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Original Article

Molluscicidal Activities and Histopatological Effects of Methanol Leaf Extracts of *Ipomea asarifolia* Against *Biomphalaria pfeifferi* In Minna, Niger State, Nigeria

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ABSTRACT

Human African schistosomiasis is a parasitic trematode disease infecting man in tropical and sub-tropical region of the world. The study evaluated the molluscicidal activities and histopathological effects of methanol leaf extracts of *Ipomea asarifolia* against *Biomphalaria pfeifferi*. The snail tissue collected for histopatholigical analysis were fixed with 10 % formaldehyde and dehydrated with descending grade of alcohol, cleared with xylene, and stained with haematoxylin and eosin and viewed under microscope using magnification of x40. Qualitative and quantitative phytochemical screening of the plant was done using standard procedures. Phytochemical screening revealed the presence of phenols, flavonoids, tannins, alkaloids and saponins. Saponins (992.31$\pm$0.41mg/100g) was the most abundant phytochemical while tanins (21.27$\pm$0.37) elicited the least phytochemicals in *I. asarifolia*. Adult snails of *B. pfeifferi* species were exposed to graded concentrations (20, 40, 80, 120, 160, 320 mg/mL) of methanol leaf extract of *I. asarifolia* at 24-, 48- and 72-hours post – exposure period, after which percentage mulluscicidal activity were recorded. The extracts exhibited dose and time dependent of molluscicidal activities with percentage molluscicidal effects. The methanol leaf extracts of *I. asarifolia* against *B. pfeifferi* at 320 mg/mL elicited 100% mortality after 24 hours post exposure. Histopathological examination showed normal cells in the digestive glands of the control group of snails (*B. pfeifferi*) species. There was marked observable tissue damage in the snail exposed to various concentrations of the extracts. Conclusively, *I. asarifolia* contained a significant amount of phytochemical and

exhibited activities thus could be considered to harbor a bioactive agent against schistosomiasis.

Keywords: *Ipomea asarifolia,* Molluscicidal, Histopathological analysis, *Biomphalaria pfeifferi*

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INTRODUCTION

Human African Schistosomiasis is a disease caused by parasitic trematodes of the genus Schistosoma infecting man in tropical and sub-tropical region of the world Viana *et al*. [1] It is among the most prevalent human parasitic infectious diseases ranking second to malaria according to Alemu *et al*. [4] There are five trematodes' species of *Schistosoma* known and these include *Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma intercalatum* and *Schistosoma mekongi* Alemu *et al*. [4] The vector species belong to the family Planorbidae these are *Bulinus globusus, Biomphalaria pfeifferi* and *Onchomelania hupensis quadrasi* Yerinya *et al*.[26] The disease is predominant in Africa, Caribbean, Middle East, South America and South Asia, etc. This endemic disease affects over 240 million people residing in rural and agricultural areas according to World Health Organization [25]. About 800 million people were at risk of infection out of which 16 million were children. Presently, there is no vaccine or drugs to protect against schistosomiasis, treatment of the disease is based on drug called Praziquantel (PRZ). This study therefore will establish the need to search for cheap and safe molluscicides from natural products by Massoud and Habib. [17]

Ipomoea asirifolia commonly known as morning glory plant is a glabrous succulent, perennial plant with large purple flowers trailing on the ground. The plant belongs to the family Convolvulaceae, most genera are weeds or creeping vines with white latex, simple alternate leaves, and showly flowers with infundibuliform corollas Lorenzi *et al*. [16] It is a native of tropical America but now pan tropical. It is also found through West Africa, from Cameroun to Senegal, Mali, Cape Verde Island and tropical Asia. In Nigeria, it is found almost in all parts of the country.

The socio-economic and health effects of schistosomiasis as a Neglected Tropical Disease cannot be emphasized as reported by Aula *et al*. [6] Infected children have retarded growth and poor school performance. The work capacity of rural dwellers is seriously reduced due to weakness and lethargy caused by this disease Massoud and Habib [17]. Current control strategies rely on chemotherapy (praziquantel), but reinfection is common due to persistent transmission from snails by Berquist *et al*. [7] Therefore, plants derived molluscicides offer a sustainable and environmentally friendly alternative to synthetic chemicals according to World Health Organization. [24]

MATERIALS AND METHODS

Study Area

The research was carried out at the Biological Science Laboratory II Department of Animal Biology, Federal University of Technology Minna, Niger State within the period eight Months for the drying of plants leaves, maintenance of the laboratory snails (*Biomphalaria pfeifferi*)

Snail Sample Collections

Snail samples were collected from the surface of the Dam, located at up El-waziri Street in Bosso Local Government Area, Minna, Niger State where they were found attached to the suspended sticks, nylon, top and back of the Dam weeds, rocks and at the bottom of water particularly where it shallow and transparent to the eyes. This method was achieved by hand picking with the aid of scoop net according to Asemota *et al*. [5] The snails were then conveyed to the Biological Science Laboratory II Federal University of Technology (FUT) Minna for maintenance using the standard method by Brown. [9]

Snail Maintenance

Snails were grouped and divided into different plastic trays containing de-chlorinated water. They were allowed to acclimatize to the laboratory environment for a period of 48 hours under room temperature (25 ^{o}c to 28 ^{o}c) and the plastic containers were covered with an incision mark on the various tops for adequate aeration and light for the survival of the organisms. The snail water in the container was changed three times in a week to prevent contamination that will lead to snail mortality by Ekwunife *et al*. [12] Snails were fed with fresh lettuce and was boiled for about 5 minutes and allowed to cool before introducing into the snail containers three times in a week after the water have been changed throughout the period of maintenance in the Laboratory.

Plant Sample Collection and Preparation

Ipomoea asarifolia was collected from its natural habitat on the basis of ethnobotanical information in Iboko Ofu Local Government Area of Kogi State. The plant sample was taken to the Department of Plant Biology Federal University of Technology (FUT) Minna for identification and authentication, after which a voucher specimen for the collections was deposited in the herbarium.

Plant Material Extraction

The extraction of the plant material was carried out according to the method described by Bimakr [8] at the Biochemistry Laboratory in the Department of Biochemistry, Federal University of Technology, Minna, Niger State, then, the resulting powdered plant was preserved separately in a clean dry dark glass bottle and stored in a cool and dry environment till use in the biological test. The crude extracts were obtained using soxhlet apparatus. Methanol was used for the extraction. Filtration was done using Whatman 1 filter paper. The extracts were concentrated using water bath at 45^{o}c to give residue. The crude extracts were weighed and stored in sealed bottles and refrigerated at 21^{o}c.

Phytochemical Screening

The plant extracts were subjected to phytochemical screening using the standard method described by Sofowora [22] in order to detect the presence of phytochemical constituents.

Extract Bioassay

The snail species used in this study was *Biomphalaria pfeifferi* and the molluscicidal evaluation of the plant's potency was done according to the established procedure by WHO. The plant extracts were evaluated for molluscicidal activity on the snail species as follows: Group of ten snails (*Biomphalaria pfeifferi*) were placed in plastic containers holding 500ml of distilled water to have working solution; The different concentrations; 20mg/L, 40mg/L, 80mg/L, 120mg/L, 160mg/L and 320mg/L were prepared by weighing 0.02g, 0.04g, 0.08g, 0.12g, 0.16g and 0.32g then dissolving each in one litre of distilled water. Positive control was prepared as 1mg/litre of ethanol, and the negative control was 500ml of distilled water only. After 24 hours, distilled was discarded from each container holding 10 snails and replaced with 500ml of the different extract concentrations, positive control and negative control. For each dosage, a duplicate set up was made for both snails and plants. The snails were not fed and after 48 hours the extracts were discarded and replaced with distilled water for recovery. After 24 hours of recovery period, snails were observed, and mortality counts were recorded by Asemota *et al*. [5] Dead snails remained retracted inside their shells with discolored body at the bottom of the plastic container. Mortality was also confirmed by the absence or no reaction of the foot with a blunt wooden probe to elicit typical withdrawal movement Labe *et al*. [15]

Percentage mortality = (No of death) / (No of Population) x 100 computed by Schneider. [20]

The values obtained were used to compute the various corresponding minimal lethal concentration (LC_{90}) and median (LC_{50}) concentrations as shown on the probit regression table 3 below.

Histhopathological Analysis of Snail Tissue

A definite number of snails from various concentrations were randomly chosen for histological and ultrastructural studies. The shell of the tested snails was removed by making a cut round the whorls in a continuous manner starting at the aperture opening using a bone scissors and broken fragments of the shells were carefully removed. Dissection was carried out under Zeiss binocular microscope as reported by Hamed *et al*. [14] The histological study involving collection of digestive tissues (gland) from the intestine of treated and untreated (control) snails was performed according to Choji *et al*. [10] Tissue was harvested and fixed in 10 % formalin for 3 days, cut into thin slices of 5 mm x 2 mm x 1mm thick and then processed in the following Spin Tissue Processor (STP) 120 (Thermoscientiic Germany) through; fixation, dehydration, clearing, Impregnation and staining. The staining methods are categorized into two methods: Haematoxylin and Eosin (H & E). Tissues were taken to the water and were embedded in molten paraffin wax using

embedded moulds and embedding casettes on a tissue tek embedding centre and cooled on the cooling component. Sections were dewaxed and hydrated by passing through 3 -changes of xylene treat with descending grade of alcohol absolute (100 %), 90 %, and 70 % for 3 minutes each and then washed in water, stained with haris haematoxylin for 5 minutes and washed in running water. They were differentiated in 1 % acid- alcohol and then washed again in water, blued in scott's tap water substitute for 2 minutes and rinsed briefly in distilled water, counter stain with 1 % eosin for 3 minutes wash well in water, dehydrated in ascending grade of alcohol, 70 %, 90 %, and 100 %, the 100 % grade of alcohol cleared in xylene and mounted with cover slip and Dextrin Plasticizer Xylene (DPX). Sections were then placed in slides carriers and placed in a 40 oven to dry overnight. Microscopically reading; the histological changes in tissue sections of snail were mounted in Canada balsam and finally viewed with light microscope using x10, x40 and x100 objectives according to Okete *et al*. [18]

Data Analysis

Probit analysis of the raw data was carried out using Statistical Package for Social Science (SPSS) software (Version 17.0) designed by Finney and Steven [13] and adopted by Philomina *et al*. [19] to obtain the lethal concentration levels of all tested extracts for *Biomphalaria pfeifferi*. Analysis of Variance (ANOVA) was used to test the significance differences in mean percentage mortality with plant extract concentration as reported by Okete *et al*. [18]

RESULTS

The results obtained from the phytochemical screening of the methanolic extract of *Ipomoea asarifolia* revealed the presence of some phytochemical and a few absences of others as shown in Table 1

Table 1: Phytochemical constituents of methanolic extract of *Ipomoea asarifolia*

Phytochemicals	Test results	Inference
Alkaloids	+	Present
Flavonoids	+	Present
Phenols	+	Present
Saponins	+	Present
Tannins	+	Present
Anthraquinones	_	Absent
Glycosides	_	Absent
Phlobotanins	_	Absent

Steroids	_	Absent

Table 2 showed the molluscicidal activity of methanol extracts of *Ipomoea asarifolia* on adult *B. pfeifferi* after 24, 48, and 72 hours of exposure, and it indicates certain quantities of plant extracts to have 0 – 100% mortality of the experimental organisms.

Table 2: Molluscicidal Activity of Methanol Leaves Extracts of *Ipomoea asarifolia* against *Biomphalaria pfeifferi*

	24 hours	48 hours	72 hours
Conc.mg/L	*I. asarifolia*	*I. asarifolia*	*I. asarifolia*
Control	0.00 ±0.00^{a*}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
20	0.33 ±0.33^{ab}	0.33 ±0.33^{a}	0.33 ±0.33^{a}
40	1.33 ±0.67^{ab}	1.33 ±0.67^{ab}	2.33 ±0.33^{b}
80	2.00 ±0.58^{ab}	2.33 ±o,67^{ab}	4.00 ±0.58^{c}
120	5.67 ±0.88^{d}	6.67 ±0.88^{e}	7.00 ±0.58^{d}
160	8.67 ±1.33^{c}	9.33 ±0.67^{f}	9.67 ±0.33^{e}
320	10.00 ±0.00^{e}	10.00 ±00.00^{f}	10.00 ±0.00^{e}

Table 3: Medial LC50 and LC90 Lethal Concentration of the Molluscicidal Activity of Methanol extract of *I. asarifolia* against *B. pfeifferi*

Exposure Time (Hours)	LC_{50}	LC_{90}	R^2	REGRESSION EQUATION
24	134.38	249.72	0.8407	Y= 0.3468x + 3.3968
48	124.60	239.28	0.7832	Y= 0.3482x + 6.6137
72	107.7	231.28	0.7599	Y= 0.3237x + 15.134

Table 3 showed the LC_{50} and LC_{90} of methanol extracts of *I. asarifolia* exposure time of 24, 48 and 72 hours are 134.38. 124.60, 107.70 and 249.72, 239.48 and 231.28 respectively. The R^2 values (coefficient of determinant) are 0.8407, 0.7832 and 0.7599. This showed a strong relationship (correlation) between the extract concentration and snail mortality.

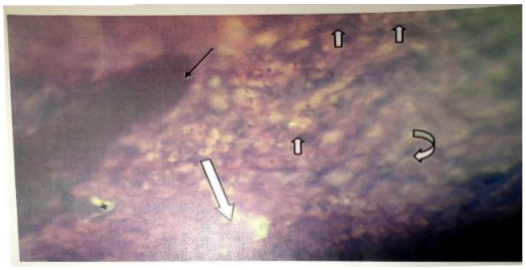

Plate I Control of *Biomphalaria pfeifferi*

Plate I Control of *Biomphalaria pfeifferi*: Transverse section of the digestive gland of *B. pfeifferi* subjected to de-chlorinated water for 24 hours of post exposure. The tissue, basement membrane, excretory cells and other impotant organs were all norma. Haematoxylin & Eosin X400 (H&E X400).

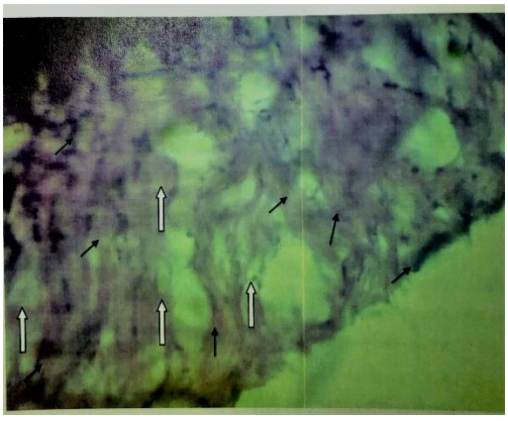

Plate II of *Biomphalaria pfeifferi*

Plate II: Section of the digestive gland of *B. pfeifferi* exposed to concentration of 320mg/L of the Methanol Extracts of *I. asarifolia*.

There was severe inflammation and mass destruction of the cell as a result of the effects of the concentration of the plant extracts on the tissues and glands of the snail species.

DISCUSSION

This study aimed at testing the activity of methanol leave extracts of *Ipomoea asarifolia* and histopathological effects on *B. pfeifferi* (the snail intermediate host) which may offer a sustainable and environmentally friendly alternative to synthetic chemicals in order to reduce the effects of the disease in the population where it is prevalent. In table 2, at 320mg/mL of methanol leaves extract of *I. asarifolia*, recorded 100 % mortality after 24-, 48- and 72-hours post exposure with the control groups recorded no mortality at the three different time post exposure. Also, at 160mg/mL, recorded 80-96 % mortality after 24, 48, 72 hours post exposure respectively.

Table 3 showed the LC_{50} and LC_{90} of methanol extracts of *I. asarifolia* exposure time of 24, 48 and 72 hours and their corresponding values are 134.38. 124.60, 107.70 and 249.72, 239.48, 231. 28 respectively. The R^2 values (coefficient of determination) are 0.8407, 0.7832 and 0.7599 as all these values showed a strong relationship (correlation) between the extract concentration and snail mortality ($p<0.05$) when compared to untreated group, this relationship could be assigned to the fact that the plant is rich in some of the phytochemical components such as saponins and flavonoids which has

antihelminthic properties according to Cowan. [10] This phytochemical screening in this study revealed the presence of phenols, flavonoids, tannins, alkaloids and saponins in the plant extracts. This result is similar to the findings described by Singh *et al*. [21] who reported that, phytochemical studies of plants for the their molluscicidal activities in different geographical locations revealed the presence of saponins, alkaloids and flavonoids. This study also agrees with Labe *et al*. [15] who reported the presence of saponins, cardiac glycosides, anthroquinones, steroids, flavonoids, tannins and alkaloids as the principal constituents of the methanol extracts and aqueous extract of *Jatropha curcas.*

The methanol extracts of *I. asarifolia* against *B. pfeifferi* exhibited no significant mortality at lower concentration of 20-120 mg/L, however all the concentrations of methanol above 120 mg/L extracts yielded various levels of mortality against the snail species. A similar observation was reported by Okete *et al*. [17] for methanol extracts of *Talinum triangulare B. globosus*. Adetunji and Salawu [23] observed similar findings to this study that, the ability of the snails to crawl out of the test extract solution and then aggregate at the water air interface on snails exposed to sub-lethal concentration where their body is not in close and continuous contact to the plant extracts. The histological analysis from this study showed cells in the digestive glands of the control group in (Plate I), while there was a tissue damage, mass destruction and the appearance of the dead cells (Plate II). This finding agrees with Akinpelu *et al*. [3] who reported that, saponins extracted from many plants' sources have been tested to exhibit molluscicidal properties and its targeted sites in the snail intermediate are muscles, tissues, hemolymph, intestine and several organs in the snail. Ahmed *et al*. [2] observed that, the pH of *Euphorbia splendens, Ziziphussspina – christ and Abrosia maritime* were reported to have histopathological effects on the digestive gland of the *Biomphalaria alexandrina* and *Bulinus truncata* where they cause damage to tissues, excretory cells and several organs. This finding agrees with the prent study reported in the research.

CONCLUSION

In conclusion, the study demonstrated significant reduction in control of *B. pfeifferi* snail population with concise effects on its glands and tissues as a result of plant extracts treatment at different time intervals. Conclusively, it can be deduced that, the use of sub-lethal concentration of the plant extracts will be of help in the control of schistosomiasis.

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