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Original article

Molluscicidal activity of Toad (Bufo Spp.) venom crude extract on the multi-species of Snail intermediate hosts of *Schistosoma*

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ABSTRACT

Schistosomiasis remains a significant public health concern in many developing nations, particularly in tropical and subtropical regions. While synthetic compounds such as Niclosamide have been developed to combat snail-borne diseases, these synthetic molluscicides are either scarce, expensive or toxic to non-target organisms in the snail habitat. In contrast, plant-derived products have been extensively studied for their molluscicidal properties, whereas animal-derived compounds, notably toad venom, have received less attention. This study explores the potential of toad venom as an alternative molluscicide for controlling snail intermediate hosts of Schistosoma. Toad venom was extracted and analyzed for zoochemicals and Gas Chromatography-Mass Spectrometry *(GC-MS) Analysis*. Molluscicidal evaluation of toad venom crude extract was conducted following World Health Organization (WHO) guidelines. Adult snails of *Biomphalaria pfeifferi, Bulinus forskalii,* and *Bulinus globosus* species were exposed to graded concentrations (200, 100, 50, 25, 12.5, and 6.5 mg/L) of the toad venom crude extract. Analysis of results was performed using ANOVA and Probit Regression analysis. Results on qualitative zoochemical screening of crude extract of toad venom revealed the presence of cardiac glycoside, carbohydrate, saponins, steroids

and terpenoids. GCMS analysis of crude toad venom revealed 30 peaks representing 25 bioactive compounds, with 9-Octadecenoic acid, methyl ester being the most abundant constituents in the crude toad venom. The toad venom extract showed a dose and time-dependent activity against the exposed mollusks. Exposure of *B. pfeifferi, B. globosus,* and *B. forskalii* to 100 mg/L concentrations resulted in mortality rates of 73.3%, 66.6%, and 60%, respectively, after 24 hours. After 24 hours of exposure period, the lethal concentrations required to kill 50% (LC50) of *B. pfeifferi, B. globosus,* and *B. forskalii* snails were 46.77, 70.79, and 79.43 mg/L, respectively. Notably, the effective doses of toad venom against the three snail species exhibited minor variations despite their coexistence in common habitats. In conclusion, this study underscores the molluscicidal potential of toad venom as a promising lead agent for controlling snail intermediate hosts of Schistosoma.

Keywords: Toad venom, *Schistosoma*, *Biomphalaria pfeifferi*, *Bulinus forskalii* and *Bulinus globosus*, Molluscicides.

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INTRODUCTION

Schistosomiasis, a helminthic parasitic disease, is caused by a blood fluke of the genus *Schistosoma* [1,2], which in turn is transmitted to humans through their intermediate hosts, such as the planorbids snails. Schistosomiasis is still considered a public health problem in many developing countries, including Nigeria [2]. According to a report by Adenowo and others in 2015, Among African countries with the highest prevalence of schistosomiasis, Nigeria has the highest number (29 million) of cases with an estimated 101.28 million people at risk of infection, followed by the United Republic of Tanzania, Ghana, and the democratic republic of Congo with 19 and 15 million cases each [2, 3]. Nonetheless, it is believed that most disease cases are not reported. The true incidence of this disease might be 400-600 million cases worldwide [3]

In Nigeria, schistosomiasis is endemic in rural areas. Its geographical conditions are suitable for the infestation of this parasite due to the presence of creeks, lakes, ponds and rivers [2]. Many individuals involved in agricultural activities in irrigated fields are at risk of constant exposure to cercaria. The transmission of schistosomiasis mainly depends on the presence of the infected person and the faecal or urinary release of eggs from helminths into water environments containing the snail host, thus maintaining the life cycle of the parasite [4,5].

Various genera of these snails have been associated with specific parasitic types. For example, the *Bulinus* species is responsible for hosting the *Schistosoma haematobium* parasite, while the *Biomphalaria* species is responsible for *S. mansoni* [2,6]. Snails, as they are economically important in the environment and food web, threaten the environment. An increase in the snail population equals an increase in the transmission rate of the parasitic disease, as they play an important role in disease transmission [7].

Control of the snail population is an alternative way to prevent parasitic diseases with molluscs in the parasite's life cycle [8]. Controlling the snail population through synthetic compounds such as Niclosamide has been one of many

alternative ways to decrease the incidence of schistosomiasis, as it reduces the risk of cercaria exposure during routine freshwater canal-related tasks. However, these synthetic molluscicides are either scarce, expensive or toxic to non-target organisms in the snail habitat. Plant-derived products have been extensively studied for their molluscicidal activity [8], whereas animal-derived compounds have received comparatively little attention. Therefore, this study attempts to provide an alternative towards controlling snail intermediate hosts of *Schistosoma* using toad venom.

The amphibian family Bufonidae is characterized by parotid macro glands behind the eyes and ears, primarily known for their toxic secretions [9]. The venom is used as a chemical defence against predators and microbial infection [10, 11, 12]. Toad venom contains toxins and bioactive alkaloids, such as bufadienolides and cardioactive steroids. In the past few decades, researchers have diverted interest into exploiting animal-based therapeutic agents [13,14,15]. According to modern medical analysis, toad venom has a variety of pharmacological activities such as cardiotonic, anaesthesia, detoxification, analgesia, antimicrobial, antitumor, and immune enhancement activities [16, 17].

Despite the therapeutic potentials of toad venoms, little has been done on the molluscicidal assessment of Nigerian toad which may be due to scarcity of information and material to explore the venom. This research will provide a deeper understanding of the active compounds found in Minna's toad venom, revealing its potential for molluscicidal purposes.

MATERIALS AND METHODS

Study Area

Ethical clearance for the use of experimental animals (Toads, Snails) was obtained from Ministry of Livestock and Fisheries, Minna, Niger State. The study was conducted in Minna, Niger State. Minna, Niger State experiences distinct dry and wet seasons. The highest monthly temperature is recorded in March with an average daily temperature of 30°C and the lowest daily temperature is recorded in August at about 22°C. Niger State has a vegetation type classified as Guinea Savannah characterized by the presence of few scattered trees and dense grass cover. However, within the Niger trough and flood plains occur taller trees and a few oil palm trees. Generally, the fertile soil and hydrology of the State permit the farming/cultivation of most of Nigeria's staple crops and still allow sufficient opportunities for grazing, freshwater fishing and forestry development [18].

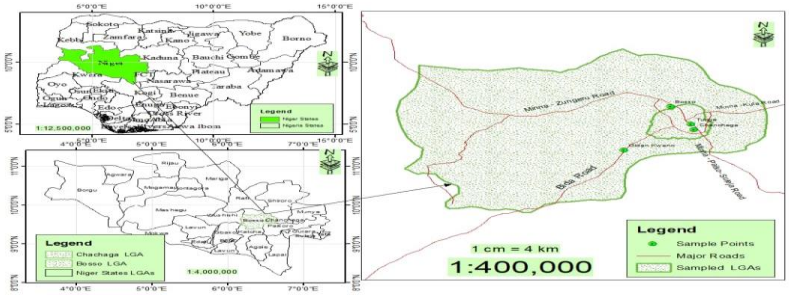

Figure.1: Map showing the sampling sites within Minna
Source: Field photograph (2021)

Biochemical Characterization of Toad Venom

Toad Collection and Venom Extraction

Toads were collected from their habitats and cleansed with distilled water before venom extraction was done to avoid contamination. The process of extraction was achieved by massaging and pressing of paratoid macroglands to release a whitish-sticky substance (i.e the venom). The collected venom was dispensed in an eppendorf tube, lyophilized and stored in a freezer for further analysis [19].

Plate 1: Massaging the parotoid gland of a Toad B: Venom in an Eppendorf Tube
Source: Field photograph (2021)

Zoochemical analysis of the crude extract of toad venom

Zoochemical analysis was conducted on the crude toad venom extract to test for the presence or absence of zoo-chemical such as tannins, saponins, terpenoids, carbohydrates, and steroids [20, 21].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Toad Venom Crude Extract

To identify and quantify the compounds present in the crude extract of toad venom, Gas Chromatography-Mass Spectrometry (GC-MS) was conducted using a Trace GC1310-ISQ mass spectrometer from Thermo Scientific in Austin, TX, USA [19].

Snail Sampling, Collection and Laboratory Maintainance

Adult snails of *Biomphalaria pfeifferi*, *Bulinus forskalii* and *Bulinus globosus* species were sampled. Sampling of snails was done once a week during the early hours of the day. The handpick technique with surgical gloves was adopted for snail sampling. Snails were often seen near the edges of slightly deep waters, attached to a rocky surface, slightly buried in the sediment, or lodging in plant materials and discarded items, including plastics and nylon bags. Snails collected from the sample site were kept in separate labelled specimen plastics and transported to the Department of Animal Biology laboratory at the Federal University of Technology Minna. They were housed in plastic aquaria that included 30 mg/L calcium carbonate, dechlorinated tap water (pH of 7.0 ± 0.2), and a temperature (26 ± 2 ºC) with a 12/12 photoperiod. They were also fed with oven-dried lettuce leaves [22].

Preparation and Dilution for Molluscicidal Activity

The study was conducted at room temperature, and snails starved during the molluscicidal assay. Before the molluscicidal assay, a preliminary molluscicidal assay was previously done to determine the minimum effective concentration. A range of 6 concentrations were randomly assayed: 200, 300, 350, 400, 450, and 500 mg/L of distilled water. In all the concentrations, a lethal effect in 6 h was observed. After the preliminary assay, a narrower range of toad venom extract was tested, with 3 concentrations that kill < 50% of snails and 3 that kill > 50–100% to establish a dose–response curve [22]. The final working solution was obtained from the least effective concentration of the preliminary molluscicidal assay. Thus, the final working solution concentrations used for molluscicidal assay were 0.00(control), 200, 100, 50, 25, 12.5 and 6.5mg/L. For the treatment, batches of 10 snails each were exposed to each dilution at room temperature. The test was performed in three replicates with negative controls consisting of only tap water. For the positive control, 1 g of Niclosamide was dissolved in 1L of distilled water. The details of the treatments are as follows:

Negative control: 100 ml of distilled water to each of the plate. No venom extract was added.
Positive control: 1 g of Niclosamide was dissolved in 1L of distilled water, and 500 ml of the dilution was added to each plate.
1st Concentration: 6.5 mg of the toad venom was diluted in 1L of distilled water and 500 ml of the dilution was added to each plate.
2nd Concentration: 12.5 mg of the toad venom was diluted in 1L of distilled water, and 500 ml of the dilution was added to each plate.
3rd Concentration: 25 mg of the toad venom was diluted in 1L of distilled water, and 500 ml of the dilution was added to each plate.
4th Concentration: 50 mg of the toad venom was diluted in 1L of distilled water, and 500 ml of the dilution was added to each plate.
5th Concentration: 100 mg of the toad venom was diluted in 1L of distilled water, and 500 ml of the dilution was added to each plate.
6th Concentration: 200 mg of the toad venom was diluted in 1L of distilled water, and 500 ml of the dilution was added to each plate.

At the end of the 24-hour exposure period, snails were removed from treated and control water, rinsed and transferred to containers with unchlorinated water; after a 24-hour recovery period, mortality was recorded. Snails that remain completely within their shells and show no movement were suspected to be dead and transferred to a separate container. Snails alive at 24 hours were placed in freshwater with food and monitored for 48 hours [6]. After the 48 hours, the number of dead snails was recorded. Results of the mean mortality across treatments were compared using ANOVA, while post-hoc tests determined which specific treatment means were statistically comparable. The relationship between dose and mortality was analyzed using log-probit [22, 23].

RESULTS

Zoochemical compositions of crude extract of toad venom

Qualitative zoochemical screening of crude extract of toad venom revealed the presence of cardiac glycoside, carbohydrate, saponins, steroids and terpenoids, while alkaloids, flavonoids, tannins and anthraquinones were absent (Table 1)

Table 1: Zoochemical composition of crude extract of toad venom

Zoochemicals	Inferences
Phenols	-
Flavonoids	-
Alkaloids	+
Tannins	-
Phlobatannins	-
Steroids	+
Cardiac glycoside	+
Carbohydrate	+
Saponins	+
Anthraquinones	-
Terpenoids	+

Keys + = presence - = absence

Molluscicidal Efficacy of Toad Venom Crude Extract

The molluscicidal efficacy of the toad venom crude extract against *Biomphalaria, B. globosus* and *B. forskalii* was concentration and time dependent. In all the experimental groups, 100% snail mortality was mostly observed within 24h in a concentration of 100 and 200 mg/l, similar to the positive control (Table 2, 3 and 4).

Snails in all experimental groups manifested similar postmortem behaviour (e.g., secreting white sticky mucus, retracted visceral foot, no movement of siphon/ proboscis, floating in the water). However, the manifestation of these observable changes did not happen simultaneously. No mortality of snails was observed on the negative control set-up (distilled water).

When *B. pfeifferi* snails were exposed to the concentrations of 100mg/L, they stopped crawling and later started bleeding. The mean mortality of *B. pfeifferi* when exposed to a concentration of 6.25 and 100mg/L was 20% and 100%, respectively, after 72 hours (Table 2), while that of *B. globosus* when exposed to a concentration of 6.25 and 100mg/L was 10% and 100% respectively after 72 hours (Table 3).

The toad venom crude extract also had molluscicidal efficacy on *B. forskalii* snail species. when exposed to a concentration of 6.25 and 100mg/L, the mean mortality recorded was 13.33% and 96.66%, respectively, after 72 hours (Table 4). When exposed to a concentration of 0.28 mg/L of niclosamide solution as a positive control, 100% mortality rate was recorded for all snail species within 24 hours. Results showed that the mean mortality across treatments (positive control, 100mg/l and 200mg/l) were statistically comparable (Table 2,3 and 4). ANOVA result showed a p value of 2.07766e-10, 9.17e-10 and 9.18e-10 for *B. pfeifferi, B. globosus and B. forskalii* respectively, indicating a very high significant difference among the treatment means (Table 2, 3 and 4). This further showed that at least one (1) group among the treatments recorded very low mortality (i.e., the negative control) compared to the rest, thus reflecting a very high difference in mortality among the test organisms.

Table 2: Mean (SD) Percentage mortalities of *B. pfeifferi* snails exposed to different concentrations of Toad venom crude extract for 24, 48 and 72 hours

	Percentage Mortality		
Conc. (mg/L)	24hrs	48hrs	72hrs
6.5	0.00 ± 0.00^{c}	13.33 ± 0.43^{c}	20.00 ± 0.40^{c}
12.5	10.00 ± 0.30^{c}	20.00 ± 0.40^{c}	30.00 ± 0.46^{c}
25	20.00 ± 0.40^{c}	30.00 ± 0.46^{c}	40.00 ± 0.49^{c}
50	56.66 ± 0.50^{b}	66.66 ± 0.47^{b}	80.00 ± 0.43^{b}
100	73.33 ± 0.44^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
200	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Control (+ve)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Control (-ve)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

MS=473.21; df= 7; F statistics=62.06;P value = 2.07e-10
There is a significant difference among the treatment means @ $p \leq 0.05$**.** A similar letter superscript of the mean $\pm$ S.D. indicates non-significant at Tukey's post hoc test ($p \leq 0.05$)

Table 3: Mean (SD) Percentage mortalities of *B. globosus* snails exposed to different concentrations of Toad venom crude extract for 24, 48 and 72 hours

	Percentage Mortality		
Conc. (mg/L)	24hrs	48hrs	72hrs
6.5	0.00 ± 0.00^{c}	6.66 ± 0.25^{c}	10.00 ± 0.30^{c}
12.5	6.66 ± 0.25^{c}	13.33 ± 0.43^{c}	23.33 ± 0.43^{c}
25	13.33 ± 0.43^{b}	33.33 ± 0.47^{c}	40.00 ± 0.49^{b}
50	50.00 ± 1.54^{b}	60.00 ± 0.49^{b}	66.66 ± 0.47^{b}
100	66.66 ± 0.47^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
200	83.33 ± 0.37^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Control (+ve)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Control (-ve)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

MS=472.19; df= 7; F statistics=51.04;P value = 9.17e-10
There is a significant difference among the treatment means @ @ $p \leq 0.05$**.** Similar letter superscript of the mean $\pm$ S.D. indicates non-significant at Tukey's post hoc test ($p \leq 0.05$).

Table 4: Mean (SD) Percentage mortalities of *B. forskali* snails exposed to different concentration of Toad venom crude extract for 24, 48 and 72 hours

Conc. (mg/L)	24hrs	48hrs	72hrs
6.5	0.00 ±0.00^{c}	6.66 ± 0.25^{c}	13.33 ± 0.43^{c}
12.5	3.33 ± 0.18^{c}	10.00 ± 0.30^{c}	20.00 ± 0.40^{c}
25	13.33 ± 0.43^{c}	20.00 ± 0.40^{c}	30.00± 0.46^{c}
50	40.00 ± 0.49 ^{b}	50.00 ± 0.50^{b}	60.00 ± 0.49 ^{b}
100	60.00 ± 0.49^{a}	83.33± 0.37^{a}	96.66 ± 0.18a
200	76.66 ± 0.43^{a}	90.00 ± 0.30^{a}	100.00 ± 0.00^{a}
Control (+ve)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Control (-ve)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

MS=440.26; df= 7; F statistics=51.04;P value = 9.18e-10
There is a significant difference among the treatment means @ $p \leq 0.05$**.** A similar letter superscript of the mean ± S.D. indicates non-significant at Tukey's post hoc test ($p \leq 0.05$).

Toxicity of Toad Venom Crude Extract on *B. pfeifferi, B. globosus and B. forskalii*

The lethal dosages were calculated and estimated using probit analysis. The lethal concentrations of the toad venom extract that caused 50% (LC_{50}) snail mortality of *B. pfeifferi* at 24, 48 and 72hrs were 46.77, 21.37 and 17.37mg/L respectively, while the respective LC_{90} values were 89.12, 53.70 and 46.77mg/L (Table 5.). The lethal concentrations that caused 50% (LC_{50}) mortality of *B. globosus* at 24, 48 and 72hrs were 70.79, 23.98 and 20.89mg/L, respectively, while the respective LC_{90} values were 162.18, 56.23 and 52.48mg/L (Table 6). The lethal concentrations of the toad venom extract that caused 50% (LC_{50}) mortality of *B. forskalii* at 24, 48 and 72hrs were 79.43, 45.70 and 24.54mg/L, respectively, while the respective LC_{90} values were 191.82, 190.54 and 69.18mg/L (Table 7). It was revealed in the Probit analysis that the slope value is positive. The results of the regression analysis of toad venom crude extracts revealed that the mortality rate (Y) was positively correlated with the concentration of exposure (X), which had a regression coefficient (R) close to 1 in each case. Thus, there is a direct relationship between the concentration of treatment and snail mortality. As observed from the results, as the toad venom concentration increased, the snails' mortality also increased.

The LC_{50} and LC_{90} values lie within the 95% confidence limits. As it can be seen from Tables 5, 6, and 7 for 72hrs of exposure, the potency difference of the extract against the three snail species was relatively small, yet *B. pfeifferi* was more sensitive than *B. forskalii* and *B. globosus*, especially in the LC_{50} concentrations. This means that *B. pfeifferi* snails were more susceptible to the toxic effect of toad venom in comparison with *B. globosus* and *B. forskalii* snails, which indicated that the order of snails' susceptibility to the toxic effect of toad venom crude extract was in the order of *B. pfeifferi* > *B. globosus* > *B. forskalii*. The graph below (Figure 2) clarifies the trend along the entire LC levels.

Table 5: Lethal concentrations (mg/L) of Toad Venom on *B. pfeifferi* Snail

Time (hours)	LC_{50}	LC_{90}	R^2	Regression Equation
24	46.77	89.12	0.893	Y = 4.4713x - 2.4686
48	21.37	53.70	0.873	Y= 3.2099x + 0.7129
72	17.37	46.77	0.892	Y= 2.9964x + 1.2619

Coefficient of correlation of the treatments and mortality rates (R^2) is 0.893, 0.873 and 0.892, which can be interpreted as "very high correlation" Keys: LC_{50} = Lethal concentrations required to cause 50% mortality
LC_{90} = Lethal concentrations required to cause 90% mortality

Table 6: Lethal Concentration (mg/L) of Toad Venom on *B. globosus* Snail

Time (hours)	LC_{50}	LC_{90}	R^2	Regression Equation
24	70.79	162.18	0.835	Y = 3.489x - 1.4559
48	23.98	56.23	0.900	Y = 3.469x + 0.18
72	20.89	52.48	0.897	Y = 3.227x + 0.7231

Coefficient of correlation of the treatments and mortality rates (R^2) is 0.835, 0.900 and 0.897, which can be interpreted as "very high correlation". Keys: LC_{50} = Lethal concentrations required to cause 50% mortality
LC_{90} = Lethal concentrations required to cause 90% mortality

Table 7: Lethal Concentration (mg/L) of Toad Venom on *B. forskalii* Snail

Time (hours)	LC_{50}	LC_{90}	R^2	Regression Equation
24	79.43	191.82	0.8493	Y = 3.3969x - 1.475
48	45.70	190.54	0.9673	Y= 2.0577x + 1.5737
72	24.54	69.18	0.9095	Y= 2.8132x + 1.0772

Coefficient of correlation of the treatments and mortality rates (R^2) is 0.849, 0.967 and 0.909, which can be interpreted as "very high correlation".
Keys: LC_{50} = Lethal concentrations needed to cause 50% mortality
LC_{90} = Lethal Concentrations needed to cause 90% mortality

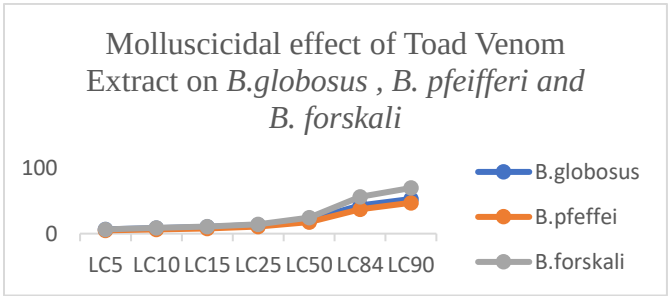

Figure 2: Relative sensitivities of *B. globosus, B. pfeifferi* and *B. forskalii* to various concentrations of toad extract

Gas chromatography and mass spectrometer analysis of toad venom crude extract

The GC-MS analysis of the crude extract of toad venom is represented in Table 8 and Figure 3. Gas chromatography and mass spectrometer analysis of crude toad venom revealed 30 peaks representing 25 bioactive compounds. The first compound identified with less retention time was Dimethyl phthalate, with a retention time of 11.327 mins, followed by Dodecanoic acid with a 12.887 mins retention time, Sulfurous acid and 2-propyl tridecyl ester had a retention time of 14.133 mins, while the last compound identified with longest retention time is 5, alpha. -Androstane-3. beta., 17. beta. -diol (21.881 mins). However, Pentacyclo [9.1.0.0(2,4).0(5,7).0(8,10)] dode (18.81 %) and 9-Octadecenoic acid, methyl ester, (E)- (14.43%) were the most abundant constituents in the crude toad venom.

Table 8: Chemical composition of crude extract of toad venom through gas chromatography and mass spectrometer analysis

Peak	Retention Time (m)	Peak Area (%)	Height (%)	Compound Name
1	11.327	0.24	0.26	Dimethyl phthalate
2	12.887	7.11	3.60	Dodecanoic acid
3	14.133	0.06	0.12	Sulfurous acid, 2-propyl tridecyl ester
4	14.208	0.10	0.17	Tetradecanoic acid, 12-methyl-, methyl ester
5	14.575	2.65	2.24	Tetradecanoic acid
6	14.952	0.17	0.15	Octadecanoic acid
7	15.787	1.15	2.33	Hexadecanoic acid, methyl ester
8	16.115	4.13	3.13	n-Hexadecanoic acid
9	16.283	1.41	1.34	Hexadecanoic acid, ethyl ester
10	16.956	1.05	1.64	9,12-Octadecadienoic acid (Z,Z)-
11	17.023	8.60	14.43	9-Octadecenoic acid, methyl ester, (E)-
12	17.190	0.98	1.47	Methyl stearate
13	17.372	10.13	6.55	Oleic Acid
14	17.405	2.35	3.56	9,12-Octadecadienoic acid (Z,Z)-
15	17.462	1.02	2.24	Ethyl Oleate
16	18.194	1.11	1.54	Hexadecanal, 2-methyl-
17	18.559	2.06	1.34	9-Octadecenamide, (Z)-
18	19.181	0.31	0.69	Hexadecanal, 2-methyl-
19	19.222	3.41	5.32	Carbamic acid, 2-(dimethylamino)ethyl este
20	19.368	1.12	1.57	Digitoxin
21	19.521	1.14	1.25	7-Hydroxyfarnesen
22	21.022	21.76	18.81	Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)]dode
23	21.559	4.95	3.83	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-e
24	21.748	1.08	0.87	2H-3,9a-Methano-1-benzoxepin, octahydro
25	21.881	9.07	9.36	5.alpha.-Androstane-3.beta.,17.beta.-diol

DISCUSSION

The eradication of schistosomiasis provided by the WHO's Road map and planned for 2030 requires a significant investment from all disciplinary fields to develop new drugs against human schistosomes and to interrupt the parasite life cycle. The search for drugs derived from natural products has accelerated in recent years. Biochemists, pharmacologists, botanists, and chemists globally are searching for natural products that could

serve as a drug lead for treating various human ailments, including schistosomiasis.

Zoo-chemicals are the animal equivalent of phytochemicals in plants, which are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms [24]. The presence of important zoo-chemicals, including saponins, cardiac glycoside, carbohydrates and terpenoids in crude extracts of toad venom, indicates its potential therapeutic effect [25]. In agreement with the zoochemical findings from this study, the use of animals' venom in the treatment of various ailments is increasing around the globe. The present zoochemical analysis showed the presence of saponins, alkaloids and terpernoids while tannins and flavonoids were absent. On the contrary, the phytochemical screening test by Mandefro *et al*. [26] has indicated the absence of alkaloids. Such differences usually arise from variations in extraction techniques and/or type of the organism used.

Molluscicidal activities of the toad venom showed that when the molluscs were exposed to high concentrations of the toad venom, high mortality was recorded within 24 hours. When *B. pfeifferi* snails were exposed to the concentrations of 100mg/L, 73.3% was recorded after 24 hours. The mean percentage mortality of *B. globosus* when exposed to a concentration of 100mg/L was 66.66%, while *B. forskali* was 60% after 24 hours. This study revealed that toad venom crude extract has a molluscicidal effect against *B. pfeifferi, B. globosus* and *B. forskali* snails with the resulting LC50 of 46.77, 70.79 and 79.43mg/L respectively in 24-hour exposure. The 24 h LC_{90} lethal dose against *B. pfeifferi, B. globosus* and *B. forskali* snails were 89.12, 162.18 and 191.82 mg/L. This result is nearly similar to the LC_{90} values (89.50 and 97.55 ppm) of mesocarp and whole fruits of *Balanites aegyptiaca* tested by [27]. It is also in line with the study carried out by [28], who worked on the Molluscicidal effect of *Achyranthes aspera* L. (Amaranthaceae) aqueous extract on adult snails of *Biomphalaria pfeifferi* and *Lymnaea natalensi* where they recorded the 24 hours LC_{90} lethal dose against *B. pfeifferi* to be 96.5 ppm. As one of the bioactive constituents of toad venom, Saponin may possess molluscicidal activity. This is consistent with the study of [29], who prepared crude saponin of two plants: fruits of wing leaf soapberry (*Sapindus saponaria*) and the leaves and stem of *Buddleia asiatica* and recorded a very strong molluscicidal potency against *Bulinus Alexandrina* at 19 and 11 ppm. The molluscicidal property of toad venom could be a result of the numerous substances that are found in them. These could be alkaloids, saponins, cardiac glycosides, carbohydrates and terpenoids. The result shows that, in the snails, as the exposure time extends from 24 to 72 h, the LC50 decreased. The reason could be the active ingredient in the toad venom is released slowly or remains stable in action for a longer time. Exposure to sub-lethal levels may also have a gradual effect on snail survival Mandefro *et al*. [26]

In this study, toad venom was selected to be tested as a promising molluscicides against three species of snail intermediate hosts schistosoma. The result showed that toad venom had good molluscicidal effects with dose-dependent variations in lethal concentration. In our experiments, *B. pfeifferi* was the most sensitive to the toad's venom molluscicidal effect compared to the other snail species tested. It was followed by *B. globosus* and *B. forskalii*. The

molluscicidal effect of the toad's venom is in accordance with previous reports on the antimicrobial and antitumor activity of toad's venom [30, 31]. It is suspected that treatment with the toad's venom may generate free radicals which could alter the snail's cell membrane and that the snail's death was due to compromised surface membrane permeability/membrane functions [32]. The extract from toad venom seems to be a promising molluscicidal candidate considering the standard criteria established by the WHO with a lethal concentration of 90% and 50% below 400 and 100 ppm respectively. The study also showed that the effective doses of toad venom against the three snails differ only slightly. According to Utzinger and Tanner [33], the three snail species usually coexist in common habitats. Their similarity in sensitivity to toad venom is a useful phenomenon, as a single effective dose could be applied to control such a mixed population.

Toad venom was analysed and observed to possess 25 bioactive metabolites; these include oleic acid and its derivatives, dodecanoic acid and 9-octadecenoic acid (Z), with Pentacyclo [9.1.0.0(2,4).0(5,7).0(8,10)]dode (18.81 %) and 9-Octadecenoic acid, methyl ester, (E)- (14.43%) being the most abundant constituents as revealed by the GC-MS analysis. These compounds have been earlier reported with several bioactivities including antimicrobial [34], anti-oxidant [35], and anti-inflammatory [36]. By implication, the recorded molluscicidal properties of the crude toad venom could be attributed to the presence of the aforementioned bioactive compounds.

CONCLUSION

The present study demonstrated the potential applications of toad venom as a molluscicide in the national schistosomiasis control programs. Indeed, toad venom is efficient in killing three species of snail, i.e. *B. pfeifferi, B. globosus, and B. forskali*., which are considered major snail intermediate hosts of the human schistosomes parasites, including *S. mansoni* and *S. haematobium.* The lethal concentration of the toad venom varied between the three species of snails tested. Therefore, it may become a new and promising molluscicide for extensive application in the field to control schistosomiasis.

Ethical approval

Ethical clearance for the use of experimental animals (Toads and Snails) was obtained from the Ministry of Livestock and Fisheries, Minna, Niger State.

Authors Contribution

IEO, OICJ, ASO and EEC conceptualized the study. IEO, OICJ, ASO and EEC designed the study. IEO, IHC, NJ and IEN participated in fieldwork and data collection. IEO, UFC and EOE performed the data analysis; IEO, UFC and EOE interpreted the data. IEO, IHC, NJ and IEN prepared the first draft of the manuscript, reviewed by OICJ, ASO and EEC. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

No conflict of interest

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