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In vitro Effects of *Schumanniophyton magnificum*, *Pseudospondias microcarpa* and *Rauvolfia vomitoria* Stem Barks Extracts, on Free Larval Stages of *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae)

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Abstract

Purpose: A comparative *in vitro* study was carried out to evaluate the potential larvicidal effect of aqueous and methanol/methylene chloride extracts of *Schumanniophyton magnificum*, *Pseudospondias microcarpa*,and *Rauvolfia vomitoria* stem barks on different life-cycle stages of *Heligmosomoides bakeri*.

Methods: The organic extracts were diluted in 2.5% dimethylsulphoxide (DMSO) and aqueous extracts in distilled water to obtain stock solutions. Serial dilutions were made to obtain four increasing concentrations of 1250, 2500, 5000 and 10000 µg/ml for incubation in Petri dishes with L1, L2 and L3 larvae of the parasite. Exposure times were 2, 4, 6 and 24 hours for L1 and L2 larvae and 24 and 48 h for the infective L3 larvae. Albendazole was used as the positive control, while 2.5% DMSO and distilled water were used as negative controls for organic and aqueous extracts, respectively.

Results: The organic extract of stem bark of R. vomitoria showed significant ($p<0.05$) mortality on L1 larvae (85%) at the 5000 µg /ml and had a 50% lethal concentration (LC_{50}) of 2404.980 µg/ml. Mortality rates of 100% were recorded at the concentrations higher or equal to 2.5 mg/ml for albendazole and 95.6 % at 5000 µg/ml for R. vomitoria extracts on L2 larvae and the LC_{50} were 1589.970 and 1272.413 µg/ml, respectively, for these products. The L3 infective larvae of the parasite were not affected by either the plant extracts or albendazole after 24 h and the effect of the products after 48 h of exposure was not significant. Organic extracts in general were more active than aqueous extracts. The larvicidal activity was dose and time dependent.

Conclusion: These results show that organic extracts of all three plants possess anti-parasitic properties. Further research, such as *in vivo* studies, need to be carried out on adult stages of *H. bakeri*.

Keywords: *Pseudospondias microcarpa*; *Schumanniophyton magnificum*; *Rauvolfia vomitoria*; Larvicidal activity; Albendazole; *Heligmosomoides bakeri*.

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Introduction

Parasitic diseases cause serious public health problems in developing countries where climatic, hygienic, and demographic conditions favor their proliferation [1,2]. About 2 billion people are infected in the world, with children (2 to 15 years old) and pregnant women (53.6%) being the most exposed groups [3-5]. Parasites cause anemia, malnutrition, growth retardation, deterioration of cognitive function and school retardation in children. In adults, parasites also can cause blockage of urinary ducts, bladder cancer and liver fibrosis [4]. For livestock breeders, the direct effect of parasites on animals may be death, which in turn leads to high losses of livestock (30 to 50%) and to the reduction of meat and milk production [4].

To control parasitic infections, synthetic drugs such as levamisol, pyranthel, mebendazole, and ivermectin are currently used [6]. Unfortunately, these drugs are expensive and often have many side effects such as itching, headache, vomiting, and swelling on various parts of the body. Moreover,

some species of parasite have developed resistance to these synthetic drugs [6-8]. Despite these shortcomings, reports show that no new synthetic drug has been developed in the last two decades against intestinal worms [9]. Due to these constraints, individuals in tropical and subtropical regions often utilize traditional plant-based remedies, which have many advantages: they are cheap, readily available, easily prepared, and may be less toxic than synthetics. Some limitations of this plant-based therapy are the potential improper dosing and imprecision in the duration of treatment. Numerous studies to confirm the claims of the anthelminthic efficacy of some plants by traditional practitioners have been carried out [9-13]. The present study was carried to evaluate the larvicidal activity of organic and aqueous extracts from *Schumanniophyton magnificum*, *Pseudospondias microcarpa*, and *Rauvolfia vomitoria* barks because different parts of these plants are used to treat worm infections by local practitioners.

Experimental Methods

Plants materials and extracts

The stem barks of *Schumanniophyton magnificum* (Rubiaceae), *Pseudospondias microcarpa* (Anacardiaceae), and *Rauvolfia vomitoria* (Apocynaceae) were obtained from market vendors in Yaoundé, Cameroon, Central Africa. These plants are shrubs (*S. magnificum*) or trees (*P. microcarpaand* and *R. vomitoria*) found in tropical and subtropical regions in Africa. They were identified at the National Herbarium of Cameroon (NHC) by comparison to voucher numbers: 107312, 107313, and 16887 NHC respectively.

Preparation of extracts

Methanol/methylene chloride extracts (organic extract): Five hundred grams (500 g) of each plant (powdered) were mixed with 3 lit of methanol/methylene chloride (l/1; v/v) for 3 days in each of 5 lit jar. The mixture was stirred twice each day. After 3 days the mixture was filtered using 150 µm mesh sieve, cotton and Whatman filter paper (#2). Filtrate was concentrated using a rotary evaporator at 82°C for 12 h. Wet extracts oven dried at 45°C for 3 days and stored in air tight containers ([modified from 14]). Two hundred mg of each extract was solubilized in 0.5 ml DMSO prior to dilution in water. Distilled water was then added to obtain a volume of 20 ml, yielding a10000 µg/ml stock solution from which successive dilutions were made to obtain working solutions with tested concentrations of 5000, 2500, 1250 and 625 µg/ml. DMSO (2.5%) served as the negative control.

Aqueous extracts: Three litres of distilled water was added to 500g of plant powder and the mixture allowed to stand for 48 h. The mixture was filtered and dried at 50°C for ~5 days. Dried extract was stored in air tight containers. The final tested concentrations of working were the same as those of organic extracts.

Albendazole: Albendazole (400 mg - VEREX, Cipharm) tablets were obtained from a pharmacy in Dschang (Menoua Division, Western Region of Cameroon, Central Africa). The choice of this drug was based on its current efficacy and wide use in deworming campaigns [15]. This divisible tablet was partitioned and each half (200 mg) ground and dissolved in 0.5 ml DMSO. Distilled water was added and the final volume brought to 20 ml to give a stock solution of 10000 µg/ml.

Heligsomoides bakeri **larvae:** L_3 larvae of a laboratory-selected strain of Heligmosomoides bakeri were obtained from Pr J. B. Githiori, of the International Livestock Research Institute (ILRI), Nairobi, Kenya and used to infect laboratory mice. After 11 days patency, fresh eggs were obtained from faeces [16]. About 3 g of feces was homogenized in a mortar, suspended in saturated Sodium Chloride solution, and cleansed of organic debris by filtration through sieves (1 mm and 150 µm mesh size). Filtrate was centrifuged at 1000 g for 5 minutes. Supernatants, containing eggs, were poured in a sieve (mesh size 45 µm) and washed with tap water to remove excess salt solution. Eggs were collected [17] and cultured using the technique described by [18]. Briefly, 3 ml of the egg suspension was put on filter paper covering the bottom of three Petri dishes. The dishes were then covered to maintain a high relative humidity (65-67%) and were stored at 24°C. After 3, 4-5, and 6-7 days of incubation L_1, L_2 and L_3 larvae, respectively, were observable. Larvae were differentiated using morphological features and were concentrated using Baermann's apparatus [18].

Evaluation of larvicidal activity

One ml of the suspension containing 10-15 larvae (L1, L_2 or L_3) was introduced into a small Petri dish and 1 ml of a working dilution of plant extract (5000, 2500, 1250, or 625 µg/ml) was added. Observations were done using a light microscope at 4X at 2, 4, 6 and 24 hours post exposure for L_1 and L_2 larvae; and 24-48 hours for L_3 infective larvae (see [19]. Mortality (M) was computed according to [20] as the percent of immobilized larvae relative to the original number of larvae incubated (M=[Num immobile larvae/Num incubated larvae]/100).

Statistical analysis

Mean mortality rates of larvae were transformed into arc sinus values [21] and analyzed using SPSS 19.0. One-way ANOVA was used to compare means and the Waller-Duncan T- test was used in post-hoc testing.

The larval mortality rates obtained 24 h post exposure to extracts were transformed into probits using a probit table and the linear regression equation of probits, according to the decimal logarithm of the concentration, was calculated. This equation is in the form: Y=A+B x. In this equation Y is the probit of the mortality rate and is equal to a value of 5 at 50% of mortality [21], A is the constant term of this equation, B is the regression coefficient, and X the decimal logarithm of the concentration of the product. This equation was used to compute the 50 per cent larvicidal concentration (LC_{50}). All

the tests were repeated four times for each treatment and control.

Results

Larval development

L_1 larvae: The effects of plant extracts and albendazole on *H. bakeri* L_1 larvae are given in Table 1. In short, at two hours post exposure all controls and products showed no effect at all tested concentrations. After 4 h, only the organic extract of *R. vomitoria* showed some effect (4.05%) at 5000 µg/ml. All aqueous extracts of all plants tested and albendazole still showed no effect 6 hours post exposure, while the organic extract of *R. vomitoria* at the highest concentration had a 35.64% mortality rate. The effects of plants extracts and albendazole were more visible after 24h of exposure, and there was a significant difference in the mortality ($p<0.05$) at the concentration 5000 µg/ml. The highest effect was recorded with *R. vomitoria* organic extract (85%) followed by albendazole (78%), while 36.65% mortality was recorded at the highest concentration for the organic extract of *S. magnificum*. The lowest effect was seen with *P. microcarpa* (13%) at the similar concentration. The calculated LC_{50}'s for organic extracts of *R. vomitoria*, albendazole, *S. magnificum* and *P. microcarpa* were at 2404.980, 1652.563, 5003.571 and 7024.282 µg/ml, respectively.

Table 1: *Mean mortality rate ± standard deviation of L1 larvae of Heligmosomoïdes bakeri according to time (hours) and extracts concentrations (µg/ml). (a, b, c, d, e, f, g, h, i and j numbers with same letters in the same line and column for each hour are not significantly different (p>0.05). (O: Organic extracts, A: Aqueous extracts PO/PA: Pseudospondias microcarpa; RO/RA: Rauvolfia vomitoria; SO/SA: Schumanniophyton magnificum; Al: Albendazole; DW: Distilled Water; DMSO: Dimethylsulphoxide).*

Time (hours)	Concentrations (mg/ml)	PO	RO	SO	PA	RA	SA	AL
4h	DMSO 2.5%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}
	DW	-	-	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	625	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2500	0.00 ± 0.00^{a}	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5000	0.00 ± 0.00^{a}	4.05 ± 1.00^{c}	2.00 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
6h	DMSO 2.5%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}
	DW	-	-	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-
	625	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2500	2.00 ± 0.01^{b}	$8.38 \pm 3.26^{d,e}$	3.58 ± 1.00^{c}	0.00 ± 0.00^{a}	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	0.00 ± 0.00^{a}
	5000	6.2 ± 2.25^{d}	35.64 ± 6.14^{f}	14 ± 5.15^{e}	0.00 ± 0.00^{a}	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	0.00 ± 0.00^{a}
24h	DMSO 2.5%	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	-	-	-	2.00 ± 0.01^{b}
	DW	-	-	-	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	2.00 ± 0.01^{b}	-
	625	0.00 ± 0.00^{a}	3.58 ± 1.25^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	27.00 ± 5.16^{f}
	1250	0.00 ± 0.00^{a}	12.24 ± 6.53^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	42.18 ± 5.25^{h}
	2500	12.09 ± 3.16^{e}	58.32 ± 10.36^{i}	26.00 ± 4.28^{f}	0.00 ± 0.00^{a}	4.00 ± 1.00^{c}	8.00 ± 1.53^{d}	59.55 ± 6.23^{i}
	5000	13.00 ± 2.14^{e}	85 ± 13.24^{j}	36.65 ± 5.41^{g}	2.00 ± 0.01^{b}	4.1 ± 1^{c}	12.32 ± 2.1^{e}	$78.00 \pm 12.25^{i,j}$

Results obtained with aqueous extracts were generally lower than that using organic extracts. A 12.32% mortality rate was obtained with aqueous extract of *S. magnificum* 24 hours post-exposure at 5000 µg/ml, while at the same concentration, 4.1 and 2% were obtained for *R. vomitoria* and *P. microcarpa*, respectively. There was a significant difference ($P<0.05$) between the mortality rate recorded with *S. magnificum* and those of the other two plants. LC_{50}'s of 7457.074, 10645.600 and 49798.535 µg/ml were recorded for aqueous extracts of *S. magnificum*, *R. vomitoria* and *P. microcarpa*, respectively

(Table 2). Comparably, the LC_{50} of aqueous extracts were far higher than those of organic extracts of the same plants.

Table 2: *Mean mortality rate±standard deviation of L_2 larvae of Heligmosomoïdes bakeri according to time (hours) and extracts concentrations (µg/ml). (a, b, c, d, e, f, g, h, i j and k 145 numbers with same letters in the same line and column for each hour are not significantly different (p>0.05), O: organic extracts, A: aqueous extracts PO/PA: Pseudospondias microcarpa; RO/RA: Rauvolfia vomitoria; SO/SA: Schumanniophyton magnificum; Al: Albendazole; DW: Distilled water; DMSO: Dimethylsulphoxide).*

Time (hours)	Concentrations (mg/ml)	PO	RO	SO	PA	RA	SA	AL
2h	DMSO 2.5%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}
	DW	-	-	-	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	625	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2500	0.00 ± 0.00^{a}	22.00 ± 3.61^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5000	0.00 ± 0.00^{a}	24.36 ± 2.39^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
4h	DMSO 2.5%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}
	DW	-	-	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-
	625	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2500	3.75 ± 0.05^{b}	23.94 ± 6.00^{d}	11.25 ± 1.25^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5000	12.58 ± 3.64^{c}	57.75 ± 10.8^{e}	21.7 ± 2.39^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
6h	DMSO 2.5%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}
	DW	-	-	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-
	625	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00a	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	4.2 ± 0.06^{c}	1.00 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2500	8.35 ± 3.9^{d}	38.78 ± 2.07^{f}	22.00 ± 2.07^{e}	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5000	26.00 ± 4.58^{e}	72.85 ± 10.59^{h}	43.00 ± 5.14^{g}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
24h	DMSO 2.5%	4.00 ± 0.97^{b}	4.00 ± 0.97^{b}	4.00 ± 0.97^{b}	-	-	-	4.00 ± 0.97^{b}
	DW	-	-	-	4.00 ± 0.97^{b}	4.00 ± 0.97^{b}	4.00 ± 0.97^{b}	-
	625	0.00 ± 0.00^{a}	18.93 ± 2.35^{d}	11.25 ± 1.35^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1250	0.00 ± 0.00^{a}	40.6 ± 5.78^{g,h}	24.15 ± 2.33^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	8.38 ± 3.5^{b,c}	34.9 ± 2.48^{f,g}
	2500	15.25 ± 2.21^{c,d}	88.56 ± 8.36^{i,j}	45.16 ± 3.29^{h}	4.00 ± 0.97^{b}	0.00 ± 0.00^{a}	12.00 ± 1.64^{c}	100 ± 0.00^{k}
	5000	34.1 ± 6.2^{f,g}	95.56 ± 13.26^{j,k}	82.00 ± 12.1^{i}	8.38 ± 3.5^{b,c}	14.12 ± 2.25^{c}	26.00 ± 5.14^{e,f}	100 ± 0.00^{k}

L_2 larvae: Unlike for L_1 larvae, where there was no effect after 2 h of exposure to all plant extracts and albendazole, 24.36% of L_2 larvae died when exposed to organic extract of *R. vomitoria* at 5000 mg/ml (Table 2). At the same concentration, after 4 h post exposure, mortalities of 57.75%, 21.7%, and 12.58% were recorded for the organic extracts of *R. vomitoria*, *S. magnificum*, and *P. microcarpa*, respectively. Mortality after 6h of exposure to organic extracts of *R. vomitoria* was 72.85% ($p<0.05$) at the highest concentration, followed by organic extracts of *S. magnificum* (43%) and *P. microcarpa* (26%). Untill this time; albendazole had no effect on the survival of larvae.

The highest mortality rates were recorded 24 h post exposure, with both *R. vomitoria* and albendazole showing mortality rates of 95.56% and 100%, respectively at 5000 µg/ml. There was a significant ($p<0.05$) difference when compared to the extracts of other plants.

The calculated LC_{50}'s were 1589.970, 1272.414, 2346,488 and 5406.080 µg/ml for the organic extracts of albendazole, *R. vomitoria, S. magnificum* and *P. microcarpa*, respectively (Table 2).

The effects of aqueous extracts were slightly higher on the L_2 larvae than L_1 larvae. Mortalities of 26, 14.12, and 8.38%

corresponding to LC_{50}'s of 5147.223, 18780.204 and 8865.517 mg/ml were recorded with the aqueous extracts of *S. magnificum*, *R. vomitoria* and *P. microcarpa* respectively (Table 2).

L_3 larvae: The effects of plant extracts and albendazole on L_3 larvae of *H. bakeri* are given in Table 1. For L_3 larvae (the infective stage of the parasite), all plant extracts and albendazole at all concentrations were inactive 24 h post-exposure. Organic extracts and Albendazole became effective only after 48 h. At the highest concentration, mortalities of 7.66, 6.67 and 14.33% were recorded for *R. vomitoria*, *S. magnificum* and albendazole, respectively, with calculated LC_{50} values being 7558.575, 25028.402, and 26748.934 µg/ml, respectively, for albendazole, *R. vomitoria* and *S. magnificum* (Tables 3 and 4)). There were no effects of the aqueous extracts of all plants tested on L_3 larvae.

Table 3: *Mean mortality rate ± standard deviation of L_3 larvae of Heligmosomoïdes bakeri according to time (hours) and extracts concentrations (µg/ml). (a, b, c, d, and e numbers with same letters in the same line and column for each hour are not significantly different (p>0.05). (O: organic extracts, PO: Pseudospondias microcarpa; RO: Rauvolfia vomitoria; SO: Schumanniophyton magnificum; Al: Albendazole; DMSO: Dimethylsulphoxide).*

Extracts				
Time (hour)	**Concentrations (µg/ml)**	**SO**	**RO**	**AL**
	DMSO 2.5%	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
48h	625	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	1250	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	2500	0.00 ± 0.00[a]	0.00 ± 0.00[a]	5.021.02[b]
	5000	6.67 ± 2.4[b,d]	7.66 ± 2.36[d]	14.33 ± 3.25[e]

Discussion

The results presented herein indicate that organic extracts of *R. vomitoria* bark have detectable *in vitro* effects on both L_1 and L_2 free-living stages of *H. bakeri*. The organic extract of *R. vomitoria* was more active on all larval stages tested than were the extracts of the other plants. According to [23] extract contains nematicidal substances, such as tannins, phenol, terpenoids, glycosides that affect the survival of these larvae. These nematicidal products likely either cross the cuticle or are ingested during their feeding. These compounds may act by paralyzing the worm or by altering their muscular cells [24]. Our results also show that the efficacy of extracts were significantly higher on L_2 larvae than on L_1 larvae, which may be related to the fact that L_1 larvae are known to be less susceptible to adverse environmental conditions than the L_2 larvae [25]. The higher toxicity observed on the L_2 larvae could be attributed to many factors, including the very low egg reserves at 4-5 days after hatching. Moreover, these larvae have just undergone molting from L_1 to L_2 and may be more vulnerable to adverse environmental conditions. Neither plant extracts nor did albendazole significantly affect the survival of the filariform larvae of this parasite, which may be the result of the sheath of the L_3 larvae inhibiting or slowing down the absorption of the products [26]. Moreover, these strongyloid larvae don't feed and thus cannot ingest bioactive compounds [18,20]. This result confirms the scale of sensibility established by [25], which shows that, in general, the third-stage larva is the least susceptible to adverse environmental conditions. This is followed by the embryonated egg, the unembryonated egg, first-stage larvae then the second-stage larvae.

Table 4: *Mean percentage of mortality of larvae after 24 h of exposure transformed into probit and LC_{50} (microgram/ml, µg/ml) resulting (10 x)*

Extracts	Concentrations	L_1	Probit	LC_{50}	L_2	Probit	LC_{50}	L_3	Probit	LC_{50}
PO	625	0		7024.28205	0		5406.080422	0		
	1250	0			0			0		
	2500	12.09	3.8300		15.25	3.9763		0		
	5000	13	3.8736		34.1	4.5903		0		
PA	625	0		49798.53479	0		8865.5173	0		
	1250	0			0			0		
	2500	0			4	3.2493		0		
	5000	2	2.9463		8.38	3.6213		0		
RO	625	3.58	3.2009	2404.9798	18.93	4.1184	1272.413952	0		25028.40236
	1250	12.24	3.8350		40.6	4.7622		0		
	2500	58.32	5.2096		88.56	6.2055		0		

	5000	85	6.0364		95.56	6.7060		7.66	3.5745	
RA	625	0		10645.60037	0		18780.20401	0		
	1250	0			0			0		
	2500	4	3.2493		0			0		
	5000	4.1	3.2608		14.12	3.9242		0		
SO	625	0		5003.571437	11.25	3.7893	2346,487709	0		26748.93429
	1250	0			24.15	4.3001		0		
	2500	26	4.3567		45.16	4.8794		0		
	5000	36.65	4.6602		82	5.9154		6.67	3.5015	
SA	625	0		7457.074459	0		5147.225995	0		
	1250	0			8.38	3.6213		0		
	2500	8	3.5949		12	3.8250		0		
	5000	12.32	3.8399		26	4.3567		0		
AL	625	27	4.3872	1652.563	0		1589.969849	0		7558.574759
	1250	42.18	4.8032		34.9	4.6120		0		
	2500	59.55	5.2430		100	8.7190		5.02	3.3551	
	5000	78	5.7722		100	8.7190		14.33	3.9331	

The bio-activity of botanical compounds found from plant materials depends on the type of solvent and the method of extraction used [27] and our organic bark extracts were obviously more effective than were aqueous extracts in killing larvae, indicating that methanol/methylene chloride extraction removed more active components from the plants than did water. The stem barks of plants are the site of the biosynthesis and storage of numerous secondary metabolites and are responsible for the biological properties of many medicinal plants extracts [12]. These plants are used by traditional healers in Yaounde (Center Region of Cameroon) and in Central Africa (Gabon, Equatorial Guinea, Republic of Central Africa) to cure intestinal worms and abdominal pains in their patients. Albendazole is known to block the absorption of glucose by the larva, leading to death. It is a lipophilic anthelmintic and thus has a greater capacity to cross the external surface of the worm [28]. The dose and time dependent response observed with our plant extracts were similar to those obtained by [9,11-13,29].

Conclusion

In summary, mehanol/methylene chloride extracts of *R. vomitoria* showed the highest anthelmintic efficacy in vitro against L_1 and L_2 larvae of *Heligmosomoides bakeri*, a nematode parasite of *Mus musculus*. The action of this extract is dose and time dependent and further *in vivo* studies are required to confirm the anthelmintic activity of these plants against the parasitic stages of the worm.

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