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PHYTOCHEMICAL CONSTITUENTS AND MOLLUSCICIDAL EFFICACY OF *ALLIUM SATIVUM* (LINN.) AGAINST *BULINUS GLOBOSUS* IN SOKOTO, NIGERIA

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ABSTRACT

Phytochemical constituents and molluscicidal effectiveness of *Allium sativum* against *Bulinus globosus* in Sokoto was investigated. The plants were purchased from Ramin Kura Market, Sokoto, identified and authenticated by botanist, fresh cloves of the plants were air dried, grinded in to powdered form and extracts were obtained by using maceration method with methanol; column purifications of the extract was conducted using silica gel as a stationary phase and 1:1 of N-hexane and ethyl acetate as mobile phase, thirteen fractions each containing ten mil (10ml) of the eluent were collected. Data obtained were analyzed by descriptive statistics, ANOVA and probate analysis. Qualitative phytochemicals analysis showed excess Saponins, flavonoids, Tannins, Glycosides and Antraqunones, while, Alkaloids, Volatile oils, Saponin-glycosides and steroids were moderately presents and Balsams were quietly presents. Mortalities of *B. globosu* snails were significanly high in fraction seven of the experimental set up, the LC_{50} was 15.599mg/l. Finally, based on finding from this research *Allium sativum* was very effective and sufficient for control of Bulinus globosus for villagers and drugs industries.

KEYWORDS: Phytochemicals, Molluscicides, *A. sativum, B. globosus*, Sokoto.

1.0 INTRODUCTION

Urinary schistosomiasis also known as Bilharziasis or snail fever, is a disease caused by infection of digenetic blood trematodes called *Schistosoma haematobium* (Downs *et al.,*2012; Behrman, 2015).

Snail fever is found in Asia, South America and Africa predominantly in rural areas supporting agriculture and inland fisheries (Bhardwaj *et al.,* 2014). Over 200 million people are estimated to be infected and 700 million people are at risk of schistosomiasis infection globally (Chitsulo *et al.,* 2014); Over 90% of schistosomiasis infection occurs in sub-Saharan Africa and almost 300,000 people died annually (Huw *et al.*, 2014). 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 Schistosomal infection (WHO, 2013). Although, In year 1988 National Schistosomiasis Control Programm 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 (Stothard *et al.,* 2011); Schistosomiasis have been found to be emerging and re-emerging diseases in some countries including Nigeria (Nmorsi *et al.,* 2010).

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 (Wynn *et al.,* 2011), but synthetic molluscicides widely used for the effective control of the intermediate host remain cost-effective and have rapid toxicity to the aquatic environment (Lima *et al.,* 2010). Moreover, most of the synthetic molluscicides such as Niclosamide, Barquat, endothall, chloramines, ozone and hydrogen peroxide are toxic to non-target animals and may have long-term detrimental effects on the aquatic life (Bukry *et al.,* 2011).

Although, several groups of compounds present in various plants were determined to have toxic effect to target organisms at acceptable doses (Khalifa, 2010); there is still an urgent need for highly toxic plants in order to avoid transmission of the parasitic disease like urinary schistosomiasis (Maillard *et al.,* 2009). Moreover, in the environment like Sokoto where a large

fraction of population is still dependent on lakes, rivers and dam water for their everyday supplement; it is highly injurious to use synthetic chemicals to control the disease, therefore, Phytochemical Constituents and Molluscicidal of *Allium sativum* efficacy against intermediate Host of *Schistosoma haematobium* (*Bulinus globosus*) was assessed in Sokoto.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of Powder

Allium sativum cloves used for this study were collected from Ramin Kura market Sokoto, each of the plant clove was identified and authenticated by taxonomist, in the herbarium, Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Voucher number was collected for the identified plant (UDUH/ANS/0102) Thereafter, fresh cloves of the plant were cut in to smaller pieces and air dried in shadow for 14 days, the dried cloves were grinded into fine powder using pistil and morta and stored in air-tight container (Sampath *et al.,* 2010).

2.2 Preparation of Methanolic Extract

The Methanolic extracts of *Allium sativum* were prepared using cold maceration method described by Handa *et al*. (2008). 100g of the plant was weighted and transferred into clean sterile bottle and soaked in to 300ml of Methanol then mixed and allowed for 72hours at room temperature, the suspension was stirred occasionally after each 24hrs then filtered in to a sterile bottle using Whatman filter paper No. 1. Filtrate of the plant was subjected for column purification.

2.3 Column Purification

The plant extract was purified according to method described by (Nesti *et al.,* 2013). The column with 95×45cm 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 1.5cm from down of the column to avoid escape of silica gel; one hundred and twenty gram (120g) 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 bobbled was 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; 80ml 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; 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.

2.4 Phytochemical Analysis

1. Saponins

Five mil (5ml) of the methanol extract of the plant was placed in a test tube; five mil of methanol was added and shakes strongly; the whole tube was filled front for several minute and observed (Harbone, 1998).

2. Flavonoids

Three mil of methanolic extract of the plant in the test tube was mixed with 10% NaOH; development of yellow color indicates the presence of flavonoid (El-Oleyi *et al.,* 1994).

3. Tannins

Ferric chloride solution was added drop by drop into the 3ml of the plant methanolic extract in the test tube, dark green and blue back colored for condensed and hydrolysable tannins were observed (Trease and Evans, 1978).

4. Alkaloids

Two milliliter (2ml) of each plant extract was stirred with 10% aqueous HCL. One mil of the volume was treated with 2 drops of Wagner's reagent, another one mil was treated with similarly with Mayer's reagent. Turbidity and precipitation with either of these reagents indicated the presence of Alkaloids (Harbone, 1973).

5. **Saponins Glycosides**

Two point five milliliter (2.5ml) of the extract was added to 2.5ml of Fehling's solution A and B in the test tubes, green precipitates color indicate the presence of saponins glycosides (El-Oley *et al.,* 1994).

6. Volatile Oils

One mil of the extract was mixed with diluted HCL. A white precipitate was formed which indicate the presence of Volatile oil in the extract (Evans, 1980).

7. Balsams

Three milliliter (3ml) of the methanolic extract of the plant was mixed with equal volume of 90% ethanol; two drops of alcoholic ferric chloride solution was added to the mixture. Dark green color indicates the presence of balsams (El-Oley *et al.,* 1994).

8. Steroids

Zero point five mil (0.5ml) of the plant methanolicextract was dissolved in 2ml of chloroform, 2ml of sulphoric acid was carefully added and lower layer was formed, a reddish brown color at the interface indicated the presence of steroid (Harbone, 19973).

9. Glycosides

Two point five mill (2.5ml) of 50% sulphoric was added to 5cm^3 of the methanolic extract of the plant in the test tube, the mixture was heated in boiling water for 15minutes; thereafter, the mixture was cooled and neutralized with 10% NaOH; five mile of Fehling's solution was added; the mixture was boiled again. A bricked red precipitate color was observed which indicated the presence of glycoside (Harbone, 1973).

10. Cardiac Glycosides

Two mil of 3.5% of ferric acid was added to the methanolic extract of the plant; the mixture was allowed to stand for 1minute, 1ml of conc. H_2SO_4 was added carefully on the wall of the test tube and lower layer was formed. Reddish brown ring was formed between the

interfaces which showed the presence of cardiac glycoside (Harbone, 1973).

2.5 Snail Collection

Adult *Bulinus globosus* snails with shells length between 9 to 11mm long were collected from Kwalkwalawa River of Wamakko Local Government, Sokoto State, from 10:00am to 12:00 noon when ever needed using a scoop as adopted by (Kanchan *et al.,* 2012). The scoop comprised wooden frame, supporting mesh and mounted handle. In the lakes, the net scoop was immersed and pushed to 16 to 20m; the scoop was lifted upward vertically to ensure proper collection; the snails were brought to the laboratory in bucket containing borehole water which was left for 24 hours after fetched.

2.6 Identification and Maintenance of the Snails

The collected snails were taken to the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University Zaria for identification of *Bulinus globosus*, the snails were identified by malacologist, 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.

2.7 Toxicity Experiment for the Intermediate Host

Each purified fractions of *Allium sativum* was tested against *Bulinus glubosus* snails according to toxicity experiment described by (Nurhayati *et al*., 2006). For experimental test of each fraction, ten adult snails were kept in plastic buckets containing concentration of the fraction 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, during experimental period, the snails were kept in starve condition. Mortality was recorded after each 24 hours (i.e. after 24hours; 48hours; 72hours and 96hours 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. The mortalities of treated groups was calculated according to Abbott's formula and recorded.

2.8 Data Analysis

Mortality of each treated group was calculated according to Abbott's formula by subtracting number of survival in the treated samples from number of survival in the untreated (control) samples multiply by one hundred. The data was analyzed to estimate the lethal concentration at 50 (LC50); lower confidence limit (LCL), upper confidence limit (UCL) and slop values of ratio at a confidence interval of 95%, using the probate analysis method in the Minitab statistical software package. Analysis of variance (ANOVA) was used to determine statistically significant differences between means at $P < 0.05$.

3.0 RESULTS

3.1 Phytochemical analysis

Allium sativum phytochemicals analysis showed excess saponins, flavonoids, tannins, saponin-glycosides, glycosides and antraqunones. Although, alkaloids, volatile oils and steroids were moderately presents, balsams were presents in least quantity nd cardiac glycosides were not detected (Table 1).

Table 1: Phytochemical Analysis of *Allium sativum.*

Phytochemical Components	*Allium sativum* L.
Saponins	+++
Flavonoids	+++
Tannins	+++
Alkaloids	++
Saponin-glycosides	+++
Volatile oils	++
Balsams	+
Steroids	++
Steroids	+++
Glycosides	+++
Cardiac glycosides	NO

Key:
Excess Present = +++
Moderate Present= ++
Quite Present = +
Not present= NO

3.2 Molluscicidal Effect of *Allium sativum* (L) Against *Bulinus globosus*

After 96 hours for the experimental set up, it was confirmed that; mortality of the snails ranged from 100.0% (10.0) to 23.3% (2.33); F_7 and F_8 showed 100.0% (10.00) mortality of *B. glubosus*, then F_6 had 93.3% (9.33) mortality and then F_5 and F_9 showed 76.7% (7.67) and 73.3% (7.33) mortality respectively, sequentially, F_{10} and F_4 showed mortality of 63.3% (6.33) and 56.7% (5.67) respectively. Nevertheless, 43.3% (4.33), 40.0% (4.00) 40.0% (4.00) and 36.7% (3.67) mortalities were observed in F_3, F_2, F_{11} and F_{12}

respectively. To the end, F_{13} showed lowest mortality of 23.30% (2.33) (Table 2) P=0.0001, there was significant difference for the mean mortality of *Bulinus globusus* snails after 96 hours into different concentrations of *Allium sativum*.

Table 2: Molluscicidal Efficacy of *A. sativum* Fractions against *B. globosus* after 96 hours (N=10).

Fraction Number	Concentration (mg/l)	Mean No. of the Snails died	Mortality of the snails (%)
F_1	14.90	$3.67{\pm}0.33^{a}$	36.7
F_2	17.70	$4.00{\pm}0.00^{d}$	40.0
F_3	21.45	$4.33{\pm}0.00^{d}$	43.3
F_4	25.10	$5.67{\pm}0.66^{c}$	56.7
F_5	30.90	$7.67{\pm}0.33^{b}$	76.7
F_6	38.15	$9.33{\pm}0.33^{a}$	93.3
F_7	42.15	$10.00{\pm}0.00^{a}$	100.0
F_8	38.75	$10.00{\pm}0.00^{a}$	100.0
F_9	35.05	$7.33{\pm}0.33^{b}$	73.3
F_{10}	34.30	$6.33{\pm}0.33^{c}$	63.3
F_{11}	24.85	$4.00{\pm}0.00^{d}$	40.0
F_{12}	22.60	$3.67{\pm}0.33^{d}$	36.7
F_{13}	11.90	$2.33{\pm}0.33^{e}$	23.3
Control	0.00	$0.00{\pm}0.00^{f}$	0.0

Values are expressed as Mean±SEM of three replicates. Values in row having different superscript differs significantly at $p{\leq}0.05$ level (One Way ANOVA followed by Duncan Multiple Range Test

3.3 DISCUSSION

Phytochemicals analysis showed excess saponins, flavonoids, tannins, saponin- glycosides, glycosides and antraqunones. Nevertheless, alkaloids, volatile-oils and steroids were moderately presents and balsams were quietly presents. The same phytochemicals were reported by several researchers. Gazuwa *et al*. (2013) reported that, alkaloids, flavonoids, cardiac glycosides, steroids and resins were present in both *Allium cepa* and *Allium sativum*. Rahman *et al.* (2017), reported that, *Allium sativum* contained, saponins, tannins, alkaloids, volatile oils and steroids. The same phytochemicals were reported by (Charlson and McFerren, 2007; Garba *et al.,* 2007).

The result of present study clearly showed that, efficacy of *Allium sativum* against *Bulinus globosus* was time and concentration defendant and this report were in the same line with the report of Kanchan *et al.* (2012), on Characterization of the molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extracts against the *fasciola* vector *lymnaea acuminate.*

Nevertheless, higher mortality was observed in fraction 7 and 10 of the plant because higher potent phytochemicals such as alkaloids, flavonoids, saponins, glycosides and tannins were eluted in these fractions as presented; this finding was in line with the report of several researchers; potency of many plants on the snails was due to presence of saponin contents (Singh and Singh, 1995), tannins compounds (Clisiane *et al.,* 2014), triterpenoid and alkaloid components (Babeet and Rekha, 2010).

In this study, LC_{50} value of the snails in column purified fractions of *Allium sativum* low due to higher toxicity the plant. Although, LC_{50} for the toxicity study after 96 hours of animal exposure to the purified fractions are 15.599 the value was in the same range with LC_{50} of niclosamide (11.8 mg/L) which is one of the standard molluscicidal drugs as reported by (Singh and, Agarwal; 1984).

3.4 Conclusion and recommendations

Column purified fractions of *Allium sativum* were very effective and sufficient in control of intermediate host of *S. haematbium* (*Bulinus globosus*); their toxicity was due to composition of active phytochemicals such as alkaloids, saponins, glycosides, tannins and flavonoids in higher amount.

The promising results obtained from column purified fractions of *Allium sativum* (cloves) encourages further investigations to establish the effectiveness of their leaves and roots, singly and in binary combinations to find out effectiveness of the plants in singly and synergistic effects of the combined (binary and tertiary combination) plants extracts on intermediate host of *S. haematobium* (*Bulinus globosus*).

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