

Versatile natural molluscicides against the snail *Biomphalaria glabrata* Say, 1818 (Pulmonata: Planorbidae), the principal vector of *Schistosoma mansoni* on the American continent

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Abstract

Schistosomiasis is a millennial disease that affects approximately 235 million people. Considering that most of the population affected by schistosomiasis faces different conditions of poverty, finding compounds from plants that can help control or eradicate this disease would be worthwhile especially if the plants are readily available for those who need to use them the most. This study aimed to evaluate the effects of fresh latex from fourteen locally cultivated Euphorbiaceae species on the freshwater snail *Biomphalaria glabrata* Say, 1818 (Gastropoda: Planorbidae), the principal vector of the *Schistosoma mansoni* trematode on the American continent. Adult *B. glabrata* snails were exposed to various concentrations of fresh latex from Euphorbiaceae species for 24 and 48 hours. Mortality data were analyzed using the probit method. To evaluate the latex ecotoxicity of the promising species against other aquatic organisms, lethality tests were performed on *Poecilia reticulata* Peter, 1859 (Cyprinodontiformes: Poeciliidae) fish and *Artemia salina* Leach (Anostraca: Artemiidae) nauplii. Three species presented significant molluscicide activity: *Euphorbia milii* var. *breonii* ($LC_{50} = 0.9 \mu\text{g/mL}$), *E. milii* var. *milii* ($LC_{50} = 1.1 \mu\text{g/mL}$) and *E. umbellata* ($LC_{50} = 9.1 \mu\text{g/mL}$). Only *E. milii* (var. *breonii* and var. *milii*) was not lethal to the evaluated fish (LC_{50} of $9.9 \mu\text{g/mL}$) and *A. salina* ($LC_{50} > 100.0 \mu\text{g/mL}$). These results qualify both the *E. milii* variety of fresh latices as versatile (active at low concentrations, biodegradable, inexpensive, and obtained from renewable resources) and alternative molluscicidal agents against *B. glabrata* snails.

1 Introduction

Schistosomiasis is a millennial disease of poverty that leads to chronic ill health. It is caused by trematodes in the *Schistosoma* genus and affects almost 235 million people worldwide, and more than 700 million people live in endemic areas. The infection is prevalent in tropical and subtropical regions and in poor communities without potable water or adequate sanitation. It is the second most important tropical disease and is responsible for mortality after malaria (WHO, 2023).

Over the years, there have been attempts to eradicate this disease. However, the number of cases of this disease has been increasing with progressive consolidation in endemic areas (Coelho & Caldeira, 2016). On the American continent, the causative agent of human schistosomiasis is the *Schistosoma mansoni* trematode. The life cycle of this parasite involves an intermediate host represented by freshwater snails from the genus *Biomphalaria*, and the *B. glabrata* snail is the principal vector in South America and the Caribbean (Coelho & Caldeira, 2016). The occurrence of mansonic schistosomiasis is closely related to precarious socioenvironmental conditions. The lack of adequate sanitary facilities is one of the major causes of the continuous emergence of new cases of this vermin disease because it is a waterborne disease (Augusto & Silva, 2018).

Several medications have already been utilized for mansonic schistosomiasis treatment, among which oxamniquine and praziquantel are available in Brazil (Coelho & Caldeira, 2016). These substances are indicated for the treatment of individuals who eliminate viable eggs in their feces so that more severe

forms can be avoided, disease propagation can be reduced, and a clinical cure can be reached (Augusto & Silva, 2018). However, there are too many side effects, and because the financial burden is low, few pharmaceutical companies have developed new drugs for treatment. While the prevalence of this disease is continuous, one of the reasonable options for reducing cases of this parasitic disease could be the control of the intermediate host using substances that have molluscicidal activity to interrupt the developmental cycle of the parasite and, consequently, the emergence of new cases of the disease (Coural & Amaral, 2004; Augusto & Silva, 2018).

Among the known synthetic molluscicides, niclosamide (Bayluscide®) is the most efficient and is recommended by the World Health Organization (WHO). However, the high expense of its application in extensive areas makes its use prohibitive in most developing countries. In addition to the cost, concerns related to the toxicity of nontarget organisms and the possible development of resistance to snails (McCullough et al. 1980) have led to a demand for alternative molluscicides of vegetal origin (Clark et al., 1997; Alves et al., 2000; Augusto & Silva, 2018).

Many species of tropical plants that have substances with molluscicidal activity, mainly among the Asteraceae, Euphorbiaceae, Fabaceae, and Phytolaccaceae, contain different kinds of substances involved in secondary vegetal metabolism with biocidal action (Kloss & McCullough, 1982; Mendes et al., 1999; Luna et al., 2005).

Considering that most of the population affected by schistosomiasis endures different classes of poverty, finding compounds from plants that can help control or eradicate this disease would be worthwhile especially if the plants are readily available for those who need to use them the most. Therefore, this study aimed to evaluate the effects of fresh latex from fourteen locally cultivated Euphorbiaceae species (native and exotics) on *B. glabrata* snails. These species were chosen according to the following criteria: wide availability, easy adaptation to tropical regions, toxicity, easy cropping (latex), and easy preparation of the molluscicide solution (fresh latex). Additional tests on ecotoxicity against nontarget organisms at molluscicidal concentrations were performed with the latices of the species that presented potential molluscicidal activity.

2 Materials and Methods

2.1 Plant material

All the species utilized in this study were collected in autumn (March-May) at the Medicinal Plant Garden of Paranaense University (Umuarama, Paraná State, Brazil), which is located at an altitude of 430 m above sea level (23°46'09.5" S-53°16'42.4" W). A voucher for each specimen was deposited at the Educational Herbarium of Paranaense University ("Herbário Educacional da Universidade Paranaense" - HEUP): *Euphorbia cotinifolia* L. (HEUP-2524); *E. milii* var. *breonii* (Nois.) Urch. & Leandri (HEUP-2471); *E. milii* Des Moul. var. *milii* (HEUP-2481); *E. pulcherrima* Willd ex. Klotzsch (HEUP-2552); *E. tirucallii* L. (HEUP-2529); *E. tithymaloides* L. [Syn. *Pedilanthus tithymaloides* (L.) Poit] (HEUP-2270); *E. umbellata*

(Pax) Bruyns [Syn. *Synadenium grantii* Hook., f.] (HEUP-2476); *Jatropha curcas* L. (HEUP-2601); *J. elliptica* (Pohl) Oken (HEUP-2506); *J. gossypifolia* L. (HEUP-2272); *J. multifida* L. (HEUP-2440); *J. podagrica* Hook. (HEUP-2271); *Manihot utilissima* Pohl. (HEUP-2603); and *Ricinus communis* L. (HEUP-2549).

2.2 Fresh latex processing

The latex from each species was extracted through incisions in the stems or leaf petioles a little before carrying out the tests. The collected latex was immediately diluted in water at a ratio of 1:9 and quickly transported to the laboratory to prepare the other utilized dilutions.

2.3 Evaluation of molluscicidal activity

In this study, *Biomphalaria glabrata* Say, 1818 (Pulmonata: Planorbidae) snails were utilized for tests of molluscicide activity. The snails were adapted to tanks at a temperature of 23–25°C and under natural light management, according to previous methods (Silva et al., 2008). Some samples of guppy fish (*Poecilia reticulata* Peter, 1859; Cyprinodontiformes: Poeciliidae) and stems of *Elodia canadensis* (Hydrocharitaceae) aquatic plants were also adapted to the tank conditions, allowing them to make up the fish tank environment. Food was provided *ad libitum* and consisted of fresh lettuce (*Lactuca sativa*) for the snails and specific feed for the fish. The bioassay with adult *B. glabrata* specimens was performed according to the procedures recommended by the WHO (1965, 1983), with minor alterations (Silva et al., 2008). First, a stock solution was prepared at three concentrations (100.0, 50.0, and 25.0 µg/mL) for the evaluation of molluscicide potential in fresh latex. Groups of adult mollusks (shell diameter 10–15 mm) were randomly selected and transferred to mini tanks filled with different latex solutions. After 24 h of exposure, the snails were removed, washed with water, and subsequently moved to other mini tanks with dechlorinated water; after 24 h, the recovery time was maintained until mortality was recorded. The control groups were provided only fish tank water without additional substances in all the assays. Assays using niclosamide (Atenase®, UCI-Farma) as the reference molluscicide (0.5 µg/mL) were performed. The snail retraction within the shell or the release of hemolymph were adopted as the death criteria. Moreover, heartbeats were observed using a stereoscopic microscope to verify mollusk mortality. The extracts that caused 100% mortality at the lowest tested concentration (25.0 µg/mL) were selected for an additional test at lower concentrations (25.0, 5.0, 1.0, and 0.5 µg/mL) to determine lethal concentration values at 50% of the tested population (LC₅₀).

2.4 Ecotoxicity assays

The ecotoxicity of fresh latices that presented promising molluscicide activity was verified against two nontarget organisms: guppy fish (*P. reticulata*) and brine shrimp (*Artemia salina* Leach, Anostraca: Artemiidae). The procedure was like that for the bioassay involving *B. glabrata*. Fish and *A. salina* nauplii were separately exposed to different concentrations of promising latices (100.0, 25.0, and 5.0 µg/mL) for 24–48 h at 24–26°C. After this period, the lethality of the strains against populations of fish and nauplii was tested.

2.4 Statistical analysis

The results relating to molluscicidal activity and ecotoxicity are expressed as the mean and percentage (%) for lethality tests on snails (*B. glabrata*), guppy fish (*P. reticulata*) and brine shrimp (*A. salina*). Comparisons between mortality rates were performed through analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$ to observe whether there was any difference between the fresh latices analyzed and the concentration. The estimated lethal concentration to kill 50% of the specimens (LC₅₀) was calculated from statistical software using probit analysis.

3 Results

The first phase of this study consisted of evaluating the molluscicide activity of fresh latex from exotic or native species known for its toxicity. In this preliminary approach, *B. glabrata* snails were subjected to only three concentrations (100.0, 50.0, and 25.0 µg/mL) of fresh latices from fourteen Euphorbiaceae species to determine the most active latices for posterior LC₅₀ tests. Table 1 shows the mortality rates of the snails in this initial evaluation. The data revealed differences in the efficacy of the molluscicidal activities of the fourteen fresh latices species evaluated ($F = 12.91$, $p < 0.05$). However, no significant difference between the concentrations was observed ($F = 2.17$, $p > 0.05$).

Table 1
Preliminary evaluation of molluscicide activity of fresh latices from several exotic species of Euphorbiaceae against *Biomphalaria glabrata* snails after 24 h of exposure and recovery.

Latex	% lethality		
	100.0 µg/mL	50.0 µg/mL	25.0 µg/mL
<i>Euphorbia cotinifolia</i>	0	0	0
<i>Euphorbia milii</i> var. <i>breonii</i>	100	100	100
<i>Euphorbia milii</i> var. <i>milii</i>	100	100	100
<i>Euphorbia pulcherrima</i>	0	0	0
<i>Euphorbia tirucallii</i>	100	10	0
<i>Euphorbia tithymaloides</i>	0	0	0
<i>Euphorbia umbellata</i>	100	100	100
<i>Jatropha curcas</i>	0	0	0
<i>Jatropha elliptica</i>	0	0	0
<i>Jatropha gossypifolia</i>	0	0	0
<i>Jatropha multifida</i>	0	0	0
<i>Jatropha podagrica</i>	0	0	0
<i>Manihot utilissima</i>	100	6.7	0
<i>Ricinus communis</i>	0	0	0

Positive control: niclosamide (0.5 µg/mL).

In this preliminary phase, *B. glabrata* snails were strongly affected when exposed to fresh latices from *Euphorbia milii* (var. *breonii* and var. *milii*) and *E. umbellata* species. Due to their high molluscicide potential, these species were reevaluated at lower concentrations to determine the LC₅₀ value. Fresh latices from *E. tirucallii* and *Manihot utilissima* were also active but only at the highest tested concentration (100 µg/mL). The other species did not exhibit activity at the highest tested concentration.

According to the recommendations of the WHO (1983), only aqueous or alcoholic extracts of vegetal materials that cause the death of 90% of the malacological population at concentrations lower than 20 µg/mL are considered potentially active and viable for field assays, while less active extracts may very well provide sources of new lead compounds with molluscicidal activities. Considering the observed results for *E. milii* var. *breonii* (LC₅₀ = 0.9 µg/mL), *E. milii* var. *milii* (LC₅₀ = 1.1 µg/mL) and *E. umbellata* (LC₅₀ = 9.1 µg/mL), their fresh latices can be classified as potential molluscicide agents (Table 2). No

significant difference between these potent fresh latices was detected ($F = 3.10$, $p > 0.05$); however, the lethality data as a function of concentration were significantly different ($F = 10.44$, $p < 0.05$).

Table 2
Complementary tests of the molluscicide activity of fresh latices from *Euphorbia milii* and *Synadenium grantii* against adult *Biomphalaria glabrata* snails after 24 h of exposure and recovery.

Latex	% lethality				LC ₅₀ (µg/mL)
	25.0 µg/mL	5.0 µg/mL	1.0 µg/mL	0.5 µg/mL	
<i>Euphorbia milii</i> var. <i>breonii</i>	100	90	50	10	0.9
<i>Euphorbia milii</i> var. <i>milii</i>	100	90	50	0	1.1
<i>Synadenium grantii</i>	100	10	0	0	9.1

Positive control: niclosamide (0.5 µg/mL).

Ecotoxicity tests were carried out to evaluate the toxicity of the fresh latices from *E. milii* (var. *breonii* and var. *milii*) and *E. umbellata* plants against nontarget organisms such as fish and small crustaceans (Table 3). Guppy fish (*P. reticulata*) was used as the nontarget organism in this study because it is sensitive to contaminants and is easily found in rivers and lagoons in Brazil, providing results with great practical significance. *A. salina*, commonly known as brine shrimp, is a small crustacean that has been the subject of many physiological studies. The brine shrimp lethality assay is one of the most practical tools for the preliminary assessment of general toxicity (McLaughlin et al., 1991); moreover, it is a good option for ecotoxicity assays because it is a sensitive nontarget organism and is usually utilized in biomonitoring or detection of toxic or bioactive compounds in vegetal extracts.

Table 3
Evaluation of the ecotoxic effects of different concentrations of fresh lattices from *S. grantii* and *E. milii* L. on guppy fish (*Poecilia reticulata*) and brine shrimp (*Artemia salina*) after 24 h of exposure and recovery.

Latex	Concentration ($\mu\text{g/mL}$)	% lethality	
		<i>P. reticulata</i>	<i>A. salina</i>
<i>Euphorbia milii</i> var. <i>breonii</i>	100.0	100	0
	25.0	100	0
	5.0	0	0
	LC ₅₀	9.9 $\mu\text{g/mL}$	> 100 $\mu\text{g/mL}$
<i>Euphorbia milii</i> var. <i>milii</i>	100.0	100	0
	25.0	100	0
	5.0	0	0
	LC ₅₀	9.9 $\mu\text{g/mL}$	> 100 $\mu\text{g/mL}$
<i>Synadenium grantii</i>	100.0	100	10
	25.0	100	0
	5.0	0	0
	LC ₅₀	9.9 $\mu\text{g/mL}$	> 100 $\mu\text{g/mL}$

E. umbellata and both varieties of *E. milii* (*breonii* and *milii*) were completely lethal to guppy fish at a concentration of 25.0 $\mu\text{g/mL}$, with an LC₅₀ = 9.9 $\mu\text{g/mL}$ (Table 3). These fresh lattices did not show toxicity against *A. salina* (LC₅₀ > 100 $\mu\text{g/mL}$). No statistically significant difference was found ($p > 0.05$).

4 Discussion

The control of snail vectors is an alternative way to prevent schistosomiasis. This strategy is particularly relevant in endemic localities in rural and urban areas, where the lack of sanitation and adequate housing show little potential for improvement in the future (Augusto & Silva, 2018). Efforts are being made to discover molluscicide products of natural origin that are potentially biodegradable to address the growing awareness of environmental pollution. In this sense, this work focused its attention on the search for plant-based molluscicides obtained by simple, inexpensive, and efficient extraction and application techniques that are capable of being used by needy communities affected by schistosomiasis, opening possibilities for sustainable improvements in the health of these communities. Generally, latex is an aqueous emulsion found in the vacuole of secretory cells and is composed of lipids, resins, sugars, proteins, and enzymes. It can be easily obtained and manipulated for the preparation of molluscicides,

dispensing the use of solvents and equipment for the preparation of extracts or extraction of active components. The use of latex from locally grown species by communities associated with health education programmes can help keep schistosomiasis at low levels since eradication is not considered realistic under current conditions in some developing countries. Among the fourteen Euphorbiaceae species evaluated in this study, only the fresh latices of three species were potentially active as molluscicides: *E. milii* var. *breonii*, *E. milii* var. *milii*, and *E. umbellata* (Table 2).

Originally from Madagascar, *Euphorbia milii* Des Moulin's species is known in Brazil, where some varieties are cultivated as ornamental plants or hedges. In the present study, fresh latex solution of *E. milii* var. *breonii* had different molluscicidal effects than did the crude extract of leaves of this same species, which did not show molluscicidal activity in a previous study ($LC_{50} > 100 \mu\text{g/mL}$) (Hartmann et al., 2011). The latex at a concentration of $5.0 \mu\text{g/mL}$ exhibited 100% lethality in the first 24 h of the experiment. After 48 h, the lethal concentration for 50% of the exposed population was calculated to be $0.9 \mu\text{g/mL}$. This difference in activity between fresh latex and the crude extract (Hartmann et al., 2011) is probably due to the concentration of substances responsible for molluscicidal activity. The concentration of the active substance in the leaf extract was likely low, or the substance could have degraded or polymerized during extract preparation. Curiously, the results observed for the *breonii* variety were very close to those obtained for the *milii* variety (Table 1; $LC_{50} = 1.1 \mu\text{g/mL}$). These concentrations are much lower than the WHO (1983) recommended concentrations for molluscicide activity. It was also noted that the calculated LC_{50} value of the latex was very close to that reported in the literature for some species and varieties known as Christ's crown or crown-of-thorns ($LC_{50} = 0.12$ to $LC_{50} = 2.0 \mu\text{g/mL}$) (Vasconcelos & Schall, 1986; Baptista et al., 1994; Carvalho et al., 1998; Oliveira-Filho & Paumgarten, 2000; Coêlho et al., 2018). *E. milii* Des Moul. Ex Boiss var. *hislopii* (syn. *E. splendens*) lyophilized latex is one of the most potent molluscicide plants tested in outlying areas. Zani et al. (1993) isolated eight categories of ingenol esters (miliamines) from *E. milii* var. *hislopii* and tested their molluscicide activities on *B. glabrata*, with miliamine L being the most efficient, with lethality occurring at concentrations as low as 1.0 ppb. The latex of this variety was also very toxic to other Planorbidae snails (Oliveira-Filho & Paumgarten, 2000), suggesting that fresh latex from *E. milii* var. *milii* and var. *breoni* could also be good molluscicides against other snail species.

Another relevant aspect of extract concentrations is concern about other components of aquatic biota. Even though natural molluscicides are biodegradable at specific concentrations, these extracts may have risks even within the values demanded by the WHO. Therefore, toxicity tests showed that the use of latices from *E. milii* (var. *breonii* and var. *milii*) as molluscicide agents would be favorable because both species are lethal to snails at lower concentrations ($LC_{50} \sim 1.0 \mu\text{g/mL}$) than are the ones that are lethal to guppy fish ($LC_{50} = 9.9 \mu\text{g/mL}$) and *A. salina* microcrustaceans ($LC_{50} > 100.0 \mu\text{g/mL}$). Therefore, these latices can be considered atoxic to the environment at molluscicide concentrations ($LC_{50} \sim 1.0 \mu\text{g/mL}$). These data corroborated those of Oliveira-Filho and Paumgarten (2000), who analyzed the ecotoxicity of lyophilized latex from *E. milii* var. *hislopii*.

E. umbellata (syn. *Synadenium grantii*) is an African species cultivated as an exotic ornamental plant in Brazil where it is considered highly toxic. The population utilizes latex to reduce warts, treat gastric diseases, and even treat cancer (Kinghorn, 1980). According to the literature, the chemical composition of latex from this species includes derivatives of phorbol esters (Kinghorn, 1980; Bagavathi et al., 1988), triterpenoids (Uzabakiliho et al., 1987), proteolytic enzymes (Menon et al., 2002), carboxylesterase enzymes (Govindappa et al., 1987), acetylcholinesterase isoenzymes (Govindappa et al., 1987) and glycoproteins (Rajesh et al., 2006). During the evaluation of the molluscicidal activity of fresh latex from this species, it was found to be potent (Table 2). As previously mentioned for *E. milli* var. *breoni*, the crude alcoholic extract of *E. umbellata* leaves is less active ($LC_{50} = 40.0 \mu\text{g/mL}$; Hartmann et al., 2011) than fresh latex is. Regarding ecotoxicity, in the case of fresh *E. umbellata* latex, the lethal concentration for *B. glabrata* ($LC_{50} = 9.1 \mu\text{g/mL}$) was close to that of guppy fish ($LC_{50} = 9.9 \mu\text{g/mL}$) (Tables 2 and 3). Thus, although the latex from this species is considered a good molluscicide agent, its use requires ample care due to its toxicity to other organisms present in the aquatic environment at the same concentration, as latex is lethal to the vector snail of *Schistosoma mansoni*. These data differed from those achieved by Pereira et al. (2017), who reported an $LC_{50} = 1.36 \mu\text{g/mL}$ for lyophilized latex. These authors also performed assays with *Danio regio* (zebrafish) to investigate the toxicity of *E. umbellata* latex to nontarget organisms, and they found that this latex was less toxic to zebrafish ($LC_{50} = 7.22 \mu\text{g/mL}$) than to *B. glabrata* ($LC_{50} = 1.36 \mu\text{g/mL}$). The difference in activity observed between our results and those of the authors (Pereira et al., 2017) may be related to different factors: type of latex (fresh/lyophilized), latex harvesting period (autumn/spring), and geography (harvesting in the area close to the Tropic of Capricorn/harvesting in the equatorial area-coast).

Another evaluated species in this study also presented latices with moderate molluscicidal activity. In Brazil, the African milkbush or *aveloz* plant (*E. tirucalli*), which is very caustic and popularly used for wart scaring, is known for its latex toxicity. As part of the screening of the molluscicide species, latex from this species was evaluated and found to have moderate activity against *B. glabrata*, with an $LC_{50} = 89.4 \mu\text{g/mL}$. Cassava (*Manihot utilissima* Pohl) is a native plant known for its great usefulness in the economy and history of Brazil. One of its varieties, “mandioca-brava”, is also known for poisoning cases in humans and animals. Considering the toxic character of your latex, its possible molluscicide activity was evaluated. Latex from leaf petioles of *M. utilissima* also presented moderate molluscicidal activity ($LC_{50} = 95.0 \mu\text{g/mL}$).

Latex from Barbados nuts (*Jatropha curcas*) did not exhibit activity at the highest tested concentration ($100 \mu\text{g/mL}$; Table 1) in the present study. Other authors have observed significant molluscicidal activity of *J. curcas* against different vector snails (Liu et al., 1997; Rug & Ruppel, 2000). According to Nath and Dutta (1991), latex from *J. curcas* has a protolithic enzyme called curcain. Another study indicated two significant activities for *J. curcas* latex: procoagulant and anticoagulant (Osoniyi & Onajobi, 2003). Therefore, even if latex contains interesting substances related to other activities, this does not indicate who is a good molluscicide. Latices from another species of this genus were evaluated in this study (*J.*

elliptica, *J. gossypifolia* L., *J. multifida* L., and *J. podagrica*); however, none of them presented molluscicidal activity at the highest tested concentration (Table 1).

5 Conclusions

Among the several Euphorbiaceae species evaluated in this study, only *E. milii* (var. *breonii* and var. *milii*) and *E. umbellata* presented potent molluscicidal effects on *B. glabrata* snails. The use of fresh latices from *E. milii* varieties represents a feasible alternative strategy for schistosomiasis control because these plants can be efficiently grown in endemic areas (not requiring soil care, fertilizers, or water); additionally, these plants yield a relatively large amount of latex throughout the year, and the latex can be easily collected. In summary, these fresh latices are active at low concentrations, are biodegradable and inexpensive, and are obtained from renewable resources. The preparation of these molluscicides is straightforward, does not require organic solvents (typically used to prepare extracts) or equipment (rotary evaporator or freeze dryer), and can be easily prepared by the population most affected by schistosomiasis. The molluscicidal activity of fresh latex from *E. milii* var. *breoni* and *E. milii* var. *milii* latices is reported here for the first time. On the other hand, the fresh latex from *E. umbellata* was strongly lethal to *B. glabrata* snails ($LC_{50} = 9.1 \mu\text{g/mL}$) and guppy fishes ($LC_{50} = 9.9 \mu\text{g/mL}$). Consequently, their use as molluscicides is contraindicated because they require special attention due to their possible toxicity against nontarget organisms.

Declarations

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Author Contributions

D. B. H., Z. C. G. and A. L. J. contributed to the conception and design of the experiments. E. J. contributed to the collection and identification of the botanical material. D. B. H., R. A. M., G. Z., I. A. S., and E. J. contributed to the data acquisition. D. B. H., R. A. M., and A. L. J. contributed to the analysis and interpretation of the data. A. L. J. supervised the project and wrote the manuscript with input from all the authors. All the authors read and agreed to the publication of this version of the article.

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Competing interests

The authors declare no conflicts of interest.

Ethical approval

Does not apply.

Informed consent

Does not apply.

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