PRELIMINARY SCREENING OF SOME NIGERIAN MEDICINAL PLANTS FOR MOLLUSCICIDAL ACTIVITIES

Amenze Asemota¹, Adesola Hassan², MacDonald Idu³

¹Department of Animal and Environmental Biology, University of Benin, P.M.B 1154, Benin City, Nigeria
²Department of Zoology, University of Ibadan, Oyo State, Nigeria
³Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.
mcdonald.idu@gmail.com.

ABSTRACT: In a search for natural products that could be used to control the vectors of tropical diseases, four medicinal plants grown in Nigeria; Moringa oleifera, Senna alata, Caesalpinia pulcherrima, and Vernonia amygdalina were screened for molluscicidal effects. Serial dilutions of 1000, 625, 500, 375, 250, and 125 ppm were prepared from the stock solution of the extracts. Adult Biomphalaria pfeifferi were exposed to the various graded concentrations. Mortalities were recorded after 24 hours. The results of mortality and the assessment of the lethal concentrations for 50% and 90% (LC50 and LC90) mortalities of snails were determined using probit analysis. There was a high degree of homogeneity between concentration and mortalities observed; R² = 0.919, 0.976, 0.963, 0.988 for ethanolic extracts of M. oleifera, Senna alata, C. pulcherrima and V. amygdalina respectively. There were positive correlations between mortalities observed in snails and extracts’ concentrations. Aqueous and ethanolic extracts of V. amygdalina had the lowest LC50 values (614.8 and 338.8 ppm) making it the most potent of the plants used in this study. All extracts caused significant mortality of B. pfeifferi (P < 0.05). These plant extracts generally showed promising molluscicidal activity and are recommended for further studies.

Keywords: Molluscicidal Effects, Biomphalaria Pfeifferi, Lethal Concentration, Medicinal Plants.

INTRODUCTION

Schistosomiasis is an endemic parasitic disease which affects tropical and subtropical regions of the world. It is caused by several species of trematodes (flukes), a parasitic worm of the genus Schistosoma [1]. The number of cases due to schistosomiasis has been estimated to be around 200 million worldwide with 650 million people being at risk of infection [2]. In Nigeria, approximately 22 million Nigerians, including 16 million children, need to be treated for schistosomiasis, making the country the most endemic in the world. The best way to break the cycle of infection and reinfection is by effective control of the snail intermediate host; attempts at this are usually made by controlling the intermediate snail hosts usually by killing them. The way this has been done overtime is by using a molluscicide called niclosamide which is quite expensive and could be toxic in the environment. Since the imported synthetic compounds are of high cost, the discovery of plant derived compounds that could help in the eradication of the disease would be of great value [3]. A number of plant species have been shown to have molluscicidal effects on snail vectors. Crude water extracts of Alternanthera sesselis showed molluscicidal activity against B. globosus [4], Adetunji and Salawu [5], also reported the efficacy of ethanolic leaf extracts of Carica papaya and Terminalia catappa against B. pfeifferi and B. globosus while Adenusi and Odaibo [6], worked on the molluscicidal, ovicidal and cercaricidal activities of the parts of Dalbergia sissoo, Otarigho and Morenikeji [7] investigated the molluscicidal effects of aqueous and ethanolic extracts of Lemongrass (Cymbopogon citratus) against different developmental stages of Biomphalaria pfeifferi.
The plants used in this study, Moringa oleifera, Senna alata, Caesalpinia pulcherrima and Vernonia amygdalina were selected on the basis of their local use in herbal treatment. The aim of this study is to test these plants for molluscicidal activity on the adult snail- Biomphalaria pfeifferi which is the major intermediate host for Schistoma mansoni in Nigeria.

MATeRIAL AND METHoDS
CollECTION and IDENTIFICATION OF PLANT MATERIAL
The selected plant species (Moringa oleifera, Senna alata, V. amygdalina and Caesalpinia pulcherrima) were collected locally from their natural habitat. Plant samples were collected from a home garden in Benin City (S. alata and V. amygdalina) and from the Botanical garden, University of Ibadan (M. oleifera and C. pulcherrima). Harvesting was done by using a sharp knife with hands properly protected with gloves in order to avoid contact with the milky latex that exudes from some of the plants which causes irritation and itching of the skin. The collected plants were covered with wet sacks to avoid the effect of direct sunlight, which could lead to dehydration, then taken to the laboratory.

Plant specimens used in this study were identified in the Herbarium, Botany and Microbiology department, University of Ibadan. The plants were cleaned to remove foreign materials and thereafter were air dried under room temperature (25-30oC) for weeks in the laboratory until they became crisp. Crisp leaves were properly ground into fine powder using Moulyneux Blender. The powdered samples were stored in moisture-free, airtight Ziploc bags and kept in a cool dry place for further use.

Snail Collection and Identification:
Snails were collected from the Eleyele Reservoir in Ibadan. This dam was the first modern water supply system for Ibadan city. The Dam at present is being fished intensively by local fishermen. Snails were collected from floating plants that were used as attachment sites by hand picking between the hours of 8.00am and 12.00 noon. Collected snails were put in a sterile plastic bowl containing sterile cotton wool soaked with Dam water. They were then taken to the Parasitology Research Laboratory, Department of Zoology, University of Ibadan. The snails used in this study were Biomphalaria pfeifferi. The snails were identified to species level by Prof. A.B. Odaibo of the Department of Zoology, Faculty of Science, University of Ibadan.

Laboratory Protocol
Snail maintenance
Snails were acclimatized for 48 hours to laboratory conditions in a transparent glass aquarium containing dechlorinated tap water. Snails were maintained in aquaria of glass troughs (32 cm diameter X 12 cm depth) with capacity of about 6 litres.

Lettuce (Lactuca sativa) was used in feeding the snails for the duration of the experiment. The midrib of a single leaf of lettuce was removed and the leafy part immersed in hot water for about 60 seconds then quickly cooled in cool water. Thereafter, the leaves were dried, powdered and stored in an airtight container. Snails were fed 3-4 times a week on this. Water in the aquaria was changed at least once a week to prevent contamination leading to death of snails.

Extraction procedure:
Five grams of each dried plant part material was weighed using Metler Digital Weighing Balance. A stock solution was prepared by placing the 5 grams of each powdered dry plant part material in 150ml of distilled water for 24 hours with occasional vigorous shaking. The extract was filtered through a cotton cloth after 24 hours and thereafter with filter paper. The marc (residue left after filtering and pressing the plant part mixture) was washed with several portions of distilled water to adjust the volume of the solution; using volumetric flasks to 200ml (25,000 ppm). The aqueous plant extracts was used immediately after the extraction to ensure its freshness.

Ethanolic extract was prepared using the same technique but after extraction, the solvent was removed by evaporation and the volume adjusted to 200ml

TreatmenT protocol for concentration-response relationship:
The molluscicidal potency tests were investigated according to the primary screening technique recommended by [8]. Different extracts of 0.0 (Control), 2.0, 3.0, 4.0, 5.0, 8.0, and 10.0 ml (six concentrations and a control) was taken from the working solution and added to equal volume of dechlorinated tap water in crystallizing dishes (5cm depth by 10 cm diameter). Then the concentration of each solution was calculated in part per million (ppm). The experiment was run in duplicates and the average taken.
Ten (10) snails of length ranging from 5.0mm to 7.0mm, which had been acclimatized for at least 10 days in the laboratory, were immersed in solution; the exposure period for each test was 24 hours. Snails were prevented from crawling out of glass troughs by covering with a light, clean, transparent fabric secured with an elastic band. The treated snails were then transferred to de-chlorinated water for a recovery period of 24 hours, thereafter mortality counts were recorded. All the snails which at the end of the recovery period remained completely within their shells, or floated upside down on the surface of the solution and/or settled at the bottom without response to mechanical prodding were considered dead. Negative control experiments were conducted using under the same conditions using only de-chlorinated water without plant extracts. Snails were not fed throughout the duration of this part of the experiment.
Mortality counts were then taken after careful observation using a light microscope and the results subjected to statistical analysis.
The data obtained was subjected to Probit analysis [9]. The LC50 and LC90 values with 95% confidence intervals of all tested extracts for B. pfeifferi adults were determined by analysis of the mortality data and logarithm of the concentration in ppm using SPSS 15.0 for Windows. Regression coefficient (R2) was calculated to show the degree of homogeneity between concentration of the plant samples and mortality of snails recorded. T-test was used to determine the statistical level of significance.

RESULTS
Snails initially retreated into their shells when placed in untreated dechlorinated water; there was subsequent activity after some minutes.
Generally, mortalities increased with extracts concentration. Table 1 shows the percentage mortality of B. pfeifferi snails exposed to different concentrations (ppm) of extracts. Ethanolic extracts of V. amygdalina showed very good molluscicidal activity at 625ppm killing off 100% of the snails, while ethanolic extracts of S. alata killed off 85% at that same concentration. Aqueous extracts of M. oleifera had no effect on B. pfeifferi snails at 250ppm and only 45% of the snails were killed at 1000ppm. Distilled water extracts of V. amygdalina still had the greatest effect on B. pfeifferi snails with 80% deaths occurring at 1000ppm.

Table 1: Percentage mortality of B. pfeifferi snails exposed to different concentrations (ppm) of extracts.

<table>
<thead>
<tr>
<th>Plant spp</th>
<th>Extract</th>
<th>1000</th>
<th>625</th>
<th>500</th>
<th>375</th>
<th>250</th>
<th>125</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. oleifera</td>
<td>E</td>
<td>85.0±0.71</td>
<td>80.0±0.00</td>
<td>70.0±0.00</td>
<td>35.0±0.71</td>
<td>30.0±0.00</td>
<td>10.0±0.00</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>45.0±0.71</td>
<td>25.0±0.71</td>
<td>15.0±0.71</td>
<td>10.0±0.00</td>
<td>-</td>
<td>-</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>S. alata</td>
<td>E</td>
<td>100.0±0.00</td>
<td>85.0±0.71</td>
<td>55.0±0.71</td>
<td>30.0±0.00</td>
<td>15.0±0.71</td>
<td>10.0±0.00</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>70.0±0.00</td>
<td>55.0±0.71</td>
<td>20.0±0.00</td>
<td>15.0±0.71</td>
<td>10.0±0.00</td>
<td>-</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>C. pulcherrima</td>
<td>E</td>
<td>100.0±0.00</td>
<td>90.0±0.00</td>
<td>55.0±0.71</td>
<td>40.0±0.00</td>
<td>40.0±0.00</td>
<td>10.0±0.00</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>60.0±0.00</td>
<td>55.0±0.71</td>
<td>40.0±0.00</td>
<td>35.0±0.71</td>
<td>30.0±0.00</td>
<td>20.0±0.00</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>V. amygdalina</td>
<td>E</td>
<td>100.0±0.00</td>
<td>100.0±0.71</td>
<td>95.0±0.00</td>
<td>60.0±0.00</td>
<td>35.0±0.00</td>
<td>-</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>80.0±0.00</td>
<td>75.0±0.00</td>
<td>40.0±0.71</td>
<td>20.0±0.00</td>
<td>10.0±0.71</td>
<td>-</td>
<td>100.0±0.00</td>
</tr>
</tbody>
</table>

E=Ethanol, D=Distilled water; - = no response SD= standard deviation; O-=negative control (dechlorinated water)

The Lethal concentration at which 50% and 90% of the snails were killed (LC50 and LC90) was calculated (Table 2). The results obtained from the toxicity tests showed that the four (4) plant extracts all had some degree of activity. Table 2 also shows the confidence limits (95%) and the regression equations for the toxicity of all extracts used on B.pfeifferi. Ethanolic extract of V. amygdalina had the lowest LC90 value at 614.8ppm, this makes it the most potent of all the plant extracts used; it also had the lowest LC50 value at 338.8ppm.
<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent used</th>
<th>Regression equations</th>
<th>R2</th>
<th>P value</th>
<th>Std. error</th>
<th>X</th>
<th>Y</th>
<th>Std. error</th>
<th>LC50 (ppm)</th>
<th>LC90 (ppm)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. oleifera</td>
<td>Ethanol</td>
<td>Y=0.003X-1.410</td>
<td>0.919</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.324</td>
<td>488.7</td>
<td>0.002</td>
<td>1057.0</td>
<td>1600.0</td>
<td>-1.734 to -1.086</td>
</tr>
<tr>
<td>D. Water</td>
<td>Ethanol</td>
<td>Y=0.002X-2.496</td>
<td>0.889</td>
<td>0.002</td>
<td>0.001</td>
<td>0.535</td>
<td>932.9</td>
<td></td>
<td></td>
<td></td>
<td>-3.035 to -1.957</td>
</tr>
<tr>
<td>S. alata</td>
<td>Ethanol</td>
<td>Y=0.005X-2.322</td>
<td>0.976</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.497</td>
<td>474.8</td>
<td>0.001</td>
<td>736.7</td>
<td></td>
<td>-2.820 to -1.825</td>
</tr>
<tr>
<td>D. Water</td>
<td>Ethanol</td>
<td>Y=0.003X-2.261</td>
<td>0.976</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.533</td>
<td>753.0</td>
<td></td>
<td>1179.8</td>
<td></td>
<td>-2.703 to -1.819</td>
</tr>
<tr>
<td>C. pulcherrima</td>
<td>Ethanol</td>
<td>Y=0.004X-1.831</td>
<td>0.963</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.417</td>
<td>408.5</td>
<td>0.002</td>
<td>694.3</td>
<td></td>
<td>-2.249 to -1.414</td>
</tr>
<tr>
<td>D. Water</td>
<td>Ethanol</td>
<td>Y=0.002X-1.174</td>
<td>0.841</td>
<td>0.002</td>
<td>0.001</td>
<td>0.539</td>
<td>712.7</td>
<td></td>
<td>1490.8</td>
<td></td>
<td>-1.470 to -0.877</td>
</tr>
<tr>
<td>V. amygdalina</td>
<td>Ethanol</td>
<td>Y=0.009X-2.982</td>
<td>0.988</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.732</td>
<td>338.8</td>
<td>0.001</td>
<td>484.4</td>
<td></td>
<td>-3.714 to -2.250</td>
</tr>
<tr>
<td>D. Water</td>
<td>Ethanol</td>
<td>Y=0.003X-2.454</td>
<td>0.961</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.511</td>
<td>614.8</td>
<td></td>
<td>990.0</td>
<td></td>
<td>-2.509 to -1.681</td>
</tr>
</tbody>
</table>

All extracts were toxic to B. pfeifferi snails, although to varying degrees. Ethanolic plant extracts were generally more potent than aqueous extracts of all plants screened.

Ethanolic extracts of V. amygdalina showed the strongest degree of homogeneity between mortalities observed and extracts’ concentration; R² = 0.988 while extracts of S. alata had the highest degree of homogeneity among aqueous extracts of all plants (R² = 0.976). V. amygdalina, Senna alata, and C. pulcherrima showed promising molluscicidal activities against adult snails, while the aqueous extract M. oleifera demonstrated weak molluscicidal activity. There were strong positive correlations between mortalities observed in snails and extracts’ concentrations; R² = 0.988, 0.963, 0.976 and 0.919 in ethanolic extracts of V. amygdalina, C. pulcherrima, S. alata, and M. oleifera respectively (Fig 1-4).

**Fig 1. Snail mortality in response to concentration (ppm) of Moringa oleifera extracts**
Fig 2. Snail mortality in response to concentration of Senna alata extracts

Fig 3. Snail mortality in response to concentration of C. pulcherrima extracts
Fig 4. Snail mortality in response to concentration of V. amygdalina extracts

The lethal concentrations (LC50) were 338.8, 408.5, 474.8, 488.7 for ethanolic extracts of V. amygdalina, C. pulcherrima, S. alata and M. oleifera respectively and the corresponding LC90 values were 484.4ppm, 694.3ppm, 736.7ppm and 932.9ppm (Fig 5-8). V. amygdalina had the lowest LC50 value (614.8ppm and 338.8ppm for aqueous and ethanol extracts respectively) making it the most potent of all the plants studied. The least mortality was recorded in aqueous extract of M. oleifera with LC50 of 1057.0ppm.

Fig 5: Probit regression graph for the determination of lethal doses of M. oleifera leaf extract on snails
Fig 6: Probit regression graph for the determination of lethal doses of S. alata extracts on snails.

Fig 7: Probit regression graph for the determination of lethal doses of C. pulcherrima extracts on snails.
Fig 8: Probit regression graph for the determination of lethal doses of V. amygdalina extracts on snails

Statistical analysis showed high significant difference at 0.05 levels between LC50 and LC90 values of all plant materials extracted. The p values of all extracts showed statistical significance (P<0.05) of mortalities. It was observed that at high concentrations (1000ppm), crawling out of ethanolic extracts of V. amygdalina, S. alata, and C. pulcherrima by snails was greatly reduced and in most cases non-existent; probably due to their acute toxic effects at this concentration. This was a welcome development since the snails died quickly thus stopping their early escape from the extract solution.

DISCUSSION
In the present study, four medicinal plant species (Moringa oleifera, Senna alata, Caesalpinial pulcherrima, Vernonia amygdalina) from Nigeria were preliminarily screened for their molluscicidal activity against Biomphalaria pfeifferi adult snails using their aqueous and ethanolic extracts.

Data obtained from this preliminary study on ethanolic extracts of M. oleifera, S. alata and C. pulcherrima showed promising molluscicidal activity. B. pfeifferi was susceptible to the various extracts at different concentrations. V. amygdalina ethanolic extract that killed 50% (LC50) and 90% (LC90) of adult snails was 338.8ppm and 484.4ppm respectively. This is considered a good rate since the potency of these extracts was much higher when compared to extracts used in other works. Adetunji and Salawu [5] reported LC90 values of 1222.8ppm and 4515.9 ppm for ethanolic extracts of Terminalia catappa and Carica papaya respectively. Aqueous extracts of V. amygdalina, S. alata, C. pulcherrimma and M. oleifera had LC90 values of 990.9ppm, 1179.8ppm, 1490.8 and 1600ppm respectively; their potency was higher than [10] who reported LC90 values of 1100ppm, 1386ppm and 2085ppm of aqueous extracts of Calotropis procera, Nicotiana tabacum and Trigonella foenum respectively.
Ethanolic extracts of V. amygdalina had the highest potency with the lowest LC50 and LC90 values (338.8ppm and 484.4ppm) while aqueous extracts of M. oleifera had the least potency with the highest LC50 and LC90 values (1057.0ppm and 1600.0ppm).

There was a marked difference in potency of the aqueous extracts and the ethanolic extracts. This may be as a result of the fact that ethanol is a much better solvent than water. It was also observed that snails initially retreated into their shells when placed in untreated dechlorinated water, there was subsequent activity after some minutes. This was also observed by [11], who stated that the snails resumed normal activity after 45 minutes. This initial inactivity and subsequent activity after a while may be due to changes in temperature and physicochemical properties of the water in the laboratory compared to the lake water. When the water is treated with the extracts, the snails again withdraw into their shells, some begin crawling up the glass trough mostly remaining at the water-air interface. Again this was also observed by [5, 12] in the pulmonate snail, Bulinus. Adenusi and Odaibo [6] properly observed the snails where their shells were partially immersed in water and their tissues a little above the water. This mechanism of B. pfeifferi leaving treated water partially has been found to increase the survival rate of some species [13]. The distress syndrome observed in this study is characteristic for all molluscicides (both natural and synthetic) and they may occur as a result of loss in the control of water balance [14]. The water imbalance caused by the introduction of plant extract creates anaerobic conditions that induce snail inactivity and its extrusion from the shell [15]. Gaseous exchange in pulmonates occurs both cutaneously and through the ‘lung’, a modified vascularized area in the mantle cavity [16]. The site of oxygen entrance may be affected by the plant extract since any damage to the cutaneous respiration would result in a change in oxygen consumption. Among the plant species that showed molluscicidal activity one has been tested for its molluscicidal activity by other researchers. Vernonia amygdalina was reported by [17] (LC90, 6241.61mg/ml). The present study, however showed V. amygdalina possessing more activity (LC50, 338.4mg/ml; LC90, 483.9mg/ml) than observed in this previous study. This difference may be due to the extraction methods or to seasonal and geographical variations of the plant. Ethanolic extracts of some extracts especially V. amygdalina had pretty quick action on the snails. This may be due to the high toxic effects at high concentrations. This is a good thing since it reduces the possibility of the snail escaping the extracts [13]. The mortality caused by the plants extracts showed a high degree of homogeneity of regression coefficients (R2) between dose and mortality, because an increase in concentration of the working solution resulted in more intake or entry of molluscicidal extract into the body of the snail. This trend is also independent of several factors such as rate of penetration, nature of slope, variability and maximal effects of active moieties [18].

In this study only leaves of M. oleifera, V. amygdalina, C. pulcherrima and S. alata were used; [19] reported that although the roots, stem, seeds and fruits were more potent in several molluscicidal plants, it will probably be necessary to use the regenerating leaves which are easier to harvest. The leaves used in this study have many advantages which support their molluscicidal potency; they are readily available in endemic areas, easily cultivated, inexpensive and can be applied with ease. Although several studies have assessed the molluscicidal activities of many plants against B. pfeifferi, no study on the toxicity of these folkloric medicinal plants has been conducted in Nigeria on the snail intermediate host of schistosomiasis. Since their medicinal activities have already been established, this new approach therefore, widens the spectrum of their medicinal values. Plant molluscicides provide cheap, locally available, biodegradable and effective control agents in rural areas of developing countries where schistosomiasis is endemic [20]. Snail control should not be overlooked even now that there is a drug Praziquantel for treatment, there are a lot of logistic problems in mass treatment. There is also the risk of reinfection. According to World Health Organization, crude organic extracts should present LC90 below 20 ppm to be considered as good molluscicide candidate for direct application in infested water [21]. However, it is possible that extracts active between 20 and 500ppm could contain small amounts of very active components, which could be isolated and/or concentrated using simple procedures, or even obtained from other plants known to produce it in larger amounts. It is also possible that using other extraction techniques (Soxhlet extraction, successive method distilled water extraction e.t.c) and solvents (chloroform, methanol, petroleum ether e.t.c) could drastically reduce the lethal concentrations of the plants used in this study especially the more active ones. Further studies could be done to validate this. The results obtained from this study yielded some promising plant molluscicide candidates and deserve further studies in order to identify and characterize their molluscicidal components and also to confirm the results obtained here.
CONCLUSION
The results gotten from this study shows that Vernonia amygdalina, Senna alata, Caesalpinia pulcherrima and Moringa oleifera should be considered as potential molluscicides that will be helpful in the control of schistosomiasis. Further studies are required for a more comprehensive evaluation of these plants.

REFERENCES