Original Article

EVALUATION OF ALLELOPATHIC POTENTIAL OF LEAF EXTRACT OF *KIELMEYERA CORIACEA* ON *LACTUCA SATIVA* L

AVALIAÇÃO DO POTENCIAL ALELOPÁTICO DE EXTRATO DE FOLHAS DE KIELMEYERA CORIÁCEA SOBRE LACTUCA SATIVA L

Valter Henrique Marinho dos SANTOS[1]; Gabriel Silva DANELUZZI[2]; Luciana Pereira SILVA[3]; Regildo Márcio Gonçalves da SILVA[4]

1. Biólogo, doutorando no Programa de Pós-Graduação em Ciências Biológicas (Botânica), Universidade Estadual Paulista - UNESP, Botucatu, SP, Brasil; 2. Biólogo, doutorando no Programa de Pós-Graduação em Fisiologia e Bioquímica de Plantas, Escola Superior de Agricultura "Luiz de Queiroz" - ESALQ, Universidade de São Paulo - USP, Piracicaba, SP, Brasil; 3. Professora, Doutora, Fundação Educacional do Município de Assis - FEMA, Assis, SP, Brasil; 4. Professor, Doutor, Faculdade de Ciências e Letras de Assis, Laboratório de Fitoterápicos - UNESP, Assis, SP, Brasil. regildos@yahoo.com.br

ABSTRACT: *Kielmeyera coriacea* Mart. (Clusiaceae), popularly known as "pau-santo", is a typical Brazilian cerrado tree known due to its varied secondary metabolites. This study aimed to determine the allelopathic potential of the hydroalcoholic extract of leaves of *K. coriacea* through bioassays of seed germination, seedling growth and mitotic index of *Lactuca sativa* L. (lettuce). In addition it was done the tetrazolium assay and a phytochemical screening. The extract concentrations caused alterations in germination parameters, in root growth and in the mitotic index. The phytochemical screening showed the presence of triterpenes, coumarins, steroids, flavonoids and condensed tannins, compounds known to confer allelopathic characteristics upon other species. These data indicate that *K. coriacea* presents an allelopathic potential because its leaf extracts interfere with germination and growth without any interference of pH and osmotic potential in the results.

KEYWORDS: *Kielmeyera coriacea*. Germination Parameters. Mitotic Index. Phytochemical Screening. Seedling Growth.

INTRODUCTION

Allelopathy is defined as the interference of plants in the growth and development of another (including microorganisms) through the release of chemical compounds into the environment (RICE, 1984). This interaction produces different answers, positive or negative, in plants sensitive to such substances, collectively known as allelochemicals, which are usually secondary plant products or waste products of main metabolic pathways (INDERJIT and NILSEN, 2003). According to Gottlieb (1982), allelochemicals are chemical signals transmitted in the environment, usually in small quantities, and account for multiple chemical interactions between different organisms.

These interactions may be indirect, when the allelopathic compounds change soil properties, or direct, when these substances interfere in the plant metabolism (FERREIRA and AQÜILA, 2000). The allelochemicals can affect the cellular ultrastructure, concentration and hormonal balance, membrane permeability affecting the uptake of ions, stomatal opening influencing photosynthesis, pigment and protein synthesis, enzyme activity, water relations, sap flow and genetic material (RIZVI and RIZVI, 1992; INDERJIT and CALLAWAY, 2003; INDERJIT et al., 2006).

In this context, allelopathy is responsible for the most varied intraspecific and interspecific interactions in the stabilization and maintenance of different life forms in the environment. Among a great number of species that use this biochemical resource as an advantage to survive in the nature, *Kielmeyera coriacea* Mart. (Clusiaceae), popularly known as "pau-santo", stands out in Brazilian cerrado because it possesses a class of substances of phytochemical interest called xanthones, besides other substances like xanthonolignoids, aucuparine, steroids, osajaxanthone, triterpenes and biphenyl compounds (CORTEZ et al., 1998).

Considering the ecological and chemical characteristics of *K. coriacea*, the aim of this work was to evaluate the allelopathic potential of the hydroalcoholic extract of its leaves through bioassays of seed germination, seedling growth and mitotic index of *Lactuca sativa* L.

MATERIAL AND METHODS

Plant and vegetal extract

The leaves of *K. coriacea* were collected in the cerrado area from the city of Patos de Minas-MG, Brazil (17°30.27'34''S and 45°31.21'17''W) in June 2009. The plant was identified and a voucher specimen was deposited in the Mandevilla

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Herbarium at the Centro Universitário de Patos de Minas (UNIPAM) under the number MGHM0632-7. After collection, the leaves were selected and dried in a forced-air oven at an average temperature of 40°C for 24 h, and shortly after, they were ground and the resulting powder was stored in dark plastic bottles.

The hydroalcoholic extract was prepared by mechanical maceration of the powdered plant material in a 70% ethanol and distilled water solution (at a ratio of 1:10 [w/v]) for 24 h at room temperature. The extract then was filtered at low pressure under vacuum, a methodology similar to that used by Rutherford and Powrie (1993), Hajhashemi et al. (2003) and Boligon et al. (2009). The extraction was performed in triplicate and the extracts were pooled and concentrated on a rotary evaporator at an average temperature of 60ºC. The resulting extract was lyophilized, and the dried residue was used in the bioassays, according to Aqüila (2000) and Sadraei et al. (2003).

Osmostic potencial teste

Measurement of the osmotic potential of the extracts was performed using dilutions of polyethylene glycol (PEG-6000) to produce the osmotic potentials of -0.02 to -1.0 MPa, as described by Villela et al. (1991). The measurement of the refractive Brix for each concentration of PEG-6000 and the extract was determined by an ABBE Refractometer and the values were used to calculate the osmotic potential, as described by Bakke et al. (2006).

Germination assay

The bioassay was conducted in Petri dishes lined with germination paper moistened with 1 mL of the extract diluted in Tween 80 (0.3%) to concentrations of 5, 10 or 20 mg mL^{-1}. Fifty achenes of *Lactuca sativa* L. cv. 'Grand Rapids' (lettuce) were sown per dish, separated into experimental and control (Tween 80 0.3%) groups, and incubated for 48 h in a growth chamber at 23±2°C. The experimental design was completely randomized, with four replicates for each treatment concentration and the control. Germination was monitored every 6 h and the root protrusion and geotropic curvature was adopted as the evaluation criterion of germination, as described by Ferreira and Aqüila (2000), Maraschin-Silva and Aqüila (2006) and Ferreira et al. (2008). From the data obtained, the following indexes were calculated: germinability or percentage of germination ([∑ni/A].100), mean germination time (Tm=[∑ni.ti]/∑ni), mean germination speed (Vm=1/Tm), and the synchronism of germination (E=-∑ [fi.log2.fi]), where A= total number of achenes put to germinate, ni= the number of achenes that germinated at each time point (ti), ti= the time between the beginning of the experiment and the i-th h of observation and fi= the relative frequency of germination (LABOURIAU, 1983; SANTANA and RANAL, 2004; PEREIRA et al., 2009).

Viability assay

The seeds that did not germinate during the germination assay were incubated in a tetrazolium solution (2,3,5-triphenyltetrazolium chloride, 0.5%) for 6 h at 30°C in the dark, as described by Delouche et al. (1976) and the Rules for Seed Analysis (BRASIL, 2009). The viability (dead or dormant seeds) was determined in order to characterize the metabolic state of the un-germinated seeds (SOUZA, 1996; PINHEIRO and BORGHETTI, 2003).

Seedling growth assay

In the growth bioassay, twenty-five *L. sativa* seedlings (primary root with approximately 2 mm) were placed in the Petri dishes moistened with 1 mL of the extract (5, 10 or 20 mg mL^{-1}) or Tween 80 solution, as described above. The dishes were placed in a BOD-type growth chamber under the same conditions of the germination assay, as described by Maraschin-Silva and Aqüila (2006) and Ferreira et al. (2008). The experimental design was completely randomized with four replicates for each treatment concentration and the control. After 24 and 48 h of the beginning of the experiment, the length of the primary roots was measured with a digital caliper.

Mitotic index determination

For the analysis of the mitotic index, the *L. sativa* achenes were put to germinate and grow in distilled water. After that, the seedlings (primary root with approximately 2 mm) were transferred to Petri dishes lined with germination paper moistened with 1 mL of the extracts at the same concentrations previously described, or Tween 80 solution (0.3%). When the roots reached approximately 5 mm, they were collected and prepared by the squash technique (GUERRA and SOUZA, 2002; MAHAJAN and SHARMA, 2008).

First, the roots were fixed in Carnoy's solution (ethanol: glacial acetic acid, 3:1) for 2 h, hydrolyzed in 5N HCl for 15 min at room temperature, washed with distilled water and stained with 5% acetic carmine. Cells were observed under a light microscope with 100x magnification. In

order to verify the number of cells in each phase of the mitosis, 2000 cells were analyzed in each treatment and in the control. The mitotic index (MI) was obtained from the equation, MI=(m/T).100, where m= the number of cells in mitosis and T= total number of cells (TABUR and ONEY, 2009).

Phytochemical screening

Samples (50 g of K. coriacea leaves) were extracted with 250 mL of water-ethanol (30:70) at 60ºC (water bath) under magnetic heating agitation for 2h. The extract solution was filtered and concentrated with a rotary evaporator.

The phytochemical tests were carried out for the above mentioned extract using standard procedures (SIVASANKARI et al., 2010) to identify components such as flavonoids, alkaloids, terpenoids, triterpenoids, hydrolysable tannins, condensed tannins, coumarins, saponins, glycosides and phenols.

Statistics

Statistical analysis was performed using the Shapiro-Wilks Normality Test and Levene's Test for homogeneity. The transformed data showed normality, and the variances were homogeneous; therefore, the data were analyzed by ANOVA and Tukey (α=0.5) parametric tests. These tests were performed using the SISVAR software, according to Santana and Ranal (2004) and Pereira et al. (2009). For the analysis of the mitotic index, the Chi-square Test was performed to identify a positive response between the experimental and control groups, according to the analysis proposed by Ribeiro et al. (2003).

RESULTS

The three concentrations of the hydroalcoholic extract reduced the germination of *L. sativa*, markedly at the concentration of 20 mg mL^{-1} (Table 1). The mean germination time was reduced only by the concentrations of 5 and 10 mg mL^{-1}; however these concentrations increased the mean germination speed. The concentration of 20 mg mL^{-1} did not affect these two parameters when compared to the control group (Table 1). In relation to the synchronism of germination, the achenes were affected by the three concentrations of the extract, as the index was reduced (Table 1).

Table 1. Germinability (G), mean germination time (Tm), mean germination speed (Vm), and synchronism of germination (E) of seeds of *L. sativa* subjected to different concentrations of a hydroalcoholic extract of *K. coriacea*.

Treatment (mg mL^{-1})	G±sd (%)	Tm±sd (hours)	Vm±sd (seeds h^{-1})	E±sd (bits)
5	29.00±4.76a	33.21±1.35a	0.030±0.00a	1.481±0.51a
10	28.00±7.65a	33.62±3.01a	0.029±0.00a	0.963±0.36b
20	3.50±1.91b	40.00±6.32b	0.025±0.00b	0.229±0.45c
Control Tween 80	67.50±6.19c	38.27±2.80b	0.026±0.00b	2.084±0.17d

Data presented as mean±standard deviation; Means sharing the same letter in a column do not differ significantly by Tukey's test (α= 0.05).

The *K. coriacea* leaf extract reduced the root length of *L. sativa* seedlings in a dose-dependent manner after 24 and 48 h of experimentation. The reduction was greater at the concentration of 20 mg mL^{-1}, regardless of the exposition time to the extract (Table 2).

Table 2. Root length of *L. sativa* seedlings (RL) subjected to different concentrations of a hydroalcoholic extract of *K. coriacea* after 24 and 48 hours of exposure.

Treatment (mg mL^{-1})	RL(mm)	
	24 h	48 h
5	4.40±0.93a	4.83±0.64a
10	3.65±0.32b	3.65±0.15b
20	2.55±0.37c	2.64±0.18c
Control Tween 80	5.03±0.52d	9.73±1.76d

Data presented as mean±standard deviation; Means sharing the same letter in a column do not differ significantly by Tukey's test (α= 0.05).

The physicochemical characterization of the hydroalcoholic extract of *K. coriacea* leaves revealed that the pH showed little variation and low acidity values (Table 3). The values of the osmotic potential showed variation between -0.0054 and -0.0154 MPa (Table 3).

Table 3. The pH and osmotic potential of a hydroalcoholic extract of *K. coriacea* under different concentrations.

Concentration (mg mL^{-1})	pH	Osmotic potential (MPa)
5	6.43	-0.0054
10	6.57	-0.0069
20	6.61	-0.0154
Control Tween 80	6.65	-0.0098

The hydroalcoholic extract of *K. coriacea* leaves reduced the mitotic index of meristematic root cells of *L. sativa* in all three concentrations tested. The concentrations of 5 (MI=14.5) and 20 mg mL^{-1} (MI=15.5) produced similar results, whereas the concentration of 10 mg mL^{-1} (MI=11.6) was the most effective in the reduction of the mitotic index (Figure 1).

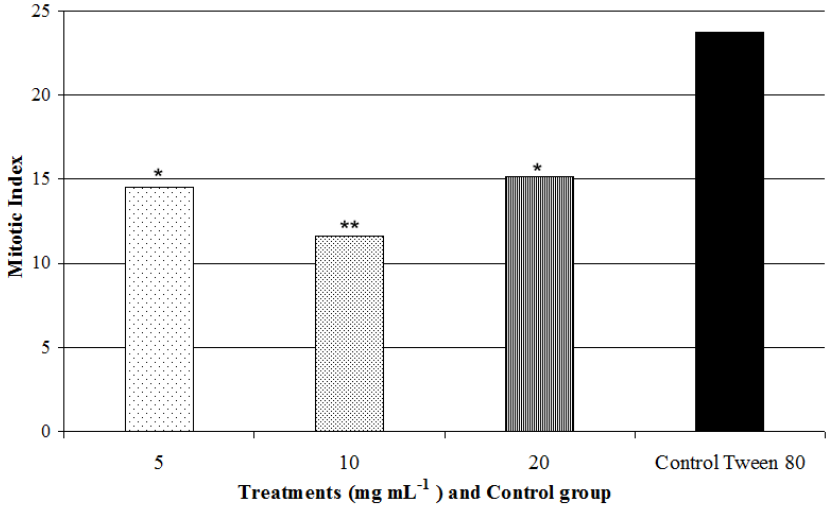


Figure 1. The mitotic index of meristematic cells from *L. sativa* roots treated with different concentrations of an extract of *K. coriacea* or Tween 80 (0.3%) (* and ** significant difference, $\chi^2 < 0.01$).

The results of the phytochemical screening showed the presence of triterpenes, coumarins, steroids and flavonoids (Table 4). Triterpenes and steroids were characterized by the less teal and red aspect on the assay tube. Flavonoids showed a less green-orange aspect in the assay, and coumarins a blue fluorescent aspect. More prevalent compounds present in *K. coriacea* hydroalcoholic extract could be attributed to condensed tannins by their heavy teal aspect in the assay. Investigation of anthraquinones, anthocyanines and amino groups was not performed.

Table 4. Results of the phytochemical screening of a hydroalcoholic extract of *K. coriacea*.

Phytochemical Compounds	Intensity	Color (aspect)
Triterpenes	+	Teal
Steroids	++	Teal
Alkaloids	-	-
Flavonoids	+	Green orange
Coumarins	+	Blue fluorescent
Hydrolysable tannins	-	-
Condensed tannins	++	Blue green

+, low visualization; ++, moderate visualization; +++, high visualization; -, not visualized

DISCUSSION

Allelochemicals may act in different ways, depending on the environment and life cycle stages, as they reflect different morphological and physiological states. Moreover, the effects can also be variable when considering the plant organ that they are acting upon (AQÜILA et al., 1999; FERREIRA; AQÜILA, 2000).

The germination is not the primary target for allelopathy but the subsequent seedling growth is more susceptible to some allelochemicals, which interfere with nutrient absorption, enzyme activity, water relations, photosynthesis, and respiration (INDERJIT; DAKSHINI, 1995). Therefore, the use of seedlings to seeds is sometimes preferred, which allows the researcher to select uniform seedlings and control their density, making the interpretation of the results easier (WU, 2001).

On the other hand, several researchers have studied the effects of allelochemicals on seed germination based only on the final number of seeds germinated under experimental conditions. The mean germination time, mean germination speed and synchronism of germination are characteristics that express the rate and degree of organization or disorder in chemical reactions that occur with seeds during the germination process and need to be analyzed together with germinability (SANTANA et al., 2006). Therefore, we evaluated all of these indexes besides the root growth to improve the quantification of the germination process when analyzing the effects of allelochemicals.

The tests of pre-emergence demonstrated effect on germinability, mean germination time, mean germination speed and synchronism of germination. The three concentrations of the extract reduced the germinability of *L. sativa* markedly whereas the concentrations of 5 and 10 mg mL^{-1} decreased the mean germination time and increased the mean germination speed, i.e., the extract interfered with the metabolic reactions that culminate in germination, as seen in the difference between the treatment and the control groups (Table 1).

The importance of germination inhibition by secondary plant metabolites is related to the search for eco-friendly herbicides (VYVYAN, 2002). The influence on germination speed has an ecological significance, because plants that germinate faster can establish in the environment taking advantage of the environmental conditions favorable to the development of new individuals (BORGHETTI and FERREIRA, 2004). On the other hand, plants that germinate more slowly may present a reduced size and, thus, may be more susceptible to stresses and have less chance in the competition for resources (JEFFERSON; PENNACHIO, 2005).

Regarding the synchronism of germination, the index interpretation is: the lower the value of E, the more synchronized is the germination, regardless of the total number of seeds that germinated (SANTANA; RANAL, 2004). In this work, as the extract concentration increased, the germination became more synchronized. The strategy to synchronize the germination process, as well as to reduce the mean germination time, is related to the fast colonization of the environment. On the other hand, the delay and the lower synchronism of germination may increase the chances that at least some seeds can germinate under favorable conditions for seedling establishment (BORGHETTI; FERREIRA, 2004).

The root growth was reduced by the three concentrations of the extract (Table 2). According to Rice (1984) and Aqüila et al. (1999), the uptake of ions and water by the roots is of great importance for the plant growth and development, and they have shown many types of allelopathic effects in this process. Studies conducted by Balke (1985) have shown that flavonoids found in plant extracts have inhibitory activity of root plasma membrane ATPases, while phenolic compounds in excised roots inhibited the uptake of minerals through changes in membrane permeability.

According to Rodrigues et al. (1992) and Ferreira and Borghetti (2004), allelochemicals are inhibitors of germination and development, because they interfere in the cell division, membrane permeability and activation of enzymes. In the present study, the inhibition of *L. sativa* seed germination and root growth was the consequence of inhibition of cell division (Figure 1). Souza et al. (2005) and Iganci et al. (2006) also found interference in the mitosis caused by the action of plant extracts.

Table 3 shows the values of pH and osmotic potential of the extract of *K. coriacea*. Experiments performed by Ferreira and Aqüila (2000) and Carmo et al. (2007) demonstrated that these physicochemical characteristics are key factors when the constitution of sugars, amino acids, organic acids, ions and other molecules contained in plant extracts are unknown. Extreme values of pH and osmotic potential can act on seeds and seedlings masking the allelopathic effect. *Lactuca sativa* has a wide pH range for germination that goes from 3.0 to 7.0 (BASKIN; BASKIN, 1998), and therefore, the pH of the *K. coriacea* extract was suitable to the germination and growth of the test plant.

Regarding the osmotic potential, Gatti et al. (2004) recommend that the extracts involved in the germination bioassays not exceed the value of -0.2 MPa, which was found in this study (Table 3). In view of that, the possibility of interference of these physicochemical factors on the results can be ruled out, reinforcing the idea that the extract presented a toxic effect on the germination and growth of *L. sativa*.

The results of the viability test (tetrazolium) revealed that the seeds did not germinate, when achenes were submitted to different concentrations of *K. coriacea* extracts, showed to be viable and/or dormant, while the ones of control group were died. These observations are according to the Rules for Seed Analysis (BRASIL, 2009).

The phytochemical analysis showed the presence of triterpenes, coumarins, steroids, flavonoids and condensed tannins, compounds of the secondary metabolism (Table 4). The presence of these substances is an indicative of allelopathic potential according to Inderjit and Dakshini (1995), Inderjit (1996), Borella et al. (2009). Moreover, Rice (1984) have demonstrated the action of secondary metabolites of the same chemical classes found in the extract of *K. coriacea* on growth regulators, being able to reduce the activity of auxin and gibberellins, which can inhibit the growth of target plants. Einhellig (1999) and Vyvyan (2002) also noted that process and cellular events, such as: mitosis, cellular respiration, photosynthesis and enzymatic activities are affected by several flavonoids, tannins, quinones, coumarins and phenolic acids.

Einhellig (1999) highlights the importance of phytochemical studies on species that have an allelopathic potential, because allelopathy rarely results from the single substance action and allelochemicals generally exert their effects when acting together.

Kielmeyera coriacea has an allelopathic potential as it influences the germination parameters and growth of *L. sativa*, besides presenting substances previously related as allelochemicals in other species. Thus, this plant can serve as model for ecological studies, allowing better understanding of chemical interactions between plants and for studies of new chemical compounds, especially the germination inhibitors and growth retardants, for use in sustainable agriculture.

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RESUMO: *Kielmeyera coriacea* Mart. (Clusiaceae), conhecida popularmente como "pau-santo", é uma árvore típica do cerrado brasileiro, conhecida pelos seus variados metabólitos secundários. O estudo teve como objetivo determinar o potencial alelopático do extrato hidroalcoólico de folhas de *K. coriacea* por meio dos bioensaios de germinação, crescimento de plântulas e índice mitótico de *Lactuca sativa* L. (alface). Além disso, foi feito o teste de tetrazólio e uma triagem fitoquímica. As concentrações de extrato causaram alterações nos parâmetros de germinação, no crescimento radicular e no índice mitótico. A triagem fitoquímica mostrou a presença de triterpenos, cumarinas, esteroides, flavonoides e taninos condensados, compostos conhecidos por conferir características alelopáticas sobre outras espécies. Estes dados indicam que *K. coriacea* apresenta potencial alelopático pois o extrato de sua folha interfere na germinação e no crescimento da planta alvo, sem qualquer interferência do pH e do potencial osmótico.

PALAVRAS-CHAVE: Crescimento de Plântulas. Índice Mitótico. *Kielmeyera coriácea*. Parâmetros de Germinação.

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