:95) with subsequent heating (1 minute) for viewing the flavonoids under an ultraviolet lamp (UV: 366 nm and 254 nm) (WAGNER; BLADT, 2009). The foaming rate (Afrosymmetric rate) test was used to estimate the amount of saponins present in the aqueous extract (ANDRADE FILHO et al., 2010).

Determination of total phenolic compounds (FC): the aqueous and ethanolic extracts at different concentrations (1, 2.5, 5, 10 and 20%) were used to determine the total phenols (FT), by interpolation from the absorbance of the samples against a constructed calibration curve ($y = 0.002x + 0.2342$, $R^2 = 0.9980$), Gallic acid standards (1, 2.5, 5, 10 and 20%) and expressed as $\mu g/g^{-1}$ of EAG (Gallic acid equivalent to % of extract) (SOUSA et al., 2007).

For the determination of total flavonoids (F) (aqueous and ethanolic extracts, 1, 2.5, 5, 10 and 20%), methodology adapted from Peixoto Sobrinho et al. (2008) was used. A standard methanolic solution of quercetin (Merck) at concentrations of 1, 2.5, 5, 10 and 20% was used to construct a calibration curve ($y = 0.0037x + 0.0859$, $R^2 = 0.9998$).

The aqueous and ethanolic extracts at different concentrations were also submitted to analysis of pH (pH DM-20, Digimed) and electrical conductivity (EC DM3, Digimed); and the concentration of soluble solids was determined using a refractometer hand, with results expressed in degrees Brix corrected to 20 °C (Model RTD-45, Refractometer).

Germination bioassays

Bio-testing was performed by using lettuce seeds (*Lactuca sativa* L.) of the Maravilha das Quatro Estações cultivar, with the seeds placed in Petri dishes (7 cm diameter), each containing 25 seeds (four replicates for each solution, 100 seeds tested) for each extract concentration studied.

In aqueous extracts, 5 mL of extract at the various concentrations (20, 10, 5, 2.5 and 1%) was placed in transparent boxes with two 'germitest' papers and compared with the control group (0%), where only distilled water was used. In the ethanolic extracts, 5 mL of extract at the various concentrations (20, 10, 5, 2.5 and 1%) was placed in transparent boxes with two 'germitest' papers and left to stand until total evaporation of ethanol; after that, the substrate was moistened again with 5 mL of distilled water and compared with the control group (0%). In the control group, the dishes with 5 mL of ethanol were left to stand until total evaporation of ethanol; after that, the substrate was moistened with 5 mL of distilled water.

The seeds were placed in germination chambers at 20 °C ± 2 °C, for a photo-period of 12 hours of light. Germinated seeds were counted every 24 h for seven days, with germinated seeds being considered those with 2 mm of root protrusion. The parameters analysed were: percentage of germination, germination speed index (GSI) (MAGUIRE, 1962) and average time of germination (ATG) (BORGHETTI; FERREIRA, 2004).

Growth bioassays

Lettuce seeds were germinated in boxes lined with two transparent sheets of 'germitest' paper moistened with 10 mL of distilled water and, after germination, 10 germinated seeds (with root size from 2 to 4 mm) were transplanted to the transparent boxes (11 x 11 x 3 cm) lined with two sheets of 'germitest' paper, with four replicates in each treatment.

In the aqueous extracts, 10 mL of extract at different concentrations (20, 10, 5, 2.5 and 1%) was placed on (two) 'germitest' papers and compared with the control group (0%), distilled water. In the ethanolic extracts, 10 mL of extract (concentrations of 20, 10, 5, 2.5 and 1%) was placed on (two) 'germitest' papers and the gerbox were left to stand until total evaporation of ethanol; after that, the substrate was moistened with 10 mL of distilled water and compared with the control group (0%). In the control group, the gerbox with 10 mL of ethanol was left to stand until total evaporation of ethanol; after that, the substrate was moistened with 10 mL of distilled water.

The boxes were maintained in germination chambers (20 ^{o}C) and the evaluation was done on the 10th day after sowing. The stems and primary root were measured, in millimeters (average), with a precision pachymeter.

Statistical analyses

The independent experiments (two) were installed in an entirely randomized design composed of six concentrations and two solvents (water and ethanol) with four repetitions (each of 25 seeds) for each treatments, for germination, or six concentrations and two solvents (water and ethanol) with four repetitions (each of 10 seedlings) for each treatments, for growing.

The results were submitted to ANOVA and means compared by Tukey test at 5% probability; these analyses were carried out using the BioEstat 5.0 statistical software.

RESULTS AND DISCUSSION

The results of analyses performed to understand the phytochemistry, using aqueous (20%) and ethanolic (20%) extract of *P. rigida* leaves, are shown in Table 1. In this work, as well as phenolic compounds, flavonoids, coumarins, triterpenes and steroids saponins and tannins were also found; the presence of these metabolites was confirmed by TLC analysis using specific developers. It can also be observed that the phenolic compounds showed a strong positive reaction in aqueous and ethanolic extracts; tannins, a strong positive reaction to aqueous extract and moderate to ethanol extract; flavonoids and alkaloids, a strong positive reaction to ethanol extract and saponins in the aqueous extract.

The presence of anthocyanins, anthraquinones, cyanogenic glycosides, cardiotonic glycosides and reducing sugars were not detected, for any of the extracts. In studies with species of the *Palicourea* genus steroids and triterpenes (BOLZANI et al., 1992), cumarins (EL-SEEDI, 1999), iridoids, alkaloids (LOPES et al., 2004) have been described, and, especially in *P. rigida* leaves, flavonoids (ROSA et al., 2010).

Table 1. The chemical analysis of *Palicourea rigida*, in reference to secondary metabolites of aqueous (20%) and ethanolic (20%) extract

Class of compounds	Aqueous (20%)	Ethanolic (20%)
Phenolic compounds	+++	+++
Tannins	+++	++
Flavonoids	+	+++
Cathechins	-	+
Coumarins	+	+/-
Steroids and triterpenes	-	+
Saponins	++	-
Alkaloids	+	+++

Presence (+) and absence (-); Strongly positive (+++); moderately positive (++); weakly positive (+) and more or less positive (+/-).

In the determination of phenolic compounds, the values of the aqueous (FC = 398.8 - 428.8 μg g^{-1}) and ethanolic extract (FC = 405.6 - 437.6 μg g^{-1}) are similar, while the average content of total flavonoids in the aqueous extract (F = 247.4 - 272.4 μg g^{-1}) was smaller than the values in the ethanolic extract (F = 260.6 - 427.7 μg g^{-1}) (Table 2).

Table 2. Content of phenolic compounds and total flavonoids in aqueous and ethanol extracts of the leaves of *P. rigida* at concentrations of 1, 2.5, 5, 10 and 20%

Concentration (%)	Phenolic compounds (μg g^{-1})		Total flavonoids (μg g^{-1})	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
1	398.8	405.6	247.4	260.6
2.5	401.4	410.8	249.0	278.4
5	408.2	416.0	257.8	307.9
10	418.6	426.8	264.0	380.9
20	428.8	437.6	272.4	427.7

The content of phenolic compounds in the ethanolic extract (20%) of this species are below the value recorded by Rosa et al. (2010), working with the crude ethanol extract of the leaves (FC = 523.20 μg g^{-1}). For flavonoids, no work recording the content of this class of secondary metabolite in the genus *Palicourea* was found.

The phenolic compounds belong to a group with the greatest allelopathic effects, acting by decreasing the length and elasticity of the cell wall as well as by blocking mitochondrial respiration (WEIR et al., 2004).

The foaming rate (Afrosymmetric Rate) for the aqueous extract was 1250, which confirms the presence of saponins in the aqueous extract; Schenkel et al. (2007) and Andrade Filho et al. (2010) considered this value high. Saponins, like tannins and flavonoids, are among the allelochemicals considered responsible for causing direct and indirect effects on the target plant and can be released under natural conditions, since they are water soluble (RICE, 1984). The class of this secondary metabolite presents action on the cell membrane, altering cell permeability and affecting the germination and growth process (RICE, 1984; RIZVI; RIZVI, 1992).

Regarding the pH of the aqueous extract, the value found was 4.0 - 4.7 ± 0.5, and for the ethanolic extract, 4.8 - 5.3 ± 0.5 (Table 3). The electrical conductivity was 25, 54, 98, 123 and 173 μS cm^{-1}, respectively, for the concentrations of 1, 2.5, 5, 10 and 20%, in aqueous extracts. In the ethanolic extract, the electrical conductivity was below 2 μS cm^{-1} (0.7, 0.8, 1.1, 1.1 and 1.3 μS cm^{-1}) for concentrations of 1, 2.5, 5, 10 and 20%, respectively (Table 3).

Table 3. Physico-chemical characteristics of aqueous and ethanol extracts of leaves of *P. rigida* at concentrations of 1, 2.5, 5, 10 and 20%

Parameters	**Aqueous**					**Ethanolic**				
Concentrations	1%	2.5%	5%	10%	20%	1%	2.5%	5%	10%	20%
pH	4.7	4.0	4.0	4.6	4.7	5.2	4.8	5.3	5.3	5.2
Electrical conductivity (μS cm^{-1})	25	54	98	123	173	0.7	0.8	1.1	1.1	1.3
Soluble solids (^{0}Brix 20 ºC)	0	0	0	0	0	0.3	0.3	0.3	0.3	0.3

The pH of the aqueous extract at 20% (200 g/1L) was lower than that found for the ethanolic extract. However, it was equivalent to that reported by Gatti et al. (2004), who worked with the aqueous extract (pH = 4.2) at a concentration of 100g 300 mL^{-1} of the same species; these authors also report that the extract reduced and delayed germination of lettuce seeds. In this work, however, the aqueous and ethanolic extracts pH of the leaves of *P. rigida* had no influence on germination (Table 4 and 5). Rice (1984) also states that lettuce is a species showing no sensitivity to differences in pH across a wide range of variation; it is only at the extremes of acidity or alkalinity where the pH is 3.0 or less (very acid) or higher than or equal to 9.0 or 11 (extremely alkaline), that depressive effects on seed germination and root growth are noted, a result that was also observed by Eberlein (1987) and Pattnaik and Misra (1987).

Electrical conductivity is used, in general, to determine the total ionic constituents of the water (cations and anions), dissolved solids and has been used as a test to evaluate the effect on seed (DIAS; MARCOS FILHO, 1995; VOLL et al., 2003). According to Wardle et al. (1992), from the electrical conductivity (EC), the ionic strength of the extract and consequently the osmotic potential are estimated. The conductivity value is a function of the amount of leaching released into the imbibed water during the germination of seeds and is directly related to the integrity of cellular membranes (VOLL et al., 2003).

The electrical conductivity of aqueous and ethanolic extracts at different concentrations, is in general maintained between 25 and 173 μS cm^{-1} for the aqueous layer and 0.7 and 1.3 μS cm^{-1}, for ethanol (Table 3), according to recommendations by Carmello (1992) for the process of germination and growth. Souza et al. (1999) evaluated the allelopathic potential of the hydro-alcoholic extract of "capim-gordura" (*Melinis minutiflora* P. Beauv.), "capim-jaraguá" (*Hyparrhenia rufa* (Nees) Stapf), "capim-colonião" (*Panicum maximum* Jacq.), "mucuna" (*Mucuna aterrima* (Piper & Tracy) Holland) and "serrapilheira de bamboo" (*Bambusa* spp.) on the germination of lettuce and carrots, determining the EC for these five extracts, which was less than 200 μS cm^{-1}. The authors emphasized that the action of allelopathic extracts evaluated is not related to the electrical conductivity, considered low.

The concentration of soluble solids, with results expressed in degrees Brix (Table 3), measured by refractometry, showed no difference between the concentrations (1, 2.5, 5, 10 and 20%) evaluated for the aqueous extract (0.0) and ethanol (0.3). These degrees indicate the total soluble solids content and sugar content, approximately, in the aqueous and ethanolic extracts; when no presence of reducing sugars is detected, degrees Brix tended to zero. Taking into account the presence of sugar is indicative of the osmotic potential of the solution, so a low degrees Brix and ECs of less than 200 μS cm^{-1} are indicative of a low osmotic potential, which does not adversely influence the process of germination and growth

In relation to % germination, the results obtained indicate that the aqueous extract (at 10 and 20%) showed significant inhibition of germination; but 1, 2.5 and 5% showed no statistical difference from the control-model (Table 5); for the ethanolic extract, all the results of different concentrations are statistically equal to the control (Table 4).

Similar results were found in studies already carried out with other species: Maraschin-Silva and Aquila (2006) maintain that aqueous leaf extracts of *Cecropia pachystachya* Trécul (Urticaceae), *Peltophorum dubium* Taub. (Fabaceae), *Psychotria leiocarpa* Mart. (Rubiaceae), *Sapium glandulatum* [Vell.] Pax (Euphorbiaceae) and *Sorocea bonplandii* [Baill.] W.C.Burger, Lanj. & Boer (Moraceae) did not affect germination in lettuce seeds. Conversely, extracts of all species, with the exception of *S. glandulatum*, presented significant differences in primary root length in comparison to the control model; a similar result was also obtained by Oliveira et al. (2011), with *Rheedia brasiliensis* Planch. &

Triana, both with similar results to those obtained in this work.

Table 4. Average value in percentage terms of germination (%), germination speed index (GSI), average time of germination (ATG) and average size of primary root and aerial part of lettuce seeds treated with ethanolic extracts, concentrations of 0 (control), 1, 2.5, 5, 10 and 20%, temperature of 20 °C

Concentrations (%)	Germination (%)	GSI	ATG (days)	Growth (mm)	
				Stems	Root
0	96 a	1.3 a	7.8 a	19.4 a	6.6 a
1	91 a	0.7 b	11.4 cd	18.6 a	5.8 a
2.5	92 a	0.7 b	10.1 b	11.2 b	5.9 a
5	93 a	0.7 b	10.8 bc	8.7 c	6.8 a
10	94 a	0.7 b	11.6 d	4.9 d	4.3 b
20	95 a	0.7 b	12.6 e	5.1 d	4.1 b

* Means followed by same letter in columns do not differ statistically among themselves by Tukey test (p> 0.05).

Although the ethanolic extract contained a higher concentration of flavonoids (Table 2) compared to the aqueous extract, it did not significantly affect germination percentage (Table 4). The aqueous extract, with a lower concentration compared to the ethanol extract, significantly affected germination, from the concentration of 10% (Table 5).

In relation to GSI, the results indicated that concentrations had a negative effect on germination speed, showing a large reduction from the concentration of 1% (ethanolic extract) and 5% (aqueous extract) (Tables 4 and 5). In relation to ATG (ethanolic and aqueous extracts), significant differences occurred between different treatments, with germination occurring over the longest time period, and the average germination being affected by the concentration of 2.5% compared with the control group (Table 4 and 5), delaying the germination time. Cândido et al. (2010), working with the fraction of hexane, ethyl acetate and ethanol-water, using aerial parts of *Senna occidentalis* (L.) Link, and investigating effects on lettuce seeds (*L. sativa*), tomato (*Lycopersicum esculentum* Mill.), onion (*Allium cepa* L.) and wheat (*Triticum* spp.), found similar results, where all fractions delayed the germination of seeds.

Table 5. Average value in percentage terms of germination (%), germination speed index (GSI), average time of germination (ATG) and average size of primary root and aerial part of lettuce seeds treated with aqueous extracts at concentrations of 0% (control), 1, 2.5, 5, 10 and 20%, temperature of 20 °C

Concentrations (%)	Germination (%)	GSI	ATG (days)	Growth (mm)	
				Stems	Root
0	96 a	1.3 a	7.9 a	19.4 a	5 a
1	92 a	1.1 a	8.5 a	3.8 b	4.4 ab
2.5	95 a	1.0 ab	9.4 b	1.8 c	4.2 ab
5	89 ab	0.7 bc	11.9 c	0 c	3.6 b
10	80 bc	0.5 cd	17.9 d	0 c	3.6 b
20	75 c	0.3 d	21.7 e	0 c	2.9 c

* Means followed by same letter in columns do not differ statistically among themselves by Tukey test (p> 0.05).

Where seeds had germinated at a high frequency, the above concentrations affected GSI and ATG – distributing the germination over a longer time frame. Therefore, it can be inferred that the higher concentrations of metabolites in these concentrations (2.5, 5, 10 and 20%) were a negative factor in the strength and vigour of the seeds. The values decreased with increasing concentration of the extract.

According to Borghetti and Ferreira (2004), germination is less sensitive to allelochemicals than is the growth of seedlings, because allelopathic substances may induce the appearance of abnormal seedlings – root decay being one of the most common symptoms of this. Thus, very often, allelopathy does not affect the germination potential per se, but rather it affects the germination speed as well as other parameters. Dry root mass, or aerial parts, such as the lower stem length or primary root,

are the parameters most used when evaluating the effects of allelopathy on growth (RICE, 1984; FUJII; HIRADATE, 2007).

The results obtained in this study for the germination bioassays are consistent with the considerations made by Borghetti e Ferreira (2004), since the percentage of germination was little affected by the extracts in the target species. On the other hand, the parameters germination speed and average germination time were significantly affected, showing a negative allelopathic effect of leaf extract at the lowest concentrations (1 and 5% - aqueous, 2.5 and 10% - ethanolic).

Significant inhibitory effects were also observed for the seedling growth medium for both shoot and root system for the target species. In lettuce seedlings, stem growth was inhibited by the aqueous extract at the lowest concentration (1%). Root growth was affected by the concentration of 5% (Table 5). Although the ethanolic extract contained a higher concentration of phenolic compounds and flavonoids (Table 2) compared to the aqueous extract, this affected the growth least (Table 4 and 5) when compared with the aqueous extract, with a lower concentration these components.

The aerial part and root of lettuce seedlings was also inhibited by the ethanolic extract, but with extracts from 2.5% (aerial) or 10% (root) (Table 4). According to Peres et al. (2009), the reduction in growth in lettuce seedlings (root and stems) treated with extracts of *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel. suggests that the allelopathic effect may be linked to harmful chemical constituents present in the extract, specifically tannin and phenolic compounds, both found in *P. rigida* leaves. Suzuki et al. (2008) show the reduction in root growth is a first apparent effect of exposure to allelochemicals, associated with premature cell wall lignification.

The greater allelopathic effects of the aqueous extract are probably linked to the presence of saponin, present in this solution (Table 1). Its presence can boost the deleterious effect of the other secondary metabolites, leading to a more intense adverse effect on the germination and growth of lettuce structures, whereas in the ethanolic extract this effect was not observed, resulting in a less deleterious effect on the target species. In the study by Oliveira et al. (2009), with *Ziziphus joazeiro* Mart., the authors also consider that the germination rate was significantly affected by allelopathic effects related to the presence of saponin.

The alterations in germination, or primary root growth, might be the result of allelochemical effects on the permeability of membranes, transcription and translation of DNA; the functioning of secondary messengers; respiration, for oxygen loss (phenols); the conformation of enzymes and receptors, or even by a combination of these factors (BORGHETTI; FERREIRA, 2004; FUJII; HIRADATE, 2007). The cytological structures of allelochemicals can affect hormone concentrations, membrane permeability, mineral absorption, respiration, protein synthesis, enzyme activity, water relations, and changes in genetic material among others (RIZVI; RIZVI, 1992).

Based on the results and the conditions under which the experiments were conducted, it is concluded that the leaves of *P. rigida* contain chemical substances (phenolic compounds, tannins, flavonoids, cathechins, coumarins, steroids and triterpenes, saponins and alkaloids); and that the rate of lettuce seed germination was little affected by the different concentrations, with the exception of 10 and 20% (aqueous extracts). However, the frequency and speed of germination was adversely affected in almost all concentrations and the development of primary roots and aerial parts was notably impeded at some concentrations. This was mainly the case with ethanolic extracts at concentrations of 10 and 20% and aqueous extracts at 5, 10 and 20%: hence, this activity may be linked to the presence of chemical substances, which may be useful for natural herbicide programs for weed management.

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RESUMO: *Palicourea rigida* é um arbusto encontrado na América do Sul. Até o momento, não existem estudos com relação ao potencial da espécie para a alelopatia. Portanto, o presente trabalho visa verificar a fitoquímica e o potencial alelopático de extratos de folhas em diferentes concentrações de *P. rigida* sobre a germinação de sementes e crescimento de plântulas de alface. Amostras de folhas foram coletadas na região de Campo Grande, Mato Grosso do Sul e extratos preparados em etanol e água (20%) e submetidos à análise fitoquímica. Os bioensaios foram realizados em laboratório com extratos aquoso e etanólico nas concentrações de 0% (controle), 1, 2,5, 5, 10 e 20%, com delineamento experimental inteiramente casualizado, analisando-se a percentagem e tempo de germinação, além do crescimento das plântulas. A prospecção química identificou a presença de compostos fenólicos, taninos, flavonóides, catequinas, cumarinas, esteróides, triterpenos, alcalóides e saponinas, com índice de espuma (Índice Afrosimétrico) de 1250, considerado elevado. A taxa de germinação de sementes de alface foi pouco afetada pelas diferentes concentrações dos extratos. No entanto, a velocidade de germinação foi adversamente afetada em quase todas as concentrações. O desenvolvimento da raiz primária e parte aérea foi fortemente inibido nas concentrações de 5, 10 e 20% (extrato aquoso) e, 10 e 20% (extrato etanólico).

PALAVRAS-CHAVE: Aleloquímicos. Potencial alelopático. 'Dourandinha'. Índice de espuma.

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