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Molluscicidal activity of extracts of plants from the Cerrado against *Biomphalaria glabrata* (Say, 1818)

Atividade Moluscicida de extratos de plantas do Cerrado em *Biomphalaria glabrata* (Say, 1818)

Actividad Moluscicida de extractos de plantas del Cerrado en *Biomphalaria glabrata* (Say, 1818)

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Abstract
Plant-derived molluscicides have been indicated as selective and low-cost strategies for the control of *Biomphalaria glabrata* (Say, 1818), an intermediate host of schistosomes. This study aimed to evaluate the aqueous and ethanolic extracts of leaves of plants present in the Cerrado such as *Caryocar brasiliense* Camb., *Ximenia americana* L., *Piptadenia viridiflora* (Kunth) Benth., and *Schinopsis brasiliensis* Engl. as alternatives in the control of the mollusk *B. glabrata*. For this, leaves were collected from plants at the in Montes Claros-MG. Extracts at concentrations of 150, 100, 75, 50, and 25 µg/mL, a positive control containing niclosamide at 3 µg/mL, and a negative control containing dechlorinated water were used to verify the molluscicidal activity. To evaluate toxicity to non-target organisms, *Artemia salina* was treated with extract concentrations of 10, 100, and 1000 µg/mL. The tested aqueous and ethanolic extracts showed significant mortality within 24 h of exposure. At concentrations above 75 µg/mL, the aqueous extracts of *P. viridiflora, C. brasiliense*, and *S. brasiliensis* achieved mortality higher than 90%. Regarding ethanolic extracts, mortality above 80% was observed for all tested plants at concentrations above 50 µg/mL. No toxicity was observed against *A. salina*. Thus, high molluscicidal activity of ethanolic and aqueous extracts of the tested plants against adult *B. glabrata* was observed and no toxicity was observed against *A. salina.*
Keywords: Alternative control; *Schistosoma mansoni*; Plant extracts; Scrubland.

Resumo
Moluscicidas derivados de plantas têm sido indicados como estratégias biodegradáveis e de baixo custo para o controle da *Biomphalaria glabrata* (Say, 1818), hospedeira intermediária da esquistossomose. O objetivo desse trabalho foi avaliar os extratos aquosos e etanólico de folhas de plantas presentes no Cerrado como *Caryocar brasiliense* Camb., *Ximenia americana* L., *Piptadenia viridiflora* (Kunth) Benth e *Schinopsis brasiliensis* Engl. como uma alternativa no controle do molusco *B. glabrata*. Para isso, as folhas foram coletadas em Montes Claros-MG. Para verificar a atividade moluscicida dos extratos, foram utilizadas concentrações de 150, 100, 75, 50 e 25 µg/ml dos extratos, um controle positivo contendo Niclosamida a 3 µg/ml e um controle negativo contendo água desclorada. Para avaliar a toxicidade frente a organismos não alvo, utilizou-se a *Artemia salina* nas concentrações de 10, 100 e 1000 µg/ml. Observou-se que os extratos aquosos e etanólicos testados demonstraram mortalidades significativas antes de 24h de exposição. Sendo que os extratos aquosos de *P. viridiflora, C. brasiliense e S. brasiliensis* nas concentrações acima de 75 µg/ml alcançaram mortalidade superior a 90%. Já para o extrato etanólico, todas as plantas testadas nas concentrações acima de 50 µg/ml observaram-se mortalidade acima de 80%. Não foi observada toxicidade frente a *A.salina.* Dessa forma, constatou-se alta atividade moluscicida dos extratos etanólico e aquosos das plantas testadas sobre adultos *B.glabrata* e não foi observada toxicidade frente a *A.salina*.
Palavras-chave: Controle alternativo; *Schistosoma mansoni*; Extratos vegetais; Cerrado.

Resumen
Los molusquicidas derivados de plantas han sido señalados como estrategias biodegradables y de bajo costo para el control de *Biomphalaria glabrata* (Say, 1818), un huésped intermedio de la esquistosomiasis. El objetivo de este trabajo fue evaluar los extractos acuoso y etanólico de hojas de plantas presentes en el Cerrado como *Caryocar*

brasiliense Camb., *Ximenia americana* L., *Piptadenia viridiflora* (Kunth) Benth y *Schinopsis brasiliensis* Engl. como alternativa para el control del molusco *B. glabrata*. Para eso, las hojas fueron recolectadas en Montes Claros-MG. Para verificar la actividad molusquicida de los extractos se utilizaron concentraciones de 150, 100, 75, 50 y 25 µg/ml de los extractos, un control positivo que contenía Niclosamida a 3 µg/ml y un control negativo que contenía agua declorada. Para evaluar la toxicidad frente a organismos no diana, se utilizó *Artemia salina* en concentraciones de 10, 100 y 1000 µg/ml. Se observó que los extractos acuoso y etanólico ensayados presentaron mortalidades significativas antes de las 24h de exposición. Los extractos acuosos de *P. viridiflora*, *C. brasiliense* y *S. brasiliensis* a concentraciones superiores a 75 µg/ml lograron una mortalidad superior al 90%. En cuanto al extracto etanólico, todas las plantas ensayadas a concentraciones superiores a 50 µg/ml presentaron una mortalidad superior al 80%. No se observó toxicidad frente a *A. salina*. Así, hubo alta actividad molusquicida de los extractos etanólico y acuoso de las plantas probadas sobre adultos de *B. glabrata* y no se observó toxicidad contra *A. salina*.
Palabras clave: Control alternativo; *Schistosoma mansoni*; Extractos de plantas; Matorral.

1. Introduction

Schistosomiasis mansoni is a parasitic disease caused mainly by the trematode helminth *Schistosoma mansoni*. Considered a major public health problem in several countries, it has a considerable social impact (Dawaki et al., 2016). In the Americas, humans are the main definitive host and planorbids of *Biomphalaria* are intermediate hosts of schistosomiasis in its life cycle (Holanda et al., 2020). As the species most found in the Americas, *Biomphalaria glabrata* (SAY, 1818) is highly efficient in transmitting the parasite owing to its extensive geographic distribution (Carvalho et al., 2018).

Some measures have been taken to prevent the spread of the disease, such as treatment of the affected individuals, improved access to education and health, basic sanitation, and mollusk control (Katz, 2018), with the last measure interrupting the transmission cycle through the control of intermediate host populations (Katz, 2018). The disease is mainly controlled using synthetic molluscicides to prevent the proliferation of snails and consequently reduce the cases of the disease (Rocha-Filho et al., 2015). However, the control has not being effective, because despite causing the death of mollusks in low concentrations, synthetic molluscicides also influence the life cycle of other species in the same ecological niche, causing environmental imbalance (Rollinson et al., 2013; Rocha- Filho et al., 2015; Martins et al., 2021).

Thus, the search for alternative control agents using plants is increasing as they possess several substances, such as secondary metabolites, which provide them with important biological actions (Simões et al., 2017; Cantanhede et al., 2010). Moreover, many species of tropical plants, mainly of the Asteraceae, Euphorbiaceae, Fabaceae, and Phytolacaceae families, contain substances with molluscicidal activity (Al-Zanbagi et al., 2001; Afonso-Neto et al., 2010; Vieira et al., 2016).

The Cerrado is known to have a wide diversity of plant species with great variation in secondary metabolites. It is considered the second largest ecological domain in Brazil and a promising environment for the scientific and technological development of the country (Rodrigues et al., 2016). In this sense, evaluation of natural molluscicidal activity is one of the promising complementary alternatives to traditional chemistry in the reduction of the populations of the schistosomiasis-transmitting mollusk.

Plants found in this biome, such as *Caryocar brasiliense* Camb., *Ximenia americana* L., *Piptadenia viridiflora* (Kunth) Benth, and *Schinopsis brasiliensis* Engl., popularly known as *pequi* (souari nut), tallow wood, *surucucu*, and *braúna*, respectively, have several biological applications such as anthelmintic, acaricidal, and anti-inflammatory agents (Moraes Costa et al., 2015; Vasconcelos et al., 2018; James et al., 2007; Bezerra et al., 2015). Thus, this study aimed to evaluate the viability of aqueous and ethanolic extracts of leaves of plants prevalent in the Cerrado such as *C. brasiliense*, *X. americana*, *P. viridiflora*, and *S. brasiliensis* as alternatives to control the mollusk *B. glabrata.*

2. Methodology

The present study was conducted at the Laboratory of Parasitology of the Institute of Agricultural Sciences in the

Montes Claros Campus of the Federal University of Minas Gerais. The municipality of Montes Claros (16°44′6″ S, 43°51′43″ W) located in the North of Minas Gerais is part of the Cerrado domain, with transition areas between the Cerrado and Caatinga.

2.1 Obtaining plant material

Dehydrated leaves of *C. brasiliense*, *P. viridiflora*, *X. americana*, and *S. brasiliensis* were collected in June at the Institute of Agricultural Sciences in the Montes Claros Campus of the Federal University of Minas Gerais (16°41′10.05″ S, 43°50′33.56″ W). After taxonomic identification, one exsiccate of each plant was deposited in the Herbarium Montes Claros (HMC)/UNIMONTES, with voucher numbers 156, 2283, 211, and 377, respectively, and were registered in SISGEN (A95A7D4, AFA7072, A75FA55, and AD861E4, respectively). The plant samples that did not show lesions or deterioration were washed in running water and dried with absorbent paper. Subsequently, they were dehydrated in a forced air circulation oven at a temperature of 40 ± 5 °C until constant weight was obtained. All plant material was ground in a knife mill and stored at a temperature of approximately 4 °C (Nery et al., 2010).

2.2 Obtaining aqueous (AE) and ethanolic (EE) extracts

The methodology for obtaining the extracts was adapted from Nery et al. (2010). The AE was obtained as a decoction, where 100 g of the dried and ground material was submerged in 1000 mL of sterile distilled water, homogenized, and incubated in a water bath at 30 °C for 60 min. Subsequently, the material was filtered through a funnel with gauze and cotton and stored at 4 °C until use. To obtain EE, 100 g of the dry material was placed in 1000 mL of ethanolic alcohol for approximately 7 d in amber glass. Subsequently, the liquid extract was placed in glass containers and placed in an oven with forced air circulation at a temperature of 40 ± 5 °C for approximately 3 d until constant weight was obtained. Finally, the obtained dry extract was stored in plastic bags containing silica at 4 °C. The dry matter of samples of both dry extracts was determined using a dry matter tester (AOAC, 1990).

2.3 Obtaining the mollusk *B. glabrata*

The mollusks were obtained from a colony maintained at the Renê Rachou Research Center in Belo Horizonte – Minas Gerais. The contamination-free mollusks were kindly provided by the institution. The snails of uniform size (shell diameter between 11 mm and 18 mm) were wrapped in moistened gauze and transported in a Styrofoam box to the Parasitology Laboratory of the Institute of Agricultural Sciences of Federal University of Minas Gerais (UFMG) Montes Claros Campus, where the experiments were conducted.

2.4 Experimental procedures

The effectiveness of the plant material extracts was determined following Bezerra et al. (2002), using concentrations of 150, 100, 75, 50, and 25 µg/mL, a positive control containing niclosamide (Bayluscide® WP70) at 3 µg/mL, and a negative control containing dechlorinated water. The extracts were diluted in dechlorinated water and the snails were placed in 6-well plates containing five adult snails. The experiments were conducted with six replicates per concentration.

The mollusks were exposed to the extracts for 24 h at 25 °C. Mortality counts were taken at 1, 2, 6, 12, and 24 h. During the experiment, dead mollusks were removed.

2.5 Statistical analysis

The percentage efficacy in mollusk mortality was determined using the formula adapted from Borges (2003) as below:

% Efficacy = Mean live mollusks from control - Mean live mollusks from treatments / mean live mollusks from control × 100

The experiments were conducted using a completely randomized design. Data were subjected to analysis of variance and mean values were compared using the Scott–Knott test ($p < 0.05$) using the statistical package SAEG 9.1 (2007). The concentrations of extracts capable of causing 90% mortality (LC90) were determined.

2.6 Toxicity test

The toxicity test was performed using a non-target species such as *Artemia salina* (Leach) nauplii following the methodology proposed by Meyer (1982). The AE and AA of *C. brasiliense*, *X. americana*, and *P. viridiflora* were tested at concentrations of 10, 100, and 1000 µg/mL. All tests were performed three times in 10 mL polystyrene plastic containers, with 10 larvae in the nauplius stage being transferred to each of the treatments. A total of 5 mL of saline solution was used as the control. After 24 h of exposure, the live larvae were counted, with considering as dead those that did not move during the observation, even with a slight shaking of the flask.

3. Results

The data on mortality as a function of time (Tables 1 and 2) showed significant mortalities with the AA and EE treatments with 24 h of exposure.

Table 1. Mortality counts of *Biomphalaria glabrata* at 1, 2, 6, 12, and 24 h in the aqueous extracts of *Piptadenia viridiflora*, *Ximenia americana*, *Caryocar brasiliense*, and *Schinopsis brasiliensis*

Treatment	Concentration µg/mL	1 h	2 h	6 h	12 h	24 h
C. brasiliense	25	-	-	-	18	-
	50	-	-	-	24	-
	75	-	-	27	-	-
	100	30	-	-	-	-
	150	30	-	-	-	-
X. americana	25	-	-	10	-	-
	50	-	12	-	-	-
	75	-	-	24	-	-
	100	-	-	27	-	-
	150	30	-	-	-	-
P. viridiflora	25	-	-	18	-	-
	50	-	12	-	-	-
	75	-	-	24	-	-
	100	-	-	27	-	-
	150	30	-	-	-	-
S. brasiliensis	25	-	18	-	-	-
	50	-	24	-	-	-
	75	27	-	-	-	-
	100	30	-	-	-	-
	150	30	-	-	-	-
Dechlorinated water		-				
Niclosamide		30				

Total number of individuals = 30 mollusks. Source: Authors.

Table 2. Mortality counts of *Biomphalaria glabrata* at 1, 2, 6, 12, and 24 h in the ethanolic extracts of *Piptadenia viridiflora*, *Ximenia americana*, *Caryocar brasiliense*, and *Schinopsis brasiliensis*.

Treatment	Concentration µg/mL	1 h	2 h	6 h	12 h	24 h
C. brasiliense	25	-	-	-	18	-
	50	-	-	-	24	-
	75	-	-	27	-	-
	100	27	-	-	-	-
	150	30	-	-	-	-
X. americana	25	7	9	2	-	-
	50	11	8	11	-	-
	75	20	4	6	-	-
	100	21	6	3	-	-
	150	30	-	-	-	-
P. viridiflora	25	-	30	-	-	-
	50	-	30	-	-	-
	75	-	30	-	-	-
	100	30	-	-	-	-
	150	30	-	-	-	-
S. brasiliensis	25	24	-	-	-	-
	50	24	-	-	-	-
	75	27	-	-	-	-
	100	30	-	-	-	-
	150	30	-	-	-	-
Dechlorinated water		-				
Niclosamide		30				

Total number of individuals = 30 mollusks. Source: Authors.

The AE of *P. viridiflora*, *C. brasiliense*, and *S. brasiliensis* achieved a mortality higher than 90% in adult snails of *B. glabrata* at concentrations above 75 µg/mL (Table 1). The EE of all tested plants achieved a mortality above 80% at concentrations above 50 µg/mL (Table 2).

A dose-dependent response was observed with all extracts. EE and AE from leaves of *P. viridiflora*, *X. americana*, *C. brasiliense*, and *S. brasiliensis* were effective in controlling adult snails of *B. glabrata* (Table 3). The LC90 of the AE of *P. viridiflora*, *S. brasiliensis*, *X. americana*, and *C. brasiliense* were 105.18 µg/mL (Confidence interval [CI] = 111.38–99.91); 61.80 µg/mL (CI = 66.73–57.69); 157.80 µg/mL (CI = 169.26–148.45), and 61.80 µg/mL (CI = 66.73–57.69), respectively. The LC90 of the EE of *S. brasiliensis* and *C. brasiliense* was 67.40 µg/mL (CI = 74.85–61.46) and 66.45 µg/mL (CI = 75.54–60.54), respectively. The LC90 of EE from *P. viridiflora* and *X. americana* were not calculated, as 100% efficacy was obtained with almost all tested concentrations.

Table 3. Percentage efficacy of the aqueous and ethanolic extracts of leaves of *Piptadenia viridiflora*, *Ximenia americana*, *Caryocar brasiliense*, and *Schinopsis brasiliensis* on adult snails of *Biomphalaria glabrata*

Concentrations µg/mL[1]	*P. viridiflora*	*X. americana*	*C. brasiliense*	*S. brasiliensis*
Aqueous extract				
150	100[a]	100a	100a	100a
100	90[a]	90a	100a	100a
75	80b	80b	90a	90a
50	40d	40c	80b	80b
25	60c	33c	60c	60c
Niclosamide *	100[a]	100a	100a	100a
Dechlorinated water	0	0	0	0
CV	17,35	33,26	9,19	15,83
Ethanolic extract				
150	100a	100a	100a	100a
100	100a	100a	90a	100a
75	100a	100a	90a	90a
50	100a	100a	80b	80b
25	100a	60b	60c	80b
Niclosamide *	100a	100a	100a	100a
Dechlorinated water	0	0	0	0
CV	0	7,38	10,93	9,39

Different letters in the same column differ statistically according to Duncan's test at 5% probability. % Efficacy = Mean live snails from control – Mean live snails from treatments / mean live snails from control × 100. Source: Authors.

The data obtained from the toxicity test on *A. salina* did not show significant mortalities in the extracts with 24 h of exposure (Table 4). It was not possible to obtain the LC50, as there was no significant mortality differences in any of the treatments.

Table 4. Evaluation of the mortality percentage of aqueous and ethanolic extracts of leaves of *Piptadenia viridiflora*, *Ximenia americana*, and *Caryocar brasiliense* on *Artemia salina*.

Concentrations µg/mL	*P. viridiflora*	*X. americana*	*C. brasiliense*
Aqueous extract			
1000	1,5	0	0
100	2,25	0	0
10	0	0	0
Distilled water	0	0	0
Ethanolic extract			
1000	35,5	18,25	3,5
100	11,25	0	0
10	0	0	0
Distilled water	0	0	0

Source: Authors.

4. Discussion

Niclosamide is the only synthetic molluscicide recommended by the World Health Organization (WHO) because it has low toxicity in mammals (Silva Filho et al., 2009; Jin et al., 2010) with insignificant absorption in the gastrointestinal tract (Costa, 2015). However, niclosamide has low selectivity, which can affect species of the local fauna, causing an ecological imbalance. Moreover, it can stimulate the development of resistance mechanisms in mollusks and its high cost of application in extensive areas limits its use in developing countries, which are the countries most affected by schistosomiasis (Silva Filho et al., 2009; Coelho & Caldeira, 2016). Thus, discovering new molluscicides that are more selective for species of *Biomphalaria* and less harmful to the aquatic ecosystem is critical (Coelho & Caldeira, 2016). Therefore, there is great interest in the use of molluscicides of plant origin for a self-sustaining system of the schistosomiasis control program (Ibrahim et al., 2004).

The activities of AE and EE from leaves of *C. brasiliense* were considered significant, with 90% mortality at a concentration of 75 µg/mL in 6 h of exposure to the extracts. This biological activity was also confirmed by Lopes et al. (2011), who obtained 100% mortality in 24 h using a hydroalcoholic extract of the leaves of *C. brasiliense* at a concentration of 0.17 mg/mL against the adults of *B. glabrata*. However, the results obtained in the present study demonstrated that *C. brasiliense* leaf extracts were effective at lower concentrations and showed responses within short periods of exposure to the extracts. One-hundred percent mortality was achieved when using the EE from the leaves of *X. americana* at the concentration of 50 µg/mL. Uchoa et al. (2006) obtained similar results (80% and 90% mortality) when using the ethanolic extract of the bark of *X. americana* at the concentration of 50 µg/mL against adults of *B. glabrata*.

In the present experiment, 100% mortality at a concentration of 100 µg/mL was obtained in 1 h with both *S. brasiliensis* extracts tested. Santos et al. (2014) noted that the ethyl acetate fraction from the shell of *S. brasiliensis* has a toxic effect on *B. glabrata* mollusks, obtaining 90% mortality at all tested concentrations (25, 50, and 100 µg/mL) in a 48 h experiment.

Although some metabolites have been previously described in the literature as having molluscicidal action, their toxicity against the mollusk *B. glabrata* has been associated with different mechanisms of action (Singab et al., 2006, Faria et al., 2018, Mendes et al., 2018). Flavonoids, for example, are known to induce disruption of cell membranes (Faria et al., 2018) and reduce heart rate in this host (Singab et al., 2006). Tannins and saponins, in turn, decrease reproductive capacity and induce behavioral changes such as lethargy and decreased feeding capacity (Mendes et al., 2018). Bahgat et al. (2018) found

that the molluscicidal activity of saponins may also be associated with their characteristic detergent effect on the soft body membranes of mollusks belonging to *Biomphalaria*. Coumarins were reported to play a role in the clotting process (Kady et al., 1992).

Lopes et al. (2011) found important classes of secondary metabolites, such as tannins, saponins, steroids, flavonoids, coumarins, and resins, in the hydroalloic extracts of *C. brasiliense* leaves that may have molluscicidal activity. The compounds found in aqueous and methanolic extracts of leaves, stem bark, and root of *X. americana* were saponins, glycosides, flavonoids, tannins, phenolic compounds, alkaloids, quinines, and terpenoids (James et al., 2007; Monte et al., 2012). Phytochemical studies of *S. brasiliensis* leaves also demonstrate the presence of polyphenols (gallic and ellagic acid), flavonoids (aglycones), steroids, terpenoids, lignins, triterpenoids, condensed tannins, and leucoanthocyanidins (Saraiva et al., 2011). Condensed tannins and flavonoids were found in the EE of *P. viridiflora* leaves (Morais-Costa et al., 2015).

To ensure the safety of using these extracts in aquatic environments, and therefore, establish tolerable non-toxic concentrations to non-target species, the WHO recommends performing toxicity tests (Schiffer and Liber, 2017; WHO, 2017). The toxicity test on *Artemia salina* Linnaeus (1758) is a widely used biological assay because it is fast, reliable, and low cost (Amarante, 2011). The research conducted to date using the method involving *A. salina* aims at identifying the biological actions of a certain natural extract, whether antifungal, insecticide, or molluscicide (Rosa et al., 2016; Cansian et al., 2016; Cansian et al., 2017). The test with *A. salina* performed in the present study did not show toxicity, as the LC50 of dead nauplii during the period of exposure of the extracts could not obtained owing to the low percentage of mortality in the treatments. Moreover, a low toxicity can be considered an interesting feature for the use of plant extracts in natural environments to control the snail population, because niclosamide is toxic to aquatic species such as *A. salina* at low concentration (LC50 of 0.18 mg/L) with variable effects (LC50 ranging between 0.25 and 1.23 mg/L) on other organisms, such as fish and zooplankton crustacean algae (Oliveira Filho; Paumgartten, 2000; Rocha and Filho, 2015).

Ascari et al. (2011) evaluated the toxicity of crude ethanol extract, fractions, and substances isolated from the epicarp and external mesocarp of *C. brasiliense* and found that these extracts were not toxic against *A. salina*, having an LC50 = 160.80 µg/mL. According to Meyer et al. (1982), extracts with LD50 > 1000 µg/mL are not considered toxic. Alves et al. (2000) tested fractions obtained from leaves and stem bark extracted with ethyl acetate from *C. brasiliense* and observed an LC50 = 90 µg/mL after 24 h of exposure to *A. salina*. Furthermore, Duavy et al. (2012) obtained LC50 values of 18.5 µg/mL and 14.9 µg/mL when testing aqueous and ethanolic extracts of *C. coriaceum* on *A. salina*.

Thus, the activity observed, the low toxicity, and the ease of preparation and solubility in water of the tested extracts, suggest that they can be used to control *B. glabrata* populations. These plants are known to occur naturally in virtually the entire Brazilian territory and can be easily cultivated, features considered as important by Clark et al. (1997) in choosing a molluscicide.

5. Conclusion

The aqueous and ethanolic extracts of *Piptadenia viridiflora*, *Ximenia americana*, *Caryocar brasiliense*, and *Schinopsis brasiliensis* were effective in controlling adult mollusks of *Biomphalaria glabrata* at concentrations of 50, 75, 100, and 150 µg/mL. None of the extracts showed toxicity against *Artemia salina*.

Finally, it is suggested that these extracts be tested in other non-target organisms, in addition to *Artemia salina*, to have more reliability in their use and advance to *in vivo* experiments.

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