

Evaluation of Molluscicidal Activities of Aqueous and Ethanolic Extracts of Onion Bulb (*Allium Sativum*) Against *Bulinus Wrighti*

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ABSTRACT

Introduction: Schistosomiasis is a disease of public health importance in the tropics and subtropics. However, the synthetic materials for prevention of the intermediate host of the disease are harmful, scarce, and toxic. Thus, an evaluation of molluscicidal activities of aqueous and ethanolic extracts of the bulb of *Allium sativum* against *Bulinus wrighti* were carried out.

Methods: Snails were exposed to various concentrations of plant preparations in laboratory conditions in a plastic aquarium containing 3L of de-chlorinated water for 96h continuously. Mortality was recorded at every 24hours interval for 96hours.

Results: The study shows that, molluscicidal activities are time and dose dependent against snails. The ethanolic extract was more toxic than aqueous extract. Ethanolic extract of *A. sativum* was found highly toxic to *B. wrighti* (24hrs. LC₅₀: 97.07mg/l; 96hrs: 21.70mg/l). Chemical profile of aqueous extracts of *A. sativum* showed the presence of some secondary metabolites. *A. sativum* extracts showed histopathological signs to hermaphrodite glands and the digestive tract of the treated snails.

Conclusion: This study showed that, this plant can be used as molluscicides. This study recommends the use of ethanolic and aqueous extract of *A. sativum* for the control of *B. wrighti*.

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Introduction

Schistosomiasis remained as a chronic and debilitating diseases due to the causative agent *Schistosoma*. It is a worldwide common infection occurring due to parasitic activity and is the most common after malaria in terms of effects on public health and socioeconomic burden in the tropical and subtropical areas of the world. On the other hand, it is still the most predominant waterborne disease, and a great risk to public health in rural settings of developing countries (Bala et al., 2012; In & Lee, 2017; Jibia & Sani, 2019). A free swimming form of the parasite at larval stage (cerariae) because of contact finds its way into the human skin and turned to mature form. Thereof, the parasite eggs are released through the urine or feces. Then hatched in the freshwater around and invade an aquatic snail (the intermediate host) (Dawaki et al., 2015; Gamde et al., 2022). For the continuity of the lifecycle, the eggs when in the snail, developed into cercariae to be disgorged into the water for the purposes of infecting human host (that usually contact freshwater such as pond, and stream) (Sing et al., 2016; Umoh et al., 2020; Johnbull et al., 2021).

Unfortunately, prior to the egestion of the eggs by human host (in the blood vessels in the urinary bladder); parts of them accumulate and affect (destroy) vital organs such as intestines, bladder, and quasi (Sady et al., 2015; Dawaki et al., 2016; Anyolitho et al., 2022). Infection due to schistosomiasis is known with severe morbidity such as bladder lesions, hematuria, kidney inflammation,

urethral obstruction, and damage to internal organs (Asenahabi, 2019; Hamburg et al., 2021). It is worrisome to note that, about 700 million persons in the 74 endemic countries are at the risk of schistosomiasis probably contacted in their agricultural, recreational, and domestic activities that require contact with fresh water. Additionally, greater than the 207 million persons are infected with the disease across the various parts of the world (Dawaki et al., 2015; Dawaki et al., 2016; Aula et al., 2021). In this vein, children are affected with anemia, physical weakness, learning ability, and quasi (Akinneye et al., 2018; Anthony et al., 2019; Oyeyemi et al., 2020).

Consequently, it is pertinent to seek for ways to prevent the spread of schistosomiasis in the country, especially in Sokoto to combat the challenges of poverty, malnutrition, and double burden of diseases (Sarkingobir et al., 2023ab; Suleiman et al., 2022). Infection prevention reduces the use of antibiotics and antimicrobial resistance, helps in ensuring clean and safe, environment, and enhances public health protection (Bala et al., 2012; Shaw, 2016; Dawaki et al., 2015; Johnbull et al., 2021). For the microorganism to spread and potentially incite the intermediate host, the snail (Shaw, 2016). The easiest way to break the chain of transmission of schistosomiasis is to scuttle it through the developing of cheap and accessible plant-based material that kills the snail intermediate host (Shaw, 2016; Nelwan, 2019; Rinaldo et al. 2021); because, the synthetic products are expensive scarce, toxic (especially to non-target freshwater organisms or humans), it is pertinent to seek for an alternative (Singh & Tiwari, 2012; Suleiman et al., 2018ab). *Allium spp* is one of

the plants with the potential for the application in the prevention of schistosomiasis through killing of the snail (intermediate host) due to its phytochemical components. Because, the plant has been reported to be active on other biological beings, there is need to test it on intermediate snail of schistosomiasis for searching of cheap and effective prevention strategy in Sokoto and beyond (Noorshilawati et al., 2020). Thus, an evaluation of molluscicidal activities of aqueous and ethanolic extracts of the bulb of *Allium sativum* against *Bulinus wrighti* was the aim of this work.

Materials And Methods

Study Area

This study was carried out in Parasitology laboratory of Biological Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State. Sokoto is the capital city of Sokoto State, lies between latitude 13° 3' 490N, longitude 5°14' 890E and at an altitude of 272m the sea level above. It is located in the extreme North Western part of Sokoto North and South local government areas and also some parts of Kware LGA from the North, Dange Shuni LGA from South and Wamakko LGA to the West. Sokoto metropolis is estimated to have a population of 427,760 people (NPC/FRN,2007) and by the virtue of its origin, the state comprises mostly Hausa/Fulani and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Ibos and others. Occupation of city inhabitants include trading, commerce, with a reasonable proportion of the population working in private and public sectors (MOI, 2008). The Sokoto

township is in dry Sahel surrounded by sandy terrain and isolated hills. Rainfall starts late that is in June and ends early, in September but may sometimes extend into October. The average annual rainfall is 550 mm with peak in the month August. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February (Abdullahi et al., 2009). Modern Sokoto city is a major commerce center in leather crafts and Agricultural products (MOI, 2008).

Collection of Snails

Adult *Bulinus wrighti* were collected from their natural habitats from Kwalkwalawa local fresh water river in Sokoto metropolis. The snails were identified at the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. Size of the shell was ± 2.00 cm. The pH of the dechlorinated tap water was 7.2 and the temperature was 32.2° C. Snails were acclimatized in the laboratory conditions for 72 hours in the Plastic aquaria containing dechlorinated tap water before being used for molluscicidal tests. Dead animals were removed quickly to avoid contamination of aquarium water.

Plant Materials Used in the Research

The bulb of *Allium sativum* were purchased from market during the period of March to July, 2013. Identified and confirmed by a senior plant taxonomist from Biological Sciences Department, Usmanu Danfodiyo University Sokoto. The

voucher numbers of the plant is: *Allium sativum*-UKM-B30093.

Preparation of Plant Extracts

Powder of *A. sativum* bulbs was prepared by peeling and slicing healthy cloves into 3mm thick, air dried and pulverizing into a mortar and pestle. The powder was kept dry, stored in air tight container in refrigerator and tested for molluscicide activity.

Aqueous Extraction

For aqueous extracts, the desired weight of plant powder was soaked in 500mls dechlorinated tap water for overnight, stirred and filtered. The filtrate was used for toxicidal activity as shown in Table 1.

Ethanollic Extraction

Five hundred grams (500g) of air-dried *A. sativum* (bulbs), was extracted with 1.5 liters of ethanol. The extraction was kept in orbital shaker for 30 minutes. The extracts were filtered, using muslin cloth and concentrated to dry under reduced pressure in a rotary evaporator at 40°C which yield ethanollic extract of *A. sativum*. The extracts were kept in fridge in Laboratory for further use.

Study of Toxicity of Preparation of Plant Derived Molluscicides

Toxicity experiments were performed by the method of Singh & Singh (1997). Ten experimental animals were kept in each aquarium containing 3 liters of dechlorinated tap water, and exposed continuously for 96h to different concentrations of plant materials and preparations (Table 1). Control animals were kept in similar conditions without treatment. During experimental period snails were kept in starved condition. As it was periodic sampling the mortality was recorded after every 24 hours interval up to 96 hours during exposure of the snails. Each experiment was replicated six times (Suleiman et al., 2018ab).

The toxic effect of the molluscicides was also studied against fish *Oreochromis niloticus* (Tilapia). In these experiments a group of 10 Tilapia were exposed in 6 liters of dechlorinated tap water as carried out by (Suleiman et al., 2018ab). The fishes were exposed for 24 hours LC₉₀ (of snail) to 96 hours.

No response to a needle probe in case of snails, and no response against touch in case of fish (Tilapia) was taken as evidence of death. Dead animals were removed on each observation during exposure period to avoid any contamination of the aquarium water (Suleiman et al., 2018ab).

Table 1: Doses of plant extracts Tested on *B. wrighti* for Toxicity.

Name of Treatment	Concentration (mg/L)
Ethanollic extraction of <i>A. sativum</i>	15, 30, 40, 50
Aqueous extraction of <i>A. sativum</i>	300, 400, 500, 600

Histological Preparation

Treated and control snails were removed from their shells, and washed thoroughly with distilled water and fixed in 10% formalin. The snail tissues were processed for paraffin sectioning after embedded in paraplast at 50°C. The 7µm sections were stained with iron haematoxylin and eosin, and examined for tissue changes with light microscopy (Ahmad et al., 2014).

Statistical Analysis

Lethal concentration (LC₅₀) value, lower (LCL) and upper (UCL) confidence limits, slope value, t- ratio, g- value and heterogeneity were calculated according to the method of POLO (probit or logit) computer programme of Suleiman et al., (2018a). Tables and charts were prepared for presentation of results.

Results And Discussion

Molluscicidal Activity of Plant Products against Snail *B. wrighti*

This part of the result deals with study of molluscicidal properties of preparation of *A. sativum* against *B. wrighti*. The freshwater snails were exposed to different concentrations of preparations (Table 1). Mortality was recorded after 24h, 48h, 72h, and 96h during the exposure period. *Tilapia* Fish were exposed to (24h LC₉₀ against *B. wrighti*) of molluscicidal formulation for 96h to observe any toxic effect against non-target animals in aquatic environment. Toxicity evaluation of all the plant derived formulations and their combinations showed that the molluscicidal activity of these preparations

against *B. wrighti* was time and dose dependent. This result revealed that *B. wrighti* was susceptible to the plant extracts at different concentrations.

Aqueous and ethanolic extract of *Allium sativum* (Galic)

Table 2 and 3, showed toxicity of aqueous and ethanolic extract of *A. sativum* (Garlic) against *B. wrighti* respectively. The aqueous extracts of *A. sativum* that killed 50% (LC₅₀) *B. wrighti* decreased from 807.00mg/l (24h), to 386.00mg/l (96h). The ethanolic extracts of *A. sativum* that killed 50% *B. wrighti* (LC₅₀) decreased from 97.07 mg/l (24h) to 21.70 mg/l (96h) indicating the molluscicidal activity of this extract against *B. wrighti* was time and dose dependent. The toxicity of aqueous extracts of *A. sativum* against *B. wrighti*, in Table 2, indicated the LC₅₀ at 96h was 386.00mg/l. while the toxicity of ethanolic extracts of *A. sativum* against *B. wrighti*, (Table 3), indicating the LC₅₀ at 96h was 21.70mg/l. This result showed that ethanolic extracts of *A. sativum* possessed high molluscicidal properties compared to the aqueous extracts of *A. sativum* against *B. wrighti*.

In the control experiment, no mortality was observed. The slope value observed in this study was steep. A value of t- ratio was greater than 1.96. The 'g value is less than 0.5. Heterogeneity factor was less than 1.0. In the control experiment, no mortality was observed.

Phytochemical Screening of Plant Extracts

Phytochemical screening was carried out to determine the specific compounds present in the Plant

extract, which may be responsible for the observed biological effects. The result of phytochemical analysis of aqueous extract of *Allium sativum* (Garlic) showed that saponins, glycoside, alkaloids, steroids, volatile oils, and cardiac glycosides are present in the

Plant extract while flavonoid, tannins, saponin glycoside, balsams and anthraquinones are absent as shown in (Table 4).

Table 2: Toxicity of Aqueous Extract of *Allium Sativum* (Garlic) Against *B. 30right*.

Exposure Time	LC ₅₀ (mg/l)	LCL	UCL	Slope Value	t-ratio	g-Value	Heterogeneity
24hr.	807.00	147.40	654.00	5.76±1.39	4.12	0.22	0.26
48hr.	770.00	114.6	660.00	3.57±0.93	3.80	0.26	0.29
72hr.	530 .00	590.00	491.00	5.42±0.85	6.31	0.09	0.30
96hr.	86.00	406.00	365.00	8.95±0.99	8.96	0.04	0.45

Batches of ten snails were exposed to different concentrations of Aqueous extract of *A. sativum* powder. Mortality was recorded at every 24hrs. Each set of experiment was replicated six times. Concentrations given were the final concentration (w/v) in the aquarium water. A value of t- ratio greater than 1.96 indicate that regression is significant.

Value of heterogeneity factor was less than 1.0 denotes that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately. The indices of significance of potency estimation ‘g value’ indicate that the value of mean was within the limits at all probability

levels (90, 95, 99) as it was less than 0.5.

Table 3: Toxicity of Ethanolic Extraction of *Allium sativum* (garlic) against *B. wrighti*

Exposure Time	LC ₅₀ mg/l	LCL	UCL	Slope Value	t-ratio	g-.Value	Heterogeneity
24hr.	97.07	63.78	419.18	1.90±0.56	2.58	0.33	0.28
48hr.	58.51	44.98	113.60	1.84±0.47	3.84	0.26	0.20
72hr.	25.80	22.13	29.23	3.18±0.46	6.88	0.08	0.35
96hr.	21.70	18.92	24.26	5.25±0.57	9.20	0.06	1.31

Batches of ten snails were exposed to different concentrations of ethanolic extract of *A. sativum* powder. Mortality was recorded at every 24hr. Each set of experiment was replicated six times. Concentrations given were the final concentration (w/v) in the aquarium water.

A value of t-ratio greater than 1.96 indicate that regression is significant.

Value of heterogeneity factor was less than 1.0 denotes that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately.

The indices of significance of potency estimation ‘g value’ indicate that the value of mean was within the limits at all probability levels (90, 95, 99) as it was less than 0.5.

Table 4: Phytochemicals present in Aqueous Extract of *Allium sativum*

PHYTOCHEMICAL	TEST	OBSERVATION	INDICATION
Flavonoid	Ferric chloride	+ve (green color)	Absent
Tannins	Ferric chloride	+ve (blue-green color)	Absent
Saponins	Frothing test	+ve (frothing)	Present
Glycosides	Fehling’s solution	+ve (brick-red precipitate)	Present
Alkaloids	Wagners	+ve (turbidity or precipitate)	Present
Cardiac glycosides	Kellerkilliani	+ve (reddish-brown color)	Present
Steroids	Chloroform	+ve (reddish-brown color)	Present
Volatile oils	Dilute HCL	+ve (white precipitate)	Highly present
Saponin glycosides	Fehling’s solution A&B	+ve (bluish-green precipitate)	Absent
Balsams	Alcoholic ferric chloride	+ve (dark green color)	Absent
Anthraquinues	Borntreger’s test	+ve (pink, red or violet color)	Absent

Histopathological Changes

Treatment of snails with *A. sativum* bulb extracts showed histopathological signs (Figures 1-3) to the

hermaphrodite glands and the digestive tract of the snails. The histological changes were a function of extract concentrations.

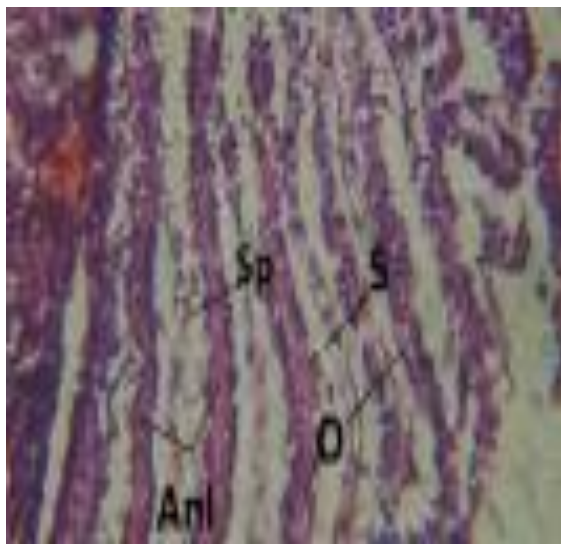


Figure 1a: T.S. in control *B. wrighti* (Hermaphrodite region). Anl= ancel's layer Sp= sperms S= spermatocytes O= oocyte X= 200

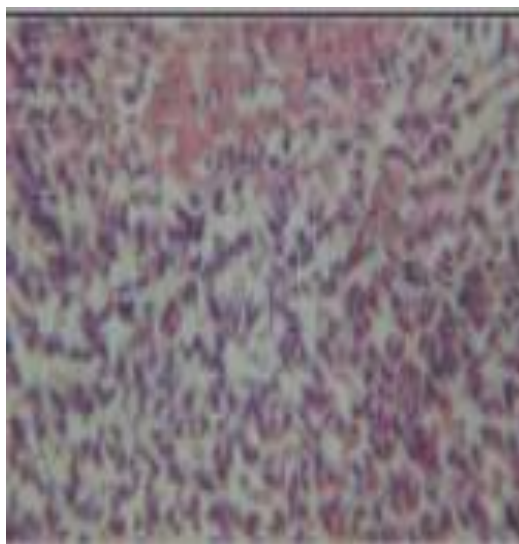


Figure 1b T.S. in control *B. wrighti* showing digestive epithelia. X = 200

Cells of hermaphrodite glands and digestive glands are all intact and normal. No changes were observed in control, all vacuoles and follicular membrane are intact and normal in shape and thickness.

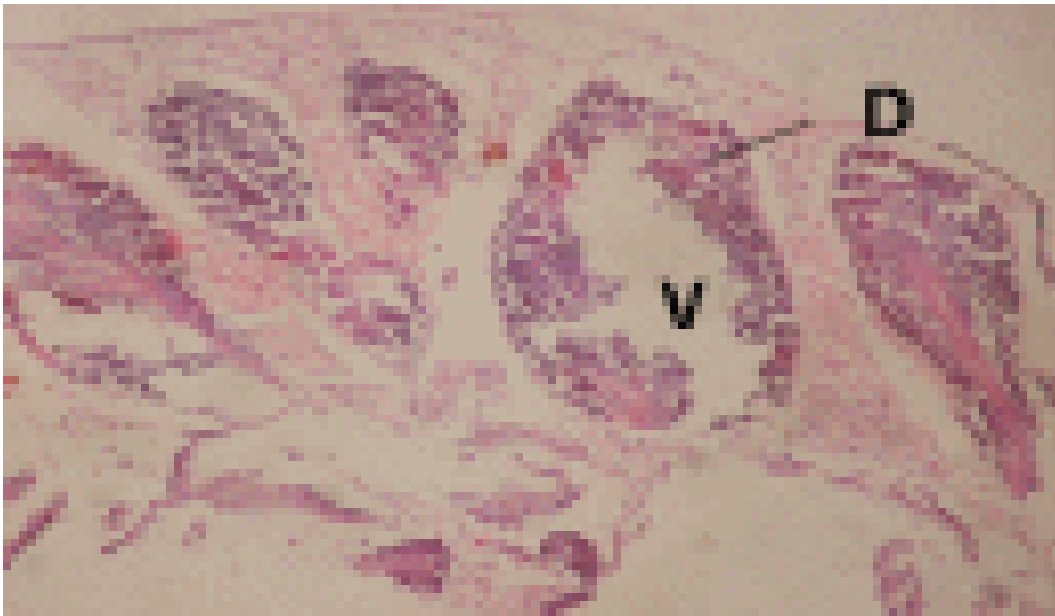


Figure 2: Ts.S. in treated *B.wrighti* with 50 mg/l ethanolic extract of *A. sativum* (Hermaphrodite region). D: degeneration V: vacuoles X= 200

Treated *B. wrighti* with a concentration 50mg/l ethanolic extraction of *A. sativum* showed large vacuoles and degeneration in the hermaphrodite

glands, destruction in the follicular membrane and the matured ovum showed losing of the nucleolus.(Figure 2).

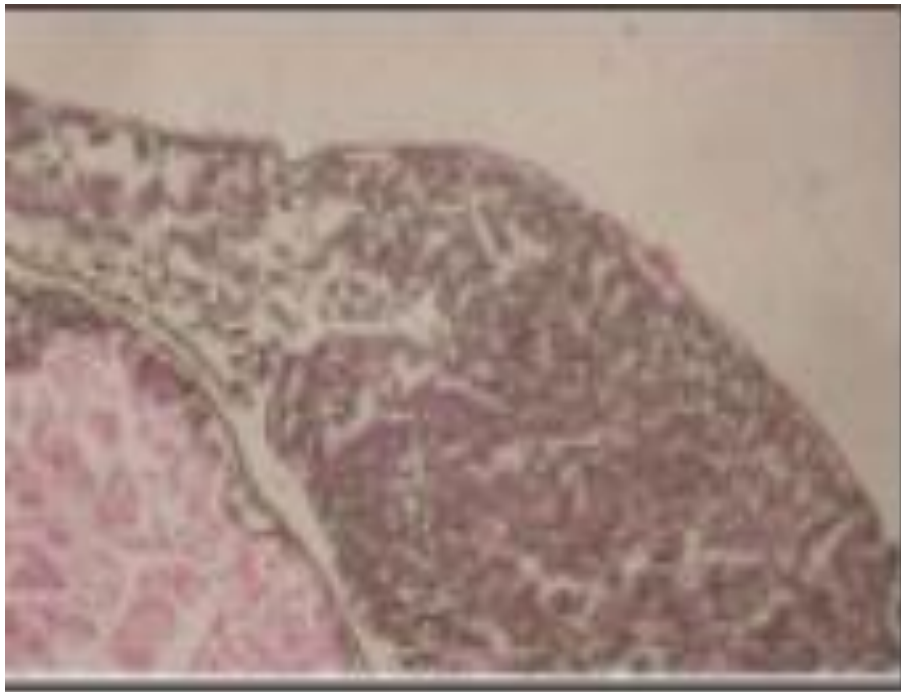


Figure 3: T.S. in treated *B. wrighti* with 50 mg/l of ethanolic extract of *A. sativum* (digestive acini).Large vacuoles and great destruction of digestive acini. X= 200

Large vacuoles and great destruction was observed in the digestive acini and the columnar epithelial cells (Figure 3) when compared with control.

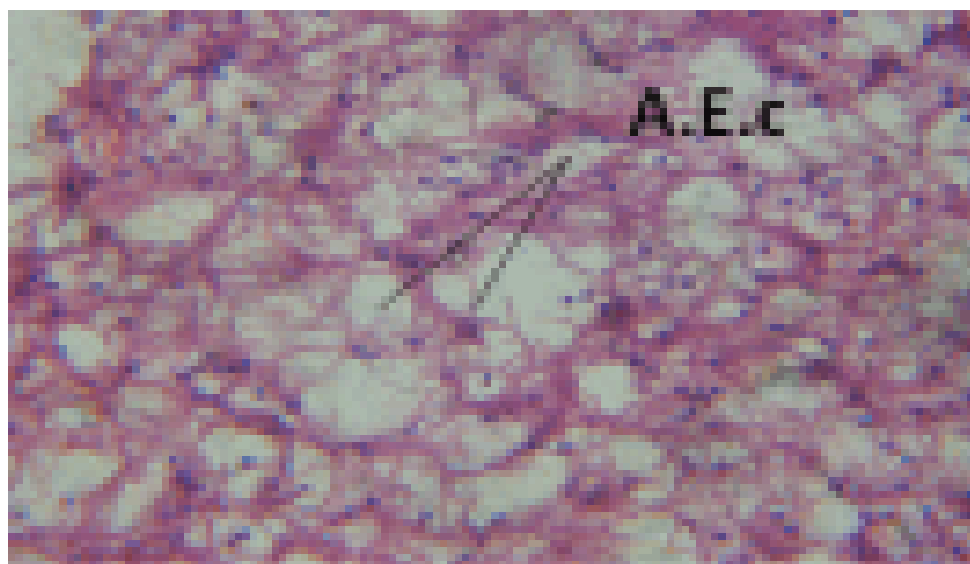


Figure 4 T.S. in treated *B. wrighti* with 50 mg/l of ethanolic extract of *Allium sativum* showing digestive epithelia. A.E.c: evacuated epithelial cells X = 200

In the digestive epithelial, there observed large evacuated epithelial cells (Figure 4).

The size of vacuoles was not affected by the decrease in concentrations of the extract. While in control snails no pathological changes were observed in the digestive gland and hermaphrodite gland.

The present study showed that aqueous extracts of *Allium sativum*, and ethanolic extracts of *Allium sativum* possessed molluscicidal properties. Their activities were time and concentration- dependent. Between the tested plants against *B. wrighti*, ethanolic extract of *A. sativum* showed the highest molluscicidal activity on the test snail species after 24hr -96hr of exposure period. The varying potencies of each plant may be due to the differences in concentration and or the type of the active ingredient (s) present in each plant (Brimer et al., 2007). Most of the plants species screened during this study for their molluscicidal activity more or less accumulate similar classes of compounds. Saponin is highly toxic and also exhibit hemolytic properties, which act as poison show cytotoxic or pesticidal activity (Hostettmann & Marston, 1995; Noorshilawati et al.,

2020). In the present study, Saponin is present in *A. sativum* which may be responsible for the death of *B. wrighti*.

However, it has been established that not only saponins but also some sesquiterpenes, flavonoids, glycosides as well as phorbol esters possess molluscicidal properties (Hostettman et al., 1982; Rug & Ruppel, 2000; Al-zanbangi et al., 2001). Flavonoids and glycosides were also present in *A. sativum* which may be responsible for the death of *B. wrighti*, in the present study. The penetration of the toxicants also has a greater significant for the aquatic environment, because their whole body is bathed in a diluted solution of toxicants. To have maximum effect the plant must penetrate the organism and then transported to active site rapidly. It seemed the high titer of plant extract in snails may be due to rapid penetration of the plant molluscicides through soft foot of snails body and / or it may be possible the plant active component may change into more toxic form in the aquarium water in snail body which is

triggered by different enzymes and cause differential mortality (Singh & Tiwari, 2012; Suleiman et al., 2018ab).

Nevertheless, no mortality was recorded in Tilapia fish (*Oreochromis niloticus*) even at 24 hrs LC₉₀ of the molluscicides investigated in this study which indicated that the use of these molluscicides may probably be safe to non-target animals such as Tilapia fish. It is reasonable to conclude that the concentrations of molluscicides does not produce symptoms of toxicity (mortality) in fish (*Oreochromis niloticus*) which possibly because the amount of drug was rapidly detoxified by fish or the fish has a different metabolic pathway than the snails.

The steep slope value observed in the toxicity studies demonstrated that a small increase in concentration of molluscicides cause a large mortality in the snails. A value of t- ratio greater than 1.96 indicated that regression is significant. Value of heterogeneity factor was less than 1.0 denoted that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately. The indices of significance of potency estimation 'g value' indicated that the value of mean was within the limits at all probability levels (90, 95, 99) as it was less than 0.5.

The results of phytochemical analysis of aqueous extract of *A. sativum* (bulb) showed the presence of saponins, glycoside, alkaloid, steroids, volatile oils and cardiac, glycoside while flavonoid, tannins, saponin glycoside, balsams and anthraquinones are absent. This result was similar to the findings of

Pavni et al. (2011) who reported that *A. sativum* contains flavonoids, monosaccharides, glycosides, saponins, tannin and free reducing sugar.

The molluscicidal activity of the plant extracts on the histology of the digestive gland of snails acting as intermediate host have been suggested in scattered cases in the literature (Brackenbury, 1999; Suleiman et al., 2018ab). In addition, several investigations have studied the histological and histochemical changes induced by molluscicides on the digestive gland of aquatic gastropods (Mulley & Mane, 1990; Sing & Tiwari, 2012; Noorshilawati et al., 2020).

Figures 1-4 shows the histopathological studies of the effects of the extracts on the *B. wrighti* snail. During the histological test of treated *B. wrighti* snail on 50mg of ethanolic extract *A. sativum*, large vacuoles, degeneration in the hermaphrodite glands and destruction in the follicular membrane were observed, which was similar to the observation of Ahmad et al., (2014), when *Biomphalaria alexandrina* snail was treated with methanolic extract of *Callistemon viminalis* fruits, bark and leaves. Thus, the plant extract of *Allium sativum* can served as a cheap, affordable, available, and effective bio-friendly material for the prevention of bilharziasis in the area.

Conclusion

The results of this study showed that *A. sativum* possessed molluscicidal properties against *Bulinus wrighti* which is the vector for urinary schistosomiasis. Ethanolic extract of *Allium sativum* was the most effective. This plant molluscicides are readily available, inexpensive and environmentally

safer for controlling human urinary schistosomiasis. This will not only eliminate the economic burden of importing expensive synthetic molluscicides, but also stimulate growth of small-scale industries in Nigeria.

Recommendations

1. The study also recommends the use of ethanolic extract of *A. sativum* in controlling the *Bulinus wrighti* because the study reveals that ethanolic extracts of *A. sativum* are highly toxic against *B. wrighti*.
2. Government should encourage the use of indigenous small-scale industries of Plant molluscicides instead of importing expensive synthetic molluscicide.
3. Further research is recommended using different solvents for extraction and comparing their activity.

Conflicts of Interest

The author stated there is no conflict of interest.

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