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The molluscicidal effects of *Hyptis suaveolens* on different stages of *Bulinus globosus* in the laboratory

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The molluscicidal effect of the ethanolic extract of the plant *Hyptis suaveolens* was evaluated against different stages: eggs, juveniles and adults of the freshwater snail *Bulinus globosus*. Ten healthy one week old juveniles were exposed to different concentrations of the extract for 3 h, while three to four weeks old snails and the adults were exposed for 24 h. The egg masses were exposed for seven days. The mortality rates varied with concentrations and time of exposure. The LC₅₀ values for the eggs, of one week old juveniles, three to four week old immature snails and the adult snails were 0.614, 0.196, 0.161 and 0.077 ppm, respectively. The corresponding LC₉₀ values were 0.796, 0.353, 0.274 and 0.467 ppm, respectively. The results showed that the egg stage of *B. globosus* was the most resistant to the ethanolic extract of *H. suaveolens*.

Key words: *Hyptis suaveolens*, *Bulinus globosus*, mortality rate, LC₅₀ and LC₉₀.

INTRODUCTION

Schistosomiasis is a serious health problem in the tropical and sub-tropical region of the world and in Nigeria. The control has been targeted at the adult worms within the human definitive host through the use of praziquantel. However, its inability to kill schistosomula that are 3 to 21 days old and parasite resistance following intensive use are its limitations (Williams et al., 2001; Nissen and Walker, 2005). The second phase of the control strategy is focused on the parasite intermediate snail host. The currently chemical molluscicide of choice 'Niclosamide' which proved to be the most potent against the intermediate snail hosts of schistosomiasis has also faced the problem of recolonization of transmission foci by snail hosts (Pieri et al., 1995). Its lethal action on non target organisms such as amphibians, fishes and aquatic organisms has also limited its application on the field (Andrews et al., 1983).

The use of plant products in the battle against tropical disease is well known. This includes the use of plant extracts as molluscicides. Many plants have been screened for their intrinsic molluscicidal properties in an attempt to find an affordable alternative to niclosamide that is suited for use in self-help schistosomiasis control

programmes (Brackenbury and Appleton, 1998). However, despite the discovery of several promising plant molluscicides, none of them has yet been used in control campaigns.

In Nigeria, there have been few reports of the screening of plant materials as molluscicides. Ndifon (1980) reported that extracts from a subaquatic macrophyte, *Althernanthera sessilis*, on which the snail normally light and lay his egg have molluscicidal, ovicidal and cercariacidal properties. Adewunmi (1993) also gave a comprehensive report on the molluscicidal properties of Aridan, *Tetraplera tetraptera*. The laboratory experiment conducted showed the effectiveness of *T. tetraptera* against *Bulinus globosus*, *Lymnaea natalensis*, *Lymnaea columella*, *Blomphalaria pfeifferi*, *Biomphalaria glabrata* and *Physa waterlotti* at concentration less than 10 ppm for the methanolic extracts. Adenusi and Odaibo (2007) assessed the molluscicidal, ovicidal and cercari-cidal activities of the crude aqueous and ethanolic extracts of *Dalbergia sissoo*. The new approach to the search of plant molluscicides as reported in this study is the use of ethanolic leaf extract of *Hyptis suaveolens*.

H. suaveolens (Labiaceae) is commonly called bush-tea. It is an aromatic annual woody herbaceous plant that appears after first rain. It is a fast growing herb found to be growing in dense clumps and also along roadsides. It is considered as a weed worldwide. The stem is squarish

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leaves are opposite, petiolate, ovate, apex acute, margin dentate and base cordate. Purple flowers are borne on verticillaster inflorescence. The plant contains ethereal oil, monoterpenes, diterpenes, suaveolic acid, suaveolol, triterpenoid, hydrocyanic acid, sterol, campesterol, fucosterol, sesquiterpene alcohols and fatty acids (Mallavarapu et al., 1993; Ngassoum, 1999). It is traditionally used in the treatment of respiratory tract infection, cold, pain, fever, cramps and skin diseases (Iwu, 1993). An infusion of the plant is used to treat catarrhal conditions, infections of the uterus and parasitical cutaneous diseases. The leaf juice is taken for colic and stomach-aches. Powder of leaf is used as snuff to stop bleeding of the nose. In Philippines, the leaves are used as antispasmodic. A decoction of the roots is used as appetizer and the root is chewed with betel nuts as a stomachic (Dalziel, 1937). This work therefore is designed to evaluate molluscicidal properties of *H. suaveolens* in Nigeria.

MATERIALS AND METHODS

Sampling of *Bulinus globosus*

Young adult of *B. globosus* were collected from the littoral zone of Awba reservoir 'a man made lake' in the University of Ibadan, Nigeria, using scoop net. They were properly washed in the water and transferred into a container. The snails were brought to the Parasitology Laboratory, Department of Zoology, University of Ibadan, for the study.

Snail culturing

30 sampled snails were transferred into each Haverian jar (aquarium) lined with transparent polythene bag containing dechlorinated tap water filled three-quarter to the brim. The snails were fed with blanched lettuce (*Lactuca sativa*). The pale-colour egg masses laid by the adult *Bulinus* snails after about 4 to 5 days of sampling were transferred into dechlorinated tap water in Petri dishes. Incubation was done as described by Madsen (1987) at room temperature between 26 and 28°C. Hatching was observed in about 6 to 7 days. The cut polythene bags were removed and as the snails grew older, they were conveyed into larger containers.

Collection and drying of *H. suaveolens*

Leaves of *H. suaveolens* were collected along roadsides and in some dilapidated buildings in Ile-Ogbo, Osun State, Nigeria. The plant was taken to the Department of Botany and Microbiology, University of Ibadan for identification in the department's herbarium. The leaves were dried for about two weeks at room temperature. The dried leaves were then pulverized using an electric grinder.

Extraction of plant extracts using soxhlet extractor

The extraction of the plant extracts using 75% ethanol was done using soxhlet extractor in the Department of Chemistry, University of Ibadan, Ibadan, Nigeria. Boiling flasks of 250 ml capacity were washed with detergent and were properly rinsed with clean water. They were dried in the oven at 105 to 110°C for about 30 min. They

were transferred into desiccators and were allowed to cool. 5 g of the pulverized leaves were weighed into a labeled thimble. The boiling flask was filled with 200 ml of 75% ethanol and the extraction thimble was plugged tightly with cotton wool. The soxhlet apparatus was then assembled to allow for reflux for about 6 h. After 6 h, the thimble containing the sample was removed with care and the ethanol on the top was drained into a container. The extract was then concentrated using water bath, which removed the ethanol component leaving behind greenish-brown viscous oil used for the toxicity bioassays.

Toxicity bioassay

A stock solution of 1000 ppm was prepared by dissolving 0.1 g of solid extracts in dechlorinated tap water and then made up to 100 ml. Serial concentrations of 0.1, 0.2, 0.31.0 ppm were prepared from the stock solution.

Ten healthy adult of *B. globosus* of length ranging from 6.0 to 8.0 mm that have been acclimatized for at least 10 days and 3 to 4 weeks old (2.0 to 4.0 mm) cultured snails were exposed to the different concentrations for 24 h. The one week old juveniles (0.8 to 1.5 mm) were exposed for 3 h, while the egg masses were exposed for 7 days (determined by the time mortality that was first observed). The control experiments were also set up for each of the different stages. All experiments were conducted at room temperature (26 to 28°C). The snails were not fed during the course of the experiment and the tests were done in duplicate. The mean mortality rate was observed at time interval of 1 h. The photomicrography of the eggs within each of the egg masses was taken to observe the ovicidal activities of the extracts.

The data obtained from the mortality rate of the different stages of the snail were plotted on probit regression graph to obtain the LC₅₀ and LC₉₀, respectively (Finney, 1971).

RESULTS AND DISCUSSION

Effects of plant extracts on embryos within the egg masses

The toxicity effect of the plant extracts on embryos within the egg masses is shown in Figures 1 and 2, respectively. Generally, the mean mortalities increased with concentration and time of exposure. No mortality was recorded in the embryos within the egg masses exposed to the first three concentrations (0.1, 0.2 and 0.3 ppm). However, all embryos were found dead in 0.7, 0.8 and 1.0 ppm on the 7th day of exposure.

Effects of plant extracts on one week old juvenile snails

The general 'distress syndromes' observed as a result of exposure of various growing stages of the snail to the plant extracts were: extension of the cephalopodal (cephalopodan) mass from the shell aperture, inability to attach to the substrate, excessive production of mucus and swelling of the body. In addition to these, the snails tried to escape from the extract solution by crawling to the side of the container. These effects were found to be more pronounced with increase in the concentration of

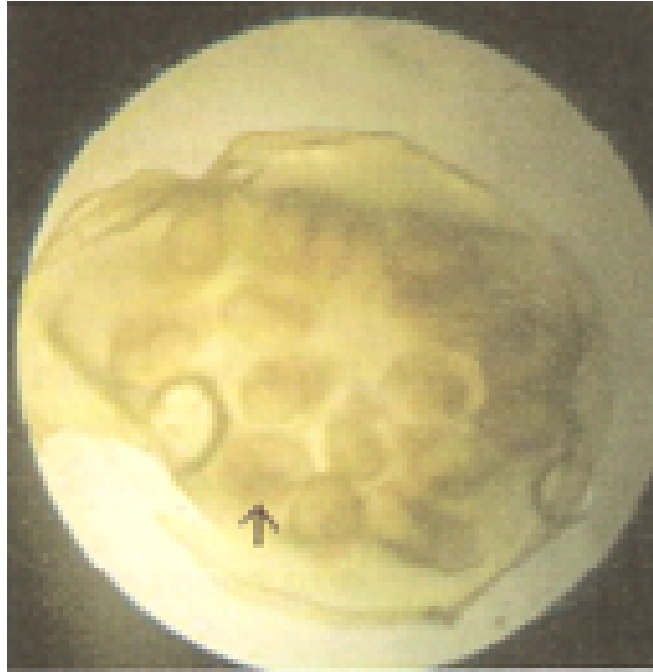


Figure 1. Control. The arrow shows active embryo.



Figure 2. Exposed egg mass. The arrow shows deformed embryo.

the plant extract.

No mortality was recorded in the first 2 h of the experiment in 0.1 ppm extract concentration. However, increase in concentration to 0.2 ppm resulted in the death of an average of 1.0 snail within 30 min to 1 h of the

experiment. A drop in mean mortality to 0.6 and 0.8 snails was recorded on 0.3 and 0.5 ppm, respectively, at the same time, while a progressive increase from an average of 0.3 to 2.6 snails was recorded in 0.6 and 1.0 ppm, respectively. The most effective concentration after

Table 1. Toxicity of extract to 1 week old juveniles of *B. globosus*

Concentration (ppm)	Mortality in time (h)				
	0.5	1.0	1.5	2.0	3.0
0.1	-	-	-	-	3.1
0.2	1.0	1.0	2.5	4.0	5.5
0.3	0.6	3.5	3.6	6.2	7.0
0.4	0.7	5.6	5.8	8.0	9.5
0.5	0.8	4.3	7.1	9.5	10.0
0.6	0.3	7.0	7.0	7.6	10.0
0.7	0.7	7.9	8.0	8.1	9.8
0.8	2.0	7.7	8.9	9.5	10.0
0.9	2.5	7.0	8.2	9.0	9.9
1.0	2.6	7.0	9.5	10.0	9.9

1 and 2 h of exposure are 0.7 and 1.0 ppm, killing an average of 7.9 and 10 snails, respectively. All snails were observed dead in 0.5, 0.6 and 0.8 ppm after 3 h (Table 1).

Effects of plant extract on 3 to 4 weeks old immature snails

Mortality was delayed till 24 h of exposure in 0.1 ppm, whereas the mean mortality was 1.7 snails in 0.2 ppm just after 2 h of exposure and there was no death in 0.3 ppm within the same time. Mortalities recorded in 0.6, 0.7 and 0.8 ppm were 5.0, 5.0 and 6.2 snails, respectively. Interestingly, 0.9 ppm recorded lower mortality of 3.5 snails. The mean mortalities were 1.7, 8.3, 8.0, 9.3 and 10 snails in 0.2, 0.4, 0.6, 0.7 and 1.0 ppm, respectively, after 4 h of exposure, while 3.5 and 5.0 snails were recorded in 0.2 and 0.3 ppm, respectively, after 6 h exposure. These increased sharply to 8.3 snails in 0.4 and 0.5 ppm and attained a mean mortality of 10 snails in 0.6, 0.9 and 1.0 ppm. Mortalities of 8.5 and 8.9 snails were recorded in 0.4 and 0.5 ppm, respectively, while all snails were found dead in other ranges of concentration (0.6 to 1.0 ppm) after 8 h of exposure. The dead snails were 3.3 in 0.1 ppm after 24 h exposure time. Mortality increased to 5.0 snails in 0.2 ppm and all snails were dead in 0.3 to 1.0 ppm (Table 2).

Effects of plant extracts on adult *B. globosus*

The adult snails reacted to the extracts as early as 2 h after exposure even in the lowest concentration (0.1 ppm). The dead snails recovered increased slightly from the average of 0.5 to 2.5 snails as concentration increased from 0.1 to 0.4 ppm. This increased gradually to 4.0 snails in 0.7 ppm and decreased slightly to 3.5 snails in 1.0 ppm. The average death recovered after 4 h were 2.0, 2.5, 3.0, 3.5 and 6.0 snails in 0.1, 0.2, 0.4, 0.6

and 0.7 ppm, respectively. The mean mortality dropped to 4.0 snails in 1.0 ppm with 0.7 and 0.9 ppm been the most effective concentrations killing an average of 6.0 snails. The mean mortalities after 6 h of exposure were 2.0, 3.0, 4.5, 5.0 snails in 0.1, 0.3, 0.4 and 0.5 ppm, respectively with 0.8 ppm been the most effective concentration recording 8.5 dead snails. All snails were observed dead in 0.7 and 0.8 ppm after 12 h of exposure. However, the mean mortality decreased from 9.5 to 8.5 snails in 0.9 and 1.0 ppm, respectively (Table 3). The mean mortality was 5.0 snails in 1.0 ppm after 24 h of exposure. A sharp increase to 8.0 snails was recorded in 0.2 and 0.3 ppm which in turn dropped to 7.0 snails in 0.4 ppm. All snails were found dead in 0.6 to 1.0 ppm extract concentrations. No snail was observed dead in the control experiment.

Lethal concentrations (LCs) determination

The LC₅₀ and LC₉₀ values which are the extract concentrations required to kill 50 and 90% of the embryos enclosed in the gelatinous layer of the egg masses (after 7 days exposure) were calculated as 0.614 and 0.796 ppm, respectively. For the different growing stage of snails (one week old juvenile, 3 to 4 weeks old immature snails and the adult snails), the LC₅₀ values were calculated and shown to be at an approximate value of 0.196, 0.161 and 0.077 ppm, respectively (Table 4), corresponding to LC₉₀ values of 0.353, 0.274 and 0.467 ppm.

The confidence limits (95%) and the regression equations of the toxicity of *H. suaveolens* extract on the different stages of *B. globosus* as obtained from the probit analysis is summarized in Table 4.

The snail response to the different extract concentrations showed that the extract from *H. suaveolens* had some molluscicidal effects. The general distress syndromes observed as a result of exposure of the various stages of the snail to plant extract are in line with the

Table 2. Toxicity of extract to 3 to 4 weeks old immature snails.

Concentration (ppm)	Mortality in time (h)				
	2	4	6	8	24
0.1	-	-	-	-	3.3
0.2	1.7	1.7	3.5	3.5	5.0
0.3	1.5	8.3	8.3	8.5	10.0
0.4	1.5	8.3	8.3	8.5	10.0
0.5	2.6	8.3	8.3	8.9	10.0
0.6	5.0	8.0	10.0	10.0	10.0
0.7	5.0	9.3	9.4	10.0	10.0
0.8	6.2	7.7	8.8	10.0	10.0
0.9	3.5	9.0	10.0	10.0	10.0
1.0	8.1	10.0	10.0	10.0	10.0

Table 3. Toxicity of extract to adult *B. globosus*.

Concentration (ppm)	Mortality in time (h)				
	2	4	6	12	24
0.1	0.5	2.0	2.0	3.0	5.0
0.2	1.5	2.5	3.0	4.0	8.0
0.3	2.5	3.0	3.0	4.5	8.0
0.4	2.5	3.0	4.5	5.0	7.0
0.5	2.0	3.5	5.0	6.5	8.5
0.6	2.0	3.5	4.0	5.5	10.0
0.7	4.0	6.0	8.0	10.0	10.0
0.8	3.0	5.5	8.5	10.0	10.0
0.9	5.0	6.0	8.1	9.5	10.0
1.0	3.5	4.0	8.2	8.5	10.0

Table 4. Probit analysis of lethal concentration determination of extract on exposed stages of *B. globosus*.

Stage	Regression	Chi square (p > 0.05)	LC ₅₀	LC ₉₀	Confidence limit	
					Lower	Upper
Egg	Y=-4.327+7.050X	12.859	0.614	0.796	0.517	0.715
1 week	Y=-1.606+8.180X	211.550	0.196	0.353	-	-
3-4 week	Y=-1.822+11.311X	2.353	0.161	0.274	0.091	0.211
Adult	Y=-0.253+3.286X	4.364	0.077	0.467	-0.291	0.207

reports of Harry et al. (1957). These syndromes have been suggested to be the possible causes of mortality in snails due to molluscicides (Clark and Appleton, 1996). The distress syndromes observed in the study is characteristic for all molluscicides (both plant and synthetic) and they may occur as a result of loss in the control of water balance (Webb, 1987). The water imbalance caused by the introduction of plant extract creates anaerobic conditions that induce snail inactivity and its extrusion from the shell (Von et al., 1950). Gaseous exchange in pulmonates occurs both cutaneously and through the 'lung', a modified

vascularised area in the mantle cavity (Cheng and Sullivan, 1977). Any damage to the cutaneous respiration would therefore result in change in oxygen consumption. The site of oxygen entrance may therefore be affected by the plant extract. The resumption of activity observed in the survivor snails after they were washed and reintroduced into the dechlorinated water might be due to the accessibility of oxygen to the organs and tissues of the snail in the new environment. Sermsart et al. (2005) has also reported the recovery of *Indoplanorbis exustus* when exposed to the extract of *Euphorbia milli*.

The haemolymph of freshwater snails is hypertonic to

the external medium and this makes most molluscan tissues to be highly permeable, maintaining water balance continually (Clark and Appleton, 1996). The swelling of the body observed in the exposed snails could be due to the damage done to the epithelial membranes which could in turn lead to the accumulation of water in the tissues. This is similar to the report of Cheng and Sullivan (1977) on the exposure of snails to copper. Swelling of the body has been suggested to interfere with respiration and death by asphyxiation (Osterberg, 1987).

The results obtained from the toxicities studies showed that ethanolic extract of *H. suaveolens* was effective against the embryos with the egg masses. Sukumaran et al. (1994, 1995, 2002) also reported the potency of butanolic extract of some plants like *Sapindus trifoliatus*, *Agave americana*, *Balanites aegyptica*, *Jatropha gossypifolia* and *Vaccaria pyramidata* against freshly laid eggs of *Lymnaea luteola*. The inability to record mortality in the lower concentrations (0.1, 0.2 and 0.3 ppm) after 7 days of exposure could be due to the presence of protective covering of capsular jelly like materials that covers and protects the freshly laid eggs from the external environment. The ovicidal action was found to increase with increase in extract concentration and age of the eggs. Parashar et al. (1995) reported a similar trend in the ovicidal action of niclosamide against the eggs of *Lymnaea auricularia*. Copper sulphate also induced similar effects against the eggs of *Taphius glabrata* and the embryonic developmental stage of *Biomphalaria pfeifferi* (Shiff et al., 1970). The higher LC₅₀ and LC₉₀ required to kill embryos within the egg masses when compared to the lethal concentrations of other stages showed that the egg was the most resistant stage to the extract of *H. suaveolens*. The extracts of few plant molluscicides like *Euphorbia splendens*, *Phytolacca dodecandra* and *Tetrapleura tetraptera* were also reported to exhibited lower toxicity towards earlier developmental stages than adults (De Souza et al., 1987; Adewunmi, 1993). The result on deformation of the early embryonic developmental stages (especially the gastrula) is in agreement with Zhang and Guo (1992) results on the eggs of *Oncomelania* snails exposed to bromoacetamide.

Generally from the result, it could be deduced that the mortalities recorded varied with increase in concentration of the extract of *H. suaveolens*. The delay in mortalities to 3 h and 24 h in the 1 week old juveniles and 3 to 4 week old immature snails in 0.1 ppm extract concentration could be due to the accumulation of the molluscicide in the tissues and organs of the snails at these stages. Sukumaran et al. (2004) also reported the accumulation of niclotinilide in the body of snail resulting in slow molluscicidal action after 24 h of exposure. The LC₅₀ results showed that the 1 week old juveniles were most resistant to the plant extract having LC₅₀ value of 0.196 ppm when compared to the 3 to 4 weeks old immature snails (0.161 ppm) and the adult snail (0.077 ppm).

This may be due to the shorter period of exposure of the juveniles to the plant extract (maximum period of 3 h), while other stages were exposed for 24 h. The LC₉₀ values suggested that during the course of exposure of snails to the extract, the adult snails used for the study showed greater adaptability to unfavourable environmental conditions brought about by the extract concentrations. The LC₉₀ value was reported as 0.467 ppm against the LC₉₀ values of 0.353 and 0.274 ppm of the 1 week old juveniles and 3 to 4 week old immature snails, respectively. This indicates that the adult snails were the least susceptible to extract concentration with time. Parashar et al. (1990) and Sukumaran et al. (2004) also reported the toxicity of niclotinilide against *Indoplanorbis exustus* snails and indicated the young adult stage as least susceptible. The higher LC₉₀ value (0.353 ppm) recorded in the 1 week old juveniles when compared to that of the 3 to 4 week old immature snails (0.274 ppm) could be due to shorter period of exposure of the 1 week old juvenile to the plant extract (having maximum period of 3 h as against 24 h of the 3 to 4 week old immature snails).

No death was recorded in all the control tests involving the 3 to 4 weeks old and the adult snails within 24 h of the experiment. However, in the 1 week old juvenile snails, there was a natural response rate of 0.22. The LC₅₀ and LC₉₀ values of the extract that acted on the 1 week old juveniles (without natural response rate, that is, extract effect) were 0.196 and 0.353 ppm, respectively. Those of the control (with natural response rate, that is, death due to unfavourable environmental conditions) were 0.230 and 0.376 ppm. The smaller LCs values of the exposed juveniles when compared to the control experiments further indicated the potency of the plant extract on the target organisms as they were more susceptible to attack in the presence of plant extract.

Conclusion

The use of plant extract of *H. suaveolens* gave impressive molluscicidal effects on the various stages of the snail intermediate host of *Schistosoma haematobium* (*Bulinus globosus*). Success has been recorded in many other works and many plants have been investigated for their mollusciciding activities. Nevertheless, since the discoveries of many of these important plant molluscicides some decades ago, none has been improvised and made available like niclosamide in the market for commercial use. Further, studies on the environmental safety of *Hyptis* spp. extracts are recommended.

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