MOLLUSCICIDAL EFFECT OF *PIPER GUINEENSE*

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Abstract

The study was undertaken to assess the dosage-mortality ratio and toxic effects of *Piper guineense* fruit extracts on the adults of *Biomphalaria pfeifferi*, the snail intermediate host of *Schistosoma mansoni*, which causes intestinal schistosomiasis. The result showed significant toxic effects with crude ethanol and hot water fruits extracts. The estimated lethal dose concentration by arithmetic method (LC_{50} and LC_{90}) using both crude ethanol and hot water fruit media extracts were found to be (0.10 $\pm$ 0.04, 0.9 $\pm$ 0.2) mgl^{-1} respectively for ethanolic extract and (5.0 $\pm$ 1.4, 8.5 $\pm$ 0.7) mgl^{-1} respectively for hot water extract. Ethanolic extract was more potent than hot water extract. An all or none phenomenon appeared characteristic of the biological activity of these extracts. There was significant decrease in oviposition rate ($p < 0.02$). The extract from the fruits of this tropical plant holds promise in the control of *Biomphalaria pfeifferi*.

Keywords: Molluscicide, *Piper guineense*, fruits, *Biomphalaria pfeifferi*, ethanol and hotwater extracts

Introduction

Well over 271 million people in the world today suffer from intestinal schistosomiasis, of which 168 million appear to be located in tropical Africa (Peters, 1978). Many broad-spectrum synthetic chemical pesticides and molluscicides have been developed recently against this dreadful scourge, targeted against adult and juvenile stages in the life cycle of the intermediate host snail (Iwu, 2000). The economic benefits of these chemicals in relation to disease control, increased food production, reduced labour and costs are recognized (Coles, 1973). However, there is a dearth of concern on the fate of these compounds and their associated problems. For instance, problems like pest's resurgence, resistance and hazard on non-target components of the biosphere (Ukwandu and Okara, 1996).

Hughes (1962) and Ojo (1983) have reported some of these hazards from leaves and roots extracts on insects and environment. Expectedly, a thorough examination of some of the tissues of the target organism has revealed the physiological damage caused by the fruit extracts (Ukwandu, 1996). *Piper* is a large genus of plant with about 700 species distributed throughout tropical and sub-tropical regions of the world (Hutchinson and Dalziel, 1954; 1963). The species that have either pungent or aromatic smelling fruits find wide application in traditional medicine (Nagi and Molan, 1994).

Piper guineense (Piperaceae) is a tropical plant used as spice and for medicinal purpose (WHO, 1990). It is a vine which climbs up to 13m high (Hutchinson and Dalziel, 1954). It is found in almost all the countries in West Africa covering both the forest and savannah regions. Some organic compounds reported by Webbe and Lambert, (1983a) contained in this species of plant include alkaloids, terpenoids, phenols, alcohols and phenolic esters. Piperine is the most abundant.

In China. *P. guineense* fruit extract is used for the treatment of epilepsy (Ray, 1982). In Nigeria, *P. guineense* leaves have shown to exhibit molluscicidal and antibacterial activity amongst inhabitants of the rain forest belt of Western Nigeria (Adegbola, 1972). Hughes (1962) reported piscicidal property of the leaves and this has shown good application amongst fishermen. In Calabar, the South-eastern part of Nigeria, fruit extract of *P. guineense* is used as surf in streams and also in cleansing fresh wounds and old sores (Sofowora, 1993). In the Eastern part of Nigeria, the fruits are used as abortifacient and in the killing and capturing of edible water snails, whose shells are later used as 'corals' by traditional healers in the decoration of their shrine (Oliver-Bever, 1986). Besides, it is used to spice varied dishes for a woman in post-natal, hence this plant is vegetative propagated along the banks of streams and the ripe fruits fall inside the streams.

Watt and Merrill (1963) reported the toxicity of the leaves of *P. guineense* when used as a laboratory food composition for the African giant land snail, *Archachataina marginata* (L) This study was carried out to investigate the toxic effect of the fruit extracts of *P. guineense* on some aspects of the biology of the adults of *B. pfeifferi*.

Material and Methods

Dried fruits of *P. guineense* Schum. and Thom. (Piperaceae) (voucher No. 43129-HNC) were procured from the daily

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market-Monday market in Maiduguri, Borno State and Ekenwuan market, in Benin, Edo State of Nigeria. Lettuce leaves, *Lactuca sativa L*. (Asteraceae) were obtained from the grocery near Leventis Superstores, Ibadan, in Oyo State.

Wild adult snails of *Biomphalaria pfeifferi* were obtained from Awba-dam in the University of Ibadan, a small stream in Jos Metropolis near Terminus Market, scattered dams in Maiduguri Metropolis and Bama, Monguno and Konduga local government areas (LGAs).

Dried black fruits of *P. guineense* were washed thoroughly and dried under the sunlight for about one hr. to make crisp. Air drying method was employed in order to facilitate finer particles when pulverized. Absolute ethanol was used in the soxhlet apparatus to extract all ground/pulverized plant materials. The extracts were then concentrated in a Rotavapour under vacuum of 60 ^{o} C, while the ethanol extract solvent evaporated. Hot water extract was prepared by grinding 80 grams of the fruits and pouring a lotre of hot distilled water (40^{o}C). This was stirred using magnetic atirrer and left for 24 hr. It was from these stock solutions (ethanolic and hot water) that several other concentrations were made by dilution. The concentrations were for ethanolic extract from fruits [(0.0156, 0.025, 0.0312, 0.05, 0.625, 0.10, 0.125, 0.50, 0.75, 1.00, 1.25, 1.50) mgl^{-1}]; and hot water extract from fruits [(1.00, 4.00, 6.00, 8.00, 9.00, 10.00, 11.00, 11.50, 12.00) mgl^{-1}] were the varied lethal test concentrations employed. All controls were 0.00 mgl^{-1}.

Preparation of snail food

Experimental food consisted of blanched and dried lettuce (*Lactuca sativa*), which is normally used as a source of food for snail in the laboratory (Watt and Merill, 1963; Thomas, 1987). *Lactuca sativa* was prepared by putting the green leaves into an already boiled water and left for about 6 mins. This softened and midribs were removed. The softened leaves were them spread on a cardboard paper and dried in an oven for about 30 mins. at a 60 ^{o} C (Adegbola, 1972).

Collection of egg mass, hatchery and culturing of juvenile snails:

Adult of *B. pfeifferi* were collected with the use of scoop net, put in a petri-dish containing small quantity of water and some green leaves of plants. These snails were later transferred into a large beaker of about 2000cm^3 containing dechlorinated water inside a cellophane bag. Some crisps of the dried *L. sativa* were then dropped on the surface of the water as food. Oviposition rate was observed for 48 hrs at intervals of 4 days. The obtained egg masses from each beaker were counted and recorded using light microscope after every 4 days. Egg masses were laid on the surface of the cellophane and cut was made around each egg mass. This cellophane cut, with egg mass was incubated for 7 days in dechlorinated water inside a Petri dish for hatching to take place. Juveniles were collected using small brush into beakers containing dechlorinated water for culturing into adulthood.

Determination of adult lethal concentration:

From the pilot test, the range finding tests were determined. These concentrations of ethanolic and hot water extracts were used to determine the median lethal concentration LC_{50} of the botanical extract. Each concentration test had two replicates. Dechlorinated water was used as control standard. Ten (10) snails were used in each replicate and tests were carried out in accordance with the specifications of WHO, (1965). Each test snail was put in a litre of dechlorinated water after bred in a litre of varied concentrations of extracts. The experiment was carried out under humid conditions for 48 hrs and the snails were fed with shreds of *L. sativa*. Death was determined by removing snails from dechlorinated water, after 24 hrs, and those that did not retract from touch with the brush were considered dead.

Statistical analysis

Various concentrations were subjected to analysis of variance test to determine the differences between the molluscicidal effects of the concentrations. In case where the null hypothesis was rejected, the student's Newman-keuls test was used to determine the differences between the concentrations.

Results

Table 1 shows the mean and percentage survivors of *B. pfeifferi* subjected to different concentrations of ethanolic extract of the fruits of *P. guineense*. The correlation coefficient (r) as calculated was 0.81. This was negative and fairly large while the r as tabulated was 0.602 (n – 2) = 9. The difference between the two values of r was significant (p= 0.05).

The mean and percentage survivors of the adults of *B. pfeifferi* subjected to different concentrations of hot water extract of fruits of *P. guineense* are shown in table 2. The r as calculated was 0.66. This was negative and large while r as tabulated was 0.602 (n – 2) = 9. The difference between the two values of r was significant (p = 0.05). Significant level of difference ($p<0.03$) existed comparing values for calculated r for both media extracts while non significant level of difference ($p>0.05$) existed for tabulated values of r for both media extracts. However, in all cases for the plant extracts, the null hypothesis r = o was rejected because the values for calculated r were consistently larger than the r tabulated. This significant difference between the two values ($p<0.05$) suggest inverse relationship between extract concentrations and percentage survivors of test organisms.

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Table 3 shows the arithmetic estimation of the median lethal concentration LC_{50} and LC_{90} of the ethanolic and hot water extracts of fruits of *P. guineense* on the adults of *B. pfeifferi* which were observed for 24 hrs. The estimated average values taken as final values of LC_{50} and LC_{90} were 0.10 $\pm$ 0.04 mgl and 0.9 $\pm$ 0.20 mg/1, respectively, which is significant ($p<0.04$) for ethanolic extract and 5.0 $\pm$ 1.40 mg/1 and 8.5 $\pm$ 0.70 mg/1, respectively, for hot water extract. This is not significant ($p>0.06$). However, there are much significant difference between $LC_{50's}$ and $LC_{90's}$ for both media extracts ($p<0.02$ and $p<0.01$) respectively.

Table 4 shows the effect of ethanolic extract on the rate of oviposition of the adults of *B. pfeifferi*. Both calculated and tabulated (F) were 3.71 and 3.01 respectively, which is significant (p=0.05). The values for the calculated and tabulated (t) were 2.29 and 2.01 respectively, where (n – 2) = 11. These two values were significant (p = 0.05). However, comparing values for the calculated F and t shows much significance ($p<0.01$), while the corresponding values for both showed non significance ($p>0.05$).

Table 1. Mean and percentage survivors of the adult of *B. pfeifferi* subjected to different concentrations of the ethanolic extract of the fruits of *P. guineense* for 24 hrs.

Concentrations (mgl^{-1})	Log. concentration	No. of survivors Replicates (R) R1	R2	Average	Percentage (%)survivors
0.0		10	10	10±0.0	100
0.015	-1.82	9.0	7.0	8.0±1.4	80
0.031	-1.51	7.0	6.0	6.5±0.7	65
0.062	-1.20	6.0	5.0	5.5±0.7	55
0.125	-0.90	3.0	4.0	3.5±0.7	35
0.25	-0.60	3.0	3.0	3.0±0.0	30
0.50	-0.30	2.0	3.0	2.5±0.7	25
0.75	-0.12	2.0	2.0	2.0±0.0	20
1.00	0.00	1.0	1.0	1.0±0.0	10
1.25	0.11	1.0	1.0	1.0±0.0	10
1.50	0.18	0.0	0.0	0.0±0.0	0

Correlation coefficient (r) calculated = 0.81 (negative and fairly large)
(r) tabulated = 0.602; Degree of freedom (df) = 9; Degree of probability (P) = 0.05

Table 2. Mean and percentage survivors of the adult of *B. pfeifferi* subjected to different concentration of hot water extract of the fruits of *P. guineense* for 24 hrs.

Concentrations (mgl^{-1})	Log. concentration	No. of survivors Replicates (R) R1	R2	Average	Percentage (%)survivors
0.00		10	10	10±0.0	100
1.00	0.00	10	10	10±0.0	100
4.00	0.60	7.0	8.0	7.5±0.7	75
6.00	0.78	4.0	5.0	4.5±0.7	45
7.00	0.85	3.0	4.0	3.5±0.7	35
8.00	0.90	2.0	3.0	2.5±0.7	25
9.00	0.95	1.0	1.0	1.0±0.0	10
10.00	1.00	0.0	0.0	0.0±0.0	0
11.00	1.04	0.0	0.0	0.0±0.0	0
11.50	1.06	0.0	0.0	0.0±0.0	0
12.00	1.08	0.0	0.0	0.0±0.0	0

Correlation coefficient (r) calculated = 0.66 (negative and fairly large)
(r) tabulated = 0.602; Degree of freedom (df) = 9; Degree of probability (P) = 0.05

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Table 3. Estimation of the LC_{50} and LC_{90} of *P. guineense* (ethanolic and hot water extracts of fruits) on the adults of *B. pfeifferi* observer for 24 hr. by arithmetic method.

Toxicant	Arithmetic Method	Mean	LC's (mgl^{-1})
Ethanolic	$(0.062\pm0.125)mgl^{-1}$	0.10±0.04	LC_{50}
Extract	$(0.75\pm1.00)mgl^{-1}$	0.9±0.2	LC_{90}
Hot water	$(4.00\pm6.00)mgl^{-1}$	5.0±1.4	LC_{50}
Extract	$(8.00\pm9.00)mgl^{-1}$	8.5±0.7	LC_{50}

Note: LC = Lethal concentration

Table 4. Determination of the effect of ethanolic extract of the fruits of *P. guineense* on the rate of oviposition.

Time (Days)	Infected	Control
4	90	102
8	30	133
12	28	28
16	26	26
20	26	30
24	29	115
28	40	118
32	68	121
36	-	-
40	45	96
44	-	84
48	-	92
Total mean for 26 days	31.8±27.1	78.8±45.3

f (calculated) = 3.71; f (tabulated) = 3.01; P(probability) = 0.05; t (calculated) = 2.29
t (tabulated) = 2.01; P (probability) = 0.05; d/f (degree of freedom) = 11

Discussion

The ethanolic and hot water extracts of the fruits had generally a lethal effect on the adults of *B. pfeifferi*. The lethal concentration (LC_{50} and LC_{90}) for the adults were 0.10 ± 0.04 mg/1 and 0.9 ± 0.20 mg/1 respectively, for ethanolic extract and 5.0 ± 1.4 mg/land 8.5 ± 0.70 mg/1 respectively, for hot water extract by arithmetic method. There was an increased order of potency from hot water extract to ethanolic extract. This was shown by the number of snails that died. When the cause of death and reduced rate of oviposition were studied using ethanolic extract of the sub-lethal dose concentration (SLDC), it was observed that the tissues affected evidenced by the presence of extract, included digestive glands, liver, gonads and the shells which became brittle. However, this can be subjected to further investigations, but the resultant effect of this on digestive glands and liver corroborates previous preliminary results that extracts' active ingredients of the fruits are better extracted with ethanol (Ukwandu, 1996; Arfaa, 1990; WHO, 2002). This agrees with Lemma, (1970); that there is a high degree of potency of the molluscicidal plant, *Phytolacca dodecandra*, when extracted with butanol than hot water. Adewunmi and others (1980) were also supportive of this view when they extracted another molluscicide, *Tetrapleura tetraptera* with methanol and found it more potent than other media extracts.

There was a significant reduction effect on the rate of egg laying of *B. pfeifferi* due to the effect of the extract on the gonads compared with those snails reared in normal dechlorinated water as control. This is in agreement with the results of Webbe and Lambert (1983a, 1983b; Adegbola, 1972), when the former used a potential molluscicide, aridanin on *B. glabrata*, while the later studied the molluscicidal properties of some African plants. Webbe and Lambert (1983a and b) reported on the destructive effects of aridanin on the reproductive systems of snails of *B. glabrata.* There was an observed all or non principle shown in the biological behaviour of the ethanolic crude extract as reported by Adegbola, (1972). Our results demonstrated a

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principle of all or none regarding the biological behaviours of the crude extracts of both ethanolic and hot water extracts. This would have explained the results of Webbe and Lambert (1983a and b).

Our results have emphasized an all or none principle as the differences in percentage survivors correlated with geometric increase in dosage concentrations. It is interesting to note that the snails were able to concentrate the molluscicide – *P. guineense* in its tissues, thus subjecting itself to intoxication. The high retention of this botanical in the gonads evidenced by low ovipositionary rate points to a possible toxic influences rather than reflecting an excretory process. There is the need to elucidate the structure/activity relationships of the active ingredients contained in this plant.

Since man also eats this plant in the form of spice (Trease and Evans, 2002), studies are also necessary on the toxicologic properties of the various *P. guineense* plant species on the various human tissues; organs; and systems like epithelial; liver, pancrease; and digestive, excretory; respectively to ascertain safety at intolerable concentration level. Better methods of propagation are also needed considering the fact that this plant is wild, and holds promise in the control of the snail intermediate host of intestinal schistosomiasis.

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