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Evaluation of extracts of *Caryocar brasiliense* (Caryocaraceae) for their insecticidal potential on *Lutzomyia longipalpis* (Diptera, Psychodidae), the main vector of visceral leishmaniasis in brazil.

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Abstract

Aim: In Brazil, kala-azar or Visceral Leishmaniasis (VL) is a parasitic disease with a high degree of lethality, transmitted to humans mainly by sandflies belonging to the species *Lutzomyia longipalpis.* The search for alternative products for the elimination of this vector aims to minimize the impacts caused by the synthetic insecticides used by the control programs. Therefore, the insecticidal activity of *Caryocar brasiliense* plant extracts on *Lutzomyia longipalpis* was evaluated in this study.

Methods: Hydroethanolic and cyclohexane extracts of leaves and bark were obtained at concentrations of 50, 100, 200 and 400 mg/mL. Thirty sand flies were exposed to the extracts and the mortality was evaluated at an interval of 1, 2, 4, 16, 24, 48 and 72 hours. The chemical constituents of the extracts were also identified.

Results: After 72 hrs of the experiment, the highest mortality rates were 93.3% for the hydroethanolic extract of bark at 400 mg/mL and 81.1% for the cyclohexane extract of leaves at 200 mg/mL. Triterpenes, steroids, tannins, flavonoids, alkaloids, and saponins were identified in the extracts.

Conclusion: The results obtained suggest that the hydroethanolic extracts of *C. brasiliense,* especially the bark, are promising in the search for natural compounds with insecticidal activity on *Lutzomyia longipalpis.*

Keywords: Visceral leishmaniasis, Phlebotomine sandflies, Bioinsecticides, Pequi.

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Introduction

In Brazil, kala-azar or Visceral Leishmaniasis (VL) is a serious public health problem that can lead to death if it is not treated in time [1,2]. The transmission of the causal agent occurs mainly through the bites of female sand flies, being *Lutzomyia longipalpis* the main vector species in the country [3,4].

In the last decades, the application of the pyrethroid alpha-cypermethrin insecticide has been the measure used to reduce the population of *Lutzomyia longipalpis* in the residences [5,6]. For many years, the use of this insecticide proved to be efficient [7]. However, a significant reduction in the sensitivity of *Lutzomyia longipalpis* to this insecticide has been observed [8,9], compromising the vector control program adopted in Brazil.

Plants are able to produce chemical compounds with the aim of defending themselves against herbivores. Thus, organic compounds that are biosynthesized by plants can present toxic, repellent, food deterrence and oviposition inhibitory activities, as well as to impair stages of development of insects [10,11].

Caryocar brasiliense (Camb.) is a native species of the Brazilian "cerrado" and belongs to the family Caryocaraceae, a group distributed in Central and South America. In Brazil, the species is popularly known as "pequi" and occurs mainly in the states of the Southeast and Center-West regions [12,13]. Apart from its fruits being used in the diet, essential oils and extracts from different parts of this plant are investigated for their phytochemical composition since the 1980s. This species has been shown to be capable of producing different classes of bioactive compounds [12] with different biological activities [14,15].

In this context, the objective of this work was to evaluate the insecticidal potential of extracts of *C. brasiliense* on adult sandflies of the *Lutzomyia longipalpis* species, in order to identify also the chemical constituents of these extracts.

Materials and Methods

Collection and preparation of plant material

Leaves and barks of *C. brasiliense* were collected in Diamantina/MG, in the district of Mendanha (18°5'43"S-43°3'4"W), in October 2016. The taxonomic identification was carried out by specialists belonging to the Jeanini Felfili Dendrological Herbarium of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM/Campus JK), where the voucher samples were deposited under the registry HDJF/4637.

The fresh plant material was submitted to the drying process in a forced circulation oven until complete dehydration. The dried material was ground in a knife mill (Willey SL31 Solab ®) to obtain a fine powder for extraction.

Preparation of plant extracts

Two botanical extracts were produced by maceration: hydroalcoholic (ethanol PA+distilled water, 3:1, v/v) and cyclohexane (cyclohexane PA). In this process, 200 g of pulverized plant material was added to 500 mL of each solvent and the mixture was allowed to stand for 48hr under light and then filtered through filter paper. The procedure was repeated three times, with solvent renovation. Finally, the extract was concentrated in a rotary evaporator (Fisatom ® 802) at 40-42°C under reduced pressure for the removal of the organic solvents from the sample. Additionally, the hydroalcoholic extract was placed in a lyophilizer (Terroni ® LS 3000) for the complete removal of water.

Collection and maintenance of sand flies

The sandflies used in the bioassays were collected in Aroeira (18°8'8"S-43°38'5"W), a rural community in the municipality of Diamantina/MG. HP light traps [16] were exposed in a chicken coop located in the peridomicile of a residence, being placed in the late afternoon and removed in the morning. The captured sand flies were taken to the Laboratory of Parasitology of UFVJM, where they remained in rest in cages for 24 hours until the beginning of the experiment. Cotton soaked with water and sugar solution was offered to insects. Part of the insects was used for specific identification according to the classification proposed by Young and Duncan [17].

Evaluation of insecticidal activity

Phlebotomies were transferred from the cages, using a manual grabber, to plastic pots with filter paper in the bottom. The top of the pots was covered with fine mesh fabric to allow oxygen to enter, and cotton soaked with water and sugar solution were also added. Each pot received 30 specimens, 15 males and 15 females. The extracts of *C. brasiliense* were diluted in 3% polysorbate 80 (Tween ®) solution to obtain the following concentrations: 50, 100, 200 and 400 mg/mL, in addition to three control groups: positive (alpha-cypermethrin 196 $\mu g.mL^{-1}$), negative (distilled water) and vehicle (Tween ® 80 3%).

The assay was performed in triplicate and 300 µL of each treatment was added to the filter paper at the bottom of the pots. Insect mortality was recorded after 1, 2, 4, 16, 24, 48 and 72 hours after the onset of exposure. Phlebotomies were considered dead when they lay flat on the surface and did not move when stimulated by touching the pot.

Phytochemical identification of extracts

The investigation of the classes of chemical compounds present in the extracts was carried out according to the preliminary prospection methodology proposed by Matos [18], which consists in the accomplishment of chromogenic reactions and precipitations for the identification of certain functional groups. The extracts were screened for the following classes of organic compounds: triterpenes, steroids, tannins, flavonoids, alkaloids, saponins and coumarins.

Statistical analysis

The data were analyzed through the percentages of means between triplicates. The statistical test used to compare the proportions between the groups at each exposure time was ANOVA followed by the Scott-Knott test, considering the level of significance of 95% ($p<0.05$) obtained by SISVAR software 5.6 (Ferreira 2010).

Results and Discussion

Table 1 shows the percentage of mortality of *Lutzomyia longipalpis* exposed to different concentrations of hydroethanolic and cyclohexanic extracts of leaves and barks of *C. brasiliense* in relation to the time of exposure. The insects were sensitive to extracts of leaves and barks of *C. brasiliense*, with variable sensitivity depending on the concentrations used, the part of the plant tested and the time of exposure to the treatments. The results indicate that the increase in insect mortality was not directly proportional to the increase in the concentration of the extracts.

Table 1. *Percentage of mortality of Lutzomyia longipalpis in contact with different concentrations of hydroethanolic and cyclohexanic extracts of leaves and barks of C. brasiliense during different exposure times.*

Extract	Material	Concentrations	Mortality (%)						
			1h	2h	4h	16h	24h	48h	72h
Hydro ethanolic	Leaves	50 mg/mL	2,20aA	4,43aA	4,43aA	18,86bB	30,00cB	62,20dC	75,53bD
		100 mg/mL	3,33aA	4,43aA	6,66aA	22,20bB	34,43dB	64,43dC	81,10bD
		200 mg/mL	2,20aA	2,20ªA	3,33aA	20,00bB	31,10cB	61,10dC	78,86bD
		400 mg/mL	3,33aA	4,43ªA	6,66aA	13,33aA	15,53bA	34,43bB	65,53bC
	Barks	50 mg/mL	1,10aA	1,10ªA	1,10ªA	13,33aB	25,53cB	54,43cC	87,76cD
		100 mg/mL	0,00aA	0,00aA	2,20ªA	10,00aB	16,66bB	52,20cC	87,76cD

		200 mg/mL	11,00aA	2,20aA	2,20ªA	8,86aA	22,20cB	50,00cC	87,76cD
		400 mg/mL	5,53ªA	6,66aA	6,66ªA	30,00bB	43,33dC	74,43dD	93,33cE
	Controls	Water	0,00aA	0,00aA	0,00aA	1,11ªA	2,22aA	6,67aB	33,33aB
		Tween	0,00aA	0,00aA	0,00aA	2,22ªA	6,67aA	32,22bB	43,33aB
		Cypermethrin	48,89bA	70,00bB	83,33bC	100cD	100eD	100eD	100cD
Cyclo hexanic	Leaves	50 mg/mL	4,44aA	5,56aA	5,56aA	6,67aA	7,78aA	30,00bB	63,33cC
		100 mg/mL	4,44aA	4,44aA	4,44aA	14,44aB	18,89bB	47,78cC	80,00dD
		200 mg/mL	5,56aA	6,67aA	6,67aA	7,78aA	20,00bA	48,89cB	81,11dC
		400 mg/mL	5,56aA	5,56aA	5,56aA	12,22aA	17,78bA	47,78cB	70,00cC
	Barks	50 mg/mL	2,22aA	2,22aA	3,33aA	14,44aB	24,44bB	41,11cC	74,44dD
		100 mg/mL	1,11aA	1,11aA	1,11aA	4,44aA	6,67aA	15,56aA	50,00cB
		200 mg/mL	4,44aA	4,44aA	4,44aA	7,78aA	12,22aA	33,33bB	60,00cC
		400 mg/mL	3,33aA	3,33aA	4,44aA	10,00aA	11,11aA	33,33bB	57,78cC
	Controls	Water	2,22aA	2,22aA	2,22aA	2,22aA	2,22aA	3,33aA	3,33aA
		Tween	1,11aA	1,11aA	1,11aA	3,33aA	3,33aA	8,89aA	26,67bB
		Cypermethrin	65,56bA	65,56bA	87,78bB	98,89bB	100cB	100dB	100eB

Lowercase letters compare the values in the columns. Capital letters compare the values in the rows. Different letters indicate a statistically significant difference. The letters in alphabetical order rank the values in ascending order. It was considered $p<0.05$ by the analysis of variance test followed by Scott-Knott

In general, during the first four hours of exposure to the extracts, there was no significant insect mortality. We did not observe the "knock-down" effect as seen in the control group with alpha-cypermethrin, which began its action on sand flies from the first hour of experiment, reaching a 50% mortality rate of the insects.

In all bioassays, it was possible to see that the alpha-cypermethrin was able to kill all exposed insects within a maximum of 24 hr. Although there are records of loss of sensibility of *Lutzomyia longipalpis* to this insecticide, our results have shown the opposite. The use of wild insects in toxicity experiments may generate some bias, but their use is justified because it's a condition that is closer to field responses when applying chemical control in locu [19].

From 16 hrs of exposure, the mortality rate of sandflies began to show a positive variation in the groups tested, suggesting the use of a methodology that establishes observation after 24hr of experiment [20,21]. Amongst the hydroethanolic extracts of pique, the bark was the most toxic to sand flies, reaching 93.3% of mortality in 72 hr at the concentration of 400 mg/mL (Table 1). Within the leaf extracts, the concentration of 100 mg/mL was the one with the highest mortality rate (81.1%), after 72 hrs of exposure. Cyclohexane extracts from leaves at concentrations of 100 and 200 mg/mL and bark at 50 mg/mL reached approximately 80% mortality in 72 hr of experiment. The data obtained by the negative control and Tween 80 were statistically lower than the extracts.

The phytochemical screening tests to investigate the composition of the secondary metabolites of hydroethanolic and cyclohexanic extracts of leaves and barks of *C. brasiliense* indicated the presence of triterpenes, steroids, tannins, flavonoids, alkaloids and saponins in the samples (Table 2). The phytochemical composition of pequi was also investigated by other authors [14,22] who obtained results similar to those found in this study. Small qualitative differences in the phytochemical profile of a species can be explained by the geographic distribution or the seasonal variation of the compounds [23].

Table 2. *Phytochemical screening results for the composition of hydroethanolic and cyclohexanic extracts of C. brasiliense.*

	Hydroethanolic		**Cyclohexanic**	
	Leaves	**Barks**	**Leaves**	**Barks**
Triterpens	+	+	+	-
Steroids	+a	+a	+	-
Tannins	+b	+c	-	+b
Flavonoids	+d	+e	-	+f
Alkaloids	+	-	+	+
Saponins	+	+	-	+
Coumarins	-	-	-	-

(+) present; (-) absent; (a) free steroids (b) hydrolysable tannins; (c) condensed tannins; (d) flavones, flavonols and xanthones; (e) chalcones and aurones; (f) flavanones

In relation to the phytochemical compounds found in the extracts of *C. brasiliense*, the presence of terpenes suggests that this class of substances may be related to the lethality of phlebotomies, since its insecticidal activity has already been well demonstrated by other authors [24-28], including *Lutzomyia longipalpis* [20,29,30] at different stages of development.

Some tannins may exhibit toxic activity against herbivorous insects, but toxicity may depend on the species from which they originate. The toxic action of these compounds, which have also been found in leaves and barks extracts of *C. brasiliense*, has been demonstrated against several insects, such as *Aedes aegypti* [31] and some coleopteran and lepidopteran species [32], suggesting some participation in the mortality of *Lutzomyia longipalpis* of this study.

Saponins extracted from plants showed potential to cause mortality of insect vectors, such as larvae of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes [33,34]. Similarly, studies have shown that some compounds, such as alkaloids and flavonoids, extracted from plants are promising in the search for a natural substance with insecticidal activity [35,36].

This preliminary study indicated that *C. brasiliense* has compounds that may be lethal to sand flies. These results are promising in the search for substances of natural origin for the formulation of a botanical insecticide applicable in control programs, since they indicate that leaves and barks of pequi contain active principles with insecticidal activity on *Lutzomyia longipalpis.*

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