

# Molluscicidal and ovicidal activities of plant extracts of the Piperaceae on *Biomphalaria glabrata* (Say, 1818)

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(Accepted 16 March 2010; First Published Online 6 May 2010)

## Abstract

Schistosomiasis is a tropical disease caused by *Schistosoma* and occurs in 54 countries, mainly in South America, the Caribbean region, Africa and the eastern Mediterranean. Currently, 5 to 6 million Brazilian people are infected and 30,000 are under infection risk. Typical of poor regions, this disease is associated with the lack of basic sanitation and very frequently to the use of contaminated water in agriculture, housework and leisure. One of the most efficient methods of controlling the disease is application of molluscicides to eliminate or to reduce the population of the intermediate host snail *Biomphalaria glabrata*. Studies on molluscicidal activity of plant extracts have been stimulated by issues such as environmental preservation, high cost and recurrent resistance of snails to synthetic molluscicides. The aim of this study was to determine the molluscicide action of extracts from Piperaceae species on adult and embryonic stages of *B. glabrata*. Fifteen extracts from 13 Piperaceae species were obtained from stems, leaves and roots. Toxicity of extracts was evaluated against snails at two different concentrations (500 and 100 ppm) and those causing 100% mortality at 100 ppm concentration were selected to obtain the LC<sub>90</sub> (lethal concentration of 90% mortality). *Piper aduncum*, *P. crassinervium*, *P. cuyabanum*, *P. diospyrifolium* and *P. hostmannianum* gave 100% mortality of adult snails at concentrations ranging from 10 to 60 ppm. These extracts were also assayed on embryonic stages of *B. glabrata* and those from *P. cuyabanum* and *P. hostmannianum* showed 100% ovicidal action at 20 ppm.

## Introduction

Schistosomiasis remains one of the most prevalent parasitic infections in the world and has significant economic and public health consequences. It is estimated

that 200 million people are infected and 600 million people are at risk of infection (WHO, 1993; Chitsulo *et al.*, 2000).

Three main schistosome species infect humans: *Schistosoma mansoni*, present mainly in Africa and South America; *S. haematobium*, found in Africa and the Middle East; and *S. japonicum*, which is endemic in Asia. Freshwater snails of the genus *Biomphalaria* play a major role as intermediate hosts in the transmission of

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*S. mansoni*. An intense multiplication of parasites occurs in these snails, making strategies to control snail populations a high priority for reduction of schistosomiasis transmission in endemic regions (WHO, 1984; Rey, 2008).

At present, niclosamide (Bayluscide<sup>®</sup>, Bayer, Leverkusen, Germany) is the only commercially available molluscicide recommended by the World Health Organization (WHO) for large-scale use in schistosomiasis control programmes (WHO, 1993). Niclosamide is not toxic to humans, domestic animals or crops, although it is costly and ictiotoxic (WHO, 1973; McCullough & Mott, 1983). Moreover, it is rapidly decomposed by sunlight and its application does not prevent recolonization of sites by surviving snails, which could lead to selection of molluscicide-resistant populations. Thus, novel natural molluscicidal compounds from plants should be investigated as possible alternatives to synthetic products (Luna *et al.*, 2005).

Members of the Piperaceae family are among a wide variety of species under study. The family comprises 14 genera and 1950 species (Mabberley, 1997); *Piper* and *Peperomia* are the most abundant genera, with approximately 700 and 600 species, respectively (Joly, 1993). Plants from the genus *Piper* are widely distributed throughout tropical and subtropical regions. Brazil has several species and promising studies on biological properties of plants of the Piperaceae family have been published (Navickiene *et al.*, 2000; Danelutte *et al.*, 2003, 2005; Cysne *et al.*, 2005; Yamaguchi *et al.*, 2006).

Phytochemical investigations on *Piper* species have revealed many bioactive compounds such as amides, alkaloids, lignans, benzoic acids and chromenes (Parmar *et al.*, 1997; Alécio *et al.*, 1998; Navickiene *et al.*, 2000; Silva *et al.*, 2002). Chromenes and benzoic acid derivatives are frequently isolated from *Piper* species showing antimicrobial, antitumoral, germination inhibition, fungicidal (Nair & Burke, 1990), insecticidal (Miranda *et al.*, 2002) and anti-leishmania activities (Torres-Santos *et al.*, 1999). In the case of molluscicidal activity, only a few reports have addressed *Piper* species, including essential oil from *P. marginatum* (Rouquayrol *et al.*, 1980) and *P. tuberculatum* (Sousa & Rouquayrol, 1974), chromenes and benzoic acid

from *P. aduncum* (Orjala *et al.*, 1993a, b) and extracts from *P. cubeba* (Pandey & Singh, 2009).

In spite of the variety of secondary compounds described, no systematic evaluation of molluscicidal activity of Piperaceae extracts has been carried out. Thus, in this work, several crude extracts were assessed regarding molluscicidal and ovicidal properties.

## Materials and methods

Molluscicidal and ovicidal assays were performed according to the methodology recommended by World Health Organization (WHO, 1965, 1983) and experimental procedures were employed according to accepted principles of animal welfare in experimental science.

Adults and egg masses were obtained from the population of *Biomphalaria glabrata* (Say, 1818) originally from Belo Horizonte, MG, Brazil and reared under laboratory conditions for several years, fed with fresh lettuce *ad libitum* and a balanced ration.

### Plant material

Fresh material of each species (table 1) was collected by Dr Massuo Jorge Kato and identified by Dr Elsie F. Guimarães from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro and vouchers were deposited in the Herbarium of Jardim Botânico do Rio de Janeiro.

### Preparation of plant extracts

Selected parts (table 1) of freshly collected plant material were separated, immediately air dried and finally dried in an oven at 45°C. Material was ground and powdered materials were extracted with dichloromethane:methanol (2:1) at room temperature (25–27°C) three times and filtered. Extracts were evaporated to dryness under vacuum in a rotoevaporator and stored at –20°C.

A stock solution containing 1000 ppm of each extract was prepared by suspending 10 mg of extract in 0.1 ml of 99.9% dimethylsulphoxide (DMSO; Aldrich, Milwaukee, Wisconsin, USA) and making up to 10 ml

Table 1. Piperaceae species screened for molluscicidal activity in *B. glabrata*.

Species	Collecting sites	Popular name	Sampling date
<i>Piper aduncum</i> L.	São Paulo, SP	Aperta-ruão, Pimenta-de-macaco	10.1.1999
<i>Piper amplum</i> Kunth	São Sebastião, SP	Jaborandi	5.12.2000
<i>Piper cuyabanum</i> C.DC.	Brotas, SP	Pimenta do mato	17.9.1999
<i>Piper crassinervium</i> Kunth	Apiáí, SP	Jaborandi	12.2.1999
<i>Piper hoffmanseggianum</i> Rocker & Schulter	Ilha Grande, RJ	João-Borandinho	31.12.2000
<i>Piper macedoi</i> Yunck	Araraquara, SP		12.2.2001
<i>Peperomia macrostachya</i> (Vahl). A. Dietr.	Manaus, AM		28.3.2001
<i>Piper hostmannianum</i> R.S.	Manaus, AM		21.3.2001
<i>Piper callosum</i> Ruiz et Pav.	Presidente Figueiredo, AM	João Brandin	29.3.2001
<i>Piper tuberculatum</i> Jacq.	São Paulo, SP	Pimenta longa, Pimenta d'arda	19.6.2001
<i>Piper diospyrifolium</i> Kunth	São Paulo, SP		1.10.2004
<i>Piper solmsianum</i> C.DC.	São Paulo, SP		2.11.2004
<i>Piper corcovadensis</i> Miq.*	São Paulo, SP	João Brandinho, Falso Jaborandi	3.5.2005

Selected part for all species, leaves; \* stem, leaves and roots.  
S, São Paulo; RJ, Rio de Janeiro, AM, Amazonas.

with dechlorinated water. Stock solutions were diluted with dechlorinated water in order to provide assay solutions.

#### Assays for molluscicidal/ovicidal activities of plant extracts

Molluscicidal activities of extracts against adults of *B. glabrata* were determined according to previously described procedures (WHO, 1965, 1983). Snails with 10–18 mm shell diameter were exposed to test compounds for 24 h. After exposure, snails were washed and observed daily for 10 days to record the death rate. A negative control group was maintained in dechlorinated tap water containing 1% DMSO under the same experimental conditions. Bayluscide WP70<sup>®</sup> was used as a positive control. Extracts were tested first at concentrations of 500 and 100 ppm and those inactive were not investigated further. LC<sub>90</sub> was determined for all selected extracts with ten animals per dose group, and experiments were repeated three times.

Based on values of LC<sub>90</sub> for adults, egg masses with embryos at blastula stage (Camey & Verdonk, 1970) were exposed to lethal concentrations of extracts for 24 h. At the end of exposure, egg masses were washed and observed for mortality daily for 7 days. Assays were repeated three times with about 100 embryos for each concentration. A control group was maintained in dechlorinated water

containing 1% DMSO under the same experimental conditions.

#### Data analysis

LC<sub>90</sub> was obtained from the logistic regression adjustment using a log-dose transformation. This corresponds to a maximum likelihood estimated for the logodds in a multinomial probability model.

#### Results

All extracts were 100% lethal to *B. glabrata* at 500 ppm but at 100 ppm, only leaf extracts of *P. aduncum*, *P. crassinervium*, *P. cuyabanum*, *P. diospyrifolium* and *P. hostmannianum* showed 100% lethality during 24 h of exposure, so the LC<sub>90</sub> (table 2, figs 1–3) and ovicidal activity (table 3 and fig. 4) of these extracts were determined. Leaf extracts of *Pep. macrostachya*, *P. hoffmanseggianum*, *P. macedoi* and *P. solmsianum* did not show molluscicidal activity at 100 ppm; but mortality obtained with extracts of *P. corcovadensis* (stem, leaf and root), *P. amplum* (leaf), *P. callosum* (leaf) and *P. tuberculatum* (stem) reached a maximum of 90% at 100 ppm (table 2). The percentage of dead snails in the control group over 10 days of observation was 3.3% while dead embryos in control groups over 7 days were 1.6%.

Table 2. Molluscicidal activity of Piperaceae extracts in adult *B. glabrata*.

Species	Selected part	Concentration (ppm)	(%) Mortality in 10 days	LC <sub>90</sub> (ppm) [ ] confidence intervals
<i>P. aduncum</i>	Stem	2.5	37	6.46 [5.34; 9.51]
		5	73	
		10	100	
<i>P. crassinervium</i>	Stem	20	40	38.15 [33.98; 46.04]
		30	73	
		40	93	
		50	97	
		60	100	
<i>P. cuyabanum</i>	Stem	5	7	12.92 [11.25; 16.98]
		10	60	
		20	100	
<i>P. diospyrifolium</i>	Stem	5	23	23.30 [19.80; 26.81]
		10	27	
		20	77	
		30	100	
<i>P. hostmannianum</i>	Stem	10	20	34.29 [32.13; 36.44]
		20	43	
		30	73	
		40	100	
<i>P. corcovadensis</i> <sup>a</sup>	Stem	100	40	nd
	Leaf	100	20	nd
	Root	100	90	nd
<i>Pep. macrostachya</i> <sup>a</sup>	Leaf	100	0	nd
<i>P. amplum</i> <sup>a</sup>	Leaf	100	50	nd
<i>P. callosum</i> <sup>a</sup>	Leaf	100	80	nd
<i>P. hoffmanseggianum</i> <sup>a</sup>	Leaf	100	0	nd
<i>P. macedoi</i> <sup>a</sup>	Leaf	100	0	nd
<i>P. solmsianum</i> <sup>a</sup>	Leaf	100	0	nd
<i>P. tuberculatum</i> <sup>a</sup>	Stem	100	20	nd

*n* = 30 adult snails; *Pep.*, *Peperomia*.

<sup>a</sup> *n* = 10 adult snails.

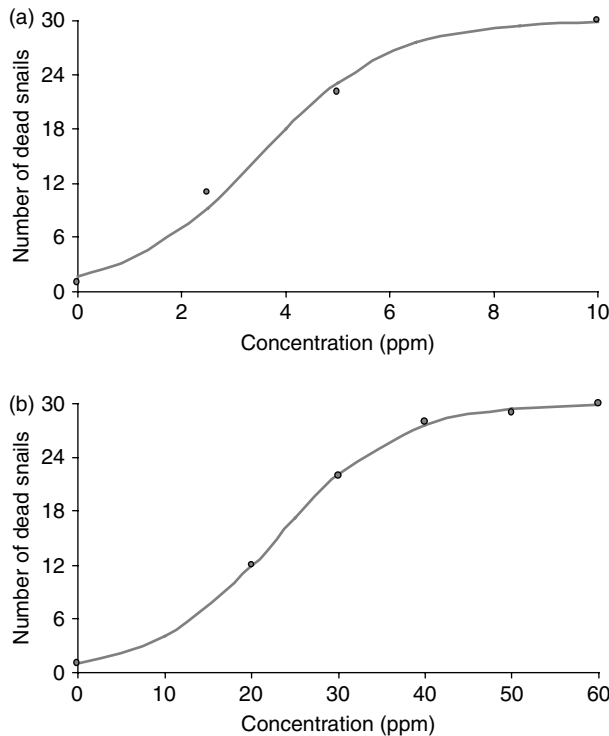


Fig. 1. Estimated mortality of *B. glabrata* exposed to (a) *P. aduncum* leaf extract; (b) *P. crassinervium* leaf extract; logistic tendency function.

### Discussion

All 15 extracts from Piperaceae species were active with 100% mortality at 500 ppm. WHO (1983) recommends that a crude preparation of plant material should be active at 100 ppm or less and kill 90% of snails exposed for 24 h. Leaf extracts of *P. aduncum*, *P. crassinervium*, *P. cuyabanum*, *P. diospyrifolium* and *P. hostmannianum* were active at concentrations of less than 100 ppm, which make them good candidates for natural molluscicides (WHO, 1965, 1983).

Adult snails showed a similar response to extracts with molluscicidal action. A common feature was low values of  $LC_{90}$  associated with death during 24 h of exposure. At the highest concentrations, death occurred in the first hours of exposure. Release of haemolymph was also frequently observed and occurred in a dose-response manner. It is quite often associated with rupture of external membranes, but haemolymph could also be liberated through the haemal pore (Duncan, 1985). At present, information on the mode of action of molluscicides is scarce. As is true for most insecticides, the action of a molluscicide may conceivably be a multi-component process affecting more than one system. Several such responses indicative of this have been reported in the literature, for example a reduction in heart rate, swelling of tissues and alteration of water balance have been described (WHO, 1983). In our work, active extracts caused death after around 4 h of exposure, differing significantly from the action of the molluscicide

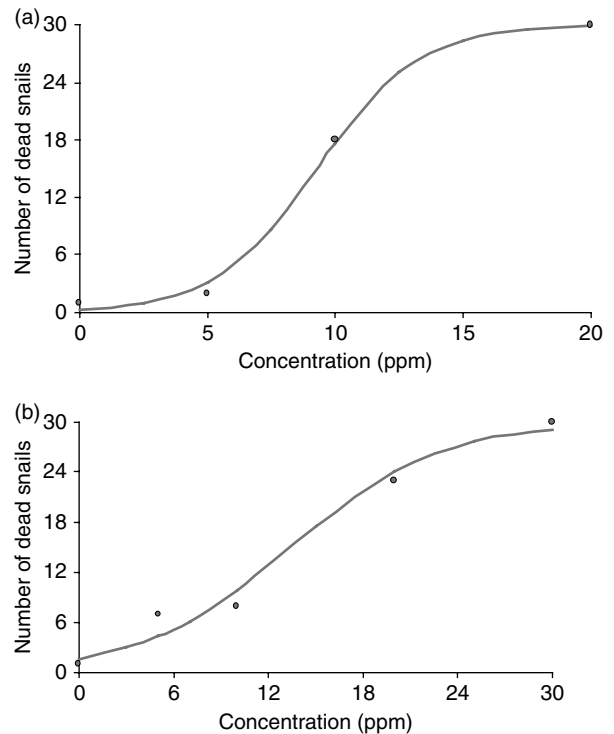


Fig. 2. Estimated mortality of *B. glabrata* exposed to (a) *P. cuyabanum* leaf extract; (b) *P. diospyrifolium* leaf extract; logistic tendency function.

Bayluscide WP70<sup>®</sup> which induces death only after 24 h, even at 100% lethal concentrations. The efficient molluscicide action observed in our study requires further investigation in order to unravel the possible mechanism of action for toxicity against *B. glabrata*.

Among the five selected Piperaceae extracts, *P. crassinervium*, *P. cuyabanum* and *P. hostmannianum* showed similar activity in adults and embryos. Sensitivity was inversely proportional to developmental stage. Mortality was highest in embryos at blastula stage, followed by gastrula, trocophore and veliger stages. This higher sensitivity could be attributed to intense

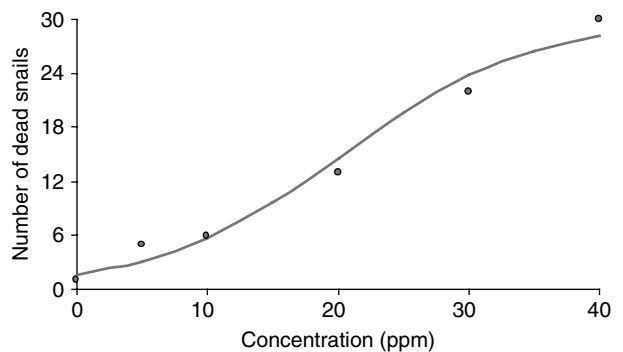


Fig. 3. Estimated mortality of *B. glabrata* exposed to *P. hostmannianum* leaf extract; logistic tendency function.

Table 3. Effect of leaf extracts from *P. aduncum*, *P. cuyabanum*, *P. crassinervium*, *P. diospyrifolium* and *P. hostmannianum* on *B. glabrata* embryos.

Plant species	Stage	Concentration (ppm)	Number of embryos	Dead embryos (%)	
<i>P. aduncum</i>	Blastula	10	111	54 (48.6)	
		20	141	69 (48.9)	
		30	110	71 (64.5)	
	Gastrula	10	123	31 (25.2)	
		Trocophore	10	101	6 (5.9)
			10	110	0
<i>P. cuyabanum</i>	Blastula	5	134	0	
		10	123	66 (53.6)	
		20	116	116 (100)	
	Gastrula	10	114	0	
		20	105	105 (100)	
		Trocophore	10	127	0
	20		120	120 (100)	
	Veliger		10	105	0
		20	106	106 (100)	
		<i>P. crassinervium</i>	Blastula	30	95
	40			99	16 (16.6)
	50			134	134 (100)
Gastrula	50		120	0	
	Trocophore		50	102	0
			50	98	0
<i>P. diospyrifolium</i>	Blastula	20	98	0	
		30	142	1 (0.7)	
		40	187	0	
	Gastrula	20	113	2 (1.7)	
		Trocophore	20	99	0
			20	129	0
<i>P. hostmannianum</i>	Blastula	10	120	0	
		20	133	133 (100)	
		30	112	112 (100)	
	Gastrula	40	133	133 (100)	
		30	90	10 (11.1)	
		40	90	90 (100)	
	Trocophore	30	129	2 (1.5)	
		40	104	104 (100)	
		Veliger	30	97	1 (0.9)
40	91		91 (100)		

cell proliferation activity at early stages; previous studies also showed a differential sensitivity according to developmental stages (Kawano *et al.*, 1979; Kawano & Simões, 1987; Kawano *et al.*, 1991; Yamamoto *et al.*, 1996).

*Piper aduncum* was more active in adults than in embryos. A similar profile was observed with crude extracts and steroidal glycoalkaloid fraction of *Lycopersicon esculentum*, which showed high activity in adults and low effect in embryos (Leyton *et al.*, 2005). Similar results were obtained in *B. glabrata*, *B. alexandrina* and *Bulinus truncatus* in a study of the molluscicidal effect of *Phytolacca dodecandra* (Lemma *et al.*, 1972). Pereira *et al.* (1978) evaluated a leaf extract of *Euphorbia cotinifolia* and observed an effect in *B. glabrata* embryos with 48 ppm, a concentration about 20 times higher than the 2.4 ppm observed to have an effect on adult specimens.

*Piper diospyrifolium* did not show molluscicidal activity in embryos at concentrations evaluated in adults. On the other hand, *P. amplum* was highly effective in embryos (LC<sub>90</sub> of 8.8 ppm) despite its low activity in adults (Teixeira, 2004).

In a comprehensive screening, the highest proportions of molluscicidal species were detected in Euphorbiaceae, Asteraceae, Leguminosae, Phytolaccaceae, Polygonaceae, Rubiaceae and Rutaceae species (WHO, 1983; Kloos & McCullough, 1987). Jurberg *et al.* (1989) describe active extracts from two species of Euphorbiaceae. In our study, five out of 13 Piperaceae species fulfilled the practical criterion for activity against *B. glabrata*. Based on results obtained with *Piper* extracts, further studies with *P. aduncum*, *P. crassinervium*, *P. cuyabanum*, *P. diospyrifolium* and *P. hostmannianum* should be carried out, looking at the isolation of bioactive compounds. At present, molluscicidal activity of *P. aduncum* and *P. diospyrifolium* fractions are being assessed. Concomitantly, we are performing bioassays with *Daphnia similis* and *Ceriodaphnia dubia* to evaluate acute and chronic toxicity of bioactive compounds isolated from the selected *Piper* extracts. Cladoceran species have been used extensively; they have several advantages, such as high sensitivity and short reproductive cycles (Farre & Barcelo, 2003). Inclusion of safety testing will ascertain whether toxic effects on non-target organisms are at acceptable levels.



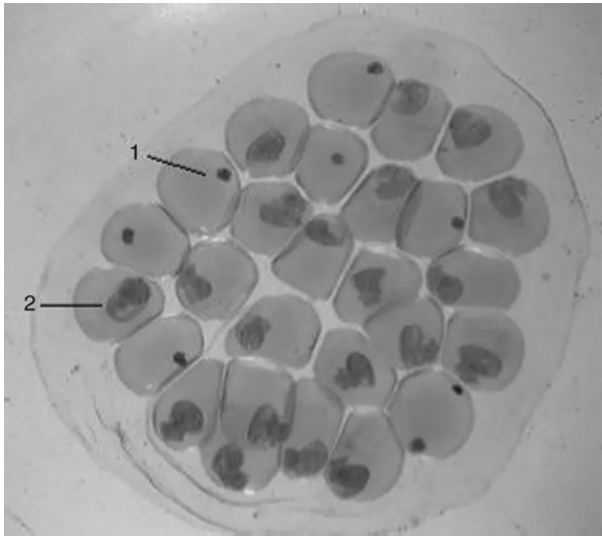


Fig. 4. Embryos of *B. glabrata* after exposure at blastula stage to 10 ppm of *P. cayabatum* leaf extract. 1, Dead embryo; 2, normal embryo at veliger stage.

### Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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