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The effect of *Ageratum fastigiatum* extract on *Rhodnius nasutus*, vector of Chagas disease

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Abstract: Control of Chagas disease is based on insecticide spraying in domiciles in order to exterminate triatomine populations. However, since the vectors differ in susceptibility to currently used insecticides, the screening of the toxic potential of Brazilian flora may identify new molecules lethal to triatomines. This study evaluated the toxicity of ethanolic extract of *Ageratum fastigiatum* (Gardner) R.M. King & H. Rob., Asteraceae, on *Rhodnius nasutus*, a known vector of Chagas disease. Ethanolic extracts of the aerial parts of *A. fastigiatum* were prepared at 25 and 50 mg/mL concentrations, and 5 µL was applied to fifth-instar nymphs of *R. nasutus* (n=30). Controls included nymphs that were treated with 5 µL ethanol (n=30) or left untreated (n=30). The percentage of dead insects in each group was observed at 24, 48, 72, 96 and 120 h after application. The extracts of *A. fastigiatum* showed a mortality rate of about 37% and 77% after 120 h, at concentrations of 25 and 50 mg/mL, respectively. In control groups, the mortality rate remained under 7%. The extract of *A. fastigiatum* contains a coumarin, a molecule with recognized toxicity in insects, and which may be responsible for killing the triatomines.

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Introduction

Chagas disease is an anthropozoonosis widely spread over the American continent. The disease is endemic in 21 countries and estimated to affect 10 million people (Schofield et al., 2006). The majority of the human cases are caused by insect vector transmission, which occurs when the feces of triatomines infected by the protozoa *Trypanosoma cruzi* (Chagas 1909) come into contact with the open skin or mucous membranes of humans.

Given the predominance of vector transmission, which is responsible for 80% of the Chagas cases in humans, the chief means of controlling the endemic are based on combating triatomines in domiciles through the use of residual-action insecticides (Schofield, 1994; Coura & Dias, 2009). Since the 1980s, the pyrethroids class of insecticides has been the most utilized in the Chagas Disease Control Program in Brazil (Silveira & Dias, 2011). However, some populations of triatomines have shown resistance to these insecticides. Among these triatomines, especially noteworthy are populations of *Triatoma infestans* (Klug 1834) and *Rhodnius prolixus* (Stål 1859), two species which are greatly responsible for the transmission of *T. cruzi* (Vassena et al., 2000).

The plant genus *Ageratum fastigiatum* (Gardner) R.M. King & H. Rob., Asteraceae, belonging to the Asteraceae family, comprises approximately thirty species of tropical plants, only a few of whose phytochemical aspects have been investigated (Okunade, 2002). Among the species with biological activity studied so far, *A. conyzoide* has shown insecticidal potential against coleopterans (Moreira et al., 2007) and repellent potential against *Amblyomma cajennense* (Fabricius 1787) (Soares et al., 2010).

Little is known about the potential insecticidal properties of another member of this genus, *A. fastigiatum* popularly known as "matapasto" and employed as an anti-inflammatory agent. Despite the presence in its chemical composition of active

biological molecules such as diterpene, triterpene and their derivatives (Bohlmann et al., 1981; Bohlmann et al., 1983; Gonçalves et al., 2011), little in known about its toxic potential on insects. Thus, the aim of this study was to evaluate the toxic effects of an ethanol extract of this species on *Rhodnius nasutus* (Stål 1859), Chagas disease vector in northeastern Brazil.

Materials and Methods

Branches containing leaves and flower clusters of *Ageratum fastigiatum* (Gardner) R.M. King & H. Rob., Asteraceae, were collected in December 2006 in the J.K. campus of the Universidade Federal dos Vales de Jequitinhonha e Mucuri (S 18°12.220´ W 43°34.720´, altitude 1250 m.) in the city of Diamantina-MG. The material was deposited in the DIA/UFVJM herbarium in Diamantina under no. 1300 and its identification made by Dr. Carlos Victor Mendonça Filho and Dr. Fabiane Nepomuceno Costa of the Department of Biological Sciences of UFVJM. The collected material was dried at room temperature until reaching a constant weight. It was then ground into powder and the powder (500 g) macerated at room temperature, first in n-hexane, then in ethyl acetate and, lastly, in ethanol (3:1 p/v-Vetec analytic solvent) for a period of three days for each solvent (Gonçalves et al., 2011). For the experiments, the ethanolic extract was concentrated in a rotary evaporator (temperature below 40 °C), and the resulting raw dried extract was dissolved in ethanol at the concentrations of 25 e 50 mg/mL.

To evaluate the toxic activity of the extract, fifth-stage nymphs of *R. nasutus* that had not been nourished for a period of three days were chosen. The *Rhodnius* genus was selected due its high reproductive rate and ease of nurturing in a laboratory. It is important to point out that fifth-instar nymphs are more resistant to insecticides than nymphs in other developmental stages (Zerba et al., 1985). Nymphs individually placed into Petri dishes were divided into four experimental groups that were treated as follows: one group (n=30) received 5 µL of *A. fastigiatum* ethanolic extract at 25 mg/mL; another group (n=30) was treated with 5 µL of the ethanolic extract at 50 mg/mL; one control group (n=30) was left untreated; and in the solvent control group (n=30), nymphs were treated with 5 µL of ethanol PA. The ethanolic extract and ethanol PA were applied to the ventral thorax, in the stridulatory groove area, by using a micropipette. The experiment was carried out in one experimental series with thirty insects for each group.

The percentage of dead individuals in each group was observed at intervals of 24, 48, 72, 96 and 120 h after the extract and solvent were applied. Insects were considered deceased when they exhibited no motor activity when stimulated with a pincer (WHO, 1994). The statistical test chosen for group comparison was ANOVA *Two-way*, followed by the Bonferroni Multiple Comparison Test, which was done with a GraphPad Prism 5 program (GraphPad Software Inc., San Diego, CA, USA). The level of significance was considered 0.05.

Results and Discussion

The ethanolic extract of *Ageratum fastigiatum* (Gardner) R.M. King & H. Rob., Asteraceae, demonstrated toxic properties in concentrations of both 25 and 50 mg/mL. A mortality rate of 60% was observed among insects treated with the 50 mg/mL concentration extract in the first 24 h, demonstrating an acute effect at this concentration. For the 25 mg/mL concentration, an insect mortality rate of approximately 37% was observed after 120 h of contact with the extract, and for the 50 mg/mL concentration the mortality rate was around 77% for this time period. The mortality rate among the insects treated with the extract at a concentration of 50 mg/mL was significantly greater

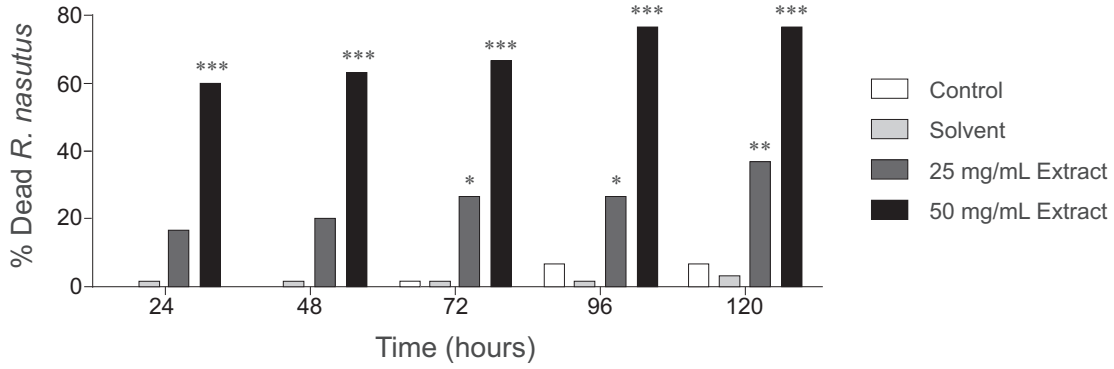

Figure 1. Percentage of dead *Rhodnius prolixus* nymphs in the presence of *Ageratum fastigiatum* ethanolic extract in two concentrations, 25 mg/mL (n=30) and 50 mg/mL (n=30); solvent group, in which the insects were treated with 5 µL of etanol (n=30); and the control group, in which the insects were left untreated (n=30). ANOVA *Two-way*, followed by the Bonferroni Multiple Comparison (*p<0.05, **p<0.01, ***p<0.001).

than the other groups at every interval of the analysis, while the 25 mg/mL concentration showed a greater toxicity than the control groups beginning 72 h after the application. The insect mortality rate of the control groups remained below 7% in all experiment periods (Figure 1).

Gonçalves et al. (2011) found the presence of coumarin 6.7-methylene-dioxide in the *A. fastigiatum* ethanolic extract. Coumarins are bioactive molecules whose toxic potential has been observed in coleoptera species and in agricultural pests (Khan et al., 2002; Razavi, 2011). It is possible that the presence of coumarin in this extract was responsible for killing the triatomines.

Other studies have demonstrated the effects of plant extracts on Chagas disease vectors. Coelho et al. (2006), studying the toxic effects of ethanolic extracts from five plant species of the Brazilian cerrado region, observed a mortality rate of up to 95% in fourth-instar nymphs of *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg 2001, 28 days after topic application of *Simarouba versicolor* St. Hil., Simaroubaceae, extract. Parra-Henao et al. (2007) noted a mortality rate of 76% and 93% in fourth-stage nymphs of *R. prolixus* and *Rhodnius pallescens* Barber 1932 treated with ethanolic extracts from four plant species commonly used by Colombian communities.

The use of residual-action insecticides, chiefly pyrethroids, to systematically combat triatomines constitutes the primary strategy of controlling Chagas disease. Although the pyrethroids are highly effective in exterminating triatomines, the appearance of resistant populations points out the necessity of finding new molecules which are toxic to these vectors (Vassena et al., 2000; Picollo et al., 2005; Germano et al., 2010; Lardeux et al., 2010; Silveira & Dias, 2011). In light of this, the significant mortality rates observed in the present study demonstrate that A. fastigiatum merits further study as to its potential use as an insecticide.

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Authors' contributions

BAAF contributed in extract preparation, assays of evaluation of insecticidal activity, analysis of data and manuscript writing. JVLD contributed to assays of evaluation of insecticidal activity, insects rearing and manuscript writing. GGT, CAR and GHBO contributed in insecticidal activity evaluation assays and insects rearing. CFFG contributed to plant sample collection, extract preparation and critical reading of the manuscript. HHRP designed the study, supervised the laboratory work and contributed to data analysis and critical reading of manuscript. All the authors have read the final manuscript and approved the submission.

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