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Insecticidal Activity of Cerrado Plant Extracts on *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg (Hemiptera: Reduviidae), under Laboratory Conditions

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Atividade Inseticida de Extratos de Plantas do Cerrado em *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg (Hemiptera: Reduviidae), em Condições de Laboratório

RESUMO - A transmissão da doença de Chagas ocorre, principalmente, por meio de fezes de hemípteros hematófagos (Triatominae), os quais ingerem *Trypanosoma cruzi* ao se alimentarem do sangue de pessoas ou animais infectados. Para o controle dos triatomíneos, os piretróides são os principais inseticidas utilizados. Entretanto, algumas populações de insetos demonstraram resistência a determinados piretróides, indicando a necessidade do desenvolvimento de novos inseticidas eficazes no controle desses vetores. Assim, foi avaliada a atividade inseticida de 24 extratos vegetais em ninfas do quarto estádio de *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg, em condições de laboratório. Para o teste tópico, foram aplicados 50 µg de cada extrato nos tergitos abdominais de dez ninfas, em duplicata. Como controles, foram utilizados insetos tratados com etanol, acetona ou sem nenhum tipo de tratamento. Os triatomíneos foram observados durante 28 dias. Extratos hexânicos e etanólicos de *Simarouba versicolor*, *Guarea kunthiana*, *Guarea guidonia* e *Talauma ovata* causaram mortalidade entre 20% e 95% de *R. milesi* em comparação com os controles, onde não houve mortalidade dos insetos. Estes dados preliminares sugerem que o extrato etanólico da casca da raiz de *S. versicolor* e o extrato hexânico da raiz de *G. guidonia*, os quais foram responsáveis pela mortalidade de 95% e 75%, respectivamente, devem ser quimicamente investigados e monitorados por ensaios biológicos a fim de determinar seus componentes inseticidas, a serem utilisados como modelos moleculares ou como compostos biorracionais nos programas de controle de insetos.

PALAVRAS-CHAVE: Triatominae, extrato vegetal, controle de insetos, *Simarouba versicolor*, *Guarea guidonia*

ABSTRACT - Chagas' disease is chiefly transmitted by feces of haematophagous bugs (Triatominae) that ingested *Trypanosoma cruzi* from blood of infected people or animals. Pyrethroids have been the main insecticides used against these insects. However, some populations of insects have shown significant levels of resistance to several pyrethroids, indicating the need of new insecticides for the control of triatomines. Insecticidal activity of 24 Cerrado plant extracts belonging to five species of four families were assayed on fourth instar nymphs of *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg (Hemiptera: Reduviidae), under laboratory conditions. For the extract application on triatomines, 50 µg of the extract were topically applied in duplicate on dorsal tergites of ten insects. Insects topically treated with acetone, ethanol, as well as insects with no treatment were used as controls. Triatomines were observed over a 28-day period. Hexanic and ethanolic extracts of *Simarouba versicolor*, *Guarea kunthiana*, *Guarea guidonia* and *Talauma ovata* caused mortality between 20% and 95% of *R. milesi* in comparison with the controls, which showed no insect mortality. These preliminary data suggest that the ethanolic extract of the root bark of *S. versicolor* and the hexanic extract of the root of *G. guidonia*, responsible for a 95% and 75% insect mortality, respectively, should be chemically investigated and monitored through biological assays in order to determine their insecticidal components, that could be used as a molecular model or as biorational compounds for use in insect control programmes.

KEY WORDS: Triatomine bug, crude extract, insect control, *Simarouba versicolor*, *Guarea guidonia*

Chagas' disease, also known as American Trypanosomiasis, is caused by the flagellated protozoan *Trypanosoma cruzi* Chagas and its transmission to vertebrate hosts is carried out by haematophagous insects, from the Triatominae subfamily, through feces contamination via mucosa or skin wounds. It is a chronic debilitating illness that is highly prevalent in Latin America and ranges from Mexico to southern Argentina (WHO 2002). Transmission through blood transfusions, orally, congenitally or by organ transplants is also possible (Miles *et al*. 2003).

In the absence of vaccines and drugs suitable for the treatment of Chagas' disease, the most important control strategy is prevention. Control of the disease is based on two main strategies: interrupting transmission by the vector, and systematic screening of blood donors in all endemic countries, as established by a World Health Assembly resolution (WHO 2002).

Despite the low resistance exhibited by triatomine bugs to insecticides when compared with mosquitoes (Flores *et al*. 2001), some populations of *Rhodnius prolixus* Stål from Venezuela, and *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) from Brazil (Zerba 1999, Vassena *et al*. 2000, Picollo 2001, Vassena & Picollo 2003), and Argentina (Zerba 1999, Picollo 2001, Vassena & Picollo 2003, Audino *et al*. 2004) have shown significant levels of resistance to deltamethrin and other pyrethroids used in the control of Chagas' disease vectors. Thus, the development of new insecticides from plant extracts sources can be an alternative for the control of triatomine bugs.

Moreover, the indiscriminate use of synthetic insecticides has caused environmental contamination and toxicity to living organisms (Raizada *et al*. 2001, Abdollahi *et al*. 2004, Nakata *et al*. 2005), indicating the need for the development of products that are not hazardous to the environment. Furthermore, the principal effects of acute or subchronic exposure to pyrethroids observed in mammals are also indicative of neurotoxicity (Soderlund *et al*. 2002, Kolaczinski & Curtis 2004, Shafer *et al*. 2005).

Plants produce secondary metabolites acting as defense mechanisms that can be used in pest control. These insecticidal substances can affect the feeding behavior and growth regulators, disrupting the endocrinological balance of the insects (Balandrin *et al*. 1985). An example is azadirachtin, a biopesticide obtained from the neem tree (*Azadirachta indica*), which could be readily biodegradable, selective, non-mutagenic, with low toxicity to mammalian, causing minimal effects on the environment (Sundaram 1996, Gupta 2004).

Several studies have shown insecticidal activity of plants extracts against triatomine bugs: *Salvia cardiophylla*, *Annona reticulada*, *Neurolaena lobata, Tagetes minuta, Erythroxylon tortuosum, Cassia* sp., *Senna occidentalis, Cabralea canjerana* elicited an increase in the mortality rate (16% to 52%) of *Rhodnius neglectus* Lent (Schmeda-Hirschmann & Rojas de Arias 1992); *Achyrocline satureioides* killed 45% of *T. infestans* which were topically treated (Rojas de Arias *et al*. 1995); *Coriandrum sativum, Chenopodium ambrosioides, Pimpinella anisum, Tagetes pusilla, Mentha arvensis, Satureja* sp., *Foeniculum vulgare, Chrysanthemum parthenium, Hedeoma mandoniana, Eucalyptus globulus* showed insecticidal activity on *T. infestans* (Laurent *et al*. 1997); and *Minthostachys andina* showed insecticidal properties on *T. infestans* and *R. neglectus* (Fournet *et al*. 1996). These studies are important for the development of new plant substances against Chagas' disease vectors. The diversity flora of Brazilian Cerrado, the country's second most important biome, has been poorly studied to evaluate the efficacy and therapeutic effects of crude extracts or isolated compounds (Espindola *et al*. 2004, Napolitano *et al*. 2005, Mesquita *et al*. 2005). In this sense, the aim of our study was to investigate the insecticidal activity of 24 plant crude extracts on fourth instar *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg nymphs.

Materials and Methods

Plant material. Plants were collected in Brasília, the Federal District of Brazil, in 2002/2003. Botanical identification was performed by Professor José Elias de Paula of the Plant Anatomy Laboratory, Institute of Biology, University of Brasília. The botanic specimens vouchers are deposited at the Herbarium (UB) of the same institution (Table 1).

Preparation of extracts. The air-dried and powdered parts of the plants were submitted first to exhaustive extractions with hexane (4 x 2 L), and successively with 95% ethanol

Table 1. Botanic voucher specimens and ethnobotanical information of the plants used in our experiments.

Plant family	Scientific name	Common name	Uses	Voucher number
Apocynaceae	*Aspidosperma macrocarpon* Mart.	Peroba-gigante-do-cerrado	Antimalaric, anti-inflammatory	(UB) 3692
Magnoliaceae	*Talauma ovata* A. St. Hil.	Baguaçu	Antidiabetic, febrifuge	(UB) 3738
Meliaceae	*Guarea guidonia* (L.) Sleumer	Açafroa	Astringent, purgative, febrifuge, abortive, emetic, anti-inflammatory	(UB) 3712
	Guarea kunthiana A. Juss.	Jatuaúba	Antimalaric, stomachache	(UB) 3710
Simaroubaceae	*Simarouba versicolor* A. St. Hil.	Mata-barata	Insecticide, vermifuge, febrifuge, antisyphilitic	(UB) 3724

(4 x 2 L) through a maceration process. The crude extracts were obtained after the evaporation of the solvents under reduced pressure at 40ºC.

Insects. Fourth instar nymphs of *R. milesi* from an asynchronous laboratory colony were fed to repletion on chickens. After 24h, individual insects were treated topically with a Gilson® 0.5–10 µl pipette, containing an aliquot of each one of the test extracts. The insects were maintained at 28°C under 60-70% relative humidity.

Topical test. A stock solution containing 50 mg of extract per ml of solvent was prepared for each sample, and 1 µl of the solution was applied directly on the abdominal tergites of ten fourth instar nymphs of *R. milesi.* The experiment was carried out in duplicate. The hexane extracts were dissolved in acetone, while the ethanolic extracts were dissolved in 95% ethanol. Three types of controls were used: insects topically treated with acetone, insects topically treated with ethanol, and insects with no treatment. The test and control insects were observed daily for 28 days, under the same conditions of temperature and humidity described above, without feeding.

Statistical analysis. The Cochran test was used to test the statistically significant differences between the treatments and the controls (Bisquerra *et al*. 2004).

Results and Discussion

Twenty four hexanic and ethanolic extracts of Cerrado plants belonging to five species of four families were assayed on fourth instar *R. milesi* nymphs. The topical application test on fourth instar nymphs is recommended by WHO (1994).

Table 2 shows that the crude extracts of *Simarouba versicolor* (root bark hexane and ethanol; stem bark ethanol; fruit hexane and ethanol), *Guarea kunthiana* (root ethanol; stem hexane and ethanol), *Guarea guidonia* (root hexane and ethanol; stem hexane and ethanol; leaves hexane) and *Talauma ovata* (stem wood hexane) caused a mortality rate between 20% and 95% ($P < 0.05$; Cochran test) of *R. milesi* in comparison with the controls, which showed no insect mortality. Significant differences were found among different plant parts of the same species. Among the 14 active extracts, seven were ethanolic and seven were hexane. However, the polarity of the substances contained in the hexanic and ethanolic extracts presented different activity rates, for exemple, with the root bark of *S. versicolor* the mortality rate was 35% for hexane and 95% for ethanol, and with the root of *G. guidonia* the mortality rate was 75% for hexane and 20% for ethanol. The different extracts of *S. versicolor*, and of *G. guidonia* were the most active, showing a good insecticidal activity when topically tested, producing better results than those reported in the literature (Rojas de Arias & Schmeda-Hirschmann 1988, Fournet *et al*. 1996, Laurent *et al*. 1997).

When the extracts were compared at different exposure times, significant differences were observed. The evaluation of the mortality rates (Table 2) shows extracts with rapid and slow activity on the insects. For the species *S. versicolor*, the ethanolic root bark extract killed practically all the insects of the sample with activity gradually increasing over time, killing 15% of the insects after 24h, 35% after 48h/72h, 50% in seven days, 90% in 14 days, and 95% of the insects in 21 days, remaining at this level until the 28th day of contact. This gradual increase in the mortality rate may represent a desired feature, since reinfestation of sprayed houses can be a major problem caused by lack of sufficient residual effect of insecticides (Schofield & Dias 1991, Moncayo 2003). The hexanic extract of the same plant part presented lower and lesser intense activity, causing mortality in 5% only after seven days of contact, reaching 35% after 28 days of treatment. The fruit of the same species presented a gradual increase in the mortality rate for the extracts of different polarities. The ethanolic extract presented a 35% increase in mortality between the 7th and 14th day of contact, while the hexanic extract increased this rate by only 10%. The stem bark presented activity only for the ethanolic extract, gradually increasing mortality from 5% to 50% between the first and the last day of treatment.

The hexanic stem wood extract of *T. ovata* presented less intense but more rapid activity, with 20% mortality after 48h, maintaining this rate until the 28th day. However, the hexanic stem extract of *G. kunthiana* presented the most rapid activity since it killed 25% of the bugs 24h after the application, and 40% after seven days; but this percentage did not increase for the remainder of the experiment. If we were to compare the hexanic root extract of the two species of *Guarea*, *G. kunthiana* and *G. guidonia*, only the latter was active, with a mortality rate of 75% after 28 days of application, thus demonstrating different sensitivities to these compounds. The evaluation of the three extracts of *Aspidosperma macrocarpon* did not show any insecticidal activity.

S. versicolor is traditionally regarded as having some insecticidal effect. *S. versicolor* is also used as a vermifuge, stimulant, febrifuge and antisyphilitic (Balbach 1995). Arriaga *et al*. (2002) isolated quassinoids, triterpenoids, a mixture of steroids, the flavonoid kaempferol, and a squalene derivative from *S. versicolor*. The medicinal use of this plant is attributed to the presence of quassinoids, which give an extremely bitter taste to all plant parts (Lorenzi & Abreu Matos 2002). Triterpenoids are known to possess insect antifeedant growth regulating activity against a variety of agricultural pests. Quassinoids, as modified triterpenoids, can be expected to show similar activity. Furthermore, quassinoids isolated from the seeds and bark of *Samadera indica* (Simaroubaceae), have been described as altering the insect tobacco cutworm feeding behavior and growth regulation (Govindachari *et al*. 2001), as well as the methanol extract of the aerial parts of *Quassia* sp. aff. *bidwillii* (Simaroubaceae) with lethal activity against the two-spotted spider mite, peach/potato aphid and the root knot nematode (Latif *et al*. 2000).

The presence of sesquiterpenes, limonoid and coumarin from the stem bark of *G. guidonia* has been demonstrated

Table 2. Mortality rate of *R. milesi* when topically applied with extracts (n = 20).

Species / control	Plant part used	Solvent	Mortality rate (%)						
			24h	48h	72h	7 days	14 days	21 days	28 days
A. macrocarpon	Root wood	Hexane	0	5	5	5	5	5	5
		Ethanol	0	0	0	0	0	0	0
	Root bark	Hexane	0	0	0	0	0	5	5
G. guidonia	Root	Hexane	20	35	35	45	45	55	75[1]
		Ethanol	0	5	5	5	10	15	20[1]
	Stem	Hexane	15	15	20	25	25	30	30[1]
		Ethanol	10	10	10	15	15	20	20[1]
	Leaves	Hexane	0	5	5	10	15	25	30[1]
G. kunthiana	Root	Hexane	0	5	5	5	5	5	5
		Ethanol	0	0	5	10	15	20	25[1]
	Stem	Hexane	25	30	35	40	40	40	40[1]
		Ethanol	10	15	15	20	20	20	20[1]
	Leaves	Ethanol	5	5	5	5	5	5	5
S. versicolor	Root bark	Hexane	0	0	0	5	10	15	35[1]
		Ethanol	15	35	35	50	90	95	95[1]
	Fruits	Hexane	0	20	20	35	45	50	65[1]
		Ethanol	5	20	20	30	65	75	80[1]
	Stem bark	Hexane	0	0	0	0	0	5	5
		Ethanol	5	10	15	20	25	35	50[1]
	Leaves	Hexane	0	0	0	0	0	0	0
T. ovata	Stem bark	Hexane	0	0	0	0	0	0	0
	Leaves	Hexane	0	0	0	0	0	0	0
	Stem wood	Hexane	5	20	20	25	25	25	25[1]
		Ethanol	0	0	0	0	0	5	10
Acetone control			0	0	0	0	0	0	0
Control with no treatment			0	0	0	0	0	0	0
Ethanolic control			0	0	0	0	0	0	0

[1]Statistically significant ($P < 0.05$; Cochran test) when compared with controls.

(Garcez *et al*. 1998). The methanolic extracts and the oil of leaves of *G. guidonia* showed the presence of terpenoids (Lago *et al*. 2002). However, our study revealed significant activity for the hexanic root extract of this species.

These preliminary data suggest that the ethanolic extract of the root bark of *S. versicolor* and the hexanic extract of the root of *G. guidonia* should be further investigated in order to establish their standard chemical composition and to identify the insecticidal components by monitoring bioassays, so that they may be used as a molecular model for a potential insecticide, or recommended as biorational compounds for use in insect control programmes.

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