Comparative Molluscicidal Activity of Aqueous and Methanolic Extracts of *Zingiber officinale* against *Bulinus globosus*

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**ABSTRACT**

Preliminary laboratory studies were conducted to compare the molluscicidal potency of aqueous and methanol extracts from rhizome of *Zingiber officinale* commonly known as ginger. *Bulinus globosus* snails, the intermediate host of *Schistosoma haematobium* in Nigeria, were exposed to varying concentrations [10-1000mg/L (ppm)] of aqueous and methanol extracts of *Zingiber officinale*. The reference molluscicide (Yomesan®) was used as standard positive control and deionized and dechlorinated water as negative control. Snail mortalities were compared between those exposed to aqueous and methanol extracts of *Zingiber officinale*. LD$_{50}$ and LD$_{90}$ values for the different extracts were computed. *Bulinus globosus* was most susceptible to methanolic extract [LD$_{50}$ 214.72 (182.56-263.49) mg/L (ppm)]. The potency of *Z. officinale* depended on relative increase in concentration of the methanol extract (p>0.05). The snails were not susceptible to the aqueous extract of *Z. officinale*, hence, no mortality was recorded at 1000ppm. In view of its many other uses, besides as a molluscicide, we recommend further studies on *Zingiber officinale*.

**Key words:** *Zingiber officinale*, molluscicidal activity, *Bulinus globosus*, Aqueous extract, Methanol extract.

**Introduction**

Urinary schistosomiasis caused by *S. haematobium* is also endemic in many parts of Nigeria [17]. Reports of Mafiana and Omotayo [11] and Anosike et al., [5] have contributed to the knowledge of the biology of snail intermediate hosts of schistosomes in some parts of Nigeria. *Bulinus globosus* serves as an intermediate host for *Schistosoma haematobium*.

The transmission of the infective stage of the parasite is accentuated through shedding of the cercariae by the snail host and the various human water contact activities [6].

The most efficient methods of controlling schistosomiasis is by the use of molluscicides to control the intermediate snail hosts. For this reason, a number of chemicals and synthetic molluscicides such copper sulphate and Bayluscide have been used to control fresh water snails with varying results [8]. Furthermore, synthetic molluscicides degrade slowly in the environment and often attack non-target organisms such as fish [14]. The high costs and toxicity of synthetic molluscicides, has stimulated renewal interest in plant molluscicides [12]. Biological control alone as a part of integrated snail control strategy stands to be a better alternative to the chemical control [9].

*Zingiber officinale* (Zingiberaceae) also called ginger is a herbaceous rhizome cultivated extensively in almost all tropical and subtropical countries. It is widely distributed all over Bangladesh, India, Jamaica, Nigeria and Taiwan. It grows up to a height of about 90cm. Ginger is a carminative, pungent stimulant used widely for dyspepsia, stomach ache, malaria and fevers [18]. Extracts of *Zingiber officinale* (ginger) rhizome showed activity against *Schistosoma mansoni* miracidia and cercariae [1].

**Materials And Methods**

**Plant Material:**

Fresh rhizomes of *Zingiber officinale* were obtained from Samaru market, Zaria, Nigeria. The authentication was done in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The rhizomes were air dried at room temperature in the laboratory for two (2) weeks. They were pulverized in a mortar and pestle. They were defatted with n-Hexane solvent and subsequently with ethyl acetate solvent. The
resultant marc was extracted with methanol as described by [13].

**Preparation Of Plant Extract:**

One kilogram (1kg) of powdered air-dried ginger was boiled for 24 hours in 2.5 litres of methanol in Soxhlet apparatus. This was concentrated in-vacuo to dryness using water bath to obtain the methanol extract. The same quantity of ginger powder was cold macerated with distilled water at room temperature for 24 hours. The resultant mixture was then filtered using Whatman’s filter paper No. 1 and the filtrate was concentrated to dryness using water bath to obtain aqueous extract of *Zingiber officinale*. This was scrapped and stored at 4°C. For the molluscicidal testing, all preparations except aqueous extract were first homogeneously suspended in TWEEN 80 then with distilled water for use on each day of the experiment.

**Phytochemical Screening:**

The methanol and aqueous extracts were phytochemically screened for secondary metabolites such as Carbohydrates, Glycosides, Anthraquinone, Cardiac glycosides, Saponins, Steroids and Triterpenes, Flavonoids, Tannins and Alkaloids [16].

**Test Snails:**

Adult snail samples of *Bulinus globosus* were obtained from Danfodio stream in Zaria, Nigeria and maintained in a glass aquarium with some sand and snail food (Pawpaw leaf, *Tridax procumbens*) in 1000ml of dechlorinated tap water bubbled with atmospheric air. Snails were prevented from crawling out of glass container by means of a fine stainless steel mesh placed above the water surface.

**Molluscicidal Activity Tests:**

Stock solutions of concentration series in gram per litre of water (1000 mg/L) were freshly prepared with distilled water from the methanol and aqueous extracts of *Zingiber officinale*. Different concentration solutions ranging from 10-1000 mg/L (ppm) were prepared from the stock solutions using deionized and dechlorinated water.

Molluscicidal evaluation of the methanol and aqueous extracts of *Zingiber officinale* was performed according to WHO [17] guidelines. The test snails (10 each) were challenged with various concentrations of both the methanol and aqueous extracts of *Z. officinale*. Each test concentration was duplicated resulting in the use of a total of 20 snails per nominal concentration. The set up was allowed to stand undisturbed for 24 hours and snails exposed to bioassay were not fed. After 24 hours of exposure to the methanol and aqueous extracts of *Z. officinale*, the snails were transferred to fresh dechlorinated and deionized water and maintained for another 24 hours. Death of snails was confirmed by the absence or no reaction to irritation of the foot with a needle to elicit typical withdrawal movement. Deionized and dechlorinated water (negative control) and niclosamide (Yomesan®) (positive control), were used to monitor the susceptibility of snails and to compare its potency with the methanol and aqueous extracts of *Z. officinale*. Lethal concentrations and their 95% confidence limits were determined by probit analysis [2,3].

**Results:**

The result of the phytochemical screening of the methanol extract of *Z. officinale* revealed the following metabolites: Carbohydrates, Glycosides, Cardiac glycosides, Saponins, Steroids and Triterpenes, others are Flavonoids and Tannins. While for aqueous extract, metabolites present were: Carbohydrates, Glycosides, Cardiac glycosides, Saponins, Steroids and Triterpenes, others are Flavonoids and Alkaloids (Table 1).

Each snail on exposure to the untreated water tanks (negative control) initially withdrew into its shell but resumed normal activity after about 30 minutes. The snails retracted immediately on application of a mechanical stimulus.

In the methanol extract of *Z. officinale*, its toxic effects became evident on the test snails. There was either partial retraction (withdrawal response) or no retraction at all (in the dead snails).

Development of hemorrhagic blisters over the foot sole was noted, with visible swelling of the cephalopodial mass. A visible change in colour of the shell was also noted (dead snails). Mucus secretion was observed in higher test concentration and was also dose-dependent. However, in the aqueous extract of *Z. officinale*, no mortality was observed in the test snails.

From this study, the LD50 for the methanol extract of *Zingiber officinale* was 214.72mg/L while no mortality was observed in the aqueous extract (Table 2).

**Discussion:**

Snails in the test solution were observed to move to the side of the glass beaker in an attempt to escape as reported by Brackenbury and Appleton, [7], Ojewole, [13] and Gehad [9]. There was increased mortality with relative increase in concentration of the methanol extract of *Zingiber officinale*. Niclosamide (Yomesan®), used as the reference molluscicide, was active at less than 1mg/L. In the negative control experiment, no mortality was recorded. The activity of the methanol extract of *Zingiber officinale* was dose-dependent using the log-dose probit analysis. The dose mortality graph
exhibit steep slope values for methanol extract unlike in aqueous extract as shown (Figure 1). The steepness of the slope line indicates that there is a large increase in the mortality of snails with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the snails to the molluscicide used. This agrees with Singh et al., [15]. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of toxicological mechanism, yet it is a useful parameter, for such a study. Adewunmi et al., [1] have also reported molluscicidal activities of Zingiber officinale. The dry rhizome of ginger contains 1-4% volatile oils which is responsible for the characteristic odour and taste [18]. The molluscicidal activity of the tested plant extract is probably due to the presence of saponins (Table 1). However, it has been established that not only saponins but also some sesquiterpenes, flavonoid, glycosides as well as phorbol esters possess molluscicidal properties [4,10,14].

Table 1: Phytochemical constituents of the methanol and aqueous extracts of Zingiber officinale.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Glycosides</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Saponins</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Steroids and Triterpenes</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Tannins</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>A</td>
<td>P</td>
</tr>
</tbody>
</table>

Key: P= Present  
A= Absent

Table 2: Molluscicidal concentration of methanolic and aqueous extracts of Zingiber officinale and Niclosamide on adult Bulinus globosus.

<table>
<thead>
<tr>
<th>Molluscicides</th>
<th>Lethal concentration Values and limits (mg/L)</th>
<th>Slope function</th>
<th>Chi-square ($\chi^2$) at p= 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of Z. officinale</td>
<td>LD$_{50}$ 214.72(182.56-263.49)</td>
<td>0.058</td>
<td>12.61</td>
</tr>
<tr>
<td>Aqueous extract of Z. officinale</td>
<td>NMR</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>Positive control Niclosamide (Yomesan®)</td>
<td>LD$_{50}$ 0.04 (0.03 – 0.082)</td>
<td>-20.2</td>
<td>4.979</td>
</tr>
</tbody>
</table>

Key: 0.1 – 10ppm = very strong molluscicidal activity, 50-100ppm = moderate to strong. 100-200ppm = mild to moderate. 200-400ppm = weak to mild.
Niclosamide (Yomesan®), used as positive control reference molluscicide killed all the snails at a dose of 1ppm. On the contrary, none of the snails (in the negative control) treated with deionized and dechlorinated water alone died.
NS -Computed $\chi^2$ not statistically significant.
() - Lower and upper limits of LD$_{50}$ values in parenthesis.
NMR - No Mortality Recorded

The inference from this observation is that the tissues of the cephalopedal mass had accumulated water, which caused haemorrhage at lethal concentrations of the active methanol plant extract, thus, preventing its normal osmoregulatory function. The toxic effect of the sublethal doses of the extract was, however, reversible after exposure of the snails when moved to non-toxic-extract-free water for a recovery period. This observation is also in agreement with the findings of Gehad et al., [9]. The results showed that the methanol extract was more potent than the aqueous extract. As the extract entered the snails’ body, a muscular twitching happened and the snails became spirally twisted, which resulted in ataxia, convulsion, paralysis and finally death of snails. Prior to death, there was complete withdrawal of the body inside the shell that indicated nerve poisoning. This was however not observed in the aqueous extract.

Conclusion:

The results of this present study have shown that Zingiber officinale possess molluscicidal properties against the intermediate host, Bulinus globosus. However, further research is recommended using different solvents for extraction and comparing their activity. Plant molluscicides are readily available, inexpensive and environmentally safer for controlling human schistosomiasis. This will not only eliminate the economic burden of importing expensive synthetic molluscicides but also stimulate growth of small-scale industries in Nigeria. In conclusion we can say that finding of present...
communication have great potential as molluscicides. Low toxicity to non-target animals, make it more suitable for snail control programme and development of indigenous molluscicides. If plant molluscicides are applied successfully, there will be sustainable control of schistosomiasis.

Fig. 1: Probit transformed responses of Log concentration (ppm) of methanol and aqueous extracts of Zingiber officinale.

References


