



## Comparison for the Effectiveness of Column Purified Fractions of *Allium cepa* Bulbs and *Allium sativum* Cloves against *Bulinus globosus* (Intermediate Host of Urinary Schistosomiasis) in Sokoto, Nigeria

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### Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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### ABSTRACT

Synthetic molluscicides widely used for the control of urinary schistosomiasis are expensive, toxic to non-target organisms, not available and may have deleterious long-term effects in the environment. The aim of this research is to compare the efficacy of column purified fractions of *Allium cepa* and *Allium sativum* against *Bulinus globosus* specimens (intermediate host of urinary schistosomiasis) under laboratory conditions as an increment for the cheaper, non-toxic, available way of controlling urinary schistosomiasis. Extracts were obtained using cold maceration method with methanol as a polar solvent; column purification was achieved using silica gel (stationary

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phase) while ethyl acetate and n-hexane (mobile phase); thirteen fractions were collected from each plant and each fraction contained 10ml of the eluent; the fractions were left open for 48 hours for evaporation of the solvents. Experiments were performed according to the methodology described by the World Health Organization for molluscicidal activity tests; each treatment was replicated three times; mortality was recorded after each 24 hours up to 96 hours. The results showed that; *A. sativum* was more effective against *B. globosus* as compared with *A. cepa* with statistical significant difference ( $P < 0.05$ ) and  $LC_{50}$  were 15.60mg/l and 19.37mg/l for the efficacy of *A. cepa* and *A. sativum* respectively. It was concluded that, although, *A. cepa* purified fractions are more effective than *A. sativum* against *B. globosus*, both of the plants species may be helpful in snail control at acceptable doses since the plant extracts caused mortality of the snail.

**Keywords:** Comparison; effectiveness; *A. cepa*; *A. sativum*; fractions; *B. globosus*.

## 1. INTRODUCTION

Schistosomiasis also known as snail fever still remains the second most prevalent tropical parasitic disease after malaria and a leading cause of severe morbidity in many parts of the world; Snail fever is found in Asia, South America and Africa [1]. Over 200 million people are infected and 700 million people are at risk of schistosomiasis infection globally [2]. Over 90% of schistosomiasis infection occurs in sub-Saharan Africa and almost 300,000 people die annually [3-4]. The highest prevalence of this infection is seen in Nigeria (29 million), which is closely followed by United Republic of Tanzania (19 million) then Ghana and Democratic Republic of Congo (15 million) making up the top five countries in Africa with *Schistosoma* infection [5]. Although, in year 1988 National Schistosomiasis Control Programme was initiated in Nigeria and the goal of the program was to deliver regular anti-helminthic treatment to at least 75% of school-age children in endemic areas in the country in line with WHO recommendation [6]; Schistosomiasis have been found to be emerging and re-emerging diseases in some countries including Nigeria [7-11] Urinary schistosomiasis cause relatively low mortality rate, but a high morbidity rate, the disease is often associated with water resource developmental projects, such as dams and irrigation schemes, where the snail intermediate hosts of the parasite live [12]. Transmission of schistosomiasis can take place in almost any type of fresh water habitat ranging from large lakes and rivers to small seasonal ponds and streams containing the infected intermediate host (snails). Snail control could be regarded as most effective way of reducing or eliminating transmission and remains among the best methods of choice for schistosomiasis control [13].

Although, several research determined toxicity of plants extract against the intermediate host

across the world [14-15]; none compared the effectiveness for the column purified fractions of *A. cepa* and *A. sativum* on intermediate host of urinary schistosomiasis (*B. globosus*), despite these plants are available, had medicinal value, none toxic and accepted among the community members for many reason. Therefore, present study was conducted to compare the efficacy of column purified fractions of *A. cepa* and *A. sativum* against *B. globosus* specimens (intermediate host of urinary schistosomiasis) in Sokoto, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Materials

Red globe onion bulbs and red cloves garlic used for this study were collected from Ramin Kura market Sokoto, each of the plant bulbs and cloves were identified and authenticated by taxonomist in the herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Voucher number collected for the identified plants were UDUH/ANS/0088 for *A. cepa* L. and UDUH/ANS/0102 for *A. sativum* L.

### 2.2 Preparation of the Plants Powder

Fresh bulbs of *A. cepa* and cloves of *A. sativum* plants were washed with borehole water, sliced in to smaller pieces and air dried under shade for 14 days, the dried bulbs and cloves were grinded into fine powder using a grinder and stored in air-tight container [16].

### 2.3 Preparation of the Plants Methanolic Extract

The Methanolic extracts of *A. cepa* and *A. sativum* were prepared using cold maceration

method described by Handa et al. [17]. One hundred grams (100 g), of each plant was weighed and transferred into clean sterile bottle. Each 100 g weighed-out of the plant powder was soaked in to 300 ml of methanol then tightly covered and allowed for 72 hours at room temperature, the suspensions were stirred occasionally after each 24 hrs. The suspensions were filtered in to a sterile bottles using whatman filter paper No. 1. Filtrate of each plant was left in a vacuum for 48 hours then finally taken for column purification.

## 2.4 Column Purification

Each of the plant extract was purified according to method described by Nesti et al. [18], column with 95×45 size was placed in the vertical position; 140mg 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; 100ml 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. 40ml:40ml) 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 evaporated and weight of each extract was measured by subtracting the initial weight of each empty bottle used from final weight of the bottle and finally weight of each fraction was recorded.

## 2.5 Snail Collection

One thousand five hundred (1500) adult *B. globosus* snails with shells length between 9 to 11mm long were collected from Kwalkwalawa River of Wamakko Local Government, Sokoto State, between 10:00 am to 12:00 noon when ever needed using a scoop as described by Kanchan et al. [19]. The scoop comprised wooden frame, supporting mesh and mounted handle. To collect the snails, the scoop net was immersed and pushed to 16 to 20m; 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 24hours 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

Initially, in the parasitology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, the snails were identified as *B. globosus* using identification key [20]. Thereafter, the snails were taken to Department of Zoology, faculty of life sciences, Ahmadu Bello University Zaria for confirmation of the animals. Snails were confirmed by malacologist as *B. globosus* at Museum of Natural History of the Department of Zoology 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 [21].

## 2.8 Toxicity Experiment for the Intermediate Host

Each purified fraction of *A. cepa* and *A. sativum* was tested against *Bulinus globosus* snails according to toxicity experiment described by WHO, [22]. For experimental test of each fraction, ten adult snails were kept in plastic buckets containing concentration of the fraction (the treatments 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/ for *A. cepa* and *A. sativum*) 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 each 24 hours (i.e. after 24 hours; 48 hours;

72 hours and 96 hours respectively) and dead snails were removed to avoid any contamination in aquarium water. Absence of response to a needle probe was considered as evidence of the snail dead. After every 24 hour of the experimental set up, mortality of each treated group was calculated according to Abbott's formula [22] by subtracting number of survival in the treated group from number of survival in the untreated (control) group multiply by one hundred and recorded.

## 2.9 Statistical Analysis

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

## 3. RESULTS AND DISCUSSION

The column purified fractions of *A. cepa* and *A. sativum* showed considerable molluscicidal effect against *B. globosus* and Identified  $LC_{50}$  was 15.60 mg/l and 19.37 mg/l for *A. cepa* and *A. sativum* respectively. These plants exhibited and acceptable toxic effect similar to that of some

plants on *B. alexandrina* snails according to WHO, [23] recommendations on plant molluscicides. Effectiveness of the two plants against *B. globosus* was time and concentration dependent. It was found that, high molluscicidal activity of *Agave celsii*, *Ammi visnaga* and *Chenopodium ambrosioides* are apparently attributed to the high concentration of active constituents [24]. This is also supported by Shoeb et al. [25], Rawi et al. [26]; Mansour et al. [27]; and Abdel Kader [28]. Column purified fractions of *A. sativum* was found to be more effective than that of *A. cepa* with statistical significant difference ( $P < 0.05$ ) due to higher toxicity of the *A. sativum* concentrations (Table 1). Similar observation was reported by Azare et al. [29]; Sakran and Bakry [30] also reported that, higher toxicity of the plant species over another plant species may be related to plant specific differences in active gradients, differences in their mode of action, and their effect on the snails.

One of the major problems in the use of plant extracts for the control of snails, is the choice of the most toxic plants. From the results presented above, the clove of garlic was more potent than the bulbs of garlic extract and use of these plants with molluscicidal properties is a simple, inexpensive and appropriate technology for control of the snail intermediate host.

**Table 1. Comparison for the efficacy of column purified fractions of *A. cepa* and *A. sativum* after 96 hours**

Fraction number	Concentration (mg/l)	Number of snails in concentration	Mean mortality in <i>Allium cepa</i>	Mean mortality in <i>Allium sativum</i>
F <sub>1</sub>	14.90	10	3.33±0.33 <sup>f</sup>	3.67±0.33 <sup>f</sup>
F <sub>2</sub>	17.70	10	3.67±0.33 <sup>f</sup>	4.00±0.00 <sup>d</sup>
F <sub>3</sub>	21.45	10	4.00±0.00 <sup>e</sup>	4.33±0.00 <sup>d</sup>
F <sub>4</sub>	25.10	10	4.33±0.33 <sup>e</sup>	5.67±0.66 <sup>c</sup>
F <sub>5</sub>	30.90	10	4.67±0.33 <sup>d</sup>	7.67±0.33 <sup>b</sup>
F <sub>6</sub>	38.15	10	7.67±0.33 <sup>b</sup>	9.33±0.33 <sup>a</sup>
F <sub>7</sub>	42.15	10	10.00±0.00 <sup>a</sup>	10.00±0.00 <sup>a</sup>
F <sub>8</sub>	38.75	10	9.33±0.33 <sup>a</sup>	10.00±0.00 <sup>a</sup>
F <sub>9</sub>	35.05	10	6.33±0.33 <sup>c</sup>	7.33±0.33 <sup>b</sup>
F <sub>10</sub>	34.30	10	5.33±0.33 <sup>d</sup>	6.33±0.33 <sup>c</sup>
F <sub>11</sub>	24.85	10	3.33±0.33 <sup>f</sup>	4.00±0.00 <sup>d</sup>
F <sub>12</sub>	22.60	10	3.00±0.00 <sup>f</sup>	3.66±0.33 <sup>d</sup>
F <sub>13</sub>	11.90	10	2.00±0.00 <sup>g</sup>	2.33±0.33 <sup>e</sup>
Control	0.00	10	0.00±0.00	0.00±0.00

Values are expressed as Mean±SEM of three replicates. Values in columns and rows having different superscript differs significantly at  $p \leq 0.05$  level (two way ANOVA followed by Duncan Multiple Range Test)

#### 4. CONCLUSION AND RECOMMENDATIONS

Present study showed that, both fractions of *A. cepa* and *A. sativum* are effective for the control of *B. globosus*. However, column purified fractions of *A. sativum* were most effective against *B. globosus* than that of *A. cepa*.

Further study should investigate the quantity of phytochemicals present in each fraction of the both plants and histopathological test should be carried out on some organs of the snails to find out the mode of action of the plants against the snails spacemen. Finally, public should be enlighten on how to use the plants for the control of the snail intermediate host of urinary schistosomiasis since the plants are available, non-toxic, cheaper and accepted among the people of Sokoto and Nigeria at large.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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