

## PHYTOCHEMICAL SCREENING AND *IN VITRO* EFFECTS OF *Azadirachta indica* METHANOL EXTRACTS ON CERCARIA OF *Biomphalaria glabrata*

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### ABSTRACT

*Biomphalaria glabrata* are freshwater snail vectors that harbor *S. mansoni* parasites, a major Neglected Tropical Disease (NTD) in Nigeria and sub-Saharan Africa; responsible for schistosomiasis. This research investigated the cercaricidal effects of *Azadirachta indica* (*A. indica*) methanolic extracts on *S. mansoni* cercaria. Fresh *A. indica* leaves and stem-bark were removed, shade-dried and pound. Phytochemical screening and Soxhlet extraction were carried out. Concentrations at 0.5-5.0 µg/ml were prepared. Cercariae from *B. glabrata* snails were shed under 100W light bulb for 2 h. These were challenged with the leaf and stem bark extracts. Mortality of each concentration was observed under a stereo microscope at 10-60 min of post exposure (PE). Results revealed that the presence of bioactive compounds include alkaloids, flavonoids, tannins, saponins, cardiac glycosides and carbohydrate. Cardiac glycosides and carbohydrates were the most abundance in the leaf, while flavonoids and tannins were the most abundant in stem bark. *A. indica* extracts demonstrated cercaricidal effect in a dose and time-dependent pattern. Mortality was recorded at 1.0 µg/ml in 30 minutes, PE. At 5.0µg/ml, there was 100% mortality in 10-20 min of PE (19.00±0.58<sup>a</sup> and 20.00±0.00<sup>a</sup>). The LC<sub>50</sub>, for stem bark and leaves were valued at 2.72 and 2.79µg/l respectively. There was no significant difference in mean mortality ( $p > 0.05$ ) of both leave and stem bark extract (LSD = 0.82 and 2.30), respectively. Control showed motility all through the 60 min of PE. Both stem bark and leaf extracts of *A. indica*, upon further investigation, could be developed into juvenile schistosomicidal agent.

**Keywords:** *Azadirachta indica*, cercaricidal, *in vitro*, phytochemical, schistosomiasis

### INTRODUCTION

*Biomphalaria glabrata* (*B. glabrata*) are freshwater snail vectors that harbor *S. mansoni* parasites, which is one of the NTDs that caused debilitating effects in human population (Mitta *et al.*, 2017). Considering the possible challenges due to molecular and biochemical relationship between the causative schistosome parasite and its hosts; may constitute barriers in terms of control and eradication of the parasites (Arican-Goktas *et al.*, 2014). These trematodes blood flukes have complicated life cycle that involve the intermediate freshwater snail hosts, and a definitive human or animals host (Hu *et al.*, 2021). *Schistosoma mansoni* and *S. japonicum* are responsible for chronic hepatic and intestinal fibrosis, while *S. haematobium* causes fibrosis in the urinary tract (World Health Organization (WHO), 2019). Praziquantel (PZQ – pyrazino-isoquinolone) is the only readily available and effective drug, widely used for the treatment of the three main parasites that cause schistosomiasis; with increasing cases of resistance especially against the larval stages (Basha & Mamo, 2021). The WHO (2019) recommended periodic PZQ treatments only as a short-term measure for the control of morbidity. Francis (2023) posited that there has been

recent increase in use of plant-based medicines even in developed world against developing world that largely rely on plant-based medicines as an alternate to orthodox medication. Moreover, with recent increase in bacterial resistance to antibiotics; efforts are being made towards new and more effective therapeutic agents.

The plant *Azadirachta indica* *A. Juss* (Meliaceae), commonly called Neem tree is well known for its multiple medicinal benefits against a wide range of ailments (Ali *et al.*, 2022). It is largely used as therapeutic medication (Abdulkadir *et al.*, 2017; Nagano & Batalini, 2021). The plant is generally called “wonder plant” (Devi & Sharma, 2023). It has long been used as an ethnopharmacological category one herbal remedy (Tembe-Fokunang *et al.*, 2019). The ethanolic leaf extract has long been established for its antibacterial activities against both gram-negative and gram-positive bacteria (Ouerfelli *et al.*, 2022). The traditional and medicinal value of neem plant has also been established and used for the treatment of various ailments such as helminthiases, respiratory disorder and constipation; depending on the plant part under use (Biswas *et al.*, 2002). This is largely, due to its ease of

accessibility as well as high economic value (Younis *et al.*, 2023). The nanoparticles and its potential use as natural biopesticide have also been established (Younis *et al.*, 2023; Zaib *et al.*, 2024). Primary chemical constituents include liminoids and terpenes that consist of azadirachtins, majorly, 3-deacetyl-3cinnamolyazadirachtin; and an antioxidant infusion extract showed at 1000 mg/L, remarkably inhibited DPPH activity (Ali *et al.*, 2022).

Acheampong *et al.* (2020), has posited the effectiveness of the cercarial and adulticidal properties of *A. indica* alongside other plants, and found it to be effective as the conventional drug – PZQ. Different parts of the plant are known to contain several bioactive compounds that are responsible for antibacterial, antiviral and antifungal activities (Herrera-Calderon *et al.*, 2019; Ouerfelli *et al.*, 2022; Francis, 2023). *Azadirachta indica* has also shown high efficacy in antibacterial activity especially against *K. pneumoniae* (Malar, *et al.*, 2020). The phenolic compounds obtained from *A. indica* have demonstrated its potency against gram-positive and gram-negative bacteria (Ouerfelli *et al.*, 2022). Akinrotoye *et al.* (2020) states the possibility of treatment of cancer and malaria with its alkaloid compounds, as well as being used as an analgesic. Further, flavonoids obtain from *A. indica* have the potential to act as antioxidants, anti-inflammatory and cardioprotective agents. Crude methanolic extracts of *A.*

*indica* is known to possess significant properties compared to other extracts and are said to be exploited for plant-based anticancer and antimicrobial agents (Muhammed & Omer, 2015; Malar *et al.*, 2020).

Sustainable control program of schistosomiasis requires an integrated approach including repeated mass chemotherapy by use of PZQ, public health education, with focus on behavior changes, improving sanitation, and provision of clean water supply (Kiros *et al.*, 2014). The affordability of neem as a remedy and ease of accessibility makes it appealing to those living in places where the disease is endemic (Biswas *et al.*, 2002). Hence, our attempt at utilizing *A. indica* could serve as alternative therapy and/or serve as part of an integrated control measures for schistosomiasis. By this, we set out to investigate its cercaricidal potential and establish the need to foster the position of integrated control measures for schistosomiasis infection especially in Nigeria and sub-Saharan Africa.

## MATERIALS AND METHODS

### Study Area

The study was conducted in the Department of Science Laboratory Technology, University of Jos, Jos-North Local Government Area (LGA) of Plateau State, Nigeria (Figure 1).

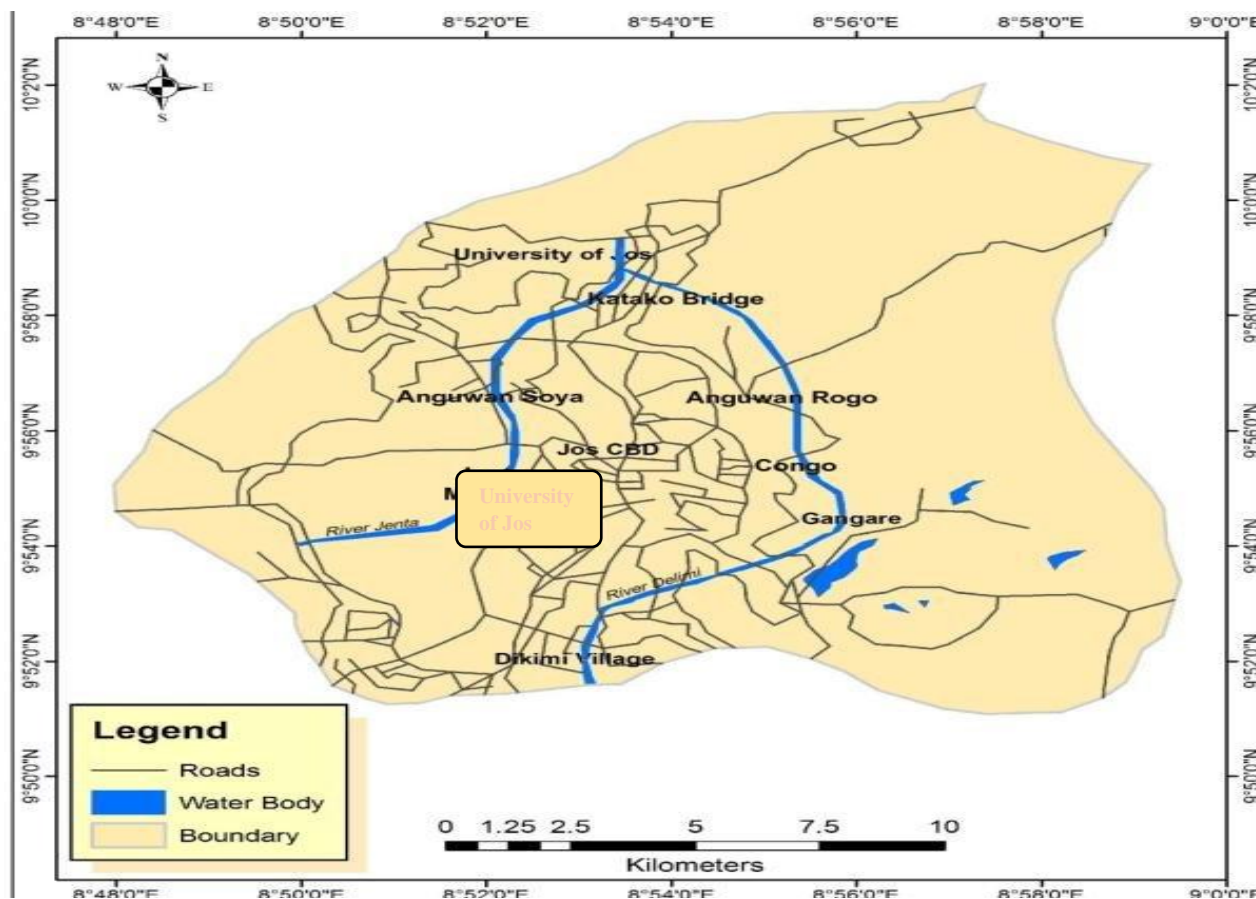


Figure 1: Map of Jos-North showing the study location

### Plant Collection

The leaves and stem bark of *A. indica* tree were collected from Liberty Boulevard, in Jos metropolis of Plateau State, Nigeria. The geographical coordinates were at latitude 090 53'54.61" N and longitude 080 53'40.93" E with an eye altitude of 1,213 m.

### Preparation of Plant Samples

The leaves and stem bark were prepared according to Daben's *et al.* (2023) description. These were cut into smaller pieces with kitchen knife and shade-dried at room temperature  $29 \pm 2$  °C, for 5 and 10 days respectively. The dried samples were pulverized using mortar and pestle, and the powder (Plates 1 & 2) was sieved with a wire mesh of sizes 0.5 mm. These were stored in a plastic jar with air tied lid, pending use.



Plate 1: Leaf powder



Plate 2: Stem bark powder

### Qualitative Phytochemical Screening

This was done according to Ali *et al.* (2022); and Daben & Mwansat (2024) descriptions. The samples were screened for the presence of secondary metabolites like flavonoids, alkaloids, tannins, steroids/terpenes, resins, anthraquinones, phenols, glycosides and saponins.

### Soxhlet Extraction and Evaporation

This was carried according to Redfern *et al.* (2014), where three hundred grams (300 g) of powdered plant sample (Plate 1 & 2) was placed inside the thimble. The thimble was then loaded into the main chamber of the Soxhlet extractor. Five hundred (500) ml of methanol was measured and transferred into a round bottom flask, placed on a heating mantle. The reflux condenser was then placed on top of the Soxhlet extractor. The solvent was heated to reflux at a temperature of 64.7 °C. The solvent vapour travels up a distillation pathway, and flooded the chamber housing the thimble of the powdered plant sample. The condenser containing constant flow of cold water, cool the solvent vapour and drips back down into the chamber housing the plant sample. The chamber containing the plant sample slowly filled up, thereby dissolved the sample with the warm solvent. The Soxhlet chamber when full was emptied by the siphon and return to the distillation flask. This process of extraction continued for a period of 8 hours, until the active component of the samples was extracted; indicated by clear appearance of the solvent at end point of the thimble (Daben *et al.*, 2023). The extracted sample (Plate 3) was kept in a water bath, which had a digital interface that allows temperature to be regulated to 40 °C for 2 hours. After the extracted sample was concentrated, it was cooled and collected in a vial. The extract was kept in an open space but close with muslin cloth, for it to be properly air-dried before use.



Plate 3: Extracted sample

### Collection and Shedding of Cercaria

*Biomphalaria glabrata* snails were collected with gloved hands, from ponds, crevices of rocks from Duala, Dogon Dutse in Jos North LGA of Plateau State, Nigeria. This was at Longitude 009° 55' 91" N, and Latitude 008° 54' 0.54" E, altitude 1,218m. Snails were placed in a plastic container with water from the source of collection and it was immediately transported to the laboratory for proper identification using standard keys (Falade & Otariho, 2015). Each snail was carefully transferred into shedding vials that contained 10ml of distilled water (Buddenborg *et al.*, 2023). The shedding of the trematodes cercariae were induced by exposure to artificial light (100W) for 2 h at room temperature, ranges between 29±2 °C. These were observed under a dissecting microscope to determine the presence of shed cercariae. Live cercariae shed from each snail were transferred to a microscopic slide and carefully observed at X40 magnification.

### Cercaricidal Bioassays

Two drops of cercarial suspension were dropped on a plane slide and iodine was applied to kill and stain the cercaria. The slide was then placed under a dissecting microscope for counting. This was done to determine the number of cercaria to be used for each concentration. Batches of about 20 cercaria were picked by use of a crown pipette attached to a rubber bulb. These were placed in 10 sterilized bottles and then exposed to each concentration of *A. indica* extracts, prepared by weighing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 µg/l respectively, and 0.1 ml of distilled water was used as control (Daben and Mwansat, 2024). These were observed under a dissecting microscope under X40 mg at time intervals of 10, 20, 30, 40, 50, and 60 minutes of post exposure (PE).

### Determination of Cercarial Mortality

Morphological changes and possible mortality were recorded at each time frame (10-60 minutes) of PE. Cercariae were presumed dead when they were immobile, lost their tail or head and/or coiled body, and sank to the bottom. The number of live cercariae at the beginning of the experiment and the number of live cercariae at the observation times were recorded. All experiments were carried out in triplicates. The LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>95</sub> of each extract were determined.

### Statistical Analysis

Data were expressed as mean ± standard deviation (M±SD). The mean and standard errors of the various mortalities observed after being challenged with methanol leave and stem bark extracts of *A. indica* at

different concentrations was computed. Data from each plant extract was subjected to One-way ANOVA to determine significant difference between the various concentrations used and increase in time of exposure. Data was subjected to Kaplan Meier test (Etikan *et al.*, 2017) to determine the survival rate of cercariae as exposed to different concentrations of the plant extract at different time intervals. The significance level was determined at  $p \leq 0.05$ ; where  $p < 0.05$  was considered significant. Cercaricidal data was subjected to linear probit regression to determine 50, 90 and 95 % lethal concentrations of the extracts versus time of exposure.

## RESULTS AND DISCUSSION

### Phytochemical Analysis

The qualitative phytochemical analysis (Table 1), showed the presence of bioactive compounds of both primary and secondary metabolites. This includes carbohydrates, alkaloids, flavonoids, glycosides, phenols, saponins, tannins, cardiac and glycosides. Cardiac glycoside and carbohydrates were found to be the most abundant in the leaf. While flavonoids and tannins were in high concentration in the stem bark; alkaloids and saponins were moderately higher in the stem bark than the leaf. Steroids and resins were not detected in the leaf extract.

**Table 1: Phytoconstituents of *A. indica* leaf and stem bark methanol extract**

Parameter	Leaf	Stem bark
Alkaloids	+	++
Flavonoids	+	+++
Phenols	+	++
Glycosides	+	+
Saponins	+	++
Steroids	-	++
Tannins	+	+++
Cardiac glycosides	+++	+
Resin	-	+
Carbohydrates	+++	++

+++ = Highly; ++ = Moderate; + = Present; - not detected

### In Vitro Cercaricidal Effects of *A. indica* Leaf Extract

The One-way ANOVA (Table 2) showed that there was a significant difference ( $p < 0.05$ ) in mean mortality at 0.5 µg/ml and 2.0-5.0 µg/ml of the *A. indica* extractson *S. mansoni* cercaria with increase in time, (95 % CI). However, there was no significant difference ( $p > 0.05$ ) in mean mortality, observed across all concentrations (LSD = 0.82). The rate of mortality was concentration and time dependent. Mortality increases with increase in concentration and exposure time. At lower concentrations between 0.5 and 1.0 µg/l, there was minimal to no death recorded. Mortality rose drastically at concentrations 2.0–5.0 µg/l, with 100% mortality at about 4.0-5.0 µg/l. The control showed motility all through the 60 minutes of post exposure.

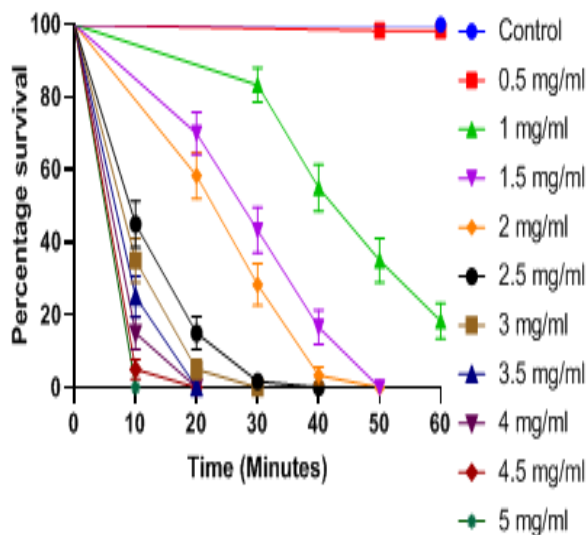
**Table 2: In vitro cercaricidal mean mortality of *A. indica* methanol leaves extract**

Concentration (µg/ml)	(Time in minutes)					
	10	20	30	40	50	60
0.5	0.00±0.00 <sup>g</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>d</sup>	0.33±0.33 <sup>c</sup>	0.00±0.00 <sup>c</sup>
1.0	0.00±0.00 <sup>g</sup>	0.00±0.00 <sup>e</sup>	3.33±0.33 <sup>d</sup>	9.00±0.00 <sup>c</sup>	13.00±0.58 <sup>b</sup>	16.33±0.67 <sup>b</sup>
1.5	0.00±0.00 <sup>g</sup>	6.00±0.00 <sup>d</sup>	11.33±0.33 <sup>c</sup>	16.67±1.21 <sup>b</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
2.0	0.00±0.00 <sup>g</sup>	8.00±0.00 <sup>c</sup>	14.00±0.00 <sup>b</sup>	19.33±0.33 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
2.5	11.00±0.58 <sup>f</sup>	17.00±0.58 <sup>b</sup>	19.67±0.33 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
3.0	13.00±0.58 <sup>e</sup>	19.00±0.58 <sup>ab</sup>	19.67±0.33 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
3.5	15.00±0.58 <sup>d</sup>	19.00±0.58 <sup>a</sup>	19.67±0.33 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
4.0	17.00±0.57 <sup>c</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
4.5	19.00±0.58 <sup>b</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
5.0	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
Control	0.00±0.00 <sup>g</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>
LSD	0.82					
P-value	≤ 0.0001					

At  $p \leq 0.05$ , there was a significant difference in the mean mortality of the *in-vitro* cercaricidal property of *A. indica* methanol leave extract on *S. mansoni* cercaria. Values are presented as mean ± standard error of means. Ranking was done across the concentrations, and values with the same super script are insignificant.

**Survival Curve of Cercaricidal Bioactivity of *A. indica* Methanol Leaves Extract**

The percentage of cercariae that survive over time after exposure to different concentrations of *A. indica* methanolic leave extract as depicted by the survival curve (Figure 2) decreases with increase in time. Lower concentrations at 0.1 µg/l, cercariae were active at 10 to about 20 minutes of post exposure. At 2.0 µg/l about 50 % of the cercaria were very sluggish and inactive. Concentrations at about 3.0 to 5.0 µg/l cercarial mortality increases to 100 %. There was a dose-dependent decline in cercarial survival, with higher concentrations and time (50-60 minutes) of post exposure; resulting in faster and higher death rates.



**Figure 2: Survival curve for *in vitro* cercaricidal property of *A. indica* methanol leaves extract.**

**In vitro Cercaricidal Mean Mortality of *A. indica* Stem Bark Bioassays**

Table 3 below showed no significant difference ( $p > 0.05$ , 95 % CI), with LSD = 2.30 in rate of mortality due to the bioactive components of the stem bark extract. The effect of concentrations on the mean mortality of the *S. mansoni* cercaria was concentration and time-dependent. The higher the concentration, the higher the rate of mortality; and the longer the exposure time, the higher the mortality rate. At lower concentrations (0.5-1.0 µg/l), parasites appear sluggish with fewer to no mortality in some of the replicates. At higher concentrations (4.0-5.0 µg/l), mortality increased significantly to 100 %. Complete mortality was observed at 4.0-5.0 µg/l. The control, recorded no mortality (0.00 %) all through the period of exposure.

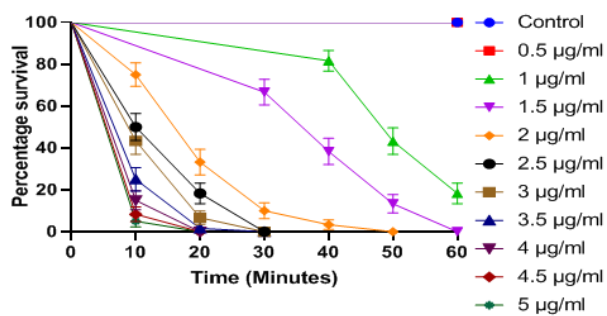
**Table 3: Mean mortality of the *in vitro* cercaricidal property of *A. indica* stem bark extract**

Conc. (µg/ml)	(Time in minutes)					
	10	20	30	40	50	60
0.5	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>
1.0	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	3.67±1.86 <sup>c</sup>	11.33±0.67 <sup>c</sup>	16.33±0.67 <sup>b</sup>
1.5	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	6.67±3.53 <sup>b</sup>	12.33±2.91 <sup>b</sup>	17.33±0.88 <sup>b</sup>	20.00±0.00 <sup>a</sup>
2.0	5.00±0.58 <sup>e</sup>	13.33±2.03 <sup>c</sup>	18.00±2.00 <sup>a</sup>	19.33±0.67 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
2.5	10.00±0.16 <sup>d</sup>	16.33±1.76 <sup>b</sup>	19.33±0.67 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
3.0	11.33±0.67 <sup>d</sup>	18.67±0.33 <sup>ab</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
3.5	15.00±1.16 <sup>c</sup>	19.67±0.33 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
4.0	17.00±1.16 <sup>bc</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
4.5	18.33±0.67 <sup>ab</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
5.0	19.00±0.58 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
Control	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>	20.00±0.00 <sup>a</sup>
LSD	2.30					
P-value	≤0.0001					

At  $p \leq 0.05$  there was no significant difference in mean mortality of the cercaricidal property of *A. indica* methanolic stem bark extract. Values are presented as mean ± standard error of means. Ranking was done across the concentrations and values with the same super script were not significant.

**Survival Curve**

The survival curve in Figure 3 below, showed that at lower concentrations, the survival rate remained relatively high. However, with increase in concentrations and time, the survival rate decreases. At higher concentrations and increase in time (50-60 minutes); the survival rate dropped sharply, indicating a significant increase in rate of mortality and very less or no survival with increase in time of exposure.



**Figure 3: Survival curve for *in vitro* cercaricidal bioactivity of *A. indica* methanolic stem bark extract**

**Probit analysis of lethal concentration and cercaricidal mortality of *A. indica* stem bark extract**  
 There was no significant difference ( $p > 0.05$ ) in the rate of mortality between the lower concentrations and period of exposure with the  $\chi$  values as reflected on Table 4, for the 60 minutes of post exposure. The concentrations required to result in 50, 90 and 95 % mortality, is as reflected on Table 4. At 10 minutes of post exposure (PE), the LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>95</sub> were valued at 2.72, 4.29 and 4.89 µg/l, respectively, 95 % CI = 5.473-7.381. While at 50 minutes PE, the LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>95</sub> valued at 0.99, 1.46 and 1.63 respectively, 95 % CI = 5.973-9.534.

**Table 4: Probit analysis of LC50, 90 and 95 of cercaricidal effects of *A. indica* stem-bark extract**

Time (minutes)	Regression equation	$\chi^2$ (p > 0.05)	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>95</sub>	Lower	Upper
10	Y=15.12*x-12.87	7.002	2.72	4.29	4.89	5.473	7.381
20	Y=16.05*x-5.73	17.141	2.02	2.68	2.89	8.638	12.474
30	Y=14.28*x+4.13	1.164	1.61	2.04	2.18	9.538	15.385
40	Y=12.17*x+13.13	3.250	1.31	1.84	2.02	7.002	10.595
50	Y=9.236*x+25.20	4.058	0.99	1.46	1.63	5.973	9.534
60	Y=7.47*x+32.33	4.866	0.82	1.19	1.31	6.214	10.041

**Table 5: Probit analysis of the LC *in vitro* cercaricidal property of *A. indica* methanol leaves extract**

Time (minutes)	Regression equation	$\chi^2$ (p > 0.05)	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>95</sub>	Lower	Upper
10	Y=16.18*x-16.00	24.913	2.79	3.94	4.35	7.286	9.910
20	Y=15.56*x-3.60	13.277	1.92	2.73	3.01	6.979	9.747
30	Y=15.82*x05.00	13.242	1.44	2.21	2.49	5.737	8.158
40	Y=14.02*x+4.93	1.513	1.08	1.61	1.81	5.731	8.794
50	Y= 8.218*x+29.40	2.137	0.88	1.26	1.40	6.258	10.136
60	Y=7.370*x+32.73	4.866	0.82	1.19	1.31	6.214	10.041

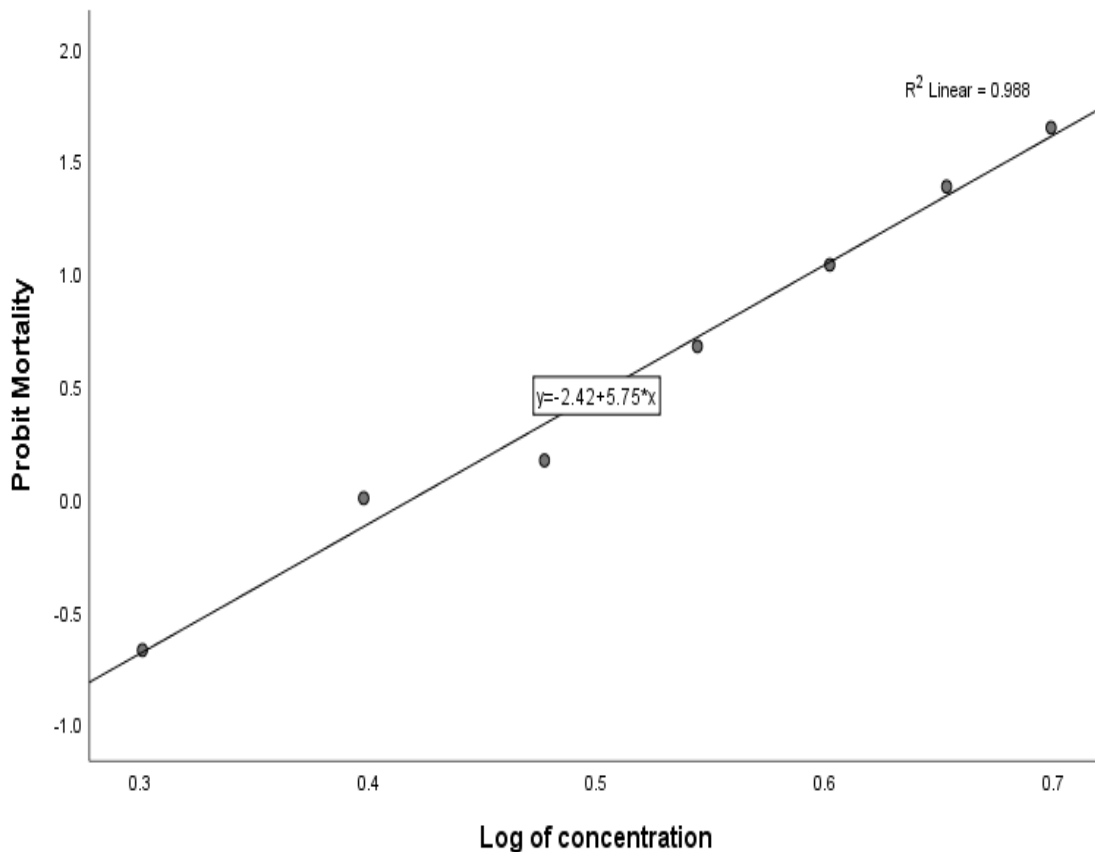
**Probit Analysis of *in vitro* Lethal Concentrations of Cercaricidal Effects of *A. indica* Leaves Extract**

The *in vitro* biopotency effects of the leave extract of *A. indica* methanol leaf extract on *B. glabrata* cercaria as shown on Table 5 below, indicated that there was no significant change (p>0.05) in rate of mortality in 10 minutes of PE. The LC<sub>50</sub> was valued at 2.79, while LC<sub>90</sub> and LC<sub>95</sub> were valued at 3.94 and 4.35 respectively. While at the maximum time of 60 minutes of post exposure, the LC<sub>50</sub>, LC<sub>90</sub>, LC<sub>95</sub> were valued at 0.82, 1.19, and 1.31, respectively.

**Logarithmic Cercaricidal Mortality Effects of *A. indica* Methanol Stem Bark Extract**

Figure 4 below, demonstrated a clear concentration-response relationship, with increase concentrations

leading to higher probabilities of cercarial death. The estimated LC<sub>50</sub> is approximated to 1.414 (Log<sub>10</sub>), indicated the concentration required to achieve 50 % cercarial mortality. The steep slope between 1.293 and 1.614 (Log<sub>10</sub>) suggests an effective concentration range for achieving significant cercarial mortality. This probit analysis models the relationship between the concentration of *A. indica* methanol stem bark extract and the probability of cercarial death and/or growth inhibition of the *S. mansoni* cercaria. The result suggests a significant positive relationship between concentration and mortality effects, with some heterogeneity in the data.



**Figure 4: Probit mortality rate of cercaricidal property of *A. indica***

In recent past, numerous studies have shown the chemo-preventive and therapeutic effects of neem extracts or their isolated bioactive compounds; which made neem trees to have received worldwide attention as “holy trees” with remarkable therapeutic benefits such as antibacterial, and hepatoprotective properties (Ujah *et al.*, 2021; Devi & Sharma, 2023). Thus, phytochemicals, being the bioactive components that include alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, steroids and carbohydrates found in the study, are likely to be associated with the *S. mansoni* cercaricidal effects. This compares favorably to the work of Malar (2020); who worked on phytochemical and bio-efficiency of *A. indica* and *M. azedarach*. However, Ibrahim *et al.* (2017), in their work on phyto-screening and *in vitro* anti-coccidial efficacy of methanolic extracts of the stem bark of *A. indica*; were not able to identify steroids in the stem bark; as identified in in the stem bark extract of this study. Notwithstanding, the findings of this work agrees with Malar *et al.* (2020); except that they were equally unable to identify steroids and carbohydrates in the stem bark as reported in this study.

Earlier investigators like Acheampong *et al.* (2020); reported that the efficacy of praziquantel (PZQ), the anti-schistosomal drug of choice, has been reduced. Hence, the need for alternative therapeutic agents with schistosomicidal activity that is biodegradable, easily accessible, as well as readily available and affordable to rural communities, who are often at the lower rung of the socioeconomic profile. Thus, the study, investigated the *in vitro* effects of the methanolic extract of *A. indica* stem bark extract on *S. mansoni* cercaria, using different concentrations. Following the fact that the candidate plant (*A. indica*), had demonstrated molluscicidal properties against *Pomacea canaliculate*, due to its major bioactive compound – adirachtin (Masaguni & Latip, 2015; Zaib *et al.*, 2024). Besides, challenges that are posed by the biochemical immunodulation of *B. glabrata* and *S. mansoni* parasites (Arican-Goktas *et al.*, 2014; Mitta *et al.*, 2017; Abdel-Salam *et al.*, 2025), potent possibility of high level of resistance in the nearest future. Hence, the search for alternative and effect antischistosomal agents that are easily accessible by the rural populace; who are often the victims of infections due to sometimes occupational hazards such as fadama farmers and anglers (Daben *et al.*, 2007). Therefore, the study established juvenile mortality in a relatively short period with the leaf extract resulting in mortality in about thirty minutes of exposure, compared to forty minutes obtained from the stem bark extract; and at

relatively low concentrations. A hundred percent mortality was recorded in about 4.5 and 5.0 µg/ml for both the leaf and stem bark; following a concentration and time dependent pattern. This is similar to the *in vivo* activities of some medicinal plants including *A. indica*, by Acheampong *et al.* (2020); and related to other plants such as *Tamarindus indica* and *Terminalia arjuna* against *Indoplanorbis exustus*; which demonstrated positive effect against liver flukes (Soni & Singh, 2015).

Howbeit, methanolic leaf extract of *A. indica* in this study, demonstrated the highest *in vitro* cercaricidal activities. The survival pattern equally reduces with increase in concentration and time. The leaf extracts proved to contain more bioactive compounds through the earlier mortality effects at about 3.0 to 5.0 µg/l, than the stem bark, which was about 4.5 to 5.0 µg/l. However, Acheampong *et al.* (2020); experience 100 % mortality in 60 minutes of post exposure; while 100 % mortality was shown in about 40 minutes of post exposure in this study, for both the leaf and stem bark extracts. Similarly, mortality occur in a much earlier time of post exposure and lower concentrations, contrary to the 30 minutes at 250 µg/mL recorded by Acheampong *et al.* (2020). Differences in mortality in the leaf and stem bark extracts, maybe due to geographical location and physicochemical conditions of the soil. This might have attributed to more potency and earlier mortality of the leaf extract in a relatively short period of exposure than the findings of Acheampong *et al.* (2020). Again, mortality rates recorded in this study was at an earlier time compared to the findings by Younis *et al.* (2023), who use *A. indica* fruit. This could possibly be due to more bioactive compounds present in the leaf and stem bark than in the fruit.

The safety profile of *A. indica* has also been emphasized, hence, its potential as an anti-schistosomal agent (Basha & Mamo, 2021). Since the extracts were seen to cause deformity such as loss of head or tail in cercaria, similar to deformity in surface architecture of the worms, particularly *Allolobophora caliginosa* as posited by Abu Hawsah *et al.* (2023). Mortality was found to be time and dose-dependent; similar to the outcome of the work by Daben *et al.* (2023), who reported the molluscicidal and cercaricidal activity of the stem bark of *E. africana* to be both time and concentration-dependent. However, complete mortality does occur at a relatively higher concentration as examination of morphological changes, and mortality of cercariae before and after exposure. The LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>95</sub>, showed reduce cercarial motility with increase in time and morphological changes, which kept

increasing with increase in concentrations and time of exposure. This can possibly be due to different phyto-composition and/or bioactive compounds of the plant extracts. Besides, it agrees with the findings of Abou El-Nour (2021), who use isolates in evaluating the biocidal activity of alkaloids, saponins and volatile oil extracted from *Nigellasativa* seeds against miracidia and cercariae of *S. mansoni*; with *Origanum majorana* extract exhibiting 100 % mortality in 30 minutes of post exposure. This discovery corroborates with the findings in this study; even though the Abou El-Nour (2021) used higher concentrations. Nevertheless, the outcome of this study was contrary to an earlier study by Aminou *et al.* (2020); who demonstrated the biocidal effect of *A. indica* along with other plant extracts; showed that the leaves had the least cercaricidal effect among the three species of plant that was utilized. On the contrary, this study demonstrated high cercaricidal properties in utilizing both the leaves and stem bark extracts, which again could be attributed to differences in physicochemical conditions of soil inherent in different geographical locations. As such, potency of plant parts maybe a function multiple factors such as physicochemical conditions of soil, rather than the biocomponent of the primary or secondary metabolites alone.

## CONCLUSION

The methanolic leave and stem bark extract of *A. indicaproved* effective in *in-vitro* cercaricidal efficacy against *S. mansoni* cercaria, harbored by *B. glabrata* that causes schistosomiasis. Thus, *A. indica* showed the ability to effectively inhibit and cause cercarial mortality at a much lower concentration. Notwithstanding, further study is needed to isolate and characterized the active compounds responsible for the cercaricidal activities of the plant part used; and to also determine the synergistic activities of the plant parts. This will lead to the development of a cercaricidal agent that is affordable to low in-come earners and those in the rural communities, where the burden of the disease is most often prevalent.

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