

BRIEF COMMUNICATION

OVICIDAL EFFECT OF PIPERACEAE SPECIES ON *Biomphalaria glabrata*, *Schistosoma mansoni* HOST

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SUMMARY

Schistosomiasis is a neglected disease with public health importance in tropical and subtropical regions. An alternative to the disease control is the use of molluscicides to eliminate or reduce the intermediate host snail population causing a reduction of transmission in endemic regions. In this study nine extracts from eight Piperaceae species were evaluated against *Biomphalaria glabrata* embryos at blastula stage. The extracts were evaluated in concentrations ranging from 100 to 10 mg/L. *Piper crassinervium* and *Piper tuberculatum* extracts were the most active (100% of mortality at 20 mg/L and 30 mg/L respectively).

KEYWORDS: Schistosomiasis; *Biomphalaria glabrata*; Embryos; Crude extracts; Piperaceae; Molluscicide.

INTRODUCTION

Schistosomiasis is one of the most prevalent, debilitating and neglected diseases of tropical and subtropical regions, such as Africa, Asia and South America. This disease is a relevant health and social-economic problem with more than 390-600 million people estimated to have been infected worldwide, while 800 million people remain under infection risk^{11,29}.

Currently, the main strategy to control schistosomiasis is based on the periodic treatment of people living in risk areas with anti-schistosomicidal drugs in order to reduce morbidity and transmission²⁸. However, evidence indicates that resistance and tolerance to praziquantel, a main drug used in *Schistosoma mansoni* treatment, have been increasing^{6,9}.

Freshwater snails of *Biomphalaria* genus play a major role as intermediate hosts in the transmission of *S. mansoni* because an intense multiplication of parasites occurs in these snails. Thus, any strategy to control snail populations for reduction of schistosomiasis transmission in endemic regions should consider some treatment at this critical stage¹⁵.

Currently, niclosamide marketed as Bayluscide® is the most important molluscicide recommended by World Health Organization (WHO) to control the host of schistosomiasis³². However, niclosamide, has plenty of adverse factors, such as, non-selective toxicity, that harms other aquatic organisms; high cost; instability in sunlight, which in addition requires frequent reapplication due to the permanence of surviving snails after its

application^{1,10,32}. Thus, novel natural molluscicidal should be investigated as a possible alternative to synthetic products^{16,17,18,25}.

The genus *Piper* belongs to the Piperaceae family and includes more than 2000 species widely distributed in the tropical and subtropical regions of the world¹³. *Piper* species are important plants used in Chinese medicine, in the Indian Ayurvedic system and folk medicine practices of Latin America and West Indies. Furthermore, studies with plants from the Piperaceae family have shown a great diversity of secondary metabolites with biological activity¹⁴.

Recently, schistosomicidal and molluscicidal activities of Piperaceae species have been described and this family is considered promising for studies of schistosomiasis prevention and control^{19,20,25}.

In this work, nine crude extracts of eight Piperaceae species were assessed for ovicidal activity against *Biomphalaria glabrata* (Say, 1818) embryos.

MATERIALS AND METHODS

Ovicidal assay was performed according to the methodology recommended by the WHO^{30,31} and the experimental procedures were according to the accepted principles of animal welfare in experimental science.

Embryos were obtained from *Biomphalaria glabrata* snail originally

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

Species	Collecting sites	Selected part	Voucher
<i>Piper callosum</i> Ruiz & Pav.	São Paulo, SP	leaf	K-161
<i>Piper crassinervium</i> Kunth	Apiá, SP	inflorescence	K-091
<i>Peperomia glabella</i> (Sw.) A. Dietr.	Apiá, SP	leaf	K-856
<i>Piper mallacophyllum</i> (C.Presl).C.DC.	Capão Bonito, SP	leaf	K-447
<i>Peperomia oreophylla</i> Hensch	Extrema, MG	leaf	K-579
<i>Piper solmsianum</i> C.DC	São Paulo, SP	leaf	K-487
<i>Peperomia tetraphylla</i> G. Forst	Apiá, SP	leaf	K-370
<i>Piper tuberculatum</i> Jacq.	São Paulo, SP	leaf and inflorescence	K-169

SP- São Paulo; MG- Minas Gerais.

from Belo Horizonte, MG, Brazil, and kept in a laboratory under light, temperature and feeding controlled.

Plant material: Fresh material of each Piperaceae species (Table 1) was collected by Dr Massuo Jorge Kato from Universidade de São Paulo (Chemical Institute) and identified by Dr Elsie F. Guimarães from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Vouchers were deposited in the Herbarium of Jardim Botânico do Rio de Janeiro, Brazil.

Preparation of extracts and samples: Selected parts of freshly collected plant material were separated, immediately air dried and finally dried in an oven at 45 °C for 24 h (Table 1). Material was ground and extracted with ethyl acetate: methanol (2:1) at room temperature (25-28 °C) three times and filtered. Extracts were concentrated to dryness under vacuum in a rotary evaporator and stored at -20 °C.

A stock solution containing 1 g/L of each extract was prepared by suspending 10 mg of extract in 0.1 mL of 99.9% dimethylsulphoxide (DMSO: Aldrich, Milwaukee, WI, USA) and making up to 10 mL with dechlorinated water. Stock solutions were diluted with dechlorinated water in order to provide assay solutions.

Assay for ovicidal activity: Egg masses with embryos at blastula stage⁵ were exposed to Piperaceae extracts (Table 1) firstly at 100 mg/L for 24 hours and observed for seven days. Inactive extracts at this concentration were not further investigated since crude preparation of plant material should be active at 100 mg/L or less, according to WHO³¹.

Tests were carried out with egg masses laid on small pieces of plastic sheet that had been left floating on the aquaria water. The pieces of plastic sheets with adhered egg masses were carefully transferred to Petri dishes, where they were further exposed to testing solutions. For each concentration, five egg masses were used and assays were repeated three times. The number of snail embryos to each concentration is indicated in Tables 2 and 3. At the end of exposure (24 hours), egg masses were washed with dechlorinated water. Petri dishes containing egg masses were kept within climatic chambers under controlled temperature (25 °C ± 1 °C). All egg masses were examined daily under a stereomicroscope up to the 7th day. Embryos were considered as dead whenever disintegrating embryonic forms were noted within the egg and or, at later developmental stages and no heartbeats were detected. A negative control group was maintained in 1% DMSO on dechlorinated

water under the same experimental conditions. Bayluscide WP70[®] was used as a positive control².

Table 2
Ovicidal effect of Piperaceae extracts at blastula stage at 100 mg/L

Species	Part	No. embryos	Mortality in 7 days (%)
<i>P. callosum</i>	leaf	99	5.05
<i>P. crassinervium</i> *	inflorescence	106	100
<i>P. glabella</i>	leaf	102	73.01
<i>P. malacophyllum</i>	leaf	143	5.59
<i>P. oreophylla</i>	leaf	108	0
<i>P. solmsianum</i>	leaf	103	1.78
<i>P. tetraphylla</i>	leaf	89	0
<i>P. tuberculatum</i> *	leaf	101	100
	inflorescence	98	100

* Death during the 24 h exposure period.

The LC₅₀ (50% lethal concentration) and the 95% confidence intervals for active extracts were estimated using Trimmed Spearman-Kärber Method¹².

RESULTS AND DISCUSSION

Three extracts from Piperaceae species were active with 100% of embryo mortality at 100 mg/L: *P. crassinervium* inflorescence extract and *P. tuberculatum* inflorescence and leaf extracts (Table 2). Both species showed 100% of lethality at 100 mg/L during the 24 h exposure period (Table 2, Fig. 1). These extracts were also evaluated at lower concentrations and inflorescence extract of *P. crassinervium* was more active than *P. tuberculatum* inflorescence and leaf extracts (100% of mortality at 20 mg/L and 30 mg/L respectively) (Table 3).

No increase of embryo lethality was observed in embryos exposed to leaf extracts of *P. solmsianum*, *P. callosum*, *P. oreophylla*, *P. tetraphylla*, *P. mallacophyllum* and *P. glabella* at 100 mg/L (Table 2). Percentage of dead embryos in control groups during all the study was not higher than 1.2%.

RAPADO *et al.* (2011) evaluated the molluscicidal effect of *P.*

Table 3
Species with ovicidal activity at the blastula stage in concentrations lower than 100 mg/L

Species	Selected part	Concentration (mg/L)	No. embryos	Mortality (%)	LC ₅₀ (mg/L)
<i>P. crassinervium</i>	inflorescence	5	94	0	12.39 [11.75-13.07]
		10	108	21.29	
		15	113	62.83	
		20	105	100	
<i>P. tuberculatum</i>	leaf	10	110	0	22.15 [21.41-22.92]
		15	125	5.6	
		20	98	31.63	
		25	107	55.14	
		30	101	100	
		5	117	0	
inflorescence	10	109	77.09		
	15	91	78.42		
	20	102	94.11		
	25	116	96.68		
	30	120	100		

[] 95% confidence intervals; Mortality obtained in 7 days.

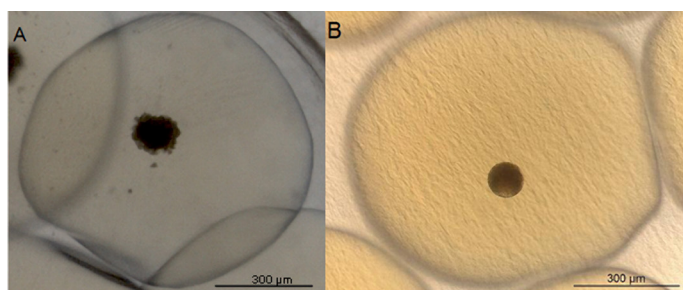


Fig. 1 - Embryos of *B. glabrata* at blastula stage during the exposure period. A- Dead embryos exposure to leaf extract of *P. tuberculatum* at 100 mg/L and B- Normal embryo (control group).

crassinervium leaf extract in *B. glabrata* adult and embryos at blastula stage obtained 100% of mortality at 60 mg/L and 50 mg/L respectively. This species has flavonoids and prenylated benzoic acid as major compound in leaf, classes of compounds with molluscicide activities already described^{8,24}. Nevertheless is not known if those compounds are responsible for the molluscicidal activity obtained in this study using inflorescence extract.

The *P. tuberculatum* is largely used in folk medicine as a sedative and antidote for snake bite. It has been shown that extracts and amides isolated from *P. tuberculatum* fruit and seeds have also a potent antifungal activity against *Cladosporium sphaerospermum* (100% active in 5 µg) and parasitic activity in *Trypanosoma cruzi* (IC₅₀ = 17.2 µg/mL in epimastigote), *Leishmania donovani* (IC₅₀ = 7.5 µg/mL in promastigote) and *S. mansoni* (100% mortality in 9.5 µM)^{4,7,19,21,22,27}. In this study, leaves and inflorescences extract showed ovicidal activity in equal concentrations, suggesting the possible presence of active compounds in both parts of the plant.

Studies with molluscicides compounds show that it is usual to obtain the death of *B. glabrata* snail but not the embryos^{18,23,25}. The

lack of ovicidal activity allows the permanence of the snail host in the environment, maintaining the transmission of schistosomiasis.

The Euphorbiaceae species are known for producing latex with molluscicidal activity restricted to *B. glabrata* adults (100% mortality at 1.5 mg/L)^{3,26}. Different from this, *Piper* species are lethal to *B. glabrata* adults and embryos in concentrations recommended by WHO as *Piper cuyabanum* (100% lethal for adults and embryos at 20 mg/L), *Piper aduncum* (100% lethal in adults at 10 mg/L and embryos at 50 mg/L) and *Piper hostmannianum* (100% lethal in adults at 40 mg/L and embryos at 20 mg/L)²⁵.

In this work, three extracts from two Piperaceae species were lethal to *B. glabrata* embryos under concentrations recommended by WHO³¹. Thus *P. tuberculatum* and *P. crassinervium* extracts were active at 30 mg/L and 20 mg/L respectively which make them species targets for isolation and identification of ovicidal compounds since these species are also active in *B. glabrata* adult²⁵.

RESUMO

Efeito ovicida de espécies de Piperaceae em *Biomphalaria glabrata*, hospedeiro do *Schistosoma mansoni*

A esquistossomose é uma doença negligenciada de importância para a saúde pública em regiões tropicais e subtropicais. Uma alternativa para o controle da doença é o uso de moluscicidas para eliminar ou reduzir a população de caramujo hospedeiro, acarretando uma redução da transmissão da doença nas regiões endêmicas. Neste estudo, nove extratos vegetais provenientes de oito espécies de Piperaceae foram expostos a embriões de *Biomphalaria glabrata* no estágio de blástula. Os extratos foram avaliados em concentrações que variaram entre 100 e 10 mg/L, sendo *Piper crassinervium* e *Piper tuberculatum* os extratos mais ativos (100% de mortalidade a 20 mg/L e 30 mg/L respectivamente).

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