

Assessment of Bioactive Compounds and Molluscicidal Activity of Neem (*Azadirachta indica*) and Bitter Leaf (*Vernonia amygdalina*) Extracts against *Biomphalaria pfeifferi*, a Schistosomiasis Vector

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ABSTRACT

Background and Objective: Schistosomiasis, a parasitic disease of major public health concern in sub-Saharan Africa, is transmitted through contact with water harboring larval *Schistosoma* species released by freshwater snails such as *Biomphalaria pfeifferi*. While chemical molluscicides are commonly used for snail control, they often pose risks to aquatic ecosystems and non-target species. This study aimed to evaluate the bioactive metabolites and molluscicidal potential of methanolic extracts of *Azadirachta indica* (neem) and *Vernonia amygdalina* (bitter leaf) as safer, plant-based alternatives for controlling *B. pfeifferi*.

Materials and Methods: Methanolic extracts of neem and bitter leaf were subjected to phytochemical screening using standard protocols to detect flavonoids, tannins, alkaloids, and saponins. Molluscicidal activity was assessed by exposing ten adult *B. pfeifferi* snails to six concentrations (10, 20, 40, 80, 160, and 240 mg/L) of each extract for 24 hours. Mortality was recorded post-exposure, and lethal concentrations (LC_{50} and LC_{90}) were calculated using probit regression analysis. Statistical significance between treatments was determined at $P < 0.05$.

Results: Both plant extracts demonstrated a dose-dependent increase in snail mortality. The LC_{50} and LC_{90} values for neem extract were 57.984 mg/L and 129.285 mg/L, respectively, while those for bitter leaf extract were 72.042 mg/L and 132.465 mg/L. Neem extract showed significantly greater molluscicidal potency than bitter leaf ($P < 0.05$).

Conclusion: The findings confirm that *A. indica* and *V. amygdalina* contain effective bioactive compounds with molluscicidal properties. Neem extract, in particular, showed strong potential for use as an eco-friendly alternative to synthetic molluscicides. Further studies are recommended to isolate active constituents and evaluate ecological safety under field conditions.

Keywords: Schistosomiasis, Molluscicidal activity, Bioactive metabolites, Efficacy, Phytochemicals.

INTRODUCTION

The trematodes of the genus *Schistosoma* cause the chronic parasitic disease known as schistosomiasis, which can affect both animals and humans [1]. Its public health impact is second only to that of malaria, making it one of the most consequential parasitic illnesses in the world [1,2]. Tropical and subtropical areas are the most common locations for the disease to occur, with over 90% of cases occurring in sub-Saharan Africa [3]. Other common locations include sections of South America, Asia, and the Middle East. Of the more than 260 million infected individuals, some 120 million are exhibiting symptoms, and 20 million are experiencing severe cases of the disease. When freshwater snail larvae (*Cercariae*) infect humans through skin-to-water contact, the disease is transmitted. *Biomphalaria*, *Bulinus*, and *Oncomelania* are only a few of the freshwater snail species that act as intermediate hosts [4]. There are five species of *Schistosoma* that can infect humans: *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. intercalatum* [5,6]. Although *S. mansoni* is common throughout Latin America, Africa, and the Caribbean, *S. haematobium* is more common in Africa.

Poverty, inadequate sanitation, and restricted access to clean water continue to impede long-term control efforts, despite efforts to eradicate the disease using environmental treatments such as mollusciciding and chemotherapy (e.g., praziquantel). Particularly in endemic regions with inadequate healthcare facilities, reinfection is a continuing concern [7,8]. School attendance, labor productivity, and overall quality of life in endemic areas are all negatively impacted by schistosomiasis, which places a large economic burden on these populations [9]. In certain parts of countries like Egypt and Nigeria, the infection rate is above 80% [10]. This is a very concerning situation. With almost 22 million cases, 16 million of which are children, Nigeria is the country in question [11]. Traditional methods of snail management, such as pharmacological therapy, have its limitations, thus scientists are now looking into alternate control tactics. Using molluscicides and antiparasitic compounds derived from plants is one method.

The bitter leaf shrub, or *Vernonia amygdalina*, is endemic to tropical Africa but has since spread over the world. Its traditional use as a spice and medicine dates back centuries [12]. Saponins, flavonoids, alkaloids, tannins, sesquiterpene lactones (especially vernodalin and vernolide), and phenolic acids are among the phytochemicals found in the leaves, which give them its characteristic bitterness [13]. The plant's broad range of biological actions, such as its ability to fight malaria, bacteria, inflammation, and antioxidants, are all attributed to these substances [14]. Bitter leaf has several uses in traditional medicine, including the treatment of parasite diseases, fever, gastrointestinal problems, and malaria [15]. Research in the field of modern pharmacology has shown that it can help lower blood glucose levels and promote liver function, which makes it a potential option for the management of diabetes and liver disease [14]. *V. amygdalina* is an important candidate in the search for plant-derived molluscicides and other therapeutic compounds due to its accessibility, medicinal potential, and abundance.

The fast-growing tree known as *Azadirachta indica*, or neem, originates originally from the Indian subcontinent but is now grown all over Africa, especially in arid areas. The tree's leaves, bark, seeds, and oil are all utilized to cure a wide range of illnesses in traditional and Ayurvedic medicine, which has brought it great fame [15].

The antibacterial, antifungal, antiviral, anti-inflammatory, insecticidal, and molluscicidal properties of neem are due in part to its abundance of bioactive chemicals, which include azadirachtin, nimbin, nimbolide, and salannin [16, 17]. The "village pharmacy" moniker comes from the widespread usage of its seeds and leaves for pest and disease management purposes [18]. Pharmaceutical researchers have recently shown a great deal of interest in neem due to its possible use in the production of drugs, especially for the treatment of cancer and infectious disorders [19, 20]. Neem extracts are a promising option for integrated vector control in regions where schistosomiasis is prevalent since they are biodegradable and have little toxicity to non-target organisms. To decrease snail populations and break the transmission cycle of schistosomiasis, eco-friendly and sustainable therapies such molluscicides based on neem and bitter leaves could offer cost-effective and locally adapted alternatives [21, 22]. These plants provide a practical substitute that is in line with traditional wisdom and environmental health objectives in areas with limited resources [23, 24]. A long-term solution to this understudied tropical disease might be found through more investigation into the snail-parasite relationship, host resistance mechanisms, and the bioefficacy of medicines derived from plants [25, 26]. *Biomphalaria pfefferi* is the primary intermediate host of *S. mansoni* in Africa; this work examines the phytochemical components and molluscicidal activity of methanolic extracts of *A. indica* and *V. amygdalina* against this parasite.

MATERIALS AND METHODS

Study Area

The study was carried out in Minna, the administrative capital of Niger State, located in the north-central region of Nigeria. Geographically, Minna lies around latitude 9.33°N and longitude 6.33°E and serves as an important urban center in the Guinea Savanna ecological zone. As of the 2007 population estimate, the city hosted approximately 1.2 million inhabitants, and its population has continued to grow due to urban expansion and migration. Minna occupies an estimated land area of about 88 km² and experiences a tropical continental climate marked by distinct wet and dry seasons. The wet season typically spans April to October, with rainfall reaching its highest levels during August and September, where monthly totals may exceed 300 mm. The dry season, dominated by dry north-easterly winds, lasts from November to March, often accompanied by the Harmattan, which brings cooler temperatures and dusty conditions. The city records an average annual rainfall of about 1334 mm, relative humidity of around 61%, and mean temperature close to 30.2°C. Vegetation in the area is representative of the Guinea Savanna, consisting mainly of grasses interspersed with shrubs and scattered medium-sized trees. This ecological setting supports both agricultural activities and peri-urban land use that characterize Minna's landscape.

Data Collection

The data for this study were obtained primarily from first-hand field investigations and laboratory-based analyses. Emphasis was placed on generating original, reliable, and scientifically valid information through systematic collection of snails, neem leaves, and bitter leaf samples. The entire data collection process was carefully planned to ensure accuracy and consistency, beginning with structured field observations and followed by controlled laboratory experiments.

This approach allowed for a thorough understanding of the materials used and provided a strong foundation for the experimental components of the research.

Field Work

Field work formed the major source of primary data for this study. The collection of snail specimens, neem leaves, and bitter leaf was carried out using appropriate scientific techniques to maintain the natural characteristics of the samples. Before the actual sampling, suitable collection sites were identified based on ecological features, abundance of target species, and accessibility. Once the sites were selected, sample gathering was performed using sterilized tools to avoid contamination. Snails were carefully picked during early morning and late evening hours when they are most active, while neem and bitter leaf samples were harvested from healthy plants showing no signs of pest or disease infestation. Following collection, the samples were properly labeled and stored in clean, airtight containers to preserve their freshness and biochemical integrity. They were then transported to the laboratory under controlled conditions to prevent deterioration. In the laboratory, each sample underwent a series of preparatory steps, including washing, sorting, and measurement, before being subjected to the experimental procedures required for the study. This systematic process ensured that all data obtained were accurate, consistent, and suitable for scientific analysis.

Collection of Snails

Snail specimens (*Biomphalaria pfeifferi*) were collected from the Bosso Dam located in Bosso Local Government Area of Niger State. Sampling was conducted early in the morning when snails are most active and easier to locate. Using a clean plastic spoon, individual snails were gently scooped from submerged vegetation, shallow water surfaces, and moist soil around the dam. A random sampling approach was employed to ensure that the collected snails represented the natural population within the area. After collection, the snails were placed in sterile containers filled with dam water to maintain their natural physiological conditions during transportation. The samples were then transferred to the Biological Science Laboratory at the Federal University of Technology, Minna. Upon arrival, the snails were maintained under controlled laboratory conditions and allowed an acclimatization period before being used for experimental procedures. This ensured that the organisms were stable, healthy, and suitable for reliable scientific analysis.

Maintenance of the Specimen (Snails)

The collected snails were maintained in perforated plastic containers filled with dechlorinated tap water kept at room temperature to replicate suitable habitat conditions. Green lettuce served as the primary feed during the maintenance period. Before feeding, the lettuce was briefly immersed in boiling water for approximately one minute to remove surface contaminants and soften the leaf tissue. After boiling, the leaves were cooled under running tap water, and the midrib was removed to ensure only the soft, tender portions were offered to the snails. These soft leaf sections were provided every two days, as they are more easily consumed and digested compared to tougher or dried parts of the plant. Previous observations indicate that snails fed with dry or fibrous midribs exhibit poor survival and reduced activity. To maintain water quality and prevent the buildup of waste materials, the water in the containers was replaced every two days.

This routine ensured a clean, stable environment for the snails throughout the acclimatization and experimental period.

Collection and Preparation of Plant Extract

The leaves of neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) were collected from the Bosso area of Minna following standard botanical sampling procedures. Upon arrival at the laboratory, the leaves were thoroughly rinsed with clean water to remove dust, sand, and any foreign particles that could compromise the quality of the extract. The cleaned leaves were then shade-dried for several weeks to preserve their bioactive components and prevent degradation from direct sunlight. Once fully dried, the leaves were manually pulverized using a clean pestle and mortar, and the resulting material was sieved to obtain a fine, uniform powder suitable for extraction. The powdered plant samples were stored in airtight containers until required for further processing. For extract preparation, 200 g of each powdered sample was immersed in 1600 mL of methanol, allowing the solvent to efficiently extract the phytochemical constituents. The mixture was then kept for further processing in accordance with standard extraction protocols.

Extract Bioassay

The bioassay of the plant extracts was carried out using the Soxhlet extraction method. A measured 50 g of the powdered leaf material was subjected to extraction in 200 mL of methanol. The resulting extract was then concentrated and air-dried using a water bath maintained at 60 °C, following established laboratory protocols [30]. From the stock solution obtained, a series of test concentrations were prepared to yield final doses of 10, 20, 40, 80, 160, and 240 mg/L of the methanolic extracts of neem and bitter leaf for assessment against *Biomphalaria pfeifferi*. Each concentration was diluted with an equal volume of distilled water to ensure uniformity, and a control treatment consisting of aged distilled water without plant extract was included in all trials. Throughout the experimental period, the snails were not fed to avoid interference with the bioassay results.

Molluscicidal Activity Test

The molluscicidal activity of the plant extracts was evaluated following the standard procedures outlined by [30]. Methanolic extracts of *Azadirachta indica* and *Vernonia amygdalina* were tested against adult *Biomphalaria pfeifferi*. For each concentration prepared, ten adult snails were exposed by immersion, and all treatments were prepared in duplicate to ensure reliability of results. The experiments were conducted at room temperature. After 24 hours of exposure, the test solutions were decanted, and the snails were thoroughly rinsed with tap water before being transferred into clean distilled water for an additional 24-hour recovery period. At the end of this period, the snails were carefully examined, and mortality was recorded. Snails were considered dead if they demonstrated no movement, failed to retract fully into their shells, or exhibited discoloration of the body or shell. Mortality counts and percentages were subsequently calculated for each treatment group.

Data Analysis

All data generated from the study were analyzed using standard statistical procedures. The results were subjected to Analysis of Variance (ANOVA) to determine significant differences among

treatment means, followed by Duncan's Multiple Range Test (DMRT) for post-hoc mean separation where applicable. Lethal concentration values (LC_{50} and LC_{90}) for the plant extracts were estimated using simple regression analysis based on mortality responses across concentrations. Statistical significance was established at $p < 0.05$, ensuring that all reported differences were scientifically reliable and meaningful.



Plate 1: collection of snails

Plate 2: Snails setup

Plate 3: Snails on exposure to extract

Results

Phytochemical Components of Methanolic Extracts of Neem and Bitter Leaf

The qualitative phytochemical analysis of the methanolic extracts of *Azadirachta indica* (neem) and *Vernonia amygdalina* (bitter leaf) revealed noticeable variations in their bioactive constituents. As shown in Table 1, both plants contained appreciable amounts of alkaloids, flavonoids, phenols, and saponins, indicating their potential biological activity and usefulness in molluscicidal applications. However, terpenoids were not detected in the neem extract, whereas they were present in the bitter leaf extract, suggesting differences in their secondary metabolite profiles. These phytochemical components are known for their toxic, antioxidant, and antimicrobial properties, which may contribute to the molluscicidal effects observed in the study.

Table 1. Phytochemical component of methanolic extracts of Neem and Bitter leaf

Phytochemicals	Neem	Bitter leaf
Alkaloid	+	+
Flavonoid	+	+
Phenol	+	+
Tannins	+	+
Terpenoids	-	+
Saponins	+	+
Cardiac glucocite	+	+

Key: + = present, - = absent

Molluscicidal Activity of Methanolic Neem Extract Against *Biomphalaria pfeifferi* After 24 Hours

The molluscicidal effects of the methanolic extract of neem (*Azadirachta indica*) on *Biomphalaria pfeifferi* after a 24-hour exposure period are summarized in Table 2. The findings revealed a clear dose-dependent response, where snail mortality increased progressively with rising extract concentrations. Notably, complete (100%) mortality was achieved at a concentration of 160 mg/L, demonstrating the strong molluscicidal potential of the extract. Statistical analysis further showed significant differences between all treatment groups and the control, which recorded no mortality, confirming that the observed effects were attributable to the neem extract.

Table 2. Molluscicidal activity of methanolic extract of Neem plant against *Biomphalaria pfeifferi* after 24 hours exposure period

Extract concentrations (mg/L)	Mortality	Percentage (%)
Control	0.00±0.00 ^a	0.00
10	1.00±0.00 ^b	10.00
20	3.20±0.09 ^c	32.00
40	4.35±0.08 ^d	43.50
80	7.56±1.23 ^e	75.60
160	10.00±10.00 ^f	100.00
240	10.00±10.00 ^f	100.00

Source: [30]

*Values are presented in mean ± standard error of 4 replicates. Values followed with the same superscript in the same column are not significantly different at $p < 0.05$.

Molluscicidal Activity of Methanolic Bitter Leaf Extract Against *Biomphalaria pfeifferi* After 24 Hours

The molluscicidal effect of the methanolic extract of bitter leaf (*Vernonia amygdalina*) on *Biomphalaria pfeifferi* after 24 hours of exposure is presented in Table 3. The results demonstrated a clear concentration-dependent pattern, as higher extract levels consistently produced increased snail mortality. Complete (100%) mortality was recorded at a concentration of 160 mg/L, indicating the strong toxic efficacy of the extract at this level. The progressive rise in mortality with increasing concentrations highlights the potency of bitter leaf extract as a viable molluscicidal agent.

Table 3. Molluscicidal activity of methanolic extract of Bitter leaf plant against *Biomphalaria pfeifferi* after 24 hours exposure period

Extract concentration (mg/L)	Mortality	Percentage (%)
Control	0.00±0.00 ^a	0.00
10	0.00±0.00 ^a	0.00
20	1.24±0.02 ^b	12.40
40	3.28±0.10 ^c	32.80
80	7.14±0.16 ^d	71.40
160	10.00±0.00 ^e	100.00
240	10.00±0.00 ^e	100.00

Source: [31]

*Values are presented in mean ± standard error of 4 replicates. Values followed with the same superscript in the same column are not significantly different at $p < 0.05$.

Medial (LC_{50}) and Upper (LC_{90}) Lethal Concentrations of Neem and Bitter Leaf Methanolic Extracts

The lethal concentration values (LC_{50} and LC_{90}) for the molluscicidal activity of neem and bitter leaf methanolic extracts against *Biomphalaria pfeifferi* after 24 hours are summarized in Table 4. Regression analysis showed that neem extract exhibited LC_{50} and LC_{90} values of 57.984 mg/L and 129.285 mg/L, respectively, indicating strong toxicity at relatively low concentrations. Similarly, bitter leaf extract recorded LC_{50} and LC_{90} values of 72.042 mg/L and 132.465 mg/L. The high coefficients of determination ($R^2 > 0.90$) for both plant extracts suggest that over 90% of the observed snail mortality can be attributed to the effects of the extracts, confirming their effectiveness as potent molluscicidal agents.

Table 4. Medial (LC_{50}) and Upper (LC_{90}) lethal concentrations of the molluscicidal activities of neem and bitter leaf methanolic extract against *Biomphalaria pfeifferi*

Extract	LC_{50} (mg/L)	LC_{90} (mg/L)	R^2	Regression Equation
Neem	57.984	129.285	0.9171	$y = 0.561x + 17.471$
Bitter leaf	72.042	132.465	0.9376	$y = 0.662x + 2.308$

DISCUSSION

The present study assessed the molluscicidal activities of methanolic extracts of *Azadirachta indica* (neem) and *Vernonia amygdalina* (bitter leaf) against *Biomphalaria pfeifferi*, a key intermediate host in the transmission of *Schistosoma mansoni*. Moreover, the environmental compatibility of neem and bitter leaf adds considerable value to their application in public health interventions, particularly in endemic regions where synthetic molluscicides like niclosamide are often inaccessible or unaffordable [32, 33, 34, 35]. Several studies have emphasized the ecological drawbacks of chemical molluscicides, such as their toxicity to non-target aquatic organisms and their poor biodegradability [36, 37, 38].

In contrast, natural molluscicides derived from endemic plants, such as *Azadirachta indica* and *Vernonia amygdalina*, are biodegradable, locally available, and pose minimal environmental risks [39, 40, 41]. This not only reduces the ecological footprint of vector control programs but also empowers local communities to participate in the production and use of plant-based solutions, enhancing sustainability and public health ownership [42, 43, 44].

Our findings, where both extracts achieved 100% mortality at 160 mg/L, indicate a higher potency compared to the study by Al-Zanbagi *et al.* (2000), where *Azadirachta indica* achieved similar mortality rates at concentrations above 250 mg/L. Similarly, [45] reported that *Vernonia amygdalina* required over 200 mg/L to produce comparable molluscicidal activity against *Biomphalaria alexandrina*, suggesting that our methanolic extracts might offer enhanced phytochemical extraction or benefit from synergistic effects of multiple compounds. [46] also observed effective molluscicidal activity of neem against *Lymnaea acuminata* at higher concentrations ranging between 200–300 mg/L. The lower concentration required in our study highlights the superior efficacy of our extract preparation or possibly regional differences in plant chemotypes. In another comparative study, [47] showed that bitter leaf extracts had a molluscicidal effect at 200 mg/L, but with only 80% mortality, again lower in efficacy compared to the full mortality observed at 160 mg/L in our study.

Our results also align with the findings of [48], who observed molluscicidal activity in other plant extracts like *Euphorbia hirta*, but only at significantly higher concentrations (>300 mg/L), further emphasizing the relative potency of neem and bitter leaf. Similarly, the widely studied *Phytolacca dodecandra*, while effective, often requires concentrations above 200 mg/L to reach complete mortality [49], placing our results on the more effective end of the spectrum. In terms of sustainability, other authors have stressed the benefits of using plant leaves over roots or bark. For instance, [50] recommended the use of leaf-based molluscicides to ensure renewable harvesting without killing the host plant. This is consistent with our study, which relied solely on leaf extracts, thus supporting environmental conservation and sustainable harvesting practices.

Such plant-based strategies are in line with integrated schistosomiasis control approaches advocated by [51], which emphasize combining chemotherapy with vector control, environmental sanitation, and education. The molluscicidal potential of neem and bitter leaf can therefore contribute meaningfully to the WHO roadmap on the elimination of neglected tropical diseases [20], which prioritizes environmentally safe, community-driven interventions. Given the promising results obtained in this study, future research should focus on formulating these extracts into standardized, user-friendly molluscicidal products. This direction is in line with the work of [21], who developed biodegradable, slow-release molluscicidal formulations using plant extracts. In addition, toxicological assessments of non-target organisms, as emphasized by [22] and [23], will be essential to validate the environmental safety of neem and bitter leaf applications. Bioaccumulation studies are also crucial to determine the long-term impact on aquatic ecosystems, molecular studies into the mechanisms of action of individual phytochemicals, such as azadirachtin from neem and vernodalinal from bitter leaf, could provide insights into the exact biochemical pathways affected in mollusks. [24]

previously used molecular docking to demonstrate the interaction of plant-based compounds with invertebrate neuroreceptors, a method that could be applied to phytochemicals identified in this study, this study highlights the molluscicidal efficacy of neem and bitter leaf methanolic extracts and confirms their superior performance when compared with other botanicals reported in the literature. The findings substantiate their ethnobotanical relevance and demonstrate their potential as sustainable, eco-compatible tools for schistosomiasis control, particularly in resource-limited settings. These results further contribute to the growing body of evidence advocating for the integration of plant-based interventions into global public health strategies.

Conclusion

This study confirms the molluscicidal potential of *Azadirachta indica* and *Vernonia amygdalina* against *Biomphalaria pfeifferi*, with 100% mortality observed at 160 mg/L. The presence of bioactive compounds suggests synergistic toxic effects on snails. Given their availability, cultural acceptance, and low environmental impact, these plants offer promising alternatives to synthetic molluscicides. Their use supports sustainable, community-based schistosomiasis control, further field studies are needed to evaluate ecological safety, stability, and non-target effects. Integrating these plant-derived agents into control programs may enhance the fight against schistosomiasis in endemic regions.

Significance Statement

This study identified the molluscicidal potential and bioactive metabolites of *Azadirachta indica* and *Vernonia amygdalina*, which could be beneficial for developing eco-friendly and sustainable strategies for snail vector control in schistosomiasis-endemic regions. This study will assist researchers in uncovering critical areas of plant-based vector management that have remained unexplored by many. Consequently, a new theory on integrating ethnobotanical knowledge into modern disease control frameworks may be developed.

Conflicts of Interest

All authors declare no conflict of interest associated with this research article.

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