CELL BIOLOGY & DEVELOPMENT
Volume 1, Number 1, June 2017
Pages: xxxx

E-ISSN: 2580-4499
DOI: 10.13057/cellbioldev/t0101xx

Toxicity of *Randia nilotica* fruit extract on *Schistosoma mansoni, Biomphalaria pfeifferi* and *Bulinus truncatus*

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Manuscript received: xxxx. Revision accepted: xxx June 2017.

Abstract. *Ebodi AYE, Ahmed MM. 2017. Toxicity of Randia nilotica fruit extract on Schistosoma mansoni, Biomphalaria pfeifferi and Bulinus truncatus. Cell Bio Dev 1 (1): xxxx.* The aqueous filtered and unfiltered extract of the fruits of *Randia nilotica* (locally name as Shagarat El-Murfaein) were assessed as molluscicides against *Biomphalaria pfeifferi* and *Bulinus truncatus* as well as their effect on cercariae and miracidia of *Schistosoma mansoni*. The plant was tested on uninfected *B. pfeifferi* and *B. truncatus*, the results showed that unfiltered extracts were found relatively more potent than filtered one (i.e. 100% was attained at 90 ppm and 80 ppm) respectively. While, filtered extract tested on uninfected *B. pfeifferi* and *B. truncatus* (100% was attained at 100 ppm and 90 ppm) respectively. The effect of unfiltered extract on infected *B. pfeifferi* produced 100% mortality in concentration of 70 ppm. The activity of the plant on cercariae and miracidia revealed that cercariae was more resistance than miracidia (i.e.50 ppm killed all cercariae within 3 hours while killed miracidia within 2 hours. The results were statistically analyzed and discussed, and the findings were promising and could open new avenues for the practical use of the plant at the field.

Keywords: Toxicity, *Randia nilotica*, fruit extract, Schistosoma mansoni, *Biomphalaria pfeifferi*, *Bulinus truncatus*

INTRODUCTION

Schistosomes are digenetic trematodes, which belong to the family Schistosomatidae.They inhabit the blood vessels of their hosts and are therefore known as blood flukes. Schistosomiasis (bilharziasis), is an important public health issue for rural communities located near and around slow-moving water-bodies in the tropics and sub tropics. It is estimated that over 200 million people in 73 countries are infected (McCullough and Mott 1983) while further 500-600 millions or 4-5% of the world populations are at risk of being infected (Basch 1991; Brown 1994). The most extensively infected areas are in Africa, China, and the new tropics. Man participated in the spread of schistosomiasis by creating habitats suitable for the snail hosts and the dispersal of the parasites through the traveling of infected people (Brown 1994).

In Africa, schistosomiasis is found in most countries and the number of infected persons was estimated to be 90 millions, while 180 million persons are at risk (Korte and Mott 1980; Inobaya et al. 2014). There are five major species of schistosomes affecting humans. These species differ not only biologically from one another, but also in their geographical distribution and in the types of infection they produce. Intestinal schistosomiasis, caused by *Schistosoma mansoni*, occur in Africa, Eastern Mediterranean, Caribbean Islands and South America. *S. japonicum* also known as Asian schistosome, is present in southeast Asia, China and the Philippines and only in small foci in other countries (Wains and McManus 1997). Another form of intestinal schistosomiasis, caused by *S. intercalatum*, has been reported in Africa in parts of Cameroon, Gabon and northeast Zaire (Doumenge et al. 1987; Lai et al. 2015). Urinary schistosomiasis is caused by *S. haematobium*, occurs in Africa and Eastern Mediterranean.

In Sudan the history of schistosomiasis began after building the Sennar Dam in 1925 and the establishment of the Gezira Agriculture Scheme. Integrated schistosomiasis control programmes were recommended by the World Health Organization (WHO) in 1994. The snail control is an important preventive strategy associated with the treatment of infected people together with environmental and socio-economic improvements and health education with community participation. Although chemical molluscicides are the most used approach of snail control (WHO 1973, 1994), yet chemicals have their hazards. In addition to their high costs, they may be toxic to aquatic funa and source to pollution. All factors make it imperative to consider using molluscicides of plant origin either naturally growing or locally cultivated.

This study is intended to determine the efficiency of the plant *Randia nilotica* on the adult snail hosts, on cercaria and miracidia of *Schistosoma mansoni.*

MATERIALS AND METHODS

The plant

Randia nilotica is a plant that belongs to the family Rubiacae. The plant is locally known as Shagarat El-Murfaein. The fruits of this plant are the parts used in this study. It was collected from Kordofan area in Western Sudan. Extraction of the plant was performed at the Department of Pathology, Faculty of Veterinary Medecine, University of Khartoum. In the laboratory the fruit were air dried under-shade before they were coarsely-powdered.

The snails

Snail collections

The snails used throughout the experiments were *Biomphalaria pfeifferi* and *Bulinus truncatus*, which are the most important vectors for the transmission of human and animal schistsomiasis in Sudan (Hussein 1973; Sulaiman and Ibrahim 1985; Jordan et al. 1993), as well as sub Sahara Africa (Hotez and Fenwick 2009; Hotez and Kamath 2009; Steinmann et al. 2006). The snails were collected from El-Seleit irrigated area on the Eastern part of the Blue Nile by deep scooping. The scoops were constructed from the kitchen sieves, supported by an iron frame and mounted on a handle (1-2 meters long). The snails were then maintained and bred in the laboratory of bilharziasis at the Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum.

Snail breeding

About 10-20 snails were put in plastic tanks, in which the water was changed even three days by normal dechlorinated water and was cleaned periodically from faecal debris. The temperature in the laboratory was kept at 25-30°C. The snails were fed dried lettuce leaves, prepared as follows, by washing green leaves of lettuce by boiling water, and allowed to dry. The water used for snail breeding was tested to determine its chemical composition using flame spectrophotometer. The same water was then used to determine the rate of development and hatching of these snails in the laboratory. The egg-masses produced by these snails were deposited on small pieces of celophane materials which were placed on the top surface of water in the tanks to collect eggs. The celophane materials contaminated with egg-masses were removed daily and placed on water in other tanks to maintain hatching of eggs and growth of embryos. The juvenil snails were fed on algae or dried lettuce, the range of growth and periods of hatching were observed.

Extract preparation

The method of the extract preparation described by Brackenbury et al. 1997) and Brackenbury (1999) was adopted in the experiment. An amount of 2.5 grams of coarsely powdered materials of *R. nilotica* fruits was soaked in 200 ml of distilled water in a flask for 24 hours. The contents of the flask were then filtered, and the volume was adjusted up to 250 ml, using distilled water. A stock solution was then prepared for future use.

Test for molluscicidal activity

The procedure of the test which was applied for the materials obtained from the fruits of *R. nilotica* was carried out according to the method recommended by WHO (1965). Ten viable snails were put in one liter of the extract concentration and left for another 24 hours. They were then removed and put in dechlorinated water for another 24 hours as a recovery period. The number of dead snails was recorded. The control was prepared by putting another group of ten snails in one liter of dechlorinated water devoid of the extract. The snails are usually considered dead when they show lack of movements, retraction or hanging out of their shells; in such cases, the snail bodies and shells will be discolored. Death is also confirmed by lack the reaction to any external stimulus from the surrounding water. The numbers of dead and living snails were recorded after 24 hours of exposure followed by 24 hours of recovery period.

Molluscicidal activity of *Randia nilotica* on *Biomphalaria pfeifferi* and *Bulinus truncatus*

From the stock solution which was prepared from *R. nilotica* fruits, different dilutions were prepared to study their molluscicidal activity on *B. pfeifferi* and *B. truncatus*. A group of ten viable *B. pfeifferi* and another group of ten *B. truncatus* snails were put each in one liter of the extract solution of known concentration in a container and exposed for 24 hours. First, a titration was used at the rate of 100, 200, 300, 400, 500, and 600 ppm. According to the results obtained from the titration, different other concentrations were used for further screening to achieve mortality percentages ranging from zero to 100% from each concentration; the experiment was repeated three times and the average obtained from the three readings was taken. Control results were similarly taken. Two types of the extract were used, the first one was the filtered extract and the other one was unfiltered. The extract was used against uninfected *Bulinus*, infected and uninfected *Biomphalaria*

Miracidicidal activity test

Production of miracidia

Miracidia were obtained using stool samples obtained from people infected with *Schistosoma mansoni* in El-Seraha village in Gezira State. Samples were examined by locally developed direct thick smear method described by Teesdale and Amin (1976). The positive samples containing eggs were put in normal saline and mixed in a conical flask. The stool sample was sieved in a wire mesh and then filtered. The filtrate was collected into one-liter conical flask. Warm dechlorinated water was then added and the flask was put under artificial light for about one hour to induce hatching.

Test for toxicity of Randia nilotica on miracidia

The test was carried out according to the method recommended by WHO (1965). A group of ten miracidia suspended in 0.5 ml distilled water, were transferred to micro-titer plates each containing one ml of the extract of a known concentration of 25, 50, 100, 250 and 500 ppm of *Randia. nilotica*. The miracidia were examined under a dissecting microscope over a period of three hours. Ten miracidia were transferred to one ml of distilled water to serve as control. Death of miracidia was determined by low of motility and by exhibiting granular shape. Each experiment was repeated three times for each concentration, and the average of three results was then taken. The time taken to kill all miracidia was observed and recorded.

Procedure of snail infection

The snails used in this procedure was *B. pfeifferi.* Each snail was placed in a micro-titer plate containing 5ml of fresh water. 3-5 miracidia were added to each micro-titer plate. The snails were exposed to schistosome miracidia under light for 24 hours. Screening for schistosome infection to detect transmission of infection in the snails started 30 days after exposure to miracidia.

Detection of the infection in snails

Firstly, the snails were washed with dechlorinated tab water 2-3 times to wash out tissue debris. They were put in a beaker containing distilled water (10 ml/snail). They were exposed to strong artificial light at a temperature of 25C. Shedding of cercariae started after about half an hour under these conditions.

For the detection of cercariae, the beaker was held against a source of light. Cercariae of *Schistosoma* species were identified under the microscope by biforked tails and the absence of eyespots. Based on the procedure mentioned above, the snails were screened and the infected ones were isolated for used in the experiment. They were then exposed to artificial light for half an hour to produce the cercariae. A volume of 0.5 ml solution containing the cercariae was randomly taken, and spread on a petri dish. The cercariae were then fixed and stained with lougols iodine and counted under a dissecting microscope. The required number for the test was then taken from the sample.

Cercaricidal activity test

The test was carried out according to the method recommended by WHO (1965). Twenty cercariae were suspended in 0.1 ml distilled water and incubated with one ml of different concentrations of the plant *R. nilotica* extract in micro-titer plates, the concentrations used were 25,50,100,250 and 500ppm. The micro-titer plates were examined under a dissecting microscope over a period of five hours after with the activity decreased. The time required to kill all cercariae was recorded. Cercariae were considered dead if they become immotile and /or their oral and ventral suckers are extended. Twenty cercariae were transferred to one ml of distilled water on the same plate to serve as control.

Statistical analysis

ANOVA, t-test and probit procedure were carried out to analyze. ANOVA test was used to assess the activity of filtered and unfiltered extracts of the plant *R. nilotica* on *B. pfeifferi*, *B. truncatus* and cercariae and miracidia of *S. mansoni* using SPSS program. The correlation coefficient that shows the relationship between the plant concentrations and the effect of this plant of the snails and cercariae and miracidia of *S. mansoni* was calculated. It was then drawn into graph for filtered and unfiltered extracts against *B. pfeifferi*, *B. truncatus;* and cercariae and miracidia of *Schistosoma mansoni*. To compare the potencies of filtered and unfiltered extracts on *B. pfeifferi* and *B. truncatus*, and to compare the potency of unfiltered extract on infected and uninfected *B. pfeifferi* T-test was used. Furthermore, the probit analysis was used to confirm the potency of *R. nilotica* filtered and unfiltered extract against *B. pfeifferi* and *B. truncatus*, and to confirm the potency of unfiltered extract on infected *B. pfeifferi* by cercaria of *S. mansoni*. The probit values were calculated using the log values of the extracts concentrations and the percentage mortalities corresponding to them.

$$Y_1 = (Y\text{-}bx) + bx_1$$

Where

(Y1) is the calculated (predictable) probit value.

(Y) = average of the% mortalities.

(X) = average of the extract concentrations. b = is a constant (least square estimate).

X = is the log of the concentration used.

RESULTS AND DISCUSSION

The activity of *Randia nilotica* filtered and unfiltered extract on *Biomphalaria pfeifferi*

The titration of the activity of filtered extract on *B. pfeifferi* revealed that concentration of 20 ppm resulted in 3% while concentration of 30% produced 13% mortality, concentration of 40 ppm resulted in 23% mortality, concentration of 50 ppm resulted in 36% mortality, concentration of 60 ppm resulted in 46%, concentration of 70 ppm produced 70% mortality, concentration of 80 ppm produced 83% mortality, concentration of 90 ppm resulted in 93% mortality, while the highest concentration of 100 ppm produced 100% mortality (Figure 1).

On the other side the activity of unfiltered extract on the snails showed that concentration of 20 ppm resulted in 6% while concentration of 30 ppm produced 16%mortality, concentration of 40 ppm resulted in 30% mortality, concentration of 50 ppm resulted in 53% mortality, concentration of 60 ppm resulted in 70%, concentration of 70 ppm produced 83% mortality, concentration of 80 ppm produced 90% mortality, while concentration of 90 ppm resulted in 100% mortality (Figure 2).

The effect of filtered extract on the snail was highly significant ($p \leq 0.001$) (Table 1), while the correlation was positive between the filtered extract concentrations and the mortality, the correlation was positive ($r = 0.960$) and it was highly significant ($p \leq 0.01$). The effect of the other unfiltered extract, was also highly significant ($p \leq 0.001$, Table 1), and the correlation was positive ($r = 0.969$), and was highly significant ($p \leq 0.01$).

There was no significant difference in potency of filtered and unfiltered extract on *B. pfeifferi*. In concentration of 10 ppm and 100 ppm there was no significant differences (p =--), while in concentration of 20 ppm the difference was found ($p = 1.000$), in concentration of 30 ppm recorded no significant difference between filtered and unfiltered extract ($p = 0.519$), in concentration of 40 ppm the result showed no significant differences ($p = 0.491$), in concentrations of 50 ppm and of 60 ppm the results was recorded as ($p = 0.152$) and as ($p = 0.091$),

respectively. In concentration of 70 ppm (p = 0.411), in concentration of 80 ppm (p = 0.492), and in concentration of 90 ppm no significant difference was shown (p = 0.116) (Figure 3)

The activity of *Randia nilotica* filtered and unfiltered extract on *Bulinus truncatus*

The titration of the activity of filtered extract revealed that concentration of 20 ppm resulted in 6% while concentration of 30 ppm produced 13%mortality, concentration of 40 ppm resulted in 30% mortality, concentration of 50 ppm resulted in 52% mortality, while concentration of 60 ppm resulted in 70%, concentration of 70 ppm produced 83% mortality, concentration of 80 ppm produced 91% mortality, concentration of 90 ppm resulted in 100% mortality (Figure 4). On the other side the activity of unfiltered extract on the snail showed that concentration of 20 ppm resulted in 7% while concentration of 30 ppm produced 23%mortality, concentration of 40 ppm resulted in 41% mortality, concentration of 50 ppm resulted in 70% mortality, while concentration of 60 ppm resulted in 86%, concentration of 70 ppm produced 93% mortality, while concentration of 80 ppm produced 100% mortality (Figure 5).

The effect of filtered extract on *B. truncatus* was highly significant (p≤0.001,Table 1).The correlation coefficient was positive (r = 0.966), and was highly significant (p≤0.01). The effect of unfiltered extract on *B. truncatus* was also highly significant (p≤0.001) (Table 1). The correlation between extract concentration and the percentage mortality was positive (r = 0.945), and was highly significant (p≤0.01).

The concentration of 10 ppm was not significant (p =-), and the same result was produced in concentrations of 90 ppm and 100 ppm. In concentration of 20 ppm the significance was (p = 1.000), while in concentration of 30 ppm, it was (p = 0.230), the significance in concentration of 40 ppm was (p = 0.288), in concentrations 50 of ppm and 60 ppm the effect showed no significance (p = 0.189) and (p = 0.067), respectively. In concentration of 70 ppm the significance was (p = 0.101), while in the last concentration of 80 ppm, the significance was (p = 0.158) (Figure 6).

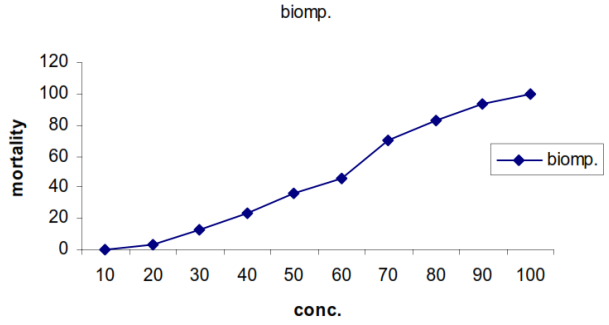

Figure 1: The effect of *R. nilotica* filtered extract concentration on *Biomphalaria pfeifferi*. Correlation Coefficient = 0.960

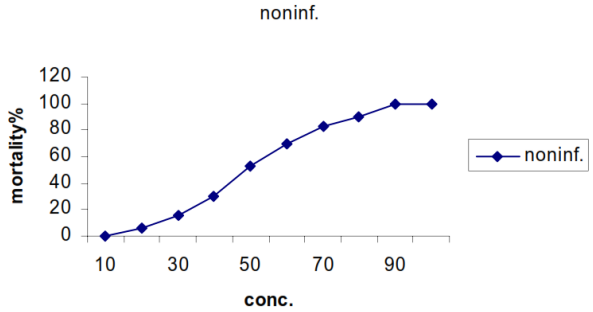

Figure 2: The effect of *R. nilotica* unfiltered extract concentration on *Biomphalaria pfeifferi*. Correlation Coefficient = 0.969

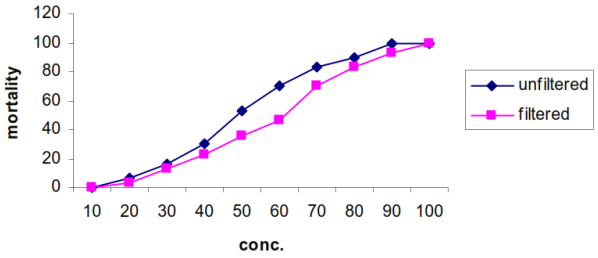

Figure 3: The effect of *R. nilotica* filtered and unfiltered extract on *Biomphalaria pfeifferi.*

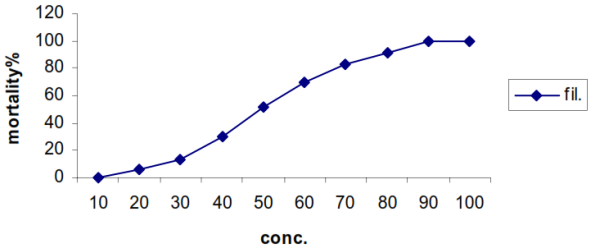

Figure 4: The effect of *R. nilotica* filtered extract concentration on *Bulinus truncatus.* Correlation Coefficient = 0.966

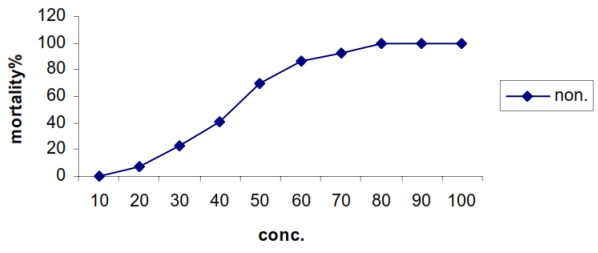

Figure 5: The effect of *R. nilotica* unfiltered extract concentration on *Bulinus truncatus.* Correlation Coefficient = 0.945

The activity of *Randia nilotica* unfiltered extract on infected and uninfected *Biomphalaria pfeifferi*

The titration of the activity of unfiltered extract of *R. nilotica* on infected *B. pfeifferi* revealed that concentration of 20 ppm resulted in 3% while concentration of 30 ppm produced 23%mortality, concentration of 40 ppm resulted in 33% mortality, concentration of 50 ppm resulted in 70% mortality, concentration of 60 ppm resulted in 90% mortality, in concentration of 70 ppm produced 100% mortality (Figure 7).

The effect of unfiltered extract on infected *B. pfeifferi* snail was highly significant ($p \leq 0.001$) (Table 1). It gave a positive correlation. The correlation coefficient was ($r = 0.934$), this correlation was highly significant ($p \leq 0.01$).

There was no significant differences in potency of unfiltered extract on infected and uninfected *Biomphalaria* snail, in concentration of 10 ppm there was no significant differences (p =--). The same result was produced in concentrations of 90 ppm and 100 ppm. There was no significant difference in concentration of 20 ppm ($p = 0.519$), while in concentration of 30 ppm the significance was ($p = 0.230$), in concentrations of 40 ppm and 50 ppm there was no significant difference ($p = 0.725$) and ($p = 0.189$), respectively. In concentration of 60 ppm gave no significance ($p = 0.070$). the significance in concentration of 70 ppm was ($p = 0.132$), while in concentration of 80 ppm was ($p = 0.158$) (Figure 8).

The activity of *Randia nilotica* filtered extract on cercariae of *Schistosoma mansoni*

The activity of *R. nilotica* filtered extract on cercariae showed that the concentration of 500 ppm killed all cercariae in a few minutes (15 minutes), and the concentration of 250 killed the cercariae at 30 minutes. In concentration of 50 ppm, the cercariae killed in about 3 hours, while concentration of 25 ppm gave no effect on cercariae for about five hours (Figure 9).

The effect of the plant on cercariae was highly significant ($p \leq 0.001$), (Table 1). The time to kill the cercariae was decreased when the used concentration increased, this means correlation was negative, the correlation coefficient was ($r = -0.751$), this correlation was highly significant ($p \leq 0.01$).

The activity of *Randia nilotica* filtered extract on miracidia of *Schistosoma mansoni*

The activity of *R. nilotica* filtered extract on miracidia showed that concentration of 500 ppm killed the miracidia just in 10 minutes, concentrations of 250 ppm and 100 ppm, killed all miracidia in 20 minutes and 45 minutes, respectively. While the concentration of 50 ppm killed all miracidia in 2 hours, the concentration of 25 ppm produced no effect on miracidia for up to 3 hours (Figure 9).

The plant was very effective on miracidia of *S. mansoni*, and was highly significant ($p \leq 0.001$) (Table 1). It was negative correlation, the correlation coefficient ($r = -0.773$), it was highly significant ($p \leq 0.01$).

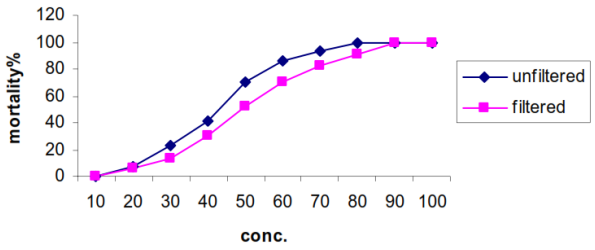

Figure 6: The effect of filtered and unfiltered extract of *R. nilotica* on *Bulinus truncatus.*

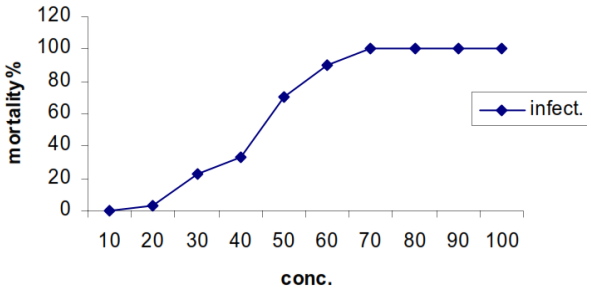

Figure 7: The effect of unfiltered extract of *R. nilotica* on infected *Biomphalaria pfeifferi.* Correlation Coefficient = 0.934

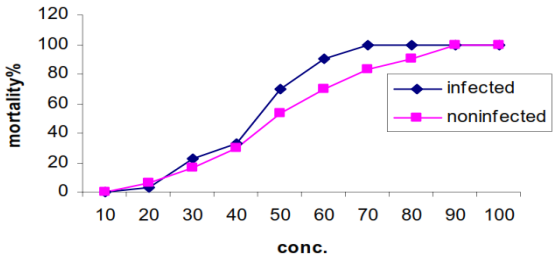

Figure 8: The effect of unfiltered extract of *R. nilotica* on infected and uninfected *Biomphalaria pfeifferi*

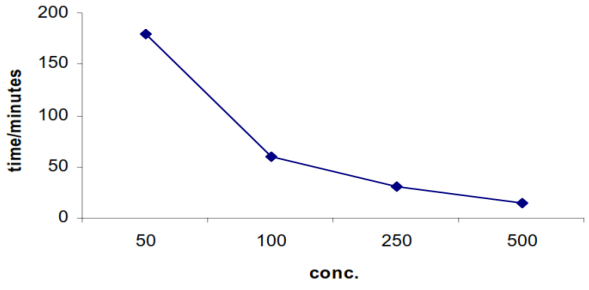

Figure 9: The effect of filtered extract concentration on Cercariae of *S. mansoni.* Correlation Coefficient =-0.751

Table 1. ANOVA degree of freedom (df),mean squares (MS),R-square (R) and F value (F) for infected and uninfected *Biomphalaria, Bulinus*, cercariae and miracidia treated by filtered and unfiltered extract of *Randia nilotica*

Extract	Source	df	MS	R	F
Filtered extract					
	Biomphalaria pfeifferi	9	42.404	0.922	34.381***
	Bulinus truncatus	9	46.756	0.932	63.758***
	Cercariae	3	16818.750	0.564	1770.395***
	Miracidia	3	7418.750	0.598	471.032***
Unfiltered extract					
	(Infected *Biomphalaria pfeifferi*)	9	54.089	0.873	135.222***
	(Uninfected *Biomphalaria pfeifferi*)	9	45.870	0.910	72.427***
	Bulinus truncatus	9	49.570	0.893	114.393***

Note: ***P≤0.001

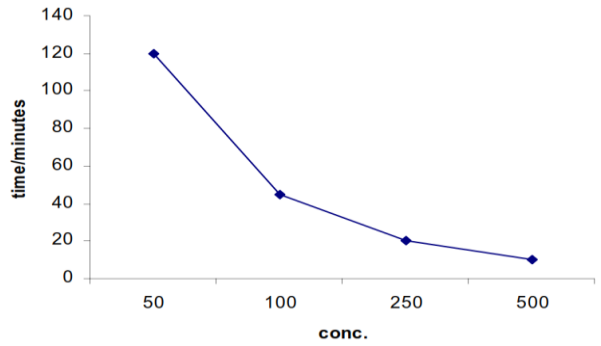

Figure 10. The effect of *R. nilotica* filtered extract concentration on Miracidia of *S. mansoni.* Correlation Coefficient = -0.773

Discussion

Aqueous extract of the plant *R. nilotica* filtered and unfiltered extract, has been used in this study for the assessment of their activities. Filtered and unfiltered extracts were used on the snails *B. pfeifferi* and *B. truncatus*, while filtered extract was used against cercariae and miracidia of *Schistosoma mansoni*. Some researchers have reported high molluscicidal activities in the alcoholic extracts of different parts of a number of species belonging to the family Rubiacae (Adewenmi 1980; Ahmed et al. 1994). One of these plants was *R. nilotica*. The stem of this plant was found very active against both snails.

The results of this study revealed that the plant extract was highly effective against *B. pfeifferi*. Filtered extract produced LD50 (probit 5.00) at concentration of 53.33 ppm and LD90 (probit 6.28) at 97.95 ppm, while 100% mortality (probit 7.33) was produced at concentration of 100 ppm. On the other side, unfiltered extract produced LD50 and LD90 at concentrations of 46.24 ppm and 85.31 ppm respectively. While 100% mortality was produced at 90 ppm and 100 ppm. That means unfiltered extract was more potent than filtered one, that effect may be due to the debris on unfiltered extract that snails fed on it. At the same time in concentration 10 ppm there was no effect on filtered and unfiltered extracts.

The effect of two extracts revealed no significant differences in each concentrations.

The effect of *R. nilotica* on *B. truncatus* revealed that filtered extract produced LD50 at concentration of 46.56 ppm, while it produced LD90 when the concentration increased to 84.33 ppm. indicates that 100% mortality was reached when 90 ppm and 100 ppm were used. On the other hand the effect of unfiltered extract on *B. truncatus* revealed that LD 50 (probit 5.00) was 39.63 ppm, while LD90 (probit 6.28) produced at concentration of 67.61 ppm. While 100% mortality was produced when concentrations 80 ppm, 90 ppm and 100 ppm were used.

The effect of each concentration revealed no significant difference between filtered and unfiltered extract on Bulinus truncatus.

The above results indicate that unfiltered extracts of *R. nilotica* were more potent than filtered one on both *B. pfeifferi* and *B. truncatus*. However, high molluscicidal activities were attained on both snails only when higher concentrations were used i.e 100% resulted in concentrations of 100 ppm and 90 ppm of filtered extract and 90 ppm and 80 ppm of unfiltered extract on *B. pfeifferi* and *Bulinus truncatus* respectively.

Concentrations of filtered extract were relatively very low as compared with the stem extracts of *R. nilotica* which produced 100% mortality when 1150 ppm and 1000 ppm were used on *B. pfeifferi* and *B. truncatus* respectively (Ibrahim 1998). The results above also revealed that the snail *B. pfeifferi* was more resistant than *B. truncatus* in both extracts, however 100% mortality was attained when 100 ppm and 90 ppm were used on *B. pfeifferi* and 90 ppm and 80 ppm on *B. truncatus* of filtered and unfiltered extracts respectively.

The plant was used also against infected *B. pfeifferi* by miracidia of *S. mansoni*, unfiltered extract was highly effective, LD50 with 40.83 ppm, while 90% mortality was produced at concentration of 65.16 ppm. Concentrations that produced 100% mortality were 70 ppm, 80 ppm,90 ppm and 100 ppm.

Table 10. Means (±SE) of the effect of time (minutes) of mortality of Miracidia and Cercariae due to the effect of *Randia nilotica* filtered extract.

Extract conc. (ppm)	Miracidia	Cercariae
50	120±2.89	180±2.89
100	45±2.89	60±1.53
250	20±1.73	30±1.00
500	10±1.15	15±1.00

These results revealed that uninfected snail was more resistant than the infected one; 100% mortality was given at concentration of 90 ppm on uninfected snails, and at concentration of 70 ppm on infected one. This difference may be due to the morbidity of infected snails by miracidia.

The effect of unfiltered extract of the plant revealed no significant difference between infected and uninfected *B. pfeifferi*.

The activity of the plant used on miracidia of *S. mansoni* was observed the time taken to kill all miracidia. In concentrations of 50 ppm and 100 ppm miracidia were killed in about 120 minutes and 45 minutes respectively. Concentration of 250 ppm killed miracidia in about 20 minutes, while concentration of 500 ppm killed all miracidia in a fewer time (10 minutes) (Tabel 10).

The effect of the plant on miracidia of S. mansoni was highly significant.

The plant was used against cercariae of *S. mansoni*, in concentration of 50 ppm killed the cercariae within 180 minutes. Concentration of 100 ppm killed the cercariae within 60 minutes, while concentrations of 250 ppm and 500 ppm killed all cercariae within 30 minutes and 15 minutes respectively.

The effect of the plant was highly significant when used against cercariae of *S. mansoni*.

These results revealed that cercariae were more resistant than miracidia. These results were attained when only lower concentration was used i.e in concentration of 50 ppm the time taken to kill cercariae and miracidia was 180 minutes and 120 minutes respectively. It was observed that concentration of 25 ppm was not active on both miracidia and cercariae within 3 hours and 5 hours respectively (Tabel 10).

Forty Sudanese plant were tested by El-Shiekh (1994) for their miracidicidal and cercaricidal activity against *S. mansoni*. In these plants *R. nilotica* was found highly effective, by killing all miracidia and cercariae of *S. mansoni* at concentration of 50 ppm within 3 hours and 5 hours respectively. The correlation coefficient (r) was negative. This means that when the concentration of the plant extract was increased, the time taken to kill all cercariae and/or miracidia decreased.

Bashir et al. (1987) reported the activity of the plant *Acacia nilotica*, with sub species *nilotica* and *adansoni* against cercariae and miracidia of *S. mansoni*. They found that high concentrations of both sub species of the plant killed both cercariae and miracidia, as concentration of 5000 ppm of *A.nilotica* with sub species *nilotica* killed cercariae and miracidia within 80 minutes and 35 minutes respectively. Similar concentration in the other sub species (*adansoni*) killed cercariae and miracidia within 100 minutes and 80 minutes respectively. Al-Sayed et al. 2014) reporeted that *Eucalyptus globulus* has a potential source for biocidal compounds against *S. mansoni* and its snail host. Ibrahim et al. 2015) stated that *Agave angustifolia* and *Pittosporum tobira* have cercaricidal and miracidicidal potencies against *S. mansoni*.

Ibrahim (1998) tested the potency of the stem of the plant *Randia nilotica* on *B. pfeifferi* and *B. truncatus*, and showed the activity of the plant against cercariae and miracidia of *S. mansoni*. The plant killed the snails at high concentrations, 100% mortality was reached when concentrations of 1200 ppm and 1300 ppm were used on *B. pfeifferi* and *B. truncatus* respectively. Where the 100% mortality of cercariae and miracidia of *S. mansoni* was taken in concentration of 100 ppm and 50 ppm respectively. These differences may be due to the part (s) used of the plant, and that the plant used in that study was obtained from Sudan National Garden in Khartoum where this plant was cultivated.

In conclusion, from molluscicidal examinations of the plant *R. nilotica* against *B. pfeifferi* and *B. truncatus* it could be concluded that the activity of this plant was high in the form of both filtered and unfiltered extract against both snails, and it gave good results even in lower concentrations. The experiments also showed that the concentration of 10 ppm did not produce any activity against the snails in all experiments. The cercaricidal and miracidicidal activity of the plant revealed that it was very effective on cercariae and miracidia of *S. mansoni*. As it gave 100% mortality in short period of time at lower concentrations. Also in these experiments the concentration of 25 ppm did not give any activity on cercariae and/or miracidia of *S. mansoni*. Based on the results of the present study it seems reasonable to conclude that the plant can be used for the control of schistosomiasis in Sudan, as it has considerable molluscicidal, cercaricidal and miracidicidal activities. In addition the plant grows naturally in different parts of the Sudan and its application requires simple technology.

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