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Short communication

Acaricidal activity of *Calea serrata* (Asteraceae) on *Boophilus microplus* and *Rhipicephalus sanguineus*

Vera Lucia Sardá Ribeiro [a], Cesar Avancini [a], Karla Gonçalves [a], Eduardo Toigo [b], Gilsane von Poser [b,*]

[a] *Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento, Gonçalves 9090, 90540-000 Porto Alegre, RS, Brazil*
[b] *Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000 Porto Alegre, RS, Brazil*

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Abstract

Calea serrata (Asteraceae) is an endemic Southern Brazilian plant species used for religious and medicinal purposes. Previous study revealed the presence of chromenes, a class of natural compounds that possess insecticidal properties. This study reports the effect of the hexane extract from the aerial parts of this plant on egg hatchability, egg production and mortality rates of newly hatched larvae of the cattle tick *Boophilus microplus*. Larvae of *Rhipicephalus sanguineus*, the brown dog tick, received the same treatment. The extract was toxic to the eggs of *B. microplus* and to the larvae of both *B. microplus* and *R. sanguineus*.

Keywords: *Calea serrata*; Chromenes; Precocenes; *Boophilus microplus*; *Rhipicephalus sanguineus*

1. Introduction

Calea serrata (Asteraceae), known by the vernacular names 'erva-de-cobra' (literally 'snake herb'), 'chá-amargo' ('bitter tea') or 'quebra-tudo' ('breaks everything'), is an endemic southern Brazilian species. The plant is used in Afro-Brazilian religious rituals and claimed to be useful for treating ulcers and liver diseases (Simões et al., 1990). Previous phytochemical study revealed the presence of chromenes (eupatoriochromene and precocene II) in the hexane extract (Steinbeck et al., 1997).

Chromenes represent a class of natural products with interesting biological properties. Several experiments have revealed the insecticidal effect of these compounds and some of them also have been shown acaricidal activity. The essential oil of *Ageratum houstonianum* flowers presented acaricidal effect on *Rhipicephalus lunulatus* and the toxicity was attributed to the chromenes or to a synergistic interaction between these components and other constituents of the essential oil (Pamo et al., 2004, 2005). A recent study has demonstrated that the extract of *Hypericum polyanthemum* (Guttiferae) containing chromenes was active against engorged females and larvae of *Boophilus microplus* (Ribeiro et al., 2007).

As the traditional approach to control tick infestations has been only partially successful due to the costs of acaricides, resistance and environmental contamination, new agents and or strategies are necessary. Searching for alternative anti-tick products a study to test the efficacy of the hexane extract of *C. serrata* against the cattle tick *B. microplus* and the brown dog tick *Rhipicephalus sanguineus* was designed.

* Corresponding author. Tel.: +55 51 33085456; fax: +55 51 33085610.
E-mail address: gilsane@farmacia.ufrgs.br (G. von Poser).

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2. Material and methods

2.1. Plant material

Aerial parts (leaves and stems) of *C. serrata* were collected in Guaiba, in March 2006. The plant was identified by Nelson Matzembacker (Programa de Pós-graduação em Botânica, Universidade Federal do Rio Grande do Sul, Brazil). Voucher specimen (ICN124883) was deposited in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN).

2.2. Extract preparation

Air dried and powdered plant material (200 g) was extracted by maceration for three times (72 h) with hexane. The extract was evaporated to dryness under reduced pressure, treated with acetone and subsequently filtered and evaporated affording an extract rich in chromenes and free of epicuticular waxes and other insoluble compounds. The solution used in the *adult immersion test* (AIT) was prepared using 1.25% Triton X-100 since ethanol was somewhat toxic to the engorged females (Gonçalves et al., 2007). For *the larval immersion test* (LIT) the hexane extract was diluted in ethanol since this solvent is not toxic to the larvae of *B. microplus*. Serial dilutions used in the AIT and in the LIT were made in water and ethanol, respectively, in order to obtain the concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. The same test-dilutions prepared for the LIT were used in the tests with *B. microplus* eggs.

2.3. Preparation of ticks

B. microplus and *R. sanguineus* females in the later stages of engorgement, measuring more than 4.5 mm, were collected from infested animals, washed with water and dried in paper toweling. The average weight of engorging ticks was 0.30 g. These females were used in the AIT or incubated at 27–28 °C and 70–80% relative humidity for ca. 2 weeks until the eggs were laid. These eggs provided the larvae used for the LIT.

2.4. Adult immersion test (AIT)

Groups of 12 (~3 g) *B. microplus* females were weighed and immersed for 5 min in the respective dilutions (10 mL) in a 50 mL Becker flask which was gently agitated (three replicates each at room temperature). The surfactant solution (1.25% Triton X-100) and water were used as the controls. Ticks were recovered from the solutions, dried and randomly placed in each Petri dish (5.5 cm diameter, 1.5 cm high). The Petri dishes were incubated at 27–28 °C, 70–80% relative humidity. After 14 days, the number of females laying eggs was recorded and the eggs were collected, weighed and observed. The eggs were placed in glass tubes, incubated at the same conditions and after 14 days the tubes were observed and the hatching rates of the different treatments were visually estimated and compared to the controls.

Percentage inhibition of egg laying was calculated as follows:

$$\text{index of egg laying (IE)} = \frac{\text{weight of eggs laid (g)}}{\text{weight of females (g)}}$$

$$\%\ \text{inhibition of egg laying} = \frac{\text{IE control group} - \text{IE treated group}}{\text{IE control group}} \times 100$$

The AIT was not performed with *R. sanguineus* due to the insufficient number of females for the experiment.

2.5. Larval immersion test (LIT)

The experiments, performed with *B. microplus* and *R. sanguineus*, were conducted by placing approximately 200 embryonated eggs (0.01 g) into bags made of TNT fabric (6 cm × 6 cm). The bags were incubated at 27–28 °C and 70–80% relative humidity for ca. 14 days, until the eggs started to hatch. After another 14 days of incubation, the bags containing the larvae ready for testing were immersed for 5 min in 10–20 mL of the test solutions (50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL). Ethanol and water were used as controls. After ca. 1 h to allow the solvents to evaporate, the bags were incubated at 27–28 °C and 70–80% relative humidity for 48 h. Larvae (alive and dead) were counted to assess percent mortality. Each treatment consisted of three replicates. Experiment with *B. microplus* larvae was repeated with lower dilutions of the extract (3.12 mg/mL and 1.56 mg/mL).

2.6. Effect on the egg hatchability

Approximately, 200 *B. microplus* embryonated eggs (0.01 g) were placed in glass tubes and immersed for 5 min in 500 μL of the test solutions. Subsequently, the solutions were decanted and after solvent evaporation the tubes closed with cotton plugs were incubated at 27–28 °C and 70–80% relative humidity for ca. 14 days, until the eggs started hatching. Ethanol and water were