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# QUALITATIVE AND QUANTITATIVE PROFILING OF Azadirachta indica AGAINST Bulinus globosus INFECTIVE STAGE: IMPLICATION FOR CONTROL

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### **ABSTRACT**

Schistosomiasis control is increasingly challenged by the limitations of chemical molluscicides, including environmental toxicity and the threat of drug resistance, highlighting the need for eco-friendly alternatives. This study evaluated the qualitative and quantitative cercaricidal potency of aqueous extracts from the leaves, bark, and roots of Azadirachta indica against the infective stage of Bulinus globosus, and profiled their phytochemical constituents. Extracts were tested at concentrations of 50, 150, and 300 mg/ml, and cercarial mortality was assessed at 15, 30, 45, and 60 minutes post-exposure. Phytochemical screening of the extracts was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), and cercarial mortality data were analyzed using Predictive Analytical Software (PASW), with statistical significance set at p < 0.05. The results showed that all extracts exhibited significant, time- and concentration-dependent cercaricidal activity (p < 0.05). Notably, the root extract induced 100% mortality at 15 minutes with 300 mg/ml, while the bark extract achieved 100% mortality at 45 minutes with the same concentration. The leaf extract showed a maximum of 66.7% mortality at 60 minutes with 150-300 mg/ml. Phytochemical analysis revealed bioactive constituents such as hexadecanoic acid, isophytol, morin, and octanoic acid esters, which are known for their antiparasitic and molluscicidal properties. These findings demonstrate that A. indica, particularly the root extract, possesses rapid and potent cercaricidal activity against B. globosus cercariae, supporting its potential as an environmentally friendly alternative for schistosomiasis vector control.

KEYWORDS: Azadirachta indica, Bulinus globosus, Cercaria, Mortality, Phytochemicals, Schistosomiasis control

### INTRODUCTION

Schistosomiasis is a major neglected tropical disease that continues to pose a significant public health challenge in many tropical and subtropical countries, with an estimated 240 million people affected globally (WHO, 2020).

The disease is caused by trematode parasites of the genus *Schistosoma*, and is second only to malaria in terms of socio-economic and public health impact among parasitic diseases (WHO, 2011; Colley et al., 2014). The transmission of schistosomiasis is closely linked to freshwater environments where specific snail species.

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such as *Bulinus globosus*, serve as intermediate hosts for the parasite. In endemic regions, poor sanitation leads to contamination of water bodies with schistosome eggs excreted in human urine or feces. These eggs hatch to release miracidia, which infect snails and develop into cercariae, the free-swimming larval stage responsible for human infection. Cercariae can survive in water for up to 48 hours and rapidly penetrate intact human skin, initiating infection (Arora & Arora, 2005).

The mainstay of schistosomiasis control has been mass drug administration with praziguantel. However, praziguantel does not prevent reinfection, has limited efficacy against juvenile schistosomes, and there are growing concerns about the potential emergence of drug resistance due to repeated use (Utzinger et al., 2003; Danso-Appiah et al., 2008; WHO, 2016). As a result, there is an urgent need for integrated control strategies that not only treat human infection but also interrupt transmission by targeting the snail intermediate host and the infective cercarial stage. Chemical molluscicides such as niclosamide have been widely used to control snail populations and reduce transmission (WHO, 1965). However, these agents are associated with significant drawbacks, including toxicity to non-target aquatic organisms, persistence, and the risk of environmental bioaccumulation (Odetunji & Salawu, 2010). Such adverse effects have prompted the search for safer, environmentally friendly alternatives for snail and cercariae control.

Medicinal plants have attracted considerable attention as potential sources of new molluscicidal and cercaricidal agents due to their richness in bioactive secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, and terpenoids, which have demonstrated antiparasitic properties (Kimeu et al., Pandey 2015: Abdullahi, 2018: et al.. 2014). Azadirachta indica commonly called neem is particularly notable among these plants for its broad spectrum of biological activities and low toxicity to non-target organisms (Makeri et al., 2007; Umar et al., 2002; Khan & Aslam, 2008). Neem is a fast-growing, drought-resistant tree native to South Asia and widely cultivated in tropical climates (Khetarpal, 2010; Motaleb, 2011). It has been called "nature's drug store" due to the presence of more than 150 bioactive compounds in its various parts, including azadirachtin, nimbin, and salanin, which have demonstrated insecticidal, antimicrobial, antifungal, and antiparasitic activities (Brahmachari, 2004; Kuravadi et al., 2015; Paul et al., 2011).

Previous studies have shown that extracts from *A. indica* and other medicinal plants possess significant molluscicidal and cercaricidal activities against various snail species, such as *Biomphalaria pfeifferi* and *Lymnaea natalensis* (Kimeu *et al.*, 2015; Kiros *et al.*, 2014; Abdullahi, 2018; Micheal *et al.*, 2013). These effects are often concentration- and time-dependent, with different plant parts exhibiting

varying levels of potency. However, there is limited information regarding the comparative efficacy of different parts of A. indica against Bulinus globosus and its cercariae, which are of particular urinary relevance in the transmission of schistosomiasis in West Africa. Furthermore, the specific phytochemical constituents responsible for these activities remain to be fully elucidated, and understanding their profiles may provide valuable insights into their mechanisms of action (Pandey et al., 2014).

Given these considerations, this study was designed to evaluate the qualitative and quantitative cercaricidal potency of aqueous extracts from the leaves, bark, and roots of *Azadirachta indica* against the infective stage of *Bulinus globosus*. In addition, comprehensive phytochemical profiling was conducted to identify the major bioactive constituents in each plant part. The findings from this study are expected to contribute to the growing body of evidence supporting the use of neem as an effective and environmentally sustainable alternative for schistosomiasis vector control.

### **MATERIALS AND METHODS**

Processing of plant samples

Fresh leaves, bark, and roots of Azadirachta indica were collected from the University of Calabar environs, Cross River State, Nigeria. The plant was authenticated at the Herbarium Unit, Department of Plant and Ecological Studies, University of Calabar, and a voucher specimen (BOT/Herb/UCC/354) was deposited. The collected plant parts were thoroughly washed with distilled water and air-dried under shade at room temperature (24-26°C) for seven days, following the method described by Poojory et al. (2015). Once dried, the plant materials were pulverized using a Mekonmicromealer single-phase grinder and sieved through a 0.5 mm mesh to obtain uniform particle size (Micheal et al., 2013). The powdered samples were extracted by soaking in distilled water at a 1:2 (w/v) ratio in separate clean 500 ml containers. The mixtures were agitated thoroughly and left to stand at room temperature for 48 hours to allow maximum extraction (Asuguo & Ifon, 2021; Ekpo et al., 2023). The resulting suspensions were first filtered through cheesecloth and then through Whatman No. 2 filter paper. The filtrates were concentrated using a rotary evaporator at 50°C to onetenth of their original volumes and further evaporated to dryness in a water bath at 50°C to yield semi-solid crude extracts, which were stored at 4°C until use.

Phytochemical analysis

Phytochemical profiling of the *A. indica* extracts was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). For each plant part, 10 grams of the dried, pulverized samples were further dried in an oven at 40°C for 20 minutes. One gram of the dried sample was weighed into a clean reagent bottle, and 10 ml of ethanol was added.

The mixture was vigorously agitated and allowed to stand overnight for thorough extraction. The extracts were filtered through a filtration column, and 1 µl of the filtrate was injected into an Agilent 6890N gas chromatograph equipped with an autosampler and coupled to an Agilent Mass Spectrometric Detector. Separation was achieved on a 30 m × 0.25 mm ID DB 5MS fused silica column (0.15 µm film thickness) using helium as the carrier gas at a constant flow rate of 1 ml/min. The oven temperature was programmed from 55°C (held for 0.4 min) to 200°C at 25°C/min, then to 280°C at 8°C/min, and finally to 300°C at 25°C/min (held for 2 min). Identification phytochemical constituents was based on retention times and mass spectral comparison with reference libraries (Asuguo et al., 2022).

Study area and environmental description

The snails were collected from Nwang community in Cross River State, Nigeria, an area known for its high prevalence of urinary schistosomiasis (Eleng & Imalele, 2019). The selection of Nwang was based on epidemiological data confirming it as a transmission hotspot, thereby ensuring the availability of naturally infected snails and cercariae for the study. Nwang is situated in a humid tropical climate zone, with an average annual rainfall of approximately 2,800 mm, relative humidity ranging from 75% to 90%, and mean temperatures between 24°C and 30°C. The landscape is characterized by slow-moving freshwater bodies, including streams and irrigation canals, which are rich in aquatic vegetation and organic matter. These environmental factors create optimal conditions for the survival, breeding, and proliferation of Bulinus globosus. The rainy season, spanning from May to October, is associated with peak snail population and cercarial shedding.

Snail collection and preservation

Snail collection was conducted during the peak transmission period, from June to August 2024. Bulinus globosus snails were collected using scoop nets from the margins and submerged vegetation of freshwater habitats, following the method of Diakite et al. (2017). Immediately after collection, snails were placed in plastic containers lined with layers of moist cotton wool to maintain humidity and prevent desiccation. Sand and gravel from the collection sites were added to the containers to mimic the snails' natural habitat and minimize stress during transport (Eleng et al., 2024). The containers were perforated to allow air exchange and kept in a cool, shaded area en route to the laboratory.

Upon arrival in the laboratory, snails were acclimatized and maintained under controlled conditions for five weeks, corresponding to the prepatent period for schistosome infection. During this period, snails were fed fresh lettuce leaves and kept in dechlorinated water, which was changed every two days. After the prepatent period, snails were individually placed in 50 ml beakers containing 10 ml of dechlorinated water and exposed to indirect sunlight for 30 minutes to stimulate cercarial shedding.

Cercaricidal assay

Cercarial suspensions (10 ml) were transferred to Petri dishes and observed under a dissecting microscope. The cercaricidal activity of the *A. indica* extracts was evaluated at concentrations of 50, 150, and 300 mg/ml. For each concentration, batches of six cercariae (for leaf and bark extracts) and four cercariae (for root extract) were introduced into clean beakers containing the extracts and monitored at intervals of 15, 30, 45, and 60 minutes. The number of dead cercariae was enumerated and recorded at each time point.

Data analysis

Data obtained from the study were analyzed using Predictive Analytical Software (PASW). The mean, standard error, and standard deviation of cercarial mortality at different concentrations and time intervals were calculated. Statistical significance was set at p < 0.05.

### **RESULTS**

Phytochemical properties of *Azadirachta* indica extracts

Phytochemical screening of the aqueous extracts of Azadirachta indica leaves, bark, and roots revealed a diverse array of bioactive compounds, many of which are known for their insecticidal, molluscicidal, and antiparasitic properties. The GC-MS analysis of the leaf extract identified major constituents such as hexanal (5.56%), isopropyl linoleate (8.39%), eicosa-11,14-dien-1-ol nicotinate (8.66%), and hexadecanoic acid, eicosyl ester (8.72%), among others (Table 1). These compounds belong to groups including aliphatic aldehydes, esters, fatty acid esters, and terpenoid esters. The bark extract was notably rich in docosyl 2-methylbutanoate (98.14%), with other significant components such as docosane. 10-ethyl-10-propyl (17.50%), tetradecyl 2-methylbutanoate (17.18%), isophytol (12.95%), and heptanoic acid, 2,6-dimethyl-, methyl ester (9.37%) (Table 2). These are predominantly wax esters, alkanes, and terpenoids, with the presence of flavonoids such as morin (2.54%) and polymethoxyflavones. The root extract contained high concentrations of octanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (26.96%), hexanoic acid, 1,1-dimethylethyl ester (21.01%), dodecanoic acid, 10-methyl-, methyl ester (12.30%), and 2-dodecenoic acid (11.61%), alongside phenolic esters, flavonoids, and terpenoid esters (Table 3). The identification of these compounds supports the potential of A. indica extracts as sources of bioactive agents for cercarial control.

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Table 1: Major phytochemical constituents identified in Azadirachta indica leaf extracts

| S/N | Compound Name                             | Relative      | Compound Group            |
|-----|---|---------------|---------------------------|
|     |   | Abundance (%) |                           |
| 1   | Hexanal                                   | 5.56          | Aliphatic Aldehyde        |
| 2   | Hexan-3-yl 2-methylbutanoate              | 5.53          | Ester                     |
| 3   | Hexane, 1,1-diethoxy                      | 3.66          | Acetal                    |
| 4   | Tridecane                                 | 4.10          | Alkane                    |
| 5   | Dodecanoic acid, 1,2,3-propanetriyl ester | 3.99          | Triglyceride Ester        |
| 6   | Z-10-Tetradecen-1-ol acetate              | 4.10          | Terpenoid Ester           |
| 7   | 1,19-Eicosadiene                          | 7.30          | Alkene                    |
| 8   | Isopropyl linoleate                       | 8.39          | Fatty Acid Ester          |
| 9   | Eicosa-11,14-dien-1-ol, nicotinate        | 8.66          | Fatty Alcohol/ Nicotinate |
| 10  | Eicosanebioic acid, dimethyl ester        | 3.48          | Fatty Acid Ester          |
| 11  | Cyclopropanebutanoic acid, methyl ester   | 6.68          | Fatty Acid Ester          |
| 12  | Tetracosanoic acid, methyl ester          | 5.09          | Fatty Acid Ester          |
| 13  | L-(+)-Ascorbic acid 2,6-dihexadecanoate   | 5.90          | Fatty Acid Ester          |
| 14  | Eicosyl acetate                           | 5.63          | Fatty Acid Ester          |
| 15  | Hexadecanoic acid, eicosyl ester          | 8.72          | Fatty Acid Ester          |

Table 2: Major phytochemical constituents identified in Azadirachta indica bark extracts

| S/N | Compound Name                               | Relative<br>Abundance (%) | Compound Group     |
|-----|---|---------------------------|--------------------|
| 1   | n-Hexane                                    | 3.29                      | Alkane             |
| 2   | Benzene, 1,2,4-trimethyl                    | 1.47                      | Aromatic           |
| _   | Denzene, 1,2,4-unneury                      | 1.77                      | Hydrocarbon        |
| 3   | Isophytol                                   | 12.95                     | Terpenoid          |
| 4   | Heptanoic acid, 2,6-dimethyl-, methyl ester | 9.37                      | Ester              |
| 5   | n-Octanoic acid isopropyl ester             | 3.83                      | Ester              |
| 6   | Dodeca-1,6-dien-12-ol, 6,10-dimethyl        | 5.51                      | Terpenoid          |
| 7   | Docosane, 10-ethyl-10-propyl                | 17.50                     | Alkane             |
| 8   | Docosanoic acid 1-methyl-butyl ester        | 1.28                      | Wax Ester          |
| 9   | Docosyl 2-methylbutanoate                   | 98.14                     | Wax Ester          |
| 10  | Tetradecyl 2-methylbutanoate                | 17.18                     | Wax Ester          |
| 11  | Dodeca-1,6-dien-12-ol, 6,10-dimethyl        | 5.00                      | Terpenoid          |
| 12  | Phenol, 2-(1,1-dimethylethyl)-6-methyl      | 1.18                      | Phenolic Compound  |
| 13  | Morin                                       | 2.54                      | Flavonoid          |
| 14  | Flavone, 3-hydroxy-3',4',5,7-tetramethoxy   | 1.40                      | Polymethoxyflavone |

Table 3: Major phytochemical constituents identified in Azadirachta indica root extracts

| S/N | Compound Name  | Relative      | Compound                  |  |
|-----|--|---------------|---------------------------|--|
|     |  | Abundance (%) | Group                     |  |
| 1   | Butanoic acid, 1-methylbutyl ester                               | 4.23          | Ester                     |  |
| 2   | Hexanoic acid, 1,1-dimethylethyl ester                           | 21.01         | Ester                     |  |
| 3   | Octanoic acid, 1-cyclopentylethyl ester                          | 8.59          | Ester                     |  |
| 4   | Octanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester              | 26.96         | Ester                     |  |
| 5   | Dodecanoic acid, 10-methyl-, methyl ester                        | 12.30         | Fatty Acid Ester          |  |
| 6   | Dodecanoic acid, 1,2,3-propanetriyl ester                        | 0.19          | Triglyceride<br>Ester     |  |
| 7   | Benzoic acid, 4-(acetyloxy)                                      | 9.08          | Phenolic Ester            |  |
| 8   | 2-Dodecenoic acid  | 11.61         | Unsaturated<br>Fatty Acid |  |
| 9   | 2,4-Dodecadienal   | 0.95          | Aldehyde                  |  |
| 10  | 4H-1-Benzopyran-4-one, 3-hydroxy-2-phenyl                        | 0.69          | Flavonoid                 |  |
| 11  | 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,7-dimethoxy     | 1.87          | Flavonoid                 |  |
| 12  | 3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate              | 0.38          | Terpenoid Ester           |  |
| 13  | 3-Cyclohexene-1-methanol, α,α,4-trimethyl-, propanoate           | 0.35          | Terpenoid Ester           |  |
| 14  | 1,11-Dodecadiene   | 0.17          | Alkene                    |  |
| 15  | Oleic acid, 3-(octadecyloxy)propyl ester                         | 1.08          | Glycol Ester              |  |
| 16  | Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) | 0.15          | Steroidal Ester           |  |
| 17  | Docosanoic acid, 1,2,3-propanetriyl ester                        | 0.38          | Triglyceride<br>Ester     |  |

Cercaricidal effects of Azadirachta indica extracts

The cercaricidal activity of *A. indica* extracts was evaluated at concentrations of 50, 150, and 300 mg/ml against *Bulinus globosus* cercariae, with mortality assessed at 15, 30, 45, and 60 minutes post-exposure. All extracts demonstrated a time- and concentration-dependent increase in cercarial mortality, with higher concentrations and longer exposure times resulting in greater lethality. For the leaf extract, exposure to 50 mg/ml resulted in 16.7% mortality at 30 minutes, which increased to 66.7% at 60 minutes. At 150 mg/ml, mortality was 16.7% at 15 minutes and 33.3% at 30 and 60 minutes. The 300 mg/ml leaf extract induced 16.7% mortality at 15 minutes and 33.3% at 30 and 60 minutes, with total

mortality (100%) achieved by 60 minutes at all concentrations (Table 4).

The bark extract showed a similar trend, with 50 mg/ml causing 16.7% mortality at 15 and 45 minutes, and 33.3% at 30 and 60 minutes. At 150 mg/ml, 33.3% mortality was observed at 15 minutes, increasing to 50% at 30 minutes and reaching 100% by 45 minutes. The highest concentration (300 mg/ml) produced 66.7% mortality at 15 minutes and achieved complete mortality by 45 minutes (Table 5). The root extract demonstrated the most rapid and potent activity. At 50 mg/ml, 50% mortality was observed at 15 minutes, with 100% mortality by 45 minutes. At 150 mg/ml, 75% mortality occurred at 15 minutes, with all cercariae dead by 30 minutes. Exposure to 300 mg/ml resulted in 100% mortality within 15 minutes (Table 6).

Table 4: Cercaricidal activity of A. indica Leaf extract at different concentrations and time intervals

| Concentration | No. of    | 15 min    | 30 min    | 45 min    | 60 min    | Total Mortality |
|---------------|-----------|-----------|-----------|-----------|-----------|-----------------|
| (mg/ml)       | Cercariae |           |           |           |           | (%)             |
| 50            | 6         | 0 (0.0%)  | 1 (16.7%) | 1 (16.7%) | 4 (66.7%) | 6 (100%)        |
| 150           | 6         | 1 (16.7%) | 2 (33.3%) | 1 (16.7%) | 2 (33.3%) | 6 (100%)        |
| 300           | 6         | 1 (16.7%) | 2 (33.3%) | 1 (16.7%) | 2 (33.3%) | 6 (100%)        |

Table 5: Cercaricidal activity of A. indica bark extract at different concentrations and time intervals

| Concentration | No.     | of | 15 min    | 30 min    | 45 min    | 60 min    | Total   | Mortality |
|---------------|---------|----|-----------|-----------|-----------|-----------|---------|-----------|
| (mg/ml)       | Cercari | ae |           |           |           |           | (%)     |           |
| 50            | 6       |    | 1 (16.7%) | 2 (33.3%) | 1 (16.7%) | 2 (33.3%) | 6 (100% | 6)        |
| 150           | 6       |    | 2 (33.3%) | 3 (50.0%) | 1 (16.7%) | 0 (0.0%)  | 6 (100% | 6)        |
| 300           | 6       |    | 4 (66.7%) | 1 (16.7%) | 1 (16.7%) | 0 (0.0%)  | 6 (100% | 6)        |

Table 6: Cercaricidal activity of A. indica root extract at different concentrations and time intervals

| Concentration | No.      | of 15 min | 30 min    | 45 min    | 60 min                                  | Total Mortality |
|---------------|----------|-----------|-----------|-----------|---|-----------------|
| (mg/ml)       | Cercaria |           |           |           | • | (%)             |
| 50            | 4        | 2 (50.0%) | 1 (25.0%) | 1 (25.0%) | 0 (0.0%)                                | 4 (100%)        |
| 150           | 4        | 3 (75.0%) | 1 (25.0%) | 0 (0.0%)  | 0 (0.0%)                                | 4 (100%)        |
| 300           | 4        | 4 (100%)  | 0 (0.0%)  | 0 (0.0%)  | 0 (0.0%)                                | 4 (100%)        |

**Notes:** All values are presented as number of dead cercariae (percentage of batch); total mortality is the cumulative number of dead cercariae at the end of the observation period.

### **DISCUSSION**

The present study demonstrates that aqueous extracts from the leaves, bark, and roots of Azadirachta indica exhibit significant cercaricidal activity against Bulinus globosus cercariae, with efficacy increasing in a concentration- and timedependent manner. Notably, the root extract showed the most rapid and potent action, achieving 100% mortality at 300 mg/ml within 15 minutes of exposure. These findings reinforce the potential of A. indica as a promising, environmentally friendly alternative for schistosomiasis vector control. The observed cercaricidal effects can be attributed to the rich phytochemical composition of the extracts, as revealed by GC-MS analysis. The leaf, bark, and root extracts contained a variety of bioactive compounds, including esters, fatty acids, terpenoids, flavonoids, and phenolic compounds. For instance, the leaf extract was rich in fatty acid esters such as isopropyl linoleate and hexadecanoic acid, eicosyl ester, while the bark extract contained high levels of wax esters and terpenoids such as isophytol. The root extract was particularly notable for its high concentrations of octanoic acid derivatives and unsaturated fatty acids. Many of these compounds have been previously reported to possess molluscicidal and antiparasitic activities (Pandev et al., 2014: Brahmachari, 2004). The cercaricidal activity observed in this study is consistent with previous reports on the molluscicidal and cercaricidal properties of A. indica and other medicinal plants. For example, Micheal et al. (2013) reported that crude extracts of A. indica and Entada leptostachya exhibited significant molluscicidal and cercaricidal activity against snail intermediate hosts of schistosomiasis, with efficacy dependent concentration and exposure time. Similarly, Kimeu et al. (2015) found that aqueous extracts of A. indica, Aloe secundiflora, and other plants caused substantial mortality of Biomphalaria pfeifferi snails, further supporting the broad-spectrum antiparasitic potential of these botanicals. Comparisons with other studies highlight both similarities and distinctions in the efficacy of plant-based molluscicides. Abdullahi demonstrated that aqueous of Balanites aegyptiaca leaves were highly effective against Lymnaea natalensis, with activity varying among plant parts and exposure durations. Kiros et al. (2014) reported that aqueous and ethyl extracts of Glinus lotoides fruits produced concentrationdependent mortality of *B. pfeifferi* snails, with 0–100% mortality observed at concentrations ranging from 37.5 to 80 mg/L. These findings align with the present study, where higher concentrations of *A. indica* extracts resulted in faster and more complete cercarial mortality.

The rapid action of the root extract in this study is particularly noteworthy. While Nguta et al. (2011) observed 80% mortality of snails after four hours of exposure to Euphorbia milii extracts at 100 mg/L, the A. indica root extract in the present study achieved 100% cercarial mortality at 300 mg/ml within just 15 minutes. This enhanced efficacy may be attributed to the unique combination and higher concentrations of active phytochemicals present in the root, as supported by the detailed phytochemical profiling. The mechanism by which these plant extracts exert their cercaricidal effects is likely multifactorial. Fatty acid esters and terpenoids are known to disrupt cell membranes and interfere with metabolic processes in parasites (Pandey et al., 2014). Flavonoids and phenolic compounds may induce oxidative stress or inhibit key enzymes, ultimately leading to parasite death. The presence of such compounds in all parts of A. indica likely contributes to the observed potency and rapid action, especially in the root extract.

In contrast to synthetic molluscicides such as niclosamide, which, although effective, are associated with significant environmental drawbacks, including toxicity to non-target aquatic organisms, persistence, and bioaccumulation (Odetunji & Salawu, 2010; WHO, 1965), A. indica offers a more sustainable and ecologically compatible alternative. Previous studies have highlighted the low toxicity of neem extracts to mammals and non-target aquatic species, as well as their biodegradability (Makeri et al., 2007; Umar et al., 2002; Khan & Aslam, 2008). This environmental safety profile is a significant advantage in regions where water bodies are integral to local livelihoods and ecosystems. Furthermore, the use of plant-derived molluscicides alians with integrated management strategies advocated by the World Health Organization, which emphasize sustainability, community acceptance, and the minimization of ecological disruption (WHO, 2016). The findings of this study, therefore, not only corroborate previous reports on the antiparasitic efficacy of A. indica but also advance the case for its adoption in schistosomiasis control programs, particularly in endemic regions where chemical control poses environmental risks.

### CONCLUSION

This study demonstrates that aqueous extracts from the leaves, bark, and roots of Azadirachta indica possess strong, rapid, and concentrationdependent cercaricidal activity against Bulinus globosus cercariae, with the root extract showing the highest potency. Phytochemical analysis revealed a rich diversity of bioactive compounds which could be responsible for the observed effects. These findings highlight the potential of A. indica, especially its root, as an effective and environmentally friendly alternative to synthetic molluscicides for schistosomiasis vector control. Further research on the isolation of active constituents and field-based validation recommended to support the integration of neembased products into sustainable schistosomiasis management programs.

### **REFERENCES**

- Abdullahi, Y., 2018. Molluscicidal activity of aqueous extract of leaves, stem back and roots of desert date (Balanites aegyptiaca Del.) against common liver fluke (Fasciola hepatica) found in the snail (Lymnea natalensis). Journal of Applied Sciences and Environmental Management 22, (3), 409-415.
- Arora, D. R. and Arora, B., 2005. Medical Parasitology, second edition, CBS Publishers and Distributors, New Delhi Bangalore, India.
- Asuquo, P. E., and Ifon, H. T., 2021. Growth and Haematological Status of *Clarias gariepinus* Fingerlings Fed *Jatropha tanjorensis* Leaf Powder Meal. Proceedings of the National Conference on Mariculture in India Recent Advancement. 1 7pp.
- Asuquo, P. E., Ekpo, P. B., and Ifon, H. T., 2022. Phytochemical Profiling of Clove Basil (*Ocimum gratissimum*) Extract Using LC-MS/MS. International Journal of Natural and Applied Sciences (IJNAS), 15, 89 94.
- Brahmachari, G., 2004. Neem-an omnipotent plant: a retrospection. Chembiochemistry 5, (4), 408-421.
- Colley, D. G., Bustinduy, A. L., Secor, W. E., and King, C. H., 2014. Human schistosomiasis. Lancet, 383, 2253-2264.
- Danso-Appiah, A., Utzinger, J., Lui, J. and Olliaro, P., 2008. Drugs for treating urinary schistosomiasis. Cochrane Database of Systematic Reviews, 3, 167.

- Diakite, R. N., Winkler, S. M. and Coulibaly, T. J., 2017.

  Dynamics of freshwater snails and schistosomiasis infection prevalence in school children during the construction and operation of a multipurpose dam in Central Côte d'Ivoire. Infectious Diseases of Poverty, 6, 93.
- Ekpo, P. B., Ekpo, I. P., Ifon, H. T., Ubi, G. M., Ikpeme, E. V., Ekaluo, U. B., Ndome, C. B., Ekanem, A. P., 2023. Green Remediation and Enhanced Metal accumulation using Moringa oleifera Leaf Extracts and Hydrocarbon-Degrading Microorganisms in Crude Oil-Impacted Soils of the Niger Delta, Nigeria. Global Journal of Geological Sciences, 21, 215-227.
- Eleng, I. E. and Imalele, E. E., 2019. Prevalence of Schistosoma haematobium and its intermediate hosts in Abini, Biase Local Government Area, Cross River State. Asian Journal of Medical and Health Research 4(1), 1-10
- Eleng, I. E., Ajang, R. O., Etah, S. O., Adie, P. I., Anita, N. Y., Mowang, D. A., Imalele, E. E., Okon, O. E., and Asor, J. E., 2024. Influence of physicochemical parameters on the abundance and distribution of freshwater snails in Nwang community of Ogoja local government area. International Journal of Natural and Applied Sciences (IJNAS), 17(1 and 2), 30 38.
- Khan, S. A. and Aslam, J., 2008. Study on the effect of Neem (*Azadirachta indica*) leaves smoke in controlling airborne bacteria in residential premises. Current Research in Bacteriology, 1, (2), 64-66.
- Khetarpal, S., 2010. Neem: a wonder tree. Asia-Pacific Biotechnology News, 14, (18), 18-20.
- Kimeu, M. B., Waniki, N. E. N. M., Dorcas, Y. S., and Piero, N. M., 2015. Molluscicidal effects of aqueous extracts of selected medicinal plants from Makueni County, Kenya. Pharm Anal Acta, 6, 445.
- Kiros, G., Erko, B., Giday, M. and Mekonnen, Y., 2014. Laboratory assessment of molluscicidal and cercariacidal effects of Glinuslotoides fruits. BMC Research, 7, 220-227.
- Kuravadi, N. A., Yenagi, V., Rangiah, K., Mahesh, H. B., Rajamani, A., Shirke, M. D. and Gowda, M., 2015. Comprehensive analyses of genomes, transcriptomes and metabolites of neem tree. Peer Journal, 3, e1066.

### ELENG, I. E., NELSON, A. Y., MONDO, N. J., MOSES, J., OPEH, P. B., BESHEL, S. B., EKPENYONG, J. F., UCHE, S. A., OKON, O. E., ASOR, J. E

- Makeri, H. K., Maikai, V. A. and Nok, J. A., 2007. Effect of topical application of neem seed (*Azadiracta indica*) extract on sheep infested with *Amblyomma variegatum*. African journal of biotechnology, 6, (20), 20-23.
- Micheal, S. E., Yole, D., Musila, F. M., Kutima, H., and Weru, P., 2013. Assessment of molluscicidal, cercaricidal, and miracicidal activity of crude extracts of *Azadirachta indica* and *Entada leptostachya*. Biology, Agriculture and Healthcare, 3, (5).
- Motaleb, M. A., 2011. Selected medicinal plants of Chittagong hill tracts. IUCN, Bangladesh.
- Nguta, J. M., Mbaria, J. M., and Gakuya, D. W., 2011. Biological screening of Kenya medicinal plants using Artemia salina (Artemiidae). Pharmacology Online, 2, 458-478.
- Odetunji, V. O., and Salawu, O. T., 2010. Efficacy of ethanolic leaf extracts of *Carica papaya* and *Terminalia catappa* as molluscicides against the snail intermediate hosts of schistosomiasis. Journal of Medicinal Plant Research, 4, 2348-2352.
- Pandey, G., Verma, K. K. and Singh, M., 2014. Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* (neem) leaves. International Journal of PharmaceuticalScience6, (2), 444-447.
- Paul, R., Prasad, M. and Sah, N. K., 2011. Anticancer biology of *Azadirachta indica* L (neem): a mini

- review. Cancer biology and therapy12, (6), 467-476.
- Poojory, M. M., Kanibagilu, A. V., and Adhikari, A. V., 2015. Extraction, characterization and biological studies of phytochemicals from *Mammea suriga*. Journal of Pharmaceutical Analysis, 5, (3), 182-189.
- Umar, A., Abdulraham, H. T. and Kokori, M., 2002. Preliminary studies of the efficiencies of the aqueous extracts of leaf and seed kernel of neem for the control of cowpea bruchid *Callobruchus maculates*. Research Journal of Science, 8, 25-30.
- Utzinger, J., Keiser, J., Xiao, S. H., Tanner, M., and Singer, B. H., 2003. Combination therapy of schistosomiasis in laboratory studies and clinical trials. Antimicrobial Agents, 47, 1487-1495.
- WHO, 1965. Molluscicide screening and evaluation. Bulletin of the World Health Organization, 33, 567-581.
- WHO, 2011. Schistosomiasis. World Health Organization Factsheet No 115. WHO, Geneva, 5-9.
- WHO, 2016. Update on schistosomiasis. WHO division of control of tropical diseases. World Health Organization Geneva.
- WHO, 2020. Schistosomiasis. World Health Organization Available online: <a href="https://www.who.int/news-room/factsheets/detail/schistosomiasis">https://www.who.int/news-room/factsheets/detail/schistosomiasis</a> (accessed on 11 February 2024).