



## Molluscicidal Potential of Column Purified Fractions of *Allium cepa* against Intermediate Host of Urinary Schistosomiasis (*Bulinus globosus*) in Sokoto, Nigeria

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### Authors' contributions

This work was carried out in collaboration between all authors. Author JS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KS and AYB managed the literature searches, while authors MTM and MSY managed the analysis of the study. All authors read and approved the final manuscript.

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### ABSTRACT

Potential of column purified fractions of *Allium cepa* bulb against intermediate hosts of urinary schistosomiasis (*Bulinus globosus*) was conducted in laboratory condition. The fresh bulbs of *A. cepa* were purchased from Ramin Kura market of Sokoto, identified and authenticated by a taxonomist. The bulbs were sliced into pieces, air dried and powdered. Extracts were obtained using methanol as polar then purified with silica gel as a stationary phase while N-hexane and ethyl acetate (1:1) as the mobile phase. Thirteen fractions each fraction containing 10 ml of the effluent was collected, the collected extracts were left open for evaporation for 48 hours. Ten adult *B. globosus* were immersed in 3liters of water containing different concentrations of the fraction and each treatment was replicated three times with control in the same condition without treatment,

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observations were recorded after 24 hours up to 96 hours. The toxicity experiment showed that fractions (F<sub>7</sub>, F<sub>8</sub>, F<sub>6</sub> and F<sub>9</sub>) were most toxic fractions, LC<sub>50</sub> after 96 hours was 19.371 mg/l. based on findings from this research it can be concluded that, *A. Cepa* was very potent and can be used for control of *B. globosus* in order to prevent urinary schistosomiasis infection in endemic areas and drugs industries may use the extracts of these plants for production of molluscicides.

**Keywords:** *Allium cepa*; *Bulinus globosus*; Ramin Kura; schistosomiasis; molluscicides.

## 1. INTRODUCTION

Urinary schistosomiasis also known as Bilharziasis or snail fever, is a disease caused by infection of digenetic blood trematode (*Schistosoma haematobium*) [1]. Urinary Schistosomiasis remains among the most important public health problems in Nigeria [2,3]. Generally, various factors that are responsible for the increment and persistent of infection with schistosomiasis in Nigeria include climatic changes, proximity to water bodies, irrigation and dam construction as well as socio-economic factors such as occupational activities and poverty as well as an abundance of the infected intermediate host such as *Bulinus* species [4]. Although, use of molluscicides to eradicate snail vector is considered to be the best method to eliminate schistosomiasis, because killing of intermediate host disrupts the life cycle of the parasite and stop the transmission of infection [5]; synthetic molluscicides widely used for the effective control of the intermediate host remain cost-effective and have rapid toxicity to the aquatic environment [6]. Moreover, most of the synthetic molluscicides such as Niclosamide, endoathal, chloramines, ozone and hydrogen peroxide are toxic to non-target animals and may have long-term detrimental effects on the aquatic life [7,8,9]. Although, several groups of compounds present in various plants were determined to have toxic effect to target organisms at acceptable doses [10,11]; there is still an urgent need for highly toxic plants to the target organism only in order to avoid transmission of the parasitic disease like urinary schistosomiasis [12]. Furthermore, in the environment like Sokoto where a large fraction of population is still dependent on lakes, rivers and dams water for their everyday supplement; it is highly injurious to use synthetic chemicals to control the disease.

In addition, *A. cepa* is available, accepted, cheap and safe plants, which have been used for many reasons (such as food, flavour, medication etc.) among Sokoto people. Therefore, a potential of column purified fractions of *A. cepa* bulb plants

on an intermediate host of urinary schistosomiasis was determined to serve as an increment for a cheaper, non-toxic and efficient way of controlling urinary schistosomiasis infections in Sokoto.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Materials

Red globe onion bulbs used for this study were purchased from Ramin Kura market Sokoto, each of the plant bulbs was identified and authenticated by taxonomist, in the herbarium, Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Voucher number collected for the identified plants was UDUH/ANS/0088.

### 2.2 Preparation of the Plants Powder

Fresh bulbs of *A. cepa* was washed with borehole water, sliced into smaller pieces and air dried under shade for 14 days, the dried bulbs and cloves were grinded into a fine powder using a grinder and stored in air-tight container [13].

### 2.3 Preparation of the Plants Methanol Extract

The methanolic extracts of *A. cepa* was prepared by using cold maceration method described by Handa et al. [14]. A total of 100g sample was weighed and transferred into clean, sterile bottle, soaked into 300 ml of Methanol, tightly covered and incubated for 72 hrs at room temperature. The suspension was stirred occasionally after each 24 hrs and filtered into a sterile bottle by using whatman filter paper (No. 1). The filtrate was incubate in a vacuum for 48 hrs and finally taken for column purification.

### 2.4 Column Purification

The plant extract was purified according to the method described by Nesti et al. [15]. The

column with 95×45 size was placed in the vertical position; 140 mg of cotton wool was inserted and pushed down to the bottom of the column which reached 1.5 cm from down of the column to avoid escape of silica gel; one hundred and twenty gram (120 g) of a dried stationary powder of silica gel (60-120 mesh) was added in to the column; 100 ml of mobile phase (hexane) was added to flushed through the column and made it wetted; while flushing through, the column was slapped several times and ensured the air bobbles were removed; dropping funnel was attached to the top of the column and extract to be purified was poured gently in to the column and sank in to the silica gel; 80 ml of ethyl acetate and hexane in ratio of 1:1 (i.e. 40 ml:40 ml) was added continuously and simultaneously through the funnel from the top of the column with carefully open stop cock until the extract eluted; from each plant extract; 13 fractions were collected and each fraction was 10 ml; the fractions were left for two days to evaporation.

## 2.5 Snail Collection

Six hundreds (600) adults *B. globosus* snails with shells length between 9 to 11 mm long were collected from Kwalkwalawa River of Wamakko Local Government, Sokoto State, between 10:00am to 12:00noon whenever needed using a scoop as described by Kanchan et al. [16]. The scoop comprised a wooden frame, supporting mesh and mounted handle. To collect the snails, the scoop net was immersed and pushed to 16 to 20 m; the scoop was lifted upward vertically to ensure proper collection; the collected snails were transferred in to plastic bucket containing borehole water which was left open for 24 hours after fetched, the snails were brought to the parasitology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto.

## 2.6 Identification of *Bulinus globosus* Snail

The snails were taken to Department of Zoology, faculty of life sciences, Ahmadu Bello University Zaria for confirmation of the animals. Snails were identified at Museum of Natural History of the Department of Zoology by malacologist as *B. globosus* and 7B was assigned as cabinet number for the animal. Finally, the snails were taken back to the parasitology laboratory Usmanu Danfodiyo University Sokoto for toxicity study.

## 2.7 Maintenance of the Snails

In the Parasitology Laboratory of Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto; identified snails were kept in groups of 40 in plastic buckets containing borehole water left for 24 hours after fetched. Water in the plastic buckets was changed twice in a week to eliminate the contaminants so as to prevent fouling. The snails were kept for a period of three weeks in the laboratory before experiment [17].

## 2.8 Toxicity Experiment for the Intermediate Host

Each purified fraction of *A. cepa* was tested against *B. globosus* snails according to toxicity experiment described by WHO [18]. For experimental test of each fraction, ten adult snails were kept in plastic buckets containing concentration of the fraction (the treatment were 14.90 mg/l; 17.70 mg/l; 21.45 mg/l; 25.10 mg/l; 30.90 mg/l; 38.15 mg/l; 42.15 mg/l; 38.75 mg/l; 35.05 mg/l; 34.30 mg/l; 24.85 mg/l; 22.60 mg/l and 11.90 mg/l) for 96 hours; each of the fraction was replicated three times; control animals were kept in similar conditions, but without treatment for each three replicates. Mortality was recorded after every 24 hours (i.e. after 24 hours; 48 hours; 72 hours and 96hours respectively) and dead snails were removed to avoid any contamination in aquarium water. The absence of response to a needle probe was considered as evidence of the snail dead. After every 24hour of the experimental setup, mortality of each treated group was calculated using Abbott's formula by subtracting a number of survival in the treated group from a number of survival in the untreated (control) group multiply by one hundred and recorded.

## 2.9 Data Analysis

The data obtained from mean mortality were analysed using one-way analysis of variance (ANOVA) to determine statistically significant differences between means at  $P < 0.05$ ; lethal concentration (LC50) at a confidence interval of 95%, using probate analysis method was estimated in the Minitab statistical software package.

## 3. RESULTS AND DISCUSSION

Potentials of column purified fractions of *A. cepa* against *B. globosus* was time and concentration

dependence, it was observed that; immediately after introducing the experimental snails in to various concentration of the fractions, the snails attempt to escape from toxicity of the plants by moving around (distressing) and becoming firmly attached to the wall of experimental container; shells of the snails also were filled up. Nevertheless, swelled pores were observed on the foot of some snails, shell's colour of most snails changed from brown to dark brown; mortality ranged between 100% (10.0) and 20% (2.0) with LC<sub>50</sub> 19.371mg/l after 96 hours into various fraction. F<sub>7</sub> had higher mortality of 100% (10.0) followed by F<sub>8</sub> with 93.3% (9.33); F<sub>6</sub> showed 76.7% (7.67) mortality and mortality of 63.3% (6.33) was observed in F<sub>9</sub>; in the same way, F<sub>10</sub> and F<sub>5</sub> showed mortality of 53.3% (5.33) and 46.7% (4.67) respectively. Although, F<sub>4</sub> and F<sub>3</sub> showed mortality of 43.3% (4.33) and 40% (4.00) respectively; also F<sub>2</sub>, F<sub>1</sub>, F<sub>11</sub> and F<sub>12</sub> showed mortality of 36.7% (3.67), 33.3% (3.33), 33.3% (3.33) and 30% (3.0) respectively. Lastly, F<sub>13</sub> had the lowest mortality of 20% (2.00) (table 1). P value was <0.05, hence, there was a significant difference for the mortality of the snails after 96 hours into different concentrations of *A. cepa*.

### 3.1 Discussion

An attempt to escape from toxicity, filling up of the shells of the experimental snails, swelled pores on the foot of some snails and changed of shells colour was due to toxicity of the plants extracts on *Bulinus globosus*; another researcher also observed movement of snails to the side of the container in an attempt to escape from the

test media containing *Althernanthera sessilis* treated water [19].

Potential of *A. cepa*, on *B. globosus* was concentration and time-dependent, because the higher the concentration in the fraction of the plants the higher mortality was observed, a similar finding was reported by Rawi et al. [20] on the toxicity of *Agave filifera* plant extracts on *Biomphalaria alexandrina*. [21], also reported similar observation while studying molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extracts against the *fasciola* vector *Lymnaea acuminata*.

Snails treated in F<sub>7</sub>, F<sub>8</sub>, F<sub>6</sub> and F<sub>9</sub>, showed higher mortality and mean mortality of these fractions were significantly different from that of other fractions; these higher mortality rate may be due to potent phytochemicals such as alkaloid, flavonoid, saponins, glycosides and tannins were eluted in higher quantities in these fractions; this was not contrary to the report of several researchers such as; [22] who reported that, potency of plants on the snails was due to presence of saponins content. [23], also reported that higher toxicity occurred due to a higher amount of tannins and flavonoid. Similarly, glycoside and alkaloid components were reported with higher toxicity to the snails by Singh and Agarwal [24]. Although LC<sub>50</sub> value in column purified fractions of *A. cepa* was 19.4mg/L, the value was in the same range with that of Niclosamide (11.8 mg/L) which is one of the standard molluscicidal drugs as reported by Singh and Agarwal [24].

**Table 1. Potential of column purified fractions of *A. cepa* (L) on *B. globosus* after 96 hours**

Fraction number	Concentration (mg/l)	No. of snails in the concentration	Mean mortality of the snails	Mortality of the snails (%)
F <sub>1</sub>	14.90	10	3.33±0.33 <sup>f</sup>	33
F <sub>2</sub>	17.70	10	3.67±0.33 <sup>f</sup>	37
F <sub>3</sub>	21.45	10	4.00±0.00 <sup>e</sup>	40
F <sub>4</sub>	25.10	10	4.33±0.33 <sup>e</sup>	43
F <sub>5</sub>	30.90	10	4.67±0.33 <sup>d</sup>	47
F <sub>6</sub>	38.15	10	7.67±0.33 <sup>b</sup>	77
F <sub>7</sub>	42.15	10	10.00±0.00 <sup>a</sup>	100
F <sub>8</sub>	38.75	10	9.33±0.33 <sup>a</sup>	93
F <sub>9</sub>	35.05	10	6.33±0.33 <sup>c</sup>	63
F <sub>10</sub>	34.30	10	5.33±0.33 <sup>d</sup>	53
F <sub>11</sub>	24.85	10	3.33±0.33 <sup>f</sup>	33
F <sub>12</sub>	22.60	10	3.00±0.00 <sup>f</sup>	30
F <sub>13</sub>	11.90	10	2.00±0.00 <sup>g</sup>	20

Values are expressed as Mean±SEM of three replicates. Values in row having different superscript differs significantly at P<0.05 level (One Way ANOVA)

#### 4. CONCLUSION AND RECOMMENDATIONS

Column purified fractions of *A. cepa* showed that, F<sub>7</sub>, F<sub>8</sub>, F<sub>6</sub> and F<sub>9</sub> were most toxic fractions; the toxicity may be due to the composition of active phytochemicals such as alkaloid, saponins, glycosides, tannins and flavonoid in a higher amount.

Governmental and nongovernmental organisations should enlighten public on the effectiveness of the plants for control of an intermediate host of urinary schistosomiasis (*Bulinus globosus*) since the plant is inexpensive, readily available and environmentally safe.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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