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Bioactivity of Brazilian plant extracts on *Oncopeltus fasciatus*

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Abstract
The search for new compounds for developing insecticide agents has stimulated interest in researching natural products from plants. This study aimed to evaluate the potential of extracts obtained from Brazilian plants – *Caesalpinia peltophoroides* (Fabaceae), "Sibipiruna"; *Cecropia catarinensis* (Urticaceae), "Embaúba"; and *Bromelia antiacantha* (Bromeliaceae), "Gravatá" – as sources of biologically active substances against *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae), the insect vector of Phytomonas. Oral treatment with *C. peltophoroides* resulted in 40% mortality rates among nymphs (500 µg/mL) and 100% toxicity in the eggs (500 µg/mL). Oral treatment with crude methanolic extract of *C. catarinensis* produced 30% nymph mortality, oviposition reduction and 100% inhibition of egg hatching of *O. fasciatus*, while topical treatment resulted in 47% nymph mortality. Topical treatment with B. *antiacantha* showed 60% nymph mortality, and toxicity in the eggs (100 µg/mL) of *O. fasciatus*. These data suggest that the plants studied here can contribute new active compounds for insect control.

Keywords: Bromelia antiacantha, Caesalpinia peltophoroides, Cecropia catarinensis, Lygaeidae, *plants extract*.

1. Introduction
Natural products are organic substances that can be extracted from different sources, such as plants, animals and microorganisms [1]. The small molecules isolated from complex mixtures of natural origin constitute an important source of new chemical structures with a wide range of biological activity [2, 3]. These data corroborate Newman and Cragg [4], who reported that there had been a significant increase in the number of new substances of plant origin, from 2009 to 2010.
The Brazilian flora is renowned for its richness and biodiversity. The various metabolites present different potentials, which include use in organic agriculture and in integrated pest control [5].
Many plant species are depredated by insects that act as disease transmission vectors or that damage the plant tissue such that plant development is affected and the biosynthesis of secondary metabolites is qualitatively and quantitatively altered [6, 7]. It is worth emphasizing that these metabolites have an important role in plant defense against various pathogens and thus constitute a strategic chemical defense mechanism against insects [7].
More than 2,000 plant species are known to synthesize these secondary metabolites, and these are effective for controlling insects [8] through acting as larvicides [9]; inhibitors of feeding [10]; inhibitors of development [11, 12]; reducers of longevity [13]; reducers of fecundity [12]; and insecticides [14].
In the present study, species of the family Fabaceae (*Caesalpinia peltophoroides* Benth, 1870), Urticaceae (*Cecropia catarinensis* Cuatrec, 1959) and Bromeliaceae (*Bromelia antiacantha* Bertol) were evaluated as sources of bioactive metabolites. The species *C. peltophoroides*, known popularly as Sibipiruna is distributed mainly in Brazil: in the Atlantic rainforest region of Rio de Janeiro, in the south of Bahia and in the Pantanal, in Mato Grosso [15].
Species of the genus *Cecropia* are found in the tropical and subtropical regions of Central America and South America [16]. In Brazil, they have been found in the north (Amazon forest), center-west, southeast and south (Atlantic rainforest) [17, 18] and are popularly known as embaúba, imbaúba or umbaúba [19]. Among the species of this genus is *C. catarinensis*, which is generally used in popular medicine to treat asthma, bronchitis and cardiac diseases [20].

The family Bromeliaceae has around 60 genera and more than 1400 species. The species *Bromelia antiacantha*, a terrestrial species known as gravatá, which is a native plant of the Atlantic rainforest in southern Brazil, can be highlighted [17]. This bromeliad has cytotoxic, antibacterial, antifungal, molluscicidal activities [21].

The insect *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Lygaeidae) has been used as an experimental model because of its characteristics, morphology, life cycle and ease of handling and rearing. *O. fasciatus* is recommended as an experimental model for bioactivity trials [22, 23]. This species of Hemiptera is found in the central and southern regions of the United States. It frequently feeds on *Asclepias curassavica* Linnaeus (tropical milkweed) and is a vector for Phytomonas [22, 24]. As phytophagous insects, hemipterans attack the leaves of these plants and cause small chlorotic stains to appear. Consequent to these lesions, the plant leaves dry out and die [23].

The objective of this study was to evaluate the potential of extracts and fractions of *C. peltophoroides*, *C. catarinensis* and *B. antiacantha* for action on the physiology and development of *O. fasciatus*, while seeking new substances that might be potentially effective in controlling insect populations.

2. Materials and Methods

2.1 Plant Material

C. peltophoroides: Sibipiruna leaves were gathered from a public garden in the municipality of Mendes, RJ. An exsiccate was prepared by Dr. Ângela Studart da F. Vaz, who then made a botanical classification of the plant material, which has been registered under the code RB 417579 (RB refers to the herbarium of the Research Institute of the Botanical Garden of Rio de Janeiro).

C. catarinensis: Embaúba leaves were gathered at Hospital Eufrásia Teixeira Leite, in the municipality of Vassouras, RJ. An exsiccate was prepared by Dr. Douglas Siqueira de Almeida Chaves and classified by Dr. Jorge Pedro Carauta, and it was deposited at the Botanical Garden of Rio de Janeiro under the code R212. 173.

B. antiacantha: Leaves of *B. antiacantha* were gathered in Vassouras, RJ. An exsiccate was prepared by the laboratory technician Marcos de Aguiar Alves and classified by the master's professor Ana Carla Pinheiro, and it was deposited at the herbarium of Severino Sombra University, under the code HUSS BR 0004.

2.2 Extraction and Purification

C. peltophoroides: The dry and fractionated leaves (38.5 g) were subjected to an extraction process by means of decoction (15 min at 100 ºC) with distilled water (1 L). Filtration of the extract was done at room temperature by means of gravity, using cotton as a physical barrier. The final aqueous extract (600 mL) was lyophilized at the Natural Product Research Center (NPPN/UFRJ), which resulted in 1.8 g of lyophilized extract, coded as CP [25].

C. catarinensis: Extraction was performed on the dry pulverized leaves (20 g) using methanol at the Pharmacological and Chemical Studies Laboratory for Natural Products of the Severino Sombra University (LAEQUIFAR-PND/USS). The extract was obtained by means of exhaustive maceration in methanol, at room temperature [26]. It was gravity-filtered using cotton as a physical barrier and then dried in a rotating drier. The dry extract (3 g) was coded as crude methanol extract (CC). A sample of the dry extract (0.180 g) was treated with chloroform (3 x 10 mL). The soluble portion was filtered and dried, and was coded as the soluble chloroform fraction (CC1; 0.077 g). The insoluble portion was resuspended in methanol and dried in a rotating evaporator, and was coded as CC2 (0.089 g).

B. antiacantha: Extraction was performed on the fresh leaves (5 g) at LAEQUIFAR- PND, by means of exhaustive maceration in methanol [27]. The methanol extract was filtered using cotton as a physical barrier and dried in a rotating evaporator (0.4 g; BA). Part of the crude methanol extract (0.29 g) was treated with chloroform (3 x 15 mL), thus producing a soluble subfraction (BA1; 0.123 g) and an insoluble subfraction (BA2; 0.876 g).

2.3 Insects

In this study, fifth-instar nymphs of *O. fasciatus* were used. These were obtained from a colony at the Vector Insect Laboratory of the Severino Sombra University, Rio de Janeiro (LIV/USS). The insects were maintained by means of sunflower seeds, water and room temperature.

2.4 Biological Assays

The bioassays were carried out at LIV/USS, using 7-10 fifth-instar nymphs, in triplicate with three repetitions. The control group consisted of nymphs without the substance and without the dilution solvent and the witness group consisted of nymphs without the substance and with addition of the dilution solvent. The insects were deprived of water and food (sunflower seeds) for 24 hours before the treatment.

The aqueous extract of *C. peltophoroides* (CP) was dissolved in water at final concentrations of 200, 400 and 500 µg/µL. The bioassays with CP were carried out by means of oral treatment. The extract was added to the diet of the fifth-instar nymphs at the same concentrations (1 µL/mL); and to the egg mass (15 mg) at the concentration of 200 µg/µL. In the control group, the treatment consisted only of water.

The methanol extract of *B. antiacantha* (BA) was dissolved in acetone and that of *C. catarinensis* (CC) in ethanol or acetone. Both of them were then dissolved in saline solution (NaCl; 1:3). Subsequently, the bioassays were performed separately, added to the water of the diet (µL/mL) (oral treatment) and by means of topical application to the ventral part of the abdomen of the nymphs (µL/nymph), both applied at concentrations of 10, 50, 100 and 200 µg/µL.

After the treatments, the groups of treated insects and their controls were maintained with their normal diet (sunflower seeds and water) in a climate-controlled BOD chamber (biochemical oxygen demand) at 24.5 ± 1 ºC and 68 ± 10% relative humidity [28]. The groups were observed over a 15-day period regarding their development and mortality and for 35 days with regard to oviposition.

2.5 Statistical Analysis

The results obtained from the biological assays were subjected to the Tukey test and to analysis of variance (F- ANOVA). The criterion for considering the results to be significant was $P < 0.05$ [29], and the analysis was performed using the Graphpad Prism software [30].

3. Results and Discussion

The oral treatment using the aqueous extract of *C. peltophoroides* (CP) produced nymph mortality of 20% to 40% ($P < 0.01$) (200, 400 and 500 µg/mL) and adult mortality of 57 to 71% (Table 1 A). The longevity of the insects was reduced by 50% at the highest concentration (500 µg/mL)

(Table 1 A). The oral treatment performed previously on female nymphs (F1) (fifth instar) resulted in oviposition of 39 mg (F2) (400 µg/mL), in comparison with 33 mg of eggs in the control group (Table 1 B). However, the viability of these eggs was only 7.6% (F2) (400 µg/mL). At the concentration of 500 µg/mL, the aqueous extract of C. *peltophoroides* inhibited 100% of the egg-hatching of *O. fasciatus* (F2) (Table 1B). Application of the aqueous extract directly onto the egg mass at the concentration of 200 µg/mL, resulted in 26% egg viability. Only 23% of these nymphs reached adulthood (Table 1 C).

In this study, the aqueous extract of *C. peltophoroides* (CP) presented toxic activity towards the eggs of *O. fasciatus*. The toxic and growth-inhibiting action of this plant towards insects adds to its other forms of action already observed: ovipositional deterrent on *Aedes fluviatilis* (Lutz) [31], molluscicide [32], antibacterial agent [33], antimalarial agent [34]. Cavalheiro *et al* [35] evaluated the activity of the water extracts of seeds from *Caesalpinia ferrea* on the development of *Aedes aegypti* (Linnaeus) and reported whitin 24 hours, the extract showed 85% larval mortality. This result showed the high larvicidal efficacy of seeds from *C. ferrea* on found by Luna *et al* [36] in ethanol extract of leaves.

Table 1: Bioassays using the crude aqueous extract of *C. peltophoroides* (CP) on fifth-instar nymphs of *O. fasciatus.* Duration of development, change, mortality and longevity, according to oral treatment (1A). Oviposition and egg viability, according to previous oral treatment (fifth instar) (1B). Topical treatment on egg mass (mg) (1C).

Treatment	**Period (days)**		**Change**		**Nymph mortality**	**Adult mortality**	**Longevity of adults**
A	X ± SD	R	X ± SD	%	%	%	%
Control	2.7 ± 1.4 a	2-6	7 ± 1.0 a	100	-	14.2	72
200 µg/mL	2.2 ± 0.4 a	2-3	8 ± 2.0 a	100	20	71.4	25
400 µg/mL	3.4 ± 2.4 a	2-7	7 ± 1.0 a	100	30	57.1	28.5
500 µg/mL	3.5 ± 2.3 a	2-7	6 ± 1.0 a	100	40	28.6	50

B	**mg of eggs/female**	**Nymph viability (F2)**		**Female/macho**
	Mg	**%**	**N**	**SR**
Control	33	29	95	0.2
200 µg/mL	22	24.5	54	0.1
400 µg/mL	39	7.6	30	0.5
500 µg/mL	20	-	-	-

C	**Eggs (mg)**	**Nymph viability (F2)**		**Adult viability**	
	X ± SD	**X ± SD**	**%**	**X ± SD**	**%**
Control	5 ± 0	6 ± 1 a	12	6 ± 1.0 a	100
200 µg/mL	5 ± 0	13 ± 2.0 a	26	3 ± 1.0 a	23

Bioassays using the crude aqueous extract of C. peltophoroides (CP) *on 10 fifth-instar nymphs of O. fasciatus at the concentrations of 200, 400 and 500 µg/mL, according to group. Mean and standard deviation values (X ± SD) from triplicates with three repetitions. R = range; SR = sex ratio; F2 = second generation. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used.*

The oral treatment using crude methanol extract of *C. catarinensis* (CC) on fifth-instar nymphs of *O. fasciatus* totally inhibited egg-hatching (50 and 100 µg/mL) and significantly reduced oviposition (9 ± 2.6) (Table 2, CC), presenting 13 eggs/female. The same extract and treatment presented 30% nymph mortality (100 µg/mL) (Table 2, CC). The methanol fraction of *C. catarinensis* (CC-2), applied orally, altered the duration of nymph development (6.1 ± 2.3) and caused mortality of 33% ($P < 0.05$) and 50% ($P < 0.05$), among nymphs and adults, respectively (Table 2, CC2). The same methanol fraction (CC2) reduced oviposition (25.6 ± 3.5; $P < 0.05$) (Table 2, CC2).

Table 2: Duration of development, change, mortality, longevity and oviposition of *O. fasciatus* in oral treatments using the crude methanol extract (CC), chloroform fraction (CC1) and methanol fraction (CC2) of *C. catarinensis.*

Treatment	**Duration (days)**		**Changes**		**Nymph mortality**	**Adult mortality**	**Oviposition**	**Egg viability**
CC	X ± SD	R	X ± SD	%	%	%	X ± SD	%
Control	9.4 ± 1.8	8-14	5.6 ± 0.5	100	23	0	23.6 ± 6 a	11
Acetone	9.8 ± 2.5	7-14	6 ±1.0	100	10	5.5	68.3 ± 6.5 b	19
10 µg/mL	9.7 ± 1.8	7-14	6.6 ± 0.5	100	4.7	10	85.6 ± 9.4 c***	9
50 µg/mL	10 ± 1.9	7-14	5.6 ± 1.5	100	15	5.8	39 ±7 d*	-
100 µg/mL	9.3 ±1.5	7-11	4.6 ± 0.5	100	30	0	9 ± 2.6 e***	-
CC1								
Control	6.8 ± 2.7	1-12	7.6 ± 0.5	100	14.1	8.7	12.6 ± 21.9	95.2
Acetone	6.3 ± 2.8	1-12	8.6 ± 0.5	100	3.7	7.6	22.3 ± 21.1	100
10 µg/mL	8.4 ± 3.6	1-12	7.6 ± 0.5	100	14.8	4.3	33 ± 32	91
50 µg/mL	7 6 ± 3.3	1-12	7 ± 1.7	100	18.5	23.8	34.6 ± 30.2	93.7
100 µg/mL	6.4 ±2.7	1-12	8 ± 1	100	11.1	12.5	34 ± 4.2	95.2
CC2								
Control	6.9 ± 0.9 [a]	6-9	10 ± 0	100	0	24	146 ± 52.1 a	79.4
Acetone	8.1 ± 2.1 [a]	6-13	9.1 ± 1	100	10	18.5	98. 9 ± 2 a	77.2
10 µg/mL	6.9 ± 1.7[a]	1-9	7 ± 1.7	95.5	26.6	18.1	22 ± 9 b*	72.2
50 µg/mL	7.6 ± 3.5 [a]	1-12	7.3 ± 1.5	88	19.3	33.3	41 ± 19.6 a	42.8
100 µg/mL	6.1 ± 24 b*	1-9	6.3 ± 2	95	33.3	50	25.6 ± 3.5 b*	60

*Bioassays on 7-10 fifth-instar nymphs of O. fasciatus. Mean and standard deviation values (X ± SD) from triplicates with three repetitions. R = range. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, ***P < 0.001 vs acetone control.*

The crude methanol extract of *C. catarinensis* (CC) used as topical treatment reduced the longevity of *O. fasciatus* by 25% and caused 33% adult mortality (Table 3, CC). The methanol fraction (CC2) with the same treatment (100 µg/nymph) caused 46.8% and 35.3% mortality among nymphs and adults, respectively. Furthermore, the longevity of the insects was reduced by 50% (Table 3, CC2).

Table 3: Duration of development, change, mortality, longevity and oviposition of *O. fasciatus* in topical treatments with the crude methanol extract (CC), chloroform fraction (CC1) and methanol fraction (CC2) of *C. catarinensis*, at the concentrations of 10, 50 and 100 µg/mL, according to group.

Treatment	Duration (days)		Change		Nymph mortality	Adult mortality	Longevity	Oviposition
CC	X ± SD	R	X ± SD	%	%	%	%	X ± SD
Control	4.4 ± 2.3	1-6	6.6 ± 1.1 a	100	5	-	75	61.3 ± 4.7 a
Ethanol	4.3 ± 2.2	1-8	6.4 ± 1.2b	100	6	3	75	14.3 ± 4 b
10 µg/nymph	5 ± 2.8	1-12	5 ± 0.5 ab***	100	12	7	75	39 ± 2 c***
100 µg/nymph	4.1 ± 2	1-11	4 ± 0 c**	92.3	13	33	25	37.6 ± 4 c***
CC1								
Control	13.7 ± 5.6	6-21	4.3 ± 1.5	100	51.8	38.4	76.9	21.3 ± 3.2 a
Acetone	10.4 ± 5.2	1-21	5.6 ± 0.5	100	37.0	58.8	75	37 ± 13.5 a
10 µg/nymph	10.3 ± 4.8	2-21	6 ± 0	100	33.3	22.3	64.2	-
50 µg/nymph	8.3 ± 3.5	1-13	6.3 ± 2.0	100	29.6	21	80	3.6 ± 6.3 b*
100 µg/nymph	9.0 ± 4.6	1-21	8 ± 1.0	100	11.1	16.6	85	3.3 ± 3.3 b*
CC2								
Control	6.2 ± 2.9 a	1-13	10.0 ± 0 a	100	23.9	38.4	71.8	144.3 ± 46.3 a
Acetone	6.3 ± 1.7 a	2-10	7.6 ± 0.5 b	100	23.3	17.39	76.9	53 ± 19.3 b
10 µg/nymph	8.3 ± 3.3 a	6-17	6.6 ± 0.5 c	95.2	30	23.81	64.2	49 ± 17.6 b
50 µg/nymph	8.2 ± 3.0 a	1-14	6.0 ± 2.0 c	81.8	26.6	27.27	80	20.6 ± 7.0 c
100 µg/nymph	8.4 ± 3.6 a	5-16	5.3 ± 2.0 c	94.1	46.8	35.29	50	11.6 ± 9.0 c

*Bioassays on 7-10 fifth-instar nymphs of O. fasciatus. Mean and standard deviation values (X ± SD) from triplicates with three repetitions. R = range. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, ***P < 0.001 vs acetone control.*

The topical treatment using crude methanol extract of *C. catarinensis* (CC), directly on the egg mass (100 µg/mL) gave rise to egg-hatching of only 5.8%, in comparison with the ethanol witness control group (Table 4).

From these results, the main efficient activity of *C. catarinensis* (CC) was through the methanol fraction (CC2), with regard to nymph mortality (47%), inhibition of oviposition (11.6 ± 9.0) and reduction of the insects' lifespan (50%). It was through the crude methanol extract with regard to hatching of *O. fasciatus* eggs (6%).

A few studies are related to Cecropia species as insecticidal activity. Mention Heal *et al.* [37] on *American cockroach*, *milkweed bug* and *German cockroach* showing 20-50% of adult mortality in bloodstream injection and immersion treatment, respectively.

Table 4: Topical treatment with crude methanol extract of *C. catarinensis* (CC) at the concentration of 100 µg/µL, on the egg mass of *O. fasciatus*.

Treatment	Eggs	Nymphs		Weight (mg)
	N	X ± SD	%	X ± SD
Control	340	16 ± 6.5 a	14.1	10 ± 0.003 a
Ethanol	340	22 ± 2.0 a	19.1	10 ± 0.003 a
100 µg/µL	360	7 ± 1.7 b**	5.8	1 ± 0.001 b*

*Bioassays on 340-360 eggs per group, in triplicates with three repetitions. Mean and standard deviation values (X ± SD) and percentages (%). Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, ***P < 0.001 vs ethanol control.*

The oral treatment with the extract of *B. anticantha* (BA) presented 60% toxicity towards the nymphs and 50% (3 ± 0.6) (P < 0.001) inhibition of ecdysis (200 µg/mL) (Table 5, BA). The same treatment with the chloroform subfraction (BA1) of *B. antiacantha* did not show inhibition of change, but extended the insects' duration of development, by 20 to 25 days at all concentrations (Table 5, BA1). The same subfraction gave rise to 57% toxicity towards the nymphs, 45% adult mortality and 45-56% reduction in lifespan for this hemipteran (Table 5, BA2).

The methanol subfraction (BA2) showed toxicity of 46% towards the nymphs of the phytophagous insect (200 µg/mL) (Table 5, BA2).

Use of the crude methanol extract of *B. antiacantha* (BA) for previous topical treatment on female fifth-instar nymphs (F1) totally inhibited egg-hatching (100 and 200 µg/nymph) (Table 6, BA). In the same treatment (50 µg/nymph), 49% of the eggs were shown to be viable, and only 10% (3 ± 2.6) of these nymphs reached adulthood (Table 6, BA).

Table 5: Duration of development, ecdysis, mortality and longevity of *O. fasciatus* in oral treatments with the crude methanol extract (BA), chloroform fraction (BA1) and methanol fraction (BA2) of *B. antiacantha,* at the concentrations of 50, 100 and 200 µg/mL, according to group.

Treatment	Duration in days		Changes		Nymph mortality	Longevity
BA	X ± SD	R	X ± SD	%	%	%
Control	7 ± 1.4 a	5-9	12 ± 1.1 a	100	20	100
Acetone	8.2 ± 0.4 a	8-9	9 ± 0.5 a	100	20	100
50 µg/mL	8.4 ± 2.5 a	5-12	9 ± 1.0 a	100	40	100
100 µg/mL	7.5 ± 1.7 a	5-9	4 ± 0.6 b*	67	60	60
200 µg/mL	9 ± 0 a	9-9	3 ± 0.6 c***	50	60	100

Treatment	Duration in days		Change		Nymph mortality	Adult mortality	Longevity
BA1	X ± SD	R	X ± SD	%	%	%	%
Control	11.6 ± 3.5	4-14	3.6 ± 1.8	100	47.7	-	100
Acetone	10.2 ± 3.4	4-13	4.6 ± 1.5	100	33.3	14.3	86
50 µg/mL	15.2 ± 7.6	4-25	3.3 ± 1.5	100	47.6	18.2	73
100 µg/mL	12.1 ± 6.1	6-20	3.6 ± 2.0	100	47.6.	9.1	45
200 µg/mL	11.7 ± 6.2	4-25	3.0 ± 1.0	100	57.1	44.4	56
BA2							
Control	12.1 ± 3.6	4-16	4.6 ± 0.5	93	28.6	7.1	50
Ethanol	12.1 ± 4.7	4-20	3.0 ± 1.7	100	33.3	14.3	64
50 µg/mL	11.1 ± 4.6	4-16	3.3 ± 1.1	77	38.1	-	100
100 µg/mL	15.1 ± 4.7	7-25	3.3 ± 1.1	100	52.4	10	70
200 µg/mL	11.6 ± 3.0	4-13	3.3 ± 2.5	91	46.6	10	70

*Bioassays on 7-10 fifth-instar nymphs of O. fasciatus. Mean and standard deviation values (X ± SD) from triplicates with three repetitions. R = range. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, ***P < 0.001 vs acetone control.*

Table 6: Mean and percentage oviposition and viability of the F2 nymphs and adults of *O. fasciatus* after previous topical treatment (F1) with the crude methanol extract (BA) chloroform fraction (BA1) of *B. antiacantha* at the concentrations of 10, 50, 100 and 200 µg/nymph.

Treatment	Egg viability		Nymph-adult viability		Egg-adult viability	
BA	X ± SD (mg)	%	X ± SD	%	X ± SD	%
Control	33 ± 14.7 a	96	31.6 ± 8.1 a	29	9.3 ± 1.1 a	28
Acetone	31 ± 3.5 a	62	19 ± 0 a	36.8	7 ± 4.3 a	23
10 µg/µL	18.3 ± 4.5 a	80	26.6 ± 6.1 a	15	5 ± 0 b	15
50 µg/µL	28.6 ± 10.1 a	49	14 ± 6.0 b	21	3 ± 2.6 b	10
100 µg/µL	29.6 ± 13.6 a	-	-	-	-	-
200 µg/µL	29.3 ± 23.5 a	-	-	-	-	-

Treatment	Egg viability		Nymph viability		Adult viability	
BA1	X ± SD (mg)	%	X ± SD	%	X ± SD	%
Control	87 ± 2.0 a	9	7.6 ± 0.6 a	82	6.3 ± 1 [a]	7
Acetone	135 ± 20.5 b	9	12 ± 0.5 b***	33	4 ± 0.6 b*	9
50 µg/µL	121 ± 4.5 c	12.3	15 ± 0.5 c***	15	2.3 ± 0.3 b	6
100 µg/µL	107 ± 4.0 c*	8.7	9.3 ± 0.3 a**	36	3.3 ± 0.3 b	3.2
200 µg/µL	103 ± 3.0 c**	0.3	1 ± 0 d***	100	1 ± 0 c**	0.3

*Treatment on fifth-instar nymphs of O. fasciatus. Mean and standard deviation values (X ± SD) from experiments in triplicate with three repetitions. R = range. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, **P < 0.01 vs acetone control.*

Use of the crude methanol extract of *B. antiacantha* (BA) for topical treatment reduced the longevity of the *O. fasciatus* adults by 50% (Table 7, BA). These data demonstrated interference by *B. antiacantha* on the lifespan of this hemipteran, and described its use in topical treatment through the crude methanol extract (BA) and methanol fraction, and in oral treatment through the chloroform subfraction (BA1). The crude methanol extract (BA) and chloroform subfraction (BA1) of *B. antiacantha* showed toxicity of around 40-60% towards the nymphs of *O. fasciatus*. Guimarães *et al* [38] confirmed that bromeliads have toxic action, through finding that the crude hexane extract of *Neoregelia compacta* (Bromeliaceae) on *A. aegypti* larvae possesses toxic activity of 45% (200 µg/mL).

Table 7: Duration of development, ecdysis, mortality and longevity of *O. fasciatus* in topical treatments using the crude methanol extract (BA), chloroform fraction (BA1) and methanol fraction of *B. antiacantha* (BA2).

Treatment	Duration (days)		Change		Nymph mortality	Adult mortality	Longevity
BA	X ± SD	R	X ± SD	%	%	%	%
Control	6.5 ± 3.5 a	3-11	5.3 ± 2.0 a	100	20	25	62
Acetone	8.8 ± 2.7 ab	3-11	3.6 ± 0.5 a	100	21	36	91
10 µg/µL	8.8 ± 2.0 ab	7-11	4.3 ± 0.5 a	100	13	7.7	77
50 µg/µL	10 ± 1.8 b*	7-11	4 ± 0 a	100	14	8.3	66
100 µg/µL	8.8 ± 3.1 ab	3-11	4.3 ± 0.5 a	100	13	-	39
200 µg/µL	7.6 ± 2.3 ab	3-11	4 ± 1 a	100	14	-	50
Treatment	**Duration (days)**		**Change**		**Mortality**		**Longevity**
BA1	X ± SD	R	X ± SD	IV	%		%
Control	9.2 ± 1.3 a	8-14	20 ± 0 a	100	-		90
Acetone	9.3 ± 1.2 a	7-11	19 ± 1 a	100	5		84
50 µg/µL	9.2 ± 0.8 a	8-11	19 ± 2.6 a	100	5		83
100 µg/µL	8.9 ± 0.6 a	8-9	16 ± 4 a	94	15		80
200 µg/µL	9 ± 0.5 a	8-11	18 ± 1 a	95	5		87
Treatment	**Duration (days)**		**Change**		**Nymph mortality**	**Adult mortality**	**Longevity**
BA2	X ± SD	R	X ± SD	%	%	%	%
Control	5.8 ± 1.3 a	2-7	6 ± 1 a	100	14.3	18	82
Methanol	4.7 ± 1.2 ab	2-7	6 ± 1 a	100	14.3	-	100
50 µg/µL	5 ± 1.5 ab	2-7	4.6 ± 1.1 a	70	4.8	-	100
100 µg/µL	4.25 ± 1.3 b	2-5	5.6 ± 0.5 a	85	4.8	6	94
200 µg/µL	4.7 ± 1.2 ab	2-7	5 ± 1.7 a	79	9.5	34	67

*Treatment on fifth-instar nymphs of O. fasciatus. Mean and standard deviation values (X ± SD) from experiments in triplicate with three repetitions. R = range. Values followed by the same letter did not have significance (P > 0.05) when the Tukey test was used. Significance levels are represented by *P < 0.05, **P < 0.01 vs acetone control.*

The plants presented in this study contain great structural diversity of natural substances that could be related to the insecticide action reported here. Chemical classes such as saponins, terpenes, steroids, flavonoids and tannins [39, 40, 41] are well known in these species.

It should be emphasized that this is the first report on the toxic activity of the species studied here, and that the process of isolation and purification of the extracts and fractions from these species is still in progress, with the objective of identifying the active substances acting against the insect model.

Substances of plant origin are generally biodegradable and do not harm the environment or humans. Thus, if a plant extract, a fraction of this extract or even a secondary metabolite that could be used in developing an insecticide formulation were to be found, this would constitute a very interesting alternative, in comparison with the synthetic compounds currently used for controlling insects.

4. Conclusion

The results from this study demonstrated the potential of the species *C. peltophoroides*, *C. catarinensis* and *B. antiacantha* for future use in insect population control.

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