Original Article

PHYTOCHEMISTRY AND LARVICIDAL ACTIVITY OF *Spermacoce latifolia* AUBL. (Rubiaceae) IN THE CONTROL OF *Aedes aegypti* L. (Culicidae)

FITOQUÍMICA E ATIVIDADE LARVICIDA DE Spermacoce latifolia *AUBL. (Rubiaceae) NO CONTROLE DE* Aedes aegypti *L. (Culicidae*)

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ABSTRACT: In the search for alternative ways to control *Aedes aegypti* with minimal environmental impact and in a manner that preserves human health, this study sought to evaluate the larvicidal effect of the invasive and antioxidant *Spermacoce latifolia* plant by performing a phytochemical study. Phytochemical screenings were done according to characterization reactions and thin layer chromatography. Phenolics compounds content (Folin-Ciocalteu's) and flavonoids ($AlCl_3$) spectrophotometric was performed, and the antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH). The phytochemical results revealed the presence of phenolic, flavonoid, tannin, steroid, free triterpene, coumarin, and alkaloid compounds. The content of total phenols (TPs) (482.7 ± 1.8 mg mgGA g^{-1}) and flavonoids (165.4 ± 1.5 mg QE g^{-1}) accounted for the antioxidant activity of 150 µg mL^{-1} methanolic extract. In the proposed bioassays, groups of 25 third-stage larvae were challenged at different concentrations of plant crude extract (1.0, 0.5, 0.25, and 0.1 mg mL^{-1}) of weight per volume in four replicates. In multiple concentration tests, the concentrations were selected to range from 0 % to 100 % mortality after 24 hours of contact with the solution. Toxicity was defined as the inhibition or total inactivity of the larvae. It was concluded that the methanol extract had an LC_{50} of 0.625 mg mL^{-1}, indicating its potential use as a larvicide against *A. aegypti* and linking its activity to its phenolic and flavonoid components.

KEYWORDS: Vector control. Plant active ingredients. Plant insecticides.

INTRODUCTION

Dengue is a viral infection transmitted by mosquitoes of the genus *Aedes* and is typical of tropical and subtropical regions. The incidence of dengue has increased due to variations in climate and rainfall. Currently, this disease affects 50 to 100 million people in over 100 countries worldwide and about two-fifths of the global population at risk of being infected by dengue virus (GARCEZ et al., 2013).

However, current measures to control dengue epidemics, such as the use of organophosphates and carbamates have not been effective due to vector resistance and plasticity that reappears under different guises during each epidemic cycle, triggering serious public health problems (TEIXEIRA et al., 2009).

The need to seek control methods with minor action toxic to humans and the environment has stimulated the search for new ways to combat *A. aegypti*, and the plants are cited as one of the effective alternatives because they are rich in bioactive ingredients. So, the high Brazilian plant diversity emerges as one way to control dengue. Their natural products are in promising vector control sources, because in addition to being rapidly degradable, renewable resources easily accessible, yet have low production cost (ROEL, 2001).

And within that botanical diversity, are the Rubiaceae, that are among the most numerous families of plants, and are noted for being bio-producer of a large number of phytochemicals such as iridoids, terpenoids, flavonoids, tannins, quinones and alkaloids. Species of the *Spermacoce* genus have an impressive number of chemical constituents, with medicinal properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, anti-ulcer and larvicidal, supporting their relevance to this study (COSTA et al., 2006).

Considering the above-mentioned aspects, this work aimed to evaluate the classes of secondary metabolites of an *S. latifolia* methanolic extract, determining larvicidal activity against *A. aegypti* larvae, and their utility as a botanical alternative to chemical agents.

MATERIAL AND METHODS

Plant sample collection and identification

Plant samples were collected at the Três Barras Farm School, Campo Grande, State of Mato

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Grosso do Sul - MS (20°33'37.44043" S, 54°32'10.3824" W), in 2008. The samples were dehydrated, and a voucher specimen was added to the Herbarium of the University Anhanguera - Uniderp under file number 1543 after botanical identification by Professor Eloty Justina Dias Schleder.

Preparation of the methanol extract

The aerial parts of the plant were cleaned, dried in an air ventilated oven at 45 °C (Model MA35, MARCON) for 4 days, weighed, pulverised in an electric grinder (Model MA048, MARCONI), and sieved (mesh No. 60). Five hundred and eighty grams of processed material was extracted with methanol (99.5%) in an ultrasonic bath for 60 minutes (Model 1450, UNIQUE), followed by room-temperature maceration; this procedure was repeated daily for 15 days. The solvent was evaporated under vacuum on a rotary evaporator (Model MA120, Tecnal) to yield 25.0 g of crude methanolic extract.

Phytochemical analysis

Phytochemicals were determined by humidification (crude extract - 20%), as per colorimetric testing and/or chemical precipitation methods described in Matos (2009). The analyses were performed in three replicates, and the results were compared with the methanolic extract (COSTA, 2002). Confirmation of the class of secondary metabolites and their elution system were performed by thin layer chromatography (TLC: silica gel $60F_{254}$) using specific reagents for terpenes, alkaloids, coumarins, flavonoids, and phenolic compounds (WAGNER; BLADT, 2009).

The methanolic extract were used to quantify the flavonoids and total phenolis. The flavonoids were determined based on method of Peixoto Sobrinho et al. (2008). The total phenolic (FT) compounds were determined based on the Folin-Ciocalteu method, as per method of Sousa et al. (2007).

Antioxidant activity

The antioxidant potential was determined based on the free radical scavenging activity of 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The 20 % methanolic extract (20 g 100 mL^{-1}) was diluted at concentrations of 250, 200, 150, 100, 50, and 25 µg mL^{-1}, and 2 mL of DPPH in methanol was added (24 mg DPPH/100 mL of methanol). After 30 minutes, the absorbance was measured in a spectrophotometer at 515 nm. DPPH in methanol solution was used as a negative control, and BHT (butylated hydroxytoluene, at the same concentrations used in the samples) was employed as a positive control (THAIPONG et al., 2006). The percentage of antioxidant activity (% AA) was calculated using the formula: $\% AA = (A_0 - A_s) / A_0) \times 100$, where A_0 is the absorbance of DPPH (control) and A_s is the absorbance of the sample in the presence of DPPH (SOUSA et al., 2007).

Bioassay

A. aegypti eggs were collected in the city of Campo Grande with the aid of an official from CCZ (Centre for Zoonoses Control) of Campo Grande - MS. Bioassays were performed at the Laboratory of Entomology of Dom Bosco Catholic University.

The eggs were allowed to mature for 1 week and then were subjected to hatching in running water (pH 6 – 7). The larvae obtained from the breeding stock were separated by age for the toxicity tests. A biochemical oxygen demand (BOD) incubator was used (adjusted to 27 ± 2 ºC), and the mosquitoes were subjected to 14 hours of photoperiod exposure during their egg and larvae stages. In the adult stage, the females were fed pigeon-blood meals three times per week, and the males were fed sweetened water.

Twenty-five third-stage larvae were used for every 25 mL solution of *S. latifolia* methanol extract, at concentrations of 1.0, 0.5, 0.25, and 0.1 mg mL^{-1}, tested in quadruplicate for 24 hours. A negative control (blank solution) and positive control (rotenone) were tested simultaneously (CONSOLI et al., 1989).

The probit analysis method was used via the POLO-PC software program to obtain the lethal concentration 50 (LC_{50}) and lethal concentration (LC) values with their respective confidence intervals.

RESULTS AND DISCUSSION

Characterized by the classification of the family as a plant insecticide, and in agreement to the results of phytochemical analysis, Table 1 shows that the methanol extract of aerial parts of *S. latifolia* was effective in controlling larvicide to be tested in four concentrations: 1.0, 0.5, 0.25, and 0. 1 mg mL^{-1}. The LC_{50} was in the range of 0.61 - 0.63 mg mL^{-1} (LC_{50} = 0.625 mg mL^{-1}). The minimum concentration capable of causing mortality (LC_{10}) was 0.125 mg mL^{-1}, and the maximum toxicity (LC_{90}) was 1.125 mg mL^{-1} after a 24-hour exposure (Figure 1). By observing the heterogeneity of the results and the range of 0.95 correlation, it is possible to observe a limit of variation between

dosages of 7.309 and the average of the responses to ± 2.619 are single.

Table 1. Larvicidal activity of *Spermacoce latifolia* (Rubiaceae) methanolic extracts against *Aedes aegypti* L. (Culicidae) third-stage larvae.

Lethal Concentration (LC);	LC_{10} 0.125 mg mL^{-1}	LC_{50} 0.62 mg mL^{-1}	LC_{90} 1.25 mg mL^{-1}
Measurement	0.118 – 0.13 mg mL^{-1}	0.61 – 0.63 mg mL^{-1}	1.117 – 1.13 mg mL^{-1}

The dispersion of data over time reveals an increasing linear regression, with R^2 value of 0.93 (Figure 1), approaching effectively the positive control rotenone which certified 100% mortality and no deaths were observed in the negative control without product

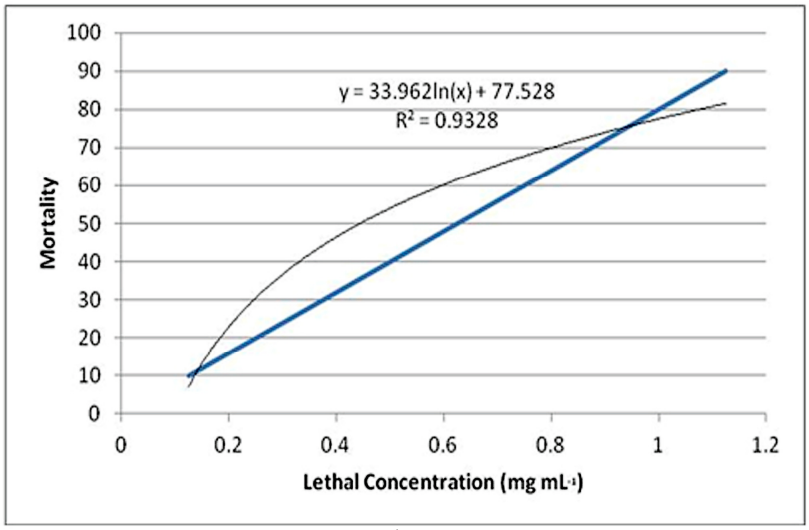

Figure 1. Toxic effect of different concentrations (mg mL^{-1}) *Spermacoce latifolia* of on larvae of *Aedes aegypti* of treatment.

The best concentration or concentrations that caused mortality above 30 %, as described in the literature (MCLAULING, 1991), were used to adjust the concentrations for the bioassay in this study. The LC_{50} was determined to be 0.62 mg mL^{-1} (Table 1, Figure 1). This result showed promise for larvicidal activity, in addition to the chemical analysis that revealed the presence of phytochemicals with similar activity responses to other Rubiaceae species.

In this study, the phytochemical analysis of the methanolic extract of *S. latifolia* aerial parts revealed the presence of phenolic compounds (482.7 ± 1.8 mg GAE g), flavonoids (165.4 ± 1.5 mg QE g), tannins, steroids, free triterpenes, coumarins and alkaloids. The % AA results from the *S. latifolia* methanol extract confirmed the presence of phenols and flavonoids, and the concentration that showed the highest oxidation potential was 150 µg mL^{-1}.

There are no reports regarding the total phenolis and total flavonoid content in Spermacoce species. The Rubiaceae family is known for the production of alkaloids, iridoids, and anthraquinones (YOUNG et al., 1996), and flavonoids have been isolated from species of this family (LOPES et al., 2004, CARDOSO et al., 2005; PINTO et al., 2008).

Noiarsa et al. (2006) identified 18 substances from the aerial parts of Spermacoce laevis Roxb. including flavonoids and iridoids, the latter being designated as a chemotaxonomic marker for species of the Rubeaceae family. The antioxidant activity of *S. articularis* was attributed to the presence of steroids and triterpenoids in the aerial parts of this species (SAHA et al., 2004) and Kaviarasan et al. (2008) determined the antioxidant activity of *S. hispida* and isolated flavonoids.

Nazar et al. (2009) and Dhanasekaran et al. (2013) investigated the larvicidal, ovicidal, and repellent potential of an *S. hispida* crude ethanol extract against *Anopheles stephensi*, *A. aegypti,* and *Culex tritaeniorhynchus*. A pronounced lethal activity (LC_{50} = 89.45 mg L^{-1}) was recorded against *Anopheles stephensi*. *S. hispida* exhibited an

ovicidal activity higher than 50 % against the mosquito eggs (at 100 mg L^{-1}). At a 200 mg L^{-1}concentration, the ethanol extract exhibited 100 % ovicidal activity against the insects tested. The extract provides 100 % repellence protection against adult female mosquitoes, at up to 120 minutes of exposure.

The ethanol extract of *S. verticillata,* collected in northeastern Brazil, was effective at a 250 mg L^{-1} concentration, with a mortality rate above 75% against the fourth-stage larvae of *A. aegypti*. Iridoids were identified in this species (SOUZA et al., 2013).

In the present study, the presence of phenolic compounds, and within this group, the flavonoids, detected in the methanolic extract are indicative of insecticidal activity. Kotkar et al. (2002) considered the phenolic compounds to be phytochemicals with insecticidal activity, based on the action of their hydroxyl groups against the larval enzyme systems (VALENCIA, 1995), which are enzyme systems similar to those involved in the respiratory chain, causing potent inhibition (BOBADILLA, 2005).

Flavonoids isolated from the leaves of *Polygonum senegalese* Meissn exhibited insecticidal and *A. aegypti* larvae growth inhibitory activity, even at low concentrations (GIKONYO et al., 1998; GARCEZ et al., 2009). The species that showed strong larvicidal activity against *A. aegypti,* with LC_{50} values between 80 and 470 g L^{-1}, belonged to the Rubiaceae family, and this activity has been related to bioactive flavonoids. It is also established that the rotenoids that belong to the isoflavones class have strong activity against *A. aegypti* larvae.

Pohlit et al. (2004) evaluated the aqueous, ethanol, and methanol extracts of various native plant species found in the Amazon region, among them, seven species of the *Piper* genus. However, only the lyophilised methanol extract of *P. aduncum* L. (leaf and root) and *P. tuberculatum* Jacq (leaf, fruit, and stem) were tested for activity against *A. aegypti* larvae; a single concentration of 0.5 g L^{-1} caused 100 % mortality of the larvae.

In addition to studies of plant extracts, recent investigations have focused on the research and use of essential oils or isolated compounds that act against mosquitoes, including *A. aegypti*. Cavalcanti et al. (2004) evaluated the larvicidal activity of nine essential oils of plants found in northeastern Brazil. It was observed that oils of *Ocimum americanum* and *Ocimun gratissimum* (Lamiaceae) have better efficacy against *A. aegypti*, with LC_{50} values of 0.067 g L^{-1} and 0.060 g L^{-1}, respectively.

An ethanol extract from *Piper nigrum* exhibited larvicidal action against *A. aegypti* at concentrations of 0.98 g L^{-1}, as did the isolated fractions piperolein-A (1.460 g L^{-1}) and piperine (1.530 g L^{-1}) (SIMAS et al., 2004). Abed et al. (2007) demonstrated the *Copaifera reticulata* oleoresin's larvicidal activity against *A. aegypti* at concentrations of 0.0089 g L^{-1} and 0.0594 g L^{-1} for the LC_{50} and LC_{90}, respectively. Based on the LC_{50} (0.625 mg mL^{-1}) obtained in this study from the methanol extract of *S. latifolia* aerial parts, this species exhibited bioinsecticidal activity against *A. aegypti* larvae, thus expanding the possibility for its use in controlling this vector.

CONCLUSION

The methanolic extract of *S. latifolia* aerial parts exhibits antioxidant activity, and this activity is linked to the presence of phenolic and flavonoid compounds, which are the predominant class of secondary metabolites. This extract has insecticidal activity for controlling *A. aegypti* larvae.

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RESUMO: Na busca de alternativas de controle do *Aedes aegypti* que minimizem os impactos ambientais e preserve a saúde humana, procurou-se neste trabalho avaliar o efeito larvicida da planta invasora *Spermacoce latifolia* e antioxidante e realizar estudo fitoquímico. As análises fitoquímicas foram realizadas por meio de reações de caracterização e cromatografia de camada delgada. O teor de compostos fenólico (Folin-Ciocalteu), flavonoides ($AlCI_3$) foram realizados utilizando a espectroscopia, e a atividade antioxidante foi determinada pelo método DPPH (2,2-difenil-1-picrilhidrazil). Os resultados fitoquímicos indicaram a presença de compostos fenólicos; flavonoides; taninos, esteroides e triterpenos livre, cumarinas e alcaloides. O conteúdo de fenóis totais (482,7 ± 1,8 mgGA g^{-1}) e flavonoides (165,4 ± 1,5 mg QE g^{-1}) justificam a atividade antioxidante de 150 µg mL^{-1} do extrato metanólico. Nos bioensaios propostos usou grupos de 25 larvas de terceiro estádio, em diferentes concentrações das soluções dos extratos brutos (1,0; 0,5; 0,25 e 0,1 mg mL^{-1}) de peso por volume para quatro réplicas. Nos testes de concentrações múltiplas foram eleitas as concentrações que

produziram mortalidade entre 0% e 100%, após o período de 24 horas de contato com as soluções. Foi definida como toxidade a paralisação ou inatividade total das larvas. Conclui-se que o extrato metanólico apresentou CL_{50} de 0,625 mg L^{-1} e indica potencialidade larvicida para o *A. aegypti* e esta atividade pode estar ligada aos compostos fenólicos e flavonoides.

PALAVRAS-CHAVE: Controle de vetores. Princípios ativos vegetais. Plantas inseticidas.

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