

Research Article

Evaluation of the Molluscicidal Activity of Aqueous and Ethanolic Extracts of the *Neorautanenia Mitis* Plant Against Snail Intermediate Hosts of Bilharziasis in the Laboratory

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Abstract

Schistosomiasis remains a major public health problem in tropical countries and especially in DR Congo. One of the main preventive measures against trematode infection is the control of the disease vector population through the use of molluscicides. The objective of this work is focused on the fight against bilharzia intermediate host snails using extracts of the *Neorautanenia mitis* plant from the East of the Democratic Republic of Congo against the snails *Biomphalaria pfeifferi* intermediate host of bilharziasis. For the identification of the different characteristics of the plant, we used phytochemical tests based on color or precipitation reactions and a test of molluscicidal activities to know the lethal concentrations of the extracts were carried out. Analysis of the plant *Neorautanenia mitis* shows that this plant is pharmacologically active against snail intermediate hosts of bilharziasis. It contains phenol, terpene and saponin which are recognized as molluscicidal and insecticidal substances. The mortality rate is greater than 50% at a concentration of up to 0.002mg/L of the crude extracts in ethanol of this plant. This plant is very poisonous than these other plants. But the responsible substance is not yet well determined and deserves very detailed studies of the extraction of the different responsible substances and their tests on molluscs before determining the structure of this substance.

Keywords

Molluscicidal Plant, *Neorautanenia Mitis*, Aqueous Extracts, Organic Extracts, Phytochemistry, Molluscicidal Activity

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Received: 20 October 2025; Accepted: 31 October 2025; Published: 11 December 2025



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1. Introduction

Schistosomiasis (bilharziasis) is a socioeconomic public health problem in tropical and subtropical countries caused by the trematode schistosoma. It is ranked as the second parasitic disease after malaria and the third most common parasitic disease in the world [1, 2]. It is caused by worms related to the order of trematodes (Distoma) of the genus schistosoma, transmitted by infested freshwater snails [3]. Trematodes are parasitic flatworms that infest different parts of the body (eg, blood vessels, digestive tract, lungs, liver) depending on the species [4].

About 200 million people in 74 tropical and subtropical countries of the world are infected with schistosomiasis, and about 20 million suffer serious consequences from the disease. An estimated 600 million people are reported to be at risk for this disease, and around one million people die each year from complications of schistosomiasis [5]. In endemic areas, particularly in Africa, schistosomiasis remains a major public health problem [6]. Although the incidence of this disease in the Democratic Republic of Congo is relatively low compared to other countries, there are however several active foci of this parasite [3, 7]. The work of Bagalwa and Baluku [8] shows that *Biomphalaria pfeifferi* is the only intermediate host snail recognized in the province of South Kivu responsible for *Schistosoma mansoni*, which is widespread in the region. This snail is widespread in several aquatic systems, including rivers, ponds, streams, marshes, lakes and especially in places frequented by the population for swimming, laundry and washing [9]. The rate of infestation in the population in the region is only increasing following the increase in risk due to poverty and the search for well-being of the population [10]. One of the main preventive measures against trematode infection is the control of the disease vector population through the use of molluscicides. This method is used in the laboratory of the Lwiro Natural Science Research Center for the preservation of the environment [10]. And also, because the use of synthetic compounds is very expensive, can cause the possibility of vector resistance to these compounds in addition to their toxicity to non-target organisms are all advantages of using plant molluscicides.

The use of plants with molluscicidal properties has become simpler and less expensive, particularly in underdeveloped countries [11, 12]. Among the work carried out in Lwiro's laboratory, the saponins isolated from the plants *Solanum syzymbriifolium*, *Polyschias fulva* and *Myrica salisifolia* contain saponins which confer this toxicity on them [13, 14] against the molluscs *Biomphalaria pfeifferi* [8]. Indeed, the potential use of molluscicidal plants has received considerable attention using techniques commonly employed in phytochemical and pharmacological studies. Plant extracts have the advantage that, in addition to being less toxic in nature, they can be degraded faster than expensive synthetic molluscicides [15]. This work is part of the same perspective as that of our predecessors to expand and diversify the plants used in the

fight against snail intermediate hosts of schistosomiasis. But it will be limited to molluscicidal activity tests of aqueous and organic extracts and to phytochemical screening of the *Neorautanenia mitis* plant from eastern Democratic Republic of Congo against the molluscs *Biomphalaria pfeifferi*, intermediate host of bilharziasis.

2. Material and Methods

2.1. Plant Material

The samples of the bulb of *Neorautanenia mitis* which are the subject of this study were collected in Kamanyola, in South Kivu in the Democratic Republic of Congo. After the harvest, the plant was identified in the Botany laboratory of the Center for Research in Natural Sciences (CRSN-Lwiro) where a specimen was kept. Then the bulb of this plant was dried in the sun then crushed using a mortar and pestle then sieved with a granulometric sieve of 250 micrometers in diameter. After this operation, a fine powder is obtained and should be kept in a labeled plastic bag. Preparation of extracts 30g of plant powder were macerated for 24 hours in 300ml of deionized water. After the required time we filtered the extract using a filter paper and a funnel. Part of the collected aqueous filter was vaporized dry and we obtained a residue. The other part of aqueous extracts was kept for the phytochemical screening test. Similarly, 30 g of plant powder was also macerated in 300ml of 70% Ethanol for 24 hours. We carried out the filtration to have the ethanolic or organic extracts. A part was evaporated to dryness and we obtained a residue which was used for subsequent tests. The other part was kept for phytochemical screening.

2.2. Phytochemical Screening

The main families of secondary metabolites were sought by either color reactions, or by formation of a precipitate, turbidity or opacity in test tubes according to the classic methods of characterization on the powder of the plant studied. Tannins and polyphenols were identified by the FeCl_3 test and Stiasny's reagent; flavonoids by test of Shibata [15]; saponins by the foam test; the triterpenes and steroids by the Liebermann-Burchard test and finally the alkaloids by the Dragendorff tests. Wagner and de Mayer [16]. Acetic anhydride and concentrated sulfuric acid were needed to search for sterols and polyterpenes by the Liebermann reaction [17]. The glycosides were identified by adding 2mL of chloroform to 3ml of the extract, the appearance of a reddish-brown color after the addition of H_2SO_4 indicates the presence of the glycosides. The presence of quinone is confirmed by adding a few drops of NaOH 1/10 to a petroleum ether extract, when the aqueous phase turns yellow, red or purple.

2.3. Preparation of Dilutions

The various residues obtained during the evaporation of the aqueous and organic extracts were redissolved in a quantity of water to make total and organic extracts. We prepared dilute solutions of 1/10 and 1/100 and intermediate solutions, if necessary, from the initial solution obtained after weighing the extraction residues. All the solutions were prepared in triplicate (three replications for each dilution of the extracts of this plant and for the controls).

2.4. Collection of Snail and Authors Aquatic Biodiversity

The molluscs were removed using a kick net, with a small 2 mm mesh, hung on a wooden handle [18]. After identification at the Malacology laboratory of the Lwiro Natural Science Research Center (CRSN), molluscs and other non-target macro-invertebrates were separated according to their morphology and counted.

The identification of the harvested molluscs was made according to the determination key of Brown [19, 20] and the macro-invertebrates by the keys of Micha and Noiset [21], Tachet and Noiset [22] and Pennak [23]. The molluscs and other macro-invertebrates thus identified were kept in aquariums in the laboratory before the experiment. The fish were also harvested in the CRSN/Lwiro water mirror and in the small streams using this kick net. The fish found were also kept in the aquaria after identification in the laboratory where the aeration conditions were permanent [13].

2.5. Activity Test

The protocol followed for the potency test is that of Ayi et al. [24] and Chifundera et al. [25]. We used 5 individuals per species of non-target molluscs and macro-invertebrates including: molluscs (*Biomphalaria pfeifferi*, *Lymnaea natalensis*, *Physa acuta*), macroinvertebrates (*Coenagrionidae*, *Libellulidae* and *Baetidae*), fish (*Oreochromis nilotica* and *Barbus* sp) for testing. The counting took place after 24 hours of exposure to the extracts. The molluscs, macro-invertebrates and fish from the test tanks were put in tap water before evaluating the mortality rate at the Malacology laboratory of the Lwiro Natural Science Research Center (CRSN). The mortality rate of molluscs, non-target macro-invertebrates and fish was calculated by the following formula:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead individuals per species} \times 100}{\text{Total number of individuals for each species}}$$

2.6. Determination of Lethal Concentration

To determine the lethal concentration, we used the regression curve on the PAST software. This curve draws a graph on the abscissa which plots the concentrations (mg/ml) and on the ordinate on the mortality rate [26]. The calculation of the LC50 is applied only to the extracts which did not give the concentration which kills 50% of the species tested. Results Phytochemical screening The results of the photochemical screening of the plant *Neorautanenia mitis* gives the group of natural organic substances presented in Table 1.

Table 1. Phytochemical screening results.

No	Substances	<i>Neorautanea metis</i>
1	Saponin	+++
2	Alkaloids	++
3	Flavonoids	++
4	Tannoids	++
5	Terpenoids	+++
6	Steroids	+++
7	Lipoids	+++
8	Glycosides	+++
9	Phenols	+++
10	Quinone	+

Legend: +++= Strong positive test ++= Test positive += Slightly positive test

It appears from this table that the plant *Neorautanenia mitis* has a strongly positive concentration in saponin, terpenoids,

steroids, glycosides, and phenol. This concentration is moderately positive in flavonoids, alkaloids and tannoids. It also has a slightly positive quinone concentration.

2.7. Molluscicidal Activity of *Neorautanenia Mitis* Plant Extracts

The molluscicidal activity of the aqueous extracts of the plant *Neorautanenia mitis* is presented in Table 2 below.

Table 2. Molluscicidal activity of aqueous extracts of *Neorautanenia mitis* in the laboratory.

	Mortality rate (%) according to concentrations		
	0.2mg/L	0.02mg/L	0.002mg/L
<i>Biomphalaria pfeifferi</i>	100	100	0
<i>Lymnaea natalensis</i>	100	100	0
<i>Physa acuta</i>	100	100	0

This table shows that the mortality rate is 100% of the snail at the concentration of 0.02mg/L for the aqueous extracts and it is 0% for the concentration of 0.002mg/L. At this concentration, the aqueous extracts of the *Neorautanenia mitis* plant

have no molluscicidal effect. As for the ethanolic extracts of *Neorautanenia mitis*, the mortality rate according to the concentration is presented in Table 3.

Table 3. Molluscicidal activity of ethanolic extracts of *Neorautanenia mitis* in the laboratory.

	Mortality rate (%) according to concentrations		
	0.2mg/L	0.02mg/L	0.002mg/L
<i>Biomphalaria pfeifferi</i>	100	100	67
<i>Lymnaea natalensis</i>	100	100	60
<i>Physa acuta</i>	100	100	60

For the ethanolic extracts the concentration of 0.002mg/L still has a molluscicidal activity. The mortality rate is over 50%. This shows that the substances responsible for the molluscicidal activity are more soluble in ethanol than in

water. Determination of lethal concentration.

The lethal concentration of *Neorautanenia mitis* plant extracts in the laboratory is shown in Table 4.

Table 4. Molluscicide LC100, LC50 and LC00 lethal concentration of the plant *Neorautanenia mitis*.

	CL ₁₀₀		CL ₅₀		CL ₀₀	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
<i>Biomphalaria pfeifferi</i>	0.02	0.02	0.11	0.006	0.002	0.002
<i>Lymnaea natalensis</i>	0.02	0.02	0.11	0.006	0.002	0.002
<i>Physa acuta</i>	0.02	0.02	0.11	0.006	0.002	0.002

This table shows that the lethal concentrations obtained in the different solvents (aqueous and organic) do not vary for the LC₁₀₀ and LC₀₀ but they vary for the LC₅₀ only.

Effect of aqueous and organic extracts of *Neorautanenia*

mitis on aquatic fauna

The evaluation of the impact of aqueous and organic extracts of *Neorautanenia mitis* on aquatic fauna is presented in Table 5.

Table 5. Effect of aqueous and organic extracts of *Neorautanenia mitis* on aquatic fauna.

	Aqueous (mg/L)			Ethanolic (mg/L)		
	0.2	0.02	0.002	0.2	0.02	0.002
Macro-invertebrates						
Coenagrionidae	100	100	0	100	100	0
Libellidae	100	100	0	100	100	0
Baetidae	100	100	0	100	100	0
Fish						
<i>Oreochromis nilotica</i>	100	100	100	100	100	100
<i>Barbus sp</i>	100	100	100	100	100	100

From this table it appears that the mortality rate of snails and macro-invertebrates is similar to the concentrations of the *Neorautanenia mitis* extracts. But fish are sensitive to all exposed concentrations.

3. Discussion

The analysis of the plant *Neorautanenia mitis* shows that this plant is pharmacologically active against the intermediate host snails of bilharziasis. Indeed, phenol, terpene and saponin are recognized as molluscicidal and insecticidal substances [25] and these substances are found in this plant. These substances contained in this plant would probably be what gives the molluscicidal activity to these extracts. The aqueous extracts of *Neorautanenia mitis* are very active than other plants such as *Maesa lanceolata*, *Solanum syzybrilifolium*, *Blighia welwitchii* and *Casia didymobotriya* recognized as molluscicides by our predecessors [13, 15]. Our results prove that *Neorautanenia mitis* extracts are toxic against snail intermediate hosts of schistosomiasis and fascioliasis at low concentrations. The mortality rate is greater than 50% at a concentration of up to 0.002mg/L of the crude extracts in ethanol of this plant. This shows that the substances responsible for the molluscicidal activity are more soluble in ethanol than in water.

The different lethal doses are lower than the concentrations of the other plants *Solanum syzybrilifolium* (0.6mg/mL for Solamargine) [13] and *Maesa lanceolata* (1mg/mL) [15]. This plant is very molluscicidal than these other plants. But the responsible substance is not yet well determined and deserves very detailed studies of the extraction of the different re-

sponsible substances and their tests on snails before determining the structure of this substance. These results are comparable to those found by our predecessors for *Solanum syzybrilifolium* extracts [13]. WHO studies [27] also show that molluscicidal plants have a higher toxicity effect on fish than on the target snails.

Abbreviations

OMS	Organisation Mondiale de la Sante
WHO	World Heath organisation

Conflicts of Interest

We declare that there is no conflict of interest with the publication of this manuscript.

References

- [1] Sofowora A., 1996, plantes médicinales et médecine traditionnelle d'Afrique, Karthala, 37 p.
- [2] Lineda D.-R., Abdelkrim C., Abbderazak M., Reddy K. H., et Govender P., 2015. Bref aperçu sur quelques molluscicides naturels. PhytoChem & BioSub Journal, 9, 3, 80-91.
- [3] OMS, 1991. Maladies tropicales, progrès de la recherche 1989-1990, 10^e rapport.
- [4] Gentilini M. et Duflo B., 1982. Médecine tropicale, Flammarion, Paris, 513 p.

- [5] OMS. 2010. Schistosomiasis. Fact Sheet No. 115, February (2010). <http://www.who.int/mediacentre/factsheets/fs115/en>
- [6] Raccurt C. P., El Samad Y., Chouaki T., Borel A., Agnamey P., Totet A., et Schmit J. L., 2007. Bilharziose a *Schistosoma mansoni* au retour de Guinée: Défaillance du sérodiagnostic. *Med Trop.*, 67, 175-178.
- [7] Devos P., 1985. Elément de Zoologie. Namur, FUNDF, 910 p.
- [8] Bagalwa M., et Baluku B., 1997. Distribution des mollusques hôtes intermédiaires des schistosomoses humains à Katana, Sud-Kivu, Est de la RDC, *Médecine Tropicale*, 57– 369 - 372.
- [9] Batumike C., Bagalwa M., Ndegeyi K., Bahizire K., Babwiriza K., Tamani M., Baluku B., 2019. Contribution à l'étude parasitologique des mollusques *Physa acuta* (Gasteropoda: Physidae) dans la région de Katana, Est de la République Démocratique du Congo. *REB-PASRES*, 4, 2, 43 -51.
- [10] Baluku B., et Bagalwa M., 2021. Schistosomiasis: Une maladie parasitaire endémique en République Démocratique du Congo. *L'Harmattan*, collection études africaines, 118 p.
- [11] Singh S. K., Yadav R. P., and Singh A., 2010. Molluscicides from some common medicinal plants of eastern Uttar Pradesh, India. *Journal of Applied Toxicology*, 30, 1, 1–7.
- [12] Al-Daihan S., 2010. Effect of plant molluscicide on selected enzymes related to energy metabolism in *Biomphalaria arabia* snails molluscan hosts to *Schistosoma mansoni* in Saudi Arabia. *J. Egypt. Soc. Parasitol.*, 40, 187-195.
- [13] Bagalwa M., Voutquenne-Nazabadioko L., Sayagh C., and Bashwira S., 2010. Evaluation of the biological activity of the molluscicidal fraction of *Solanum sisymbriifolium* against non-target organisms, *Fitoterapia*, 81, 767 – 771.
- [14] Safari H. F., Balagizi M. C., Rusimbuka B. M., et Bagalwa M., 2020. Extraction Identification and characterization of Saponins from the *Myrica Salicifolia* Hochst Plant. ex. A. Rich (Myricaceae) and Molluscicidal Activity Test in Vitro. *International Journal of Pharmacy and Chemistry*, 6(3): 26-30.
- [15] Bagalwa M., and Chifundera K., 2007. Environmental impact evaluation of the stem bark extract of *Maesa lanceolata* used in Democratic Republic of Congo. *Journal of Ethnopharmacology*, 114, 281 -284.
- [16] EL-Haoud H., Boufellous M., Berrani A., Tazougart H., Bengueddour R., 2018. Screening phytochimique d'une plante médicinale: *Mentha spicata* L. *American Journal of Innovative Research and Applied Sciences*.
- [17] N'Guessan K., Kadia B., Zirihi G. N., Traore D., Ake-Assi L., 2009. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Sciences & Nature*, 6, 1, 1-15.
- [18] Olivier L., and Scheiderman, 1956. A method for estimation of the density of aquatic snail population. *Exp. Parasitol.*, 10 p.
- [19] Brown D. S., 1980. Freshwater snails of Africa and their medical importance. London, Taylor and Francis Ltd, 487 P.
- [20] Brown D., 1994. Fresh snails of Africa and their medical importance. *Revised 2nd. Edition. Taylor and Francis*, London: 608 p.
- [21] Micha J. C., Moiset J. L., 1982. Évaluation biologique de la pollution de ruisseaux et rivières par les macro-invertébrés aquatiques, *Probio Revue* 5 num 1, 142 p.
- [22] Tachet H., Noiset J. L., 1980. Introduction à l'étude des macroinvertébrés des eaux douces, Paris, 148 p.
- [23] Pennak, R. W., 1953, Fresh water invertebrate of the United State, the Ronald press company, New York, 769 p.
- [24] Ayi I., Chandre F., Chimbari M. J., Coelho P. M. Z., El-Emam M. A., El-Harawy A. K., Gachuhi K., Garba A., Guo J., Jian-Rong D., 2020. Lignes directrices pour les essais en laboratoire et sur le terrain de molluscicides destinés à la lutte contre la schistosomiase. WHO/32 p.
- [25] Chifundera K, Baluku B; and Mashimago B. 1993. Phytochemical screening and molluscicidal potency of some zairen medicinal plants. *Pharmacological Research*. 28, 4, 333-340.
- [26] Alard F., Geerts, et Triest L., 1991. Toxicité d'*Ambrosia maritime* L, plante molluscicide, sur les organisme aquatiques non cibles. *Toxicom*, 29, 6, 745-750.
- [27] OMS, 1965, molluscicide screening and evaluation. *Bull, WHO*, 33, 56-81.