BRIEF COMMUNICATION

OVICIDAL EFFECT OF PIPERACEAE SPECIES ON \textit{Biomphalaria glabrata}, \textit{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 \textit{Biomphalaria glabrata} embryos at blastula stage. The extracts were evaluated in concentrations ranging from 100 to 10 mg/L. \textit{Piper crassinervium} and \textit{Piper tuberculatum} extracts were the most active (100% of mortality at 20 mg/L and 30 mg/L respectively).

KEYWORDS: Schistosomiasis; \textit{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\textsuperscript{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 transmission\textsuperscript{28}. However, evidence indicates that resistance and tolerance to praziquantel, a main drug used in \textit{Schistosoma mansoni} treatment, have been increasing\textsuperscript{6,9}.

Freshwater snails of \textit{Biomphalaria} genus play a major role as intermediate hosts in the transmission of \textit{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 stage\textsuperscript{15}.

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

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

MATERIALS AND METHODS

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

Embryos were obtained from \textit{Biomphalaria glabrata} snail originally

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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 stage were exposed to Piperaceae extracts (Table 1) firstly at 100 mg/L for 24 hours and observed for seven days. Embryos were considered as dead when the embryolethality was observed in embryos exposed to leaf extracts of *P. solmsianum, P. callosum, P. oreophylla, P. tretraphylla, P. mallacophyllum* and *P. globella* at 100 mg/L (Table 2). Percentage of dead embryos in control groups during all the study was not higher than 1.2%.

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 was not further investigated since crude preparation of leaf material should be active at 100 mg/L or less, according to WHO.

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

| Species          | Part           | No. embryos | Mortality in 7 days (%) |
|------------------|----------------|-------------|-------------------------|
| *P. callosum*    | leaf           | 99          | 5.05                    |
| *P. crassinervium* | inflorescence | 106         | 100                     |
| *P. globella*    | leaf           | 102         | 73.01                   |
| *P. malacophyllum* | leaf          | 143         | 5.59                    |
| *P. oreophylla*  | leaf           | 103         | 1.78                    |
| *P. solmsianum* | leaf           | 103         | 1.78                    |
| *P. tetrphyllana* | leaf           | 89          | 0                       |
| *P. tuberculatum* | leaf           | 101         | 100                     |
|                  | inflorescence | 98          | 100                     |

* Death during the 24 h exposure period.

The LC$_{50}$ (50% lethal concentration) and the 95% confidence intervals for active extracts were estimated using Trimmed Spearman-Karber Method.

**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 embryolethality was observed in embryos exposed to leaf extracts of *P. solmsianum, P. callosum, P. oreophylla, P. tetrphyllana, P. mallacophyllum* and *P. globella* 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 molluscidal effect of *P.
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. 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 (IC<sub>50</sub> = 17.2 µg/mL in epimastigote), Leishmania donovani (IC<sub>50</sub> = 7.5 µg/mL in promastigote) and S. mansoni (100% mortality in 9.5 µM)\textsuperscript{8,24}. 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 mollusicides compounds show that it is usual to obtain the death of B. glabrata snail but not the embryos\textsuperscript{18,21,25}. The lack of ovicidal activity allows the permanence of the snail host in the environment, maintaining the transmission of schistosomiasis.

In this work, three extracts from two Piperaceae species were lethal to B. glabrata embryos under concentrations recommended by WHO\textsuperscript{31}. 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 adult\textsuperscript{25}. The Euphorbiaceae species are known for producing latex with molluscicidal activity restricted to B. glabrata adults (100% mortality at 1.5 mg/L)\textsuperscript{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).\textsuperscript{25}

The 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 moluscidicidas para eliminar ou reduzir a população de caramujo hospedeiro, acarretando uma redução da transmissão da doença nas regiões endemicas. 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).

Table 3

| Species          | Selected part | Concentration (mg/L) | No. embryos | Mortality (%) | LC<sub>50</sub> (mg/L) |
|------------------|---------------|----------------------|-------------|---------------|------------------------|
| P. crassinervium| inflorescence | 5                    | 94          | 0             | 12.39 [11.75-13.07]    |
|                  |               | 10                   | 108         | 21.29         |                        |
|                  |               | 15                   | 113         | 62.83         |                        |
|                  |               | 20                   | 105         | 100           |                        |
|                  | 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           |                        |
| P. tuberculatum  | inflorescence | 5                    | 117         | 0             | 9.07 [8.57-9.60]       |
|                  |               | 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.

Table 3

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

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).
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