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Toxicity and molluscicidal activity of the essential oil *Pimenta dioica* against the snail *Biomphalaria glabrata*

Toxicidad y actividad molusccidal del aceite esencial *Pimenta dioica* contra el caracol *Biomphalaria glabrata*

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Retraction statement

We, the Editor and Editor Committee of Revista Peruana de Biología (RPB) have retracted the article: "Barros Gomes P.R., Batista Reis J., Pêssoa Fernandes R., Mouchrek Filho V.E., Gouveia de Souza A., Alves Fontenele M. and Caetano da Silva J. 2019. Toxicity and molluscicidal activity of the essential oil Pimenta dioica against the snail Biomphalaria glabrata. Revista Peruana De Biología 26 (1), 101-8. https://doi.org/10.15381/rpb.v26i1.15913." Corresponding author, Paulo Roberto Barros Gomes (August 12, 2019) requested the retraction of the article, because the information, tables and graphics of the article in RPB had already been published previously in another journal: " Everton G.O., Teles A.M., Mouchrek A.N., & M. Filho V.E. Aplicação do Óleo Essencial de Pimenta Dioica Lindl. As Molluscicide in front of Caramujo Transmissor da Esquistossomose. Revista Processos Químicos 12 (23): 85-94 (https://doi.org/10.19142/rpq.v12i23.433)".

The information included in the RPB and Revista Processos Químicos are parts of the results of the thesis: "Chemical characterization, evaluation of toxicity and molluscicidal activity two oils essenciais da folha of Lindic diode pepper, Citrus limon Linneo e Rizoma of Zingiber officinale Roscoe" (https://repositorio.ufpb.br/jspui/handle/tede/7038) (2011-05-11) by Romer Pessôa Fernandes, with advisors Victor Elias Mouchrek Filho and Antonio Gouveia de Souza. Editor Committee of RPB, on October 7 and 14, had meetings and conclude that the article will be retracted for duplicate publication and authorship ethical problems. All the authors were mailing on December. 9, 2019. Revista Peruana de Biologia calls for all readers do not cite this work, and apologises to the authors and to readers for the inconvenience caused.

Declaración de retracción

El Comité editor de la Revista Peruana de Biología (RPB) retracta el artículo: "Barros Gomes P.R., Batista Reis J., Pêssoa Fernandes R., Mouchrek Filho V.E., Gouveia de Souza A., Alves Fontenele M. and Caetano da Silva J. 2019. Toxicity and molluscicidal activity of the essential oil Pimenta dioica against the snail Biomphalaria glabrata. Revista Peruana De Biología 26 (1), 101-8. https://doi.org/10.15381/rpb.v26i1.15913." El 12 de agosto 2019, el autor de correspondencia Paulo Roberto Barros Gomes solicto la retracción del mencionado trabajo porque la informacion, figuras y tablas, ya habian sido publicadas en: " Everton G.O., Teles A.M., Mouchrek A.N., & M. Filho V.E. Aplicação do Óleo Essencial de Pimenta Dioica Lindl. As Molluscicide in front of Caramujo Transmissor da Esquistossomose. Revista Processos Químicos 12 (23): 85-94 (https://doi.org/10.19142/rpq.v12i23.433)".

La informacion enlas dos revistas son parte de los resultado de la tesis: "Chemical characterization, evaluation of toxicity and molluscicidal activity two oils essenciais da folha of Lindic diode pepper, Citrus limon Linneo e Rizoma of Zingiber officinale Roscoe" (https://repositorio.ufpb.br/jspui/handle/tede/7038) (2011-05-11) de Romer Pessôa Fernandes, con los advisors Victor Elias Mouchrek Filho y Antonio Gouveia de Souza. El 14 de octubre de 2019, el Comité editor de la RPB se reunió y acordó la retracción del artículo por publicación duplicada y problemas éticos de autorías. Los autores fueron comunicados el 9 de diciembre de 2019. La Revista Peruana de Biología solicita a los lectores a no citar este trabajo, y pide disculpas a los lectores por las inconveniencias causadas.

TRABAJOS ORIGINALES

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Palabras clave: Eugenol; Esquistosomiasis; Compuestos Volátiles; Pimenta dioica; Biomphalaria glabrata.
Keywords: Eugenol; Schistosomiasis; Volatile Compounds; Pimenta dioica; Biomphalaria glabrata.

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Introduction

The *Pimenta dioica* (L.) Merr. (Mirtácea) is native to the Antilles and Central America, where its fruits are used as seasoning and condiment (Suárez et al. 2000). Besides this utility, Central American populations use *P. dioica* as Folk medicine in treatment of indigestion, hypertension, influenza, rheumatism, fever and diabetes, due to the presence of tannins and essential oils (Vargas Chinchilla 1990, Germosen Robineau 1995, Suárez et al. 2000). Among these components, the most important is the essential oil, which can be extracted from leaves and fruits and has a yield of 1.5 to 4.5%. In addition, studies have identified the following components: eugenol (70-80%), 1,8-cineol, α-humulene, β-caryophyllene and cadinene derivatives (Tucker et al. 1991, Marongiu et al. 2005, López Hernández et al. 2008).

Due to the high eugenol composition in the essential oil of *Pimenta dioica* it is possible to consider whether it has molluscicidal activity against *Biomphalaria glabrata* (Say 1818). Since studies with eugenol, thymol and linalol have shown that they presented molluscicidal activity against *Biomphalaria alexandrina* and *Bulinus truncatus*, being these, respectively, intermediate hosts of the parasite *Schistosoma mansoni* and *Schistosoma haematobium*, transmitter of the schistosomiasis disease (T Sharaf el-Din 2006). In addition, it is also known that eugenol has other activities, such as: larvicide (Gomes et al. 2018), antimicrobial (Sanla-Ead et al. 2012), antioxidante (Yogalakshmi et al. 2010), anti-inflammatory (Yogalakshmi et al. 2010), antifungal (Wang et al. 2005), antinociceptive (Daniel et al. 2009).

In Brazil, the intermediate hosts of *Schistosoma* are species of *Biomphalaria*. To date, it is recognize that only *B. glabrata, B. tenagophila* and *B. straminea* are infected by *S. mansoni*, while *B. amazonica* and *B. peregrina* are considered potential hosts, because experimentally they have been infected. In the State of Maranhão, *B. glabrata* and *B. straminea* occur in 30 and 39 municipalities, respectively, mainly in the capital, São Luís (Carvalho et al. 2008).

Thus, one of action to combat this disease is the control of snails. Based on this principle, synthetic compounds are the most used: calcium hydroxide, Gramaxone®, Frescon® and Bayluscid® (niclosamide) (Cantanhede et al. 2010). However, the use of these is disadvantageous and this ends up limiting its use. Among these, we highlight: toxicity to other species, low selectivity, environmental contamination and resistance of *B. glabrata* molluscs. Thus, there is a great need to find new molluscicides, preferably of natural origin, which are less aggressive to the environment and more selective (Gasparotto Jr. et al. 2005, Colley et al. 2014).

According to the context, in this work, we evaluated the toxic and molluscicidal activities of the essential oil of the *Pimenta dioica* leaves against the snail *Biomphalaria glabrata*.

Material and methods

The present work was developed at the Laboratory of Physical Chemistry of the Technological Pavilion, Nucleus of Basic and Applied Immunology (NIBA) of the Federal University of Maranhão (UFMA), Analytical Central of the University of Campinas, Analytical Center of UFMA, Nucleus of Fuel, Catalysis and Environmental (NCCA).

Obtaining essential oil.- The leaves were collected in the Mixed Agricultural Cooperative of the Onça Ltda Project, in the municipality of Taperoá-BA, Brazil, in April 2009, registered in the botanical archives of the Biodynamic Institute (IBD), Botucatu according to certificate in CA021205, stored in containers plastics and transported to the Laboratory of Physical Chemistry of Food of the Technological Pavilion of the Federal University of Maranhão (UFMA). For extracting the essential oil, a glass Clevenger extractor was coupled to a 1000 mL round bottom flask and to an electric blanket as a heat source. At each extraction routine, they were weighed and crushed in an electric mill 30 g of the sample. After this step, it was mixed with distilled water in the proportion 1:10 and placed in a round bottom flask coupled to the extractor system. Then the electric blanket was connected and the temperature was maintained at 100 °C. After 5.0 hours the distillation was quenched by collecting the essential oil. This was dried by means of percolation in anhydrous sodium sulfate (Na_2SO_4). These operations were performed in triplicates and the samples stored in amber glass ampoules under refrigeration to avoid possible losses of volatile constituents. Subsequently, these oils were submitted for analyzes.

Analysis by gas chromatography coupled to mass spectrometry (GC-MS).- For the chemical analysis, the gas phase chromatography coupled to the electron impact mass spectrometer and ion trap analyzer (CG-EM-IE-Ion trap) was used. The equipment used was of the Varian 2100 brand, using helium as drag gas with flow in the column of 1mL min^{-1}; injector temperature 270 °C, split 1:50; (15mx0.25mm) with stationary phase VF-1ms (100% methylsiloxane 0.25 µm) and oven temperature programming from 60 to 200 °C with a heating rate of 8 °C min^{-1} and 200 – 290 °C with heating rate of 15 °C min^{-1}. In the Mass Spectrometer the mainfold, ion trap and transfer line temperatures were 50 °C, 190 °C and 200 °C, respectively. Aliquots of 1.0 µL (automatic injector CP-8410), of the samples diluted in the proportion of 20 µL in 1.5 mL of hexane were injected. Chromatographic peaks were identified by comparing the respective mass spectra with the spectral data (1) Wiley 139; (2) Nist107 E (3) Nist21.

Obtaining the snails.- Samples of the snails were collected in the natural breeding sites of the Sá Viana neighborhood, in the outskirts of São Luís, Maranhão. The catch was carried out during the rainy season (January to June 2009), with the use of PPE (personal protection equipment), such as a glove, boot seven leagues and metal clamp. The collection technique consists in scraping the submerged areas with the shell and the collected snails were placed in a glass container with a lid,

with water from the breeder it self (Brasil & Ministério da Saúde 2008), as shown in Figure 1. The search of the same was performed at several points in each in order to obtain a good sampling. After collection, these were labeled by nursery and taken to NIBA for further analysis.

Figure 1. Colon of snails in the breeder with metallic shell.

Parasitized test of snails.- The snails were kept in the laboratory for 30 days, being analyzed every seven days to confirm the absence of *S. mansoni* infection. After this step, five snails were placed in clear glass vials (30 mL capacity) with 25 mL of dechlorinated water, that is, 5 mL per snail, where they were exposed to light (100 W lamps), at one distance of 30 cm for 1 h to stimulate the release of cercariae (Smithers & Terry 1965). After this exposure, the glasses were taken for analysis through visualization using a stereoscopic magnifying glass, with magnification 8×. Those that were parasitized (positive) were labeled and separated for future individual analysis and those who did not show signs of infection by the trematode within 30 days were selected for molluscicidal activity test. After the positivity test, the snails were placed in polystyrene containers with dechlorinated water and fed with hydroponic lettuce for future test of molluscicidal activity.

Molluscicidal Activity Test according to the Method given by the World Health Organization.- Molluscicidal activity was performed according to a manual described by the World Health Organization (WHO), using only snails that were negative for the positivity test. For this, the essential oil obtained in this study was used in two tests, which were performed in triplicate. The first, called pilot test, was placed ten adult snails, negative for *Schistosoma mansoni* in each beaker containing 500 mL of a solution obtained from the dilution of the oil with distilled water and 0.15 mL of Tween 80 (surfactant) in the concentration of 100 mg·L^{-1}, obtaining at the end a proportion of 50 mL of solution for each snail and feeding them with hydroponic lettuce *ad libittum* (Malek 1985). They were exposed in the solution for 24 h, at room temperature. After this period, the snails were removed from the solution, washed twice with dechlorinated water, placed in each beaker containing 500 mL of dechlorinated water, fed with hydroponic lettuce and observed every 12 h (method recommended 24 h) for four days to evaluate mortality.

In the second test, called lethal concentration (CL), ten adult snails, negative for *Schistosoma mansoni* were placed in each beaker containing 500 mL of a solution obtained from the dilution of each oil with distilled water and 0.15 mL of Tween 80 (surfactant) at the concentrations of 75, 50, 25 and 10 mg·L^{-1}, obtaining at the end a proportion of 50 mL of solution for each snail and feeding them with hydroponic lettuce *ad libittum* (Malek 1985). They were exposed in the solution for 24 h, at room temperature. After this period, the snails were removed from the solution, washed twice with dechlorinated water, placed in each beaker containing 500 mL of dechlorinated water, fed with hydroponic lettuce and observed every 12 h (method recommended 24 h) for four days to evaluate mortality.

Two tests were performed for the control test. In the first, ten snails immersed in 500 mL of dechlorinated water were placed in a transparent glass; in the second, ten snails immersed in a solution with 0.15 mL of Tween 80 in 500 mL of dechlorinated water and feeding them both with hydroponic lettuce and proceeding with the same analysis carried out in the previous tests.

Molluscs are considered dead when their cephalopodal mass is retracted into the shell, releasing the hemolymph, or becoming swollen and extending the cephalopod out of the shell (McCullough et al. 1980).

Culture of *Artemia salina*.- The *Artemia saline* cysts were transferred to an aquarium containing synthetic saline solution (60 g of sea salt / liter of distilled water) and oxygen saturation, obtained with the aid of an air pump. The aquarium was divided into two interconnected compartments, the cysts remaining in one of the compartments, leaving the second compartment under artificial illumination of a 100 W lamp. After 24 h, the cysts hatched, the larvae migrated to the lighted compartment because they had phototropism positive. These were transferred to an aquarium containing synthetic saline and kept in incubation for another 24 h under the same lighting and oxygenation conditions. The methodology used was described by (Meyer et al. 1982) but with modifications.

Toxicity test.- For the evaluation of the lethality of *Artemia salina*, 20 mg of the oil was added to 0.02 mg of Tween 80, the volume was filled to 2 mL with artificial saline. This dilution was done to obtain a 10 mg·mL^{-1} stock solution and a concentration of 0.1% Tween 80. Samples of 5, 50 and 500 µL of this stock solution were transferred to vials with 5 mL of final solution, obtaining concentrations of 10, 100 and 1000 mg·L^{-1}, respectively. Ten larvae in the nauplii phase were transferred to each flask. White (saline) was made with 20 µL and the negative control (saline and 0.1% tween 80) was made with 20 µL. After 24 hours of incubation, the live larvae were counted, considering those microcrustaceans that did not move during observation and with slight agitation of the flask.

We adopted the criterion established by Dolabela (1997), which considers LC_{50} samples less than 80 mg·L^{-1}, highly toxic; with LC_{50} between 80 and 250 mg·L^{-1}, moderately toxic and LC_{50} greater than 250 mg·L^{-1}, nontoxic (Dolabela 1997).

Statistical analysis.- The statistical analysis of the data was performed according to the method of Reed-Muench (Reed & Muench 1938), which assumes that an animal that survives a certain dose will also survive in any other dose lower than that, consequently, if the animal dies with a specific dose, also will die in doses greater than that. From a table containing the mortality data for each concentration tested, a graph is constructed showing a curve for the accumulation of dead animals at each concentration and another curve for the accumulation of survivors. The point of intersection between the curves is the 50% Lethal Concentration (LC_{50}), because at this point the number of surviving animals is equal to the number of dead animals (Colegate & Molyneux 2007).

The confidence interval was calculated from the plot of the percentage of dead versus log (Log) of the dose. Next, the value of "R", which is the difference between the log of the dose that kills 75% of the larvae and the log of the dose that kills 25% of the larvae, is determined. The variable "h" is also calculated, which is the mean of the differences in the log values of the doses. With this data the log of the standard error (SE) is determined by the following formula: $(SE)^2 = 0.79 \times h \times R / 20$. Finally, the confidence interval value is equal to 2×10^{SE} (Pizzi 1950).

Results and discussion

Evaluation of the chemical characteristics of the essential oils obtained by Gas Chromatography coupled to Mass Spectroscopy (GC-MS).- The result of this study allowed the identification of seven constituents: Eugenol, Chavicol, Terpinol, Linalol, Limonene, Mirceno and Octenol. Table 1 shows the percentages and retention time of each.

From the percentages of each component, Table 1 identified the majority and minority components, which are, respectively, Eugenol (85.67%) and Linalol (0.88%). Therefore, the results of this study are in accordance with other studies that identified eugenol as a major component for the same part of the plant, although there are variations in its quantity (Lawrence 1978, Green & Espinosa 1988, Tucker et al. 1991).The explanation for differences in eugenol levels is the seasonality. This makes the content vary with the time of year and the stage of plant development. Another important factor in this variation is the role played by eugenol in pollination and in the plant defense mechanism (Mouchrek Filho 2000).

Toxicity test evaluation.- *Artemia saline* cultures were incubated at an average temperature of approximately 28 °C, and the number of dead were read after 24 hours. All dead larvae were considered as having no active movement in the observed time. Table 2 shows the mean of the toxicity tests of the oil extracted from the leaves of the *Pimenta dioica*.

According to Table 2, the concentration of 10 mg·L^{-1} of the essential oil of leaves d of *P. dioica* showed the lowest larvicidal activity, killing, on average, five larvae, corresponding to 50% mortality. At the concentration of 100 mg·L^{-1} presented a mortality of 80%. From the concentration of 1000 mg·L^{-1} of the essential oil, larvicidal activity caused the death of 100% of the tested individuals, that is, ten larvae.

Figure 2 shows the Lethal Concentration (LC_{50}). The calculated Logarithm result of the concentration was 1.15. According to the criteria adopted by Dolabela (1997), the evaluation of the toxicity of the essential oil in the tested concentrations against the microcrack *Artemia salina*, showed in the period of exposure of 24 hours, highly toxic toxicity, that is, LC_{50} (mg·L^{-1}) equal to 14, 13 mg·L^{-1}, with a confidence interval of 95% for the oil extracted from the *Pimenta dioica.*

Evaluation of the control test.- After the exposure period, that is, 24 hours, it was observed that there was no mortality of the larvae, as shown in Table 3.

The assessment of the toxicity of the essential oil in the concentration indicates the importance of the general toxicity test to prove the safety against non-target organisms in the occurrence regions of the snails (Dolabela 1997). In the literature no studies have been found concerning the toxicity of plants of this genus.

Table 1. Identification of the compounds in the essential oil sample of the leaves of *Pimenta dioica*

Peak[1]	[2] T.R (min)	Components	Percentage (%)
1	8.772	Octenol	1.186
2	9.164	Mirceno	2.762
3	10.488	Limonene	1.730
4	13.251	Linalol	0.884
5	16.122	Terpinol	0.973
6	19.026	Chavicol	6.792
7	22.755	Eugenol	85.673

Note: [1]Peak number in column elution order; [2]T.R: Retention time of the compounds.

Table 2. Mortality of larvae after tests on the different concentrations of essential oil extracted from *Pimenta dioica*

Concentration (mg·L^{-1})	Log Concentration	Dead	Live	Acumul. dead	Acumul. live	Mortality (%)
1000	3	10	0	23	0	100
100	2	8	2	13	2	80
10	1	5	5	5	7	50

Number of larvae (n = 10)

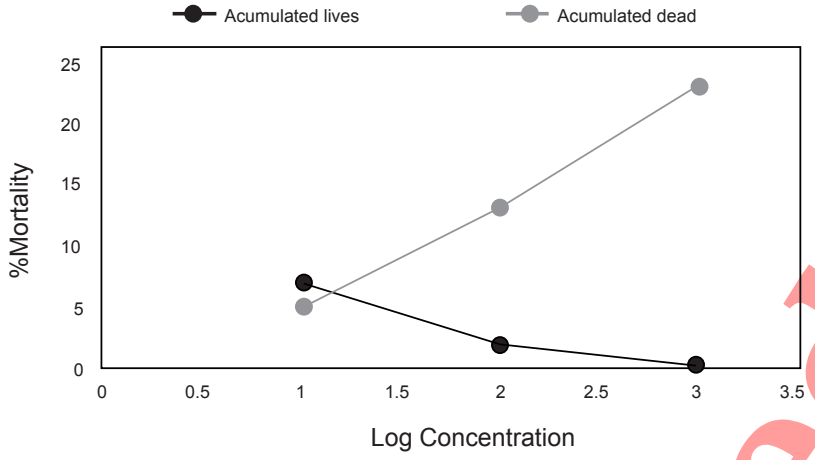

Figure 2. Estimation of the LC_{50} of the essential oil of the leaves of *Pimenta dioica* by the Reed-Muench method from the accumulated live and dead larvae as a function of the logarithm of the applied dose. The LC_{50} is the point of intersection of the two curves.

Table 3. Evaluation of the mortality of the control test for *Artemia salina*

Control	Reagents	Mortality
Blank 1	Saline solution	All active larvae
Blank 2	Saline solution + tween 80 a 0.1%	All active larvae

Capture and test positivity of snails.- The capture of the snails was carried out in rainy periods due to a greater proliferation, where the areas are flooded by the lack of sanitation, providing an excellent habitat. The positivity tests showed the presence of larval stages in some snails, a very worrying fact, since only one snail can release several cercariae daily (Neves 1992).

Evaluation of molluscicidal activity.- The methodology of the World Health Organization is the official reference for research on plants and their active principles, in order to ascertain their potential for molluscicidal activity.

Evaluation of the pilot test.- In this study a pilot test was carried out with the essential oil, in the concentration of 100 mg·L^{-1}. This test was performed to verify the existence or not of the molluscicidal activity. The study showed that the oil extracted from the leaves of *P. dioica* eliminated 70% of the snails exposed in the period of 24 hours, still noting hemorrhage; in the time superior to this, only 90% of the snails had died. Thus characterizing the oil has components capable of eliminating adult snails of the genus *B. glabrata.*

Assessment of lethal concentration.- For the essential oil of *P. dioica*, the concentrations of 75, 50, 25 and 10 mg·L^{-1} were tested in order to determine the lowest lethal concentration. The results of this study are shown in Figure 3.

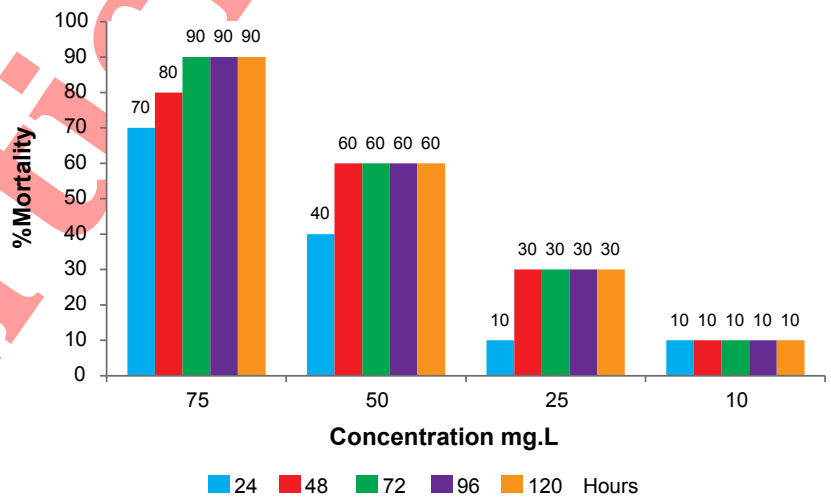

Figure 3. Simultaneous evaluation of mortality as a function of concentration and time of exposure.

Figure 3 shows a graph of the relationship between mortality and concentration and mortality and exposure time. Therefore, the molluscs evaluated at times 24, 48, 72, 96 and 120 hours of exposure at the concentration of 75 mg·L^{-1} presented mortality of 70, 80, 90, 90 and 90% respectively; for 50 mg·L^{-1} concentration was 40, 60, 60, 60 and 60%; 25 mg L^{-1} was 10, 30, 30, 30 and 30% and 10 mg·L^{-1} was 10%.

For each concentration at a given time in (hours), the test was performed in triplicate and data on the number of live and dead snails were found by averaging the three replicates for each of the five concentrations tested (Table 4).

The percentage rate of mortality of snails versus oil of *P. dioica* is directly proportional to the logarithm of the concentration, ie, when the logarithm of concentration increases, the mortality rate also increases. Figure 4 shows that the 50% Lethal Concentration (LC_{50}) was near the logarithm of concentration 1.60; calculated by intersecting the cumulative and accumulated living curves, resulting in a lethal concentration of 39.81 mg·L^{-1} over a 95% confidence interval. The LC_{50} is the point of intersection of the two curves.

However, when comparing the efficacy of molluscicidal activity relative to mortality with concentration or mortality over time, Juberg et al. mention the duality in the chapters of the book edited by the World Health Organization (WHO) and the lack of uniformity (Jurberg et al. 1989). For in this book, there is a chapter that establishes as the criterion of molluscicidal activity in aquatic mollusks the mortality in 90% of the snails in a time of exposure of 24 hours under constant temperature and a concentration of up to 100 mg L^{-1}. In another chapter, plants with a concentration mortality of up to 100 mg·L^{-1} are considered positive, whereas higher than that are negative (Jurberg et al. 1989). In Brazil, this evaluation is based on the percentage (LD_{50} and DL_{90}), or proportion of deaths to live.

Despite the dual evaluation performed by Juberg et al. (1989) there is a common point in relation to both situations. In both cases, it is observed that the concentration of the efficacy of this activity is up to 100 mg·L^{-1}. Therefore, it is stated that the essential oil of *P. dioica* leaves has molluscicidal activity both in the lethal concentration in 50% (LC50) of 39.81 mg·L^{-1} and in the concentration recommended by the WHO, which is 100 mg·L^{-1} for the crude vegetable and 20 mg·L^{-1} for extracts (World Health Organization 1983).

Evaluation of the control test.- After the exposure period, that is, 120 hours, it was observed that there was no mortality of the snails, as shown in Table 5. From this analysis, it was concluded that the snails remained active and feeding, with no interference in bioactivity.

Table 4. Mortality of snails after testing at various concentrations of essential oil extracted from *P. dioica*

Concentration (mg·L^{-1})	Log Concentration	Dead	Live	Acumul. dead	Acumul. live	Mortality (%)
100	2	9	1	28	1	90
75	1.875	9	1	19	2	90
50	1.699	6	4	10	6	60
25	1.398	3	7	4	13	30
10	1	1	9	1	22	10

Number of larvae (n = 10)

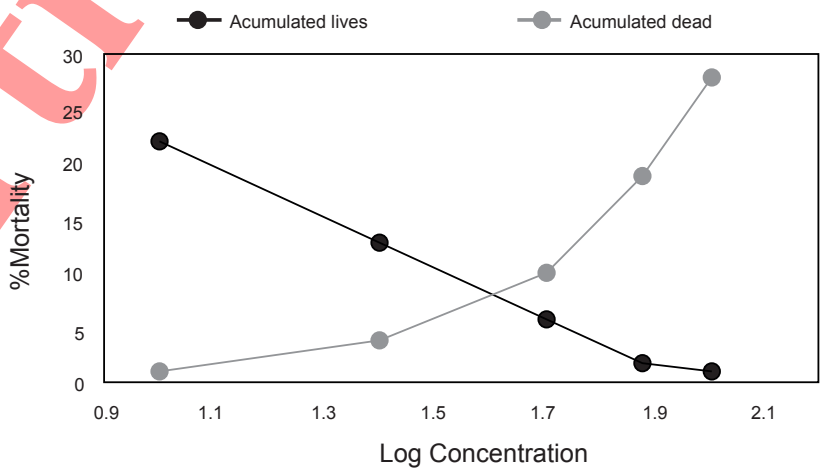

Figure 4. Estimation of LC_{50} of essential oil by the Reed-Muench method from the accumulated live and dead snails as a function of the logarithm of the applied concentration.

Table 5. Mortality of the control test for snails.

Control	Reagents	Mortality
Blank 1	Dechlorinated water	All active snails
Blank 2	Dechlorinated water + tween 80 a 0.1%	All active snails

According to the results obtained in the analytical studies and in the evaluation of the toxicity and molluscicidal activity of the essential oils extracted from the *P. dioica* leaf, it can be concluded that: GC-MS characterization allowed to identify the major and minor components of the oil in study, with eugenol containing 85.67% and linalol with 0.88% content respectively; The biological assay with *Artemia salina* for the lethal concentration (LC50) was 14.13 mg·L^{-1} in a 95% confidence interval, being considered highly toxic, according to Dolabela criteria, indicating the importance of this assay; For the pilot test, the molluscicidal activity of the oils showed a mortality of 70% in 24 hours, indicating the presence of toxic components in the oils against the snail of the species *Biomphalaria glabrata* and, finally, the molluscicidal activity of the result obtained with the lethal concentration (LC50) of 18.62 mg·L^{-1} in a 95% confidence interval, being below that recommended by the World Health Organization (WHO), which is 100 mg·L^{-1} for crude vegetable and 20 mg·L^{-1} for extracts, thus being active against the snail *Biomphalaria glabrata.*

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