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Assessment of Molluscicidal, Cercaricidal and Miracidal Activities of Crude Extracts of *Ocimum americanum, Bridelia micrantha and Chenopodium ambrosoides*

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Abstract
Schistosomiasis is a major health problem in both the tropics and subtropics. Niclosamide, the molluscicide in use is expensive, has poor water solubility and is harmful to non-target organisms such as fish. There is need of a molluscicide which is safe to a non-target host and which is capable of eliminating snail intermediate host and *Schistosoma mansoni* infective stages. This would be a better strategy of control as it would prevent infection of the definitive host and also interfere with transmission of the disease. Plants extracts have been found to be relatively safe to the environment and humans. The study was done to determine effect of selected plant extracts on *Biomphalaria pfeifferi* snails and microscopic stages of *Schistosoma mansoni*, miracidia and cercariae. Three plants: *Ocimum americanum* (whole plant); *Bridelia micrantha* (leaves and bark) and *Chenopodium ambrosoides* (leaves) were collected, dried and ground into powder. Extraction was done using methanol, hexane, and distilled water for *B. micrantha*, *O. americanum* and *C. ambrosoides* respectively. Plant extracts concentrations of 50µg/ml, 150µg/ml and 300µg/ml were prepared in the case of molluscicidal assay. Plant extract concentrations of 5µg/ml, 15µg/ml and 30µg/ml were prepared in the case of cercaricidal and miracidal assays. Data analysis was done using SPSS version 16 to calculate mortality of snails and compare effect of the plant extracts on the snails. Finney Probit analysis was used to estimate LD_{50} values of the extracts on the snails and LT_{50} values of the extracts on cercariae and miracidia. *B. micrantha* had the highest molluscicidal activity (LD_{50}, 29.775µg/ml) followed by *O. americanum* (LD_{50}, 37.5920 µg/ml); *C. ambrosoides* (LD_{50}, 1909.13µg/ml) had least molluscicidal activity. *O. americanum* had highest cercaricidal activity followed by *B. micrantha* and lastly *C. ambrosoides*. At 30 µg/ml concentration, cercaricidal LT_{50} values for *O. americanum, B. micrantha* and *C. ambrosoides* extracts were 53.85 minutes, 55.21 minutes and 79.14 minutes respectively. *O. americanum* had highest miracidal activity followed by *B. micrantha* and lastly *C. ambrosoides*. At 15 µg/ml concentration, miracidial LT_{50} values for *O. americanum*, *B. micrantha* and *C. ambrosoides* were 63.01 minutes, 69.86 minutes and 90.05 minutes respectively. In conclusion, *B. micrantha* (leaves/bark) methanol extract and whole plant hexane extract of *O. americanum* had high molluscicidal activity against *B. pfeifferi* snails which is not significantly different from the activity of Niclosamide ($p \geq 0.05$). Similarly whole plant hexane extract of *O. americanum*, methanol (leaves/bark) extract of *B. micrantha* and aqueous leaves extract of *C. ambrosoides* were shown to possess cercaricidal and micaricidal activities. The plants with best activity against *B. pfeifferi* snails, *S. mansoni* cercariae and miracidia were *B. micrantha* and *O. americanum*.

Keywords: Schistosomiasis, *Schistosoma mansoni, Biomphalaria pfeifferi*, *Ocimum americanum, Bridelia micrantha, Chenopodium ambrosoides*, Molluscicidal activity, Miracidal activity, Cercaricidal activity, Lethal dose 50 (LD_{50}), Lethal time 50 (LT_{50})

1.0 INTRODUCTION

Schistosomiasis is a chronic, debilitating disease caused by several species of trematode flatworms belonging to genus *Schistosoma*. Schistosomiasis is predominant in Africa, Asia and South America mostly in areas where water bodies contain freshwater snails (Steinauer *et al*., 2008). Schistosomiasis affects more than 1 billion people globally and around 250 million are infected. Estimates of around 200,000 deaths that occur yearly in Sub-Saharan Africa are attributed to schistosomiasis. Schistosomiasis is ranked second to malaria in terms of parasitic diseases of global health importance. Schistosomiasis is endemic in 46 out of 54 countries of Africa. In Kenya, 6 million people are infected and around 15 million are at high risk of infection in endemic areas (Hotez & Kamath, 2009). *Schistosoma mansoni* causes intestinal schistosomiasis and it is transmitted mainly by snail intermediate host, *Biomphalaria pfeifferi* (CDC, 2009).

Niclosamide the molluscicide in use, is expensive, has poor water solubility and harmful to non-target organisms such as fish (Aladesanmi, 2006). There is need of a molluscicide which is safe to a non-target host and which is capable of eliminating the intermediate host and *S. mansoni* stages which infect and emerge from the snail. This would be a better strategy of control as it would prevent infection of the definitive host and also

interfere in transmission.

S. mansoni is widely distributed in parts of South America, 36 countries in Africa, 7 countries in East Mediterranean and Middle East; however it is also found in some Caribbean islands, Venezuela and Brazil (Gryseels *et al.*, 2006). *S. mansoni* is mostly found in places with poor sanitation, because of the parasite's fecal-oral transmission (Hotez *et al*., 2008). In Kenya the localities where *S. mansoni* is found include Nyanza province, Machakos County, Busia County and Mwea in Central Kenya (Fullford *et al.,* 1992).

The life cycle of *S. mansoni* involves a definitive host and an intermediate host. The definitive host (mammals including man) is where the parasite undergoes sexual reproduction and the intermediate host is where the parasite undergoes asexual reproduction. *B. pfeifferi* is the most important intermediate host in transmission of schistosomiasis caused by *S. mansoni* in the world (Roberts *et al.*, 2009). *S. mansoni* eggs are excreted in human faeces into water. The eggs hatch in freshwater releasing larvae known as miracidia. The miracidia then searches for a suitable snail host and infects it. In the snail, mother sporocysts are formed from germ cells. Mother sporocysts later develop through asexual reproduction in the digestive glands of the snails. Daughter sporocysts then migrate to snail's hepatopancreas and develop into thousands of cercariae. Cercariae emerge from the snail through the birth pore and are capable of infecting skin of humans, definitive host (Mandal, 2011).

The aim of the study was to determine effect of selected plant extracts on *B. pfeifferi* snails, *S. mansoni* miracidia and cercariae. Plants extracts have been found to be relatively safe to the environment and humans; if they are found to be effective against the snail intermediate host and *S. mansoni* larval stages it would be a double benefit.

2.0 MATERIALS AND METHODS

2.1 Study site

The study was carried out at Institute of Primate Research (IPR) and National Museums of Kenya (NMK).

2.2 Plant collection, identification and drying

Plant samples of *Ocimum americanum* (whole plant), *Bridelia micrantha* (bark and leaves) and *Chenopodium ambrosoides* (leaves) were collected from Machakos county, Kiambu county and Nairobi county respectively. Identification of the plants was done at National Museums of Kenya. The plants were dried at room temperature (24-26ºC) for 2 months.

2.3 Plant extraction process

O. americanum was extracted using hexane extraction process. Dried plant parts were ground into powder using a Mekon- Micromealer single phase machine. The ground material was soaked in 3.5 litres of hexane for 72 hours. The solution was filtered twice using Whatman filter paper no. 1 and the filtrate concentrated in vacuo in a rotary evaporator machine. *B. micrantha* was extracted using methanol extraction process. The procedure was same as that of extraction of *O. americanum* but 3.5 litres of methanol were used in soaking the ground material. *C. ambrosoides* was extracted using aqueous extraction process. The leaves were dried and ground as for hexane. The powder was soaked in distilled water for 72 h; the solution was filtered twice using Whatman filter paper no. 1 and the filtrate frozen. The frozen material was freeze dried.

2.4 Collection and maintenance of *B. pfeifferi* snails

B. pfeifferi snails were collected from Mwea irrigation scheme using a scooper from canals and placed in a well aerated container with wet cotton wool. The snails were transported and maintained at IPR, Snail laboratory. The snails were housed in a temperature-controlled room (25-28ºC) with a period of 12 h of light and 12 h of darkness. The snails were fed on dried lettuce and daphnia was used for aeration.

2.5 Molluscicidal effect of plant extracts

Molluscicidal activity of plant extracts on adult snails was evaluated. Groups of 10 snails were placed in plastic containers containing 500ml distilled water. The snails were fed on lettuce and left for 24 h. After 24 h from the start of the experiment distilled water was poured out and lettuce removed. Plant extract dosages of 50µg/ml, 150µg/ml and 300µg/ml at a volume of 500ml were prepared and tested on groups of 10 snails for each dosage. Duplicates were set for each of the different concentrations. Positive control was 1mg/litre of Niclosamide (McCollough, 1992) and negative control was 500ml of distilled water. The setups were left for 48 h without feeding the snails. After 48 h in distilled water, the snails were observed to determine whether they were dead or alive by poking their foot with a wooden applicator stick and checking their heart beat; lack of motion signified death of the snail. The entire experiment was repeated.

2.6 Hatching of miracidia

S. mansoni eggs were obtained from faeces of *S. mansoni* chronically-infected baboons (*Papio anubis*) maintained at IPR Animal Science Department. The faeces were thoroughly mixed with saline in a 1 litre plastic container, passed through 2 sieve meshes (sizes 600 and 250µm) and the filtrate collected in a metal tray. The filtrate was then placed in urine jars and allowed to stand for 30 minutes in the dark. The clear supernatant was poured out; fresh saline added and again allowed to stand for three minutes. This process was repeated 3 times. Finally the deposit was placed under artificial light (20-25°C) for 30 minutes for miracidia to emerge (Yole *et al*., 1993). This procedure was used to prepare miracidia for snail infection and miracidia for miracicidal assay.

2.7 Miracicidal effect of plant extracts

Miracicidal effect of plant extracts was tested. Plant extract concentrations of (5µg/ml, 15µg/ml and 30µg/ml) were prepared for each plant extract and dispensed in each well of a 24 well culture plate containing an aliquote of 20 miracidia. A duplicate was setup for each concentration made. Each preparation was observed under a dissecting microscope for cercariae motility at the following time points: 5, 10, 20, 30, 45 and 60 minutes. Immobile miracidia were enumerated and recorded at every time point.

2.8 Infection of snails with miracidia

Snails were infected with *S. mansoni* miracidia. This was done by picking 3-5 miracidia using a drawn out glass pipette from a petridish containing hatched miracidia. The miracidia were dispatched into each well of a 24 well culture plate. Snails were transferred individually into these wells and the plates covered to prevent snails from crawling out. The plates were left for 30 minutes to allow miracidia penetration. The snails were then maintained in 12 hours light/ 12 hours dark cycle for 3 weeks. In the 4^{th} week, they were placed in the dark (prepatent period of snails when infected is 5 weeks) and tanks covered with dark clothes to prevent trickle shedding of cercariae.

2.9 Shedding of cercariae

After the prepatent period, snails were removed from the dark and placed in 10 millilitre beakers with snail water (unchlorinated water). The beakers were placed under light (100 watts lamp) shaded with glass to release cercariae. The cercariae suspension was pooled in a 100 millilitre beaker and mixed well.

2.10 Cercaricidal effect of plant extracts

Cercaricidal effect of plant extracts was tested. Plant extract concentrations of (5µg/ml, 15µg/ml and 30µg/ml) were prepared for each plant extract and dispensed in each well of a 24 well culture plate containing an aliquote of 20 cercariae. A duplicate was setup for each concentration made. Each preparation was observed under a dissecting microscope for cercariae motility at the following time points: 5, 10, 20, 30, 45 and 60 minutes. Immobile cercariae were enumerated and recorded at every time point.

2.11 Data analysis

Data analysis was done using SPSS (Statistical Package for the Social Science) version 16 to calculate statistical mean, standard errors and standard deviations of snail mortalities after exposure to the plant extracts. One way ANOVA was used to determine if there were significant differences between various concentrations used for the case of molluscicidal assay. Dunnett test was used to compare two concentrations at a go to determine whether they were significantly different or not for the case of molluscicidal assay. Biostat 2009 program was used to determine Lethal dose 50 (concentration of extract which killed 50 % snails) and Lethal Time 50 (LT_{50}) for miracidia and cercariae by subjecting the data to Finney Probit analysis.

3.0 RESULTS

3.1 Moluscicidal assay results

3.1.1 Mortality of snails

Effect of different concentrations (50µg/ml, 150µg/ml and 300µg/ml) of plant extracts of *O. americanum*, *B. micrantha*, and *C. ambrosoides* on *B. pfeifferi* snails were evaluated. 4 sets of 10 snails were tested in each case. Figure 1 below shows effect of *B. micrantha*, *O. americanum* and *C. ambrosoides* on *B. pfeifferi* snails. *B. micrantha* extract caused highest mortality of *B. pfeifferi* snails followed by *O. americanum* extract; *C. ambrosoides* extract caused least mortality of the snails.

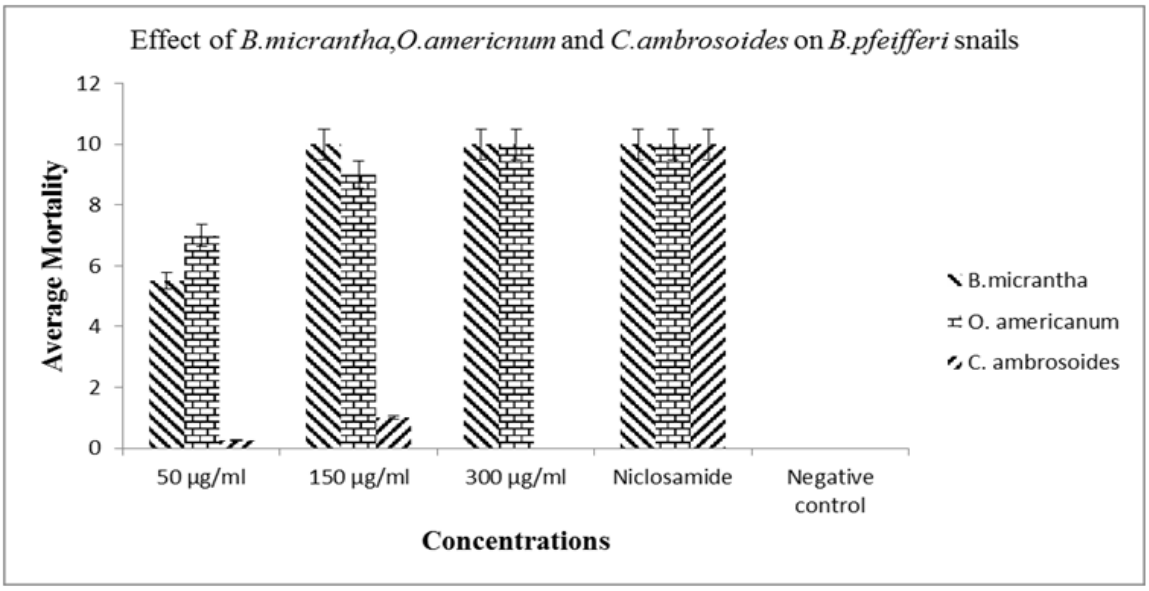

Figure 1: Effect of *B. micrantha*, *O. americanum* and *C. ambrosoides* on *B. pfeifferi* snails

All the three concentrations of *B. micrantha* (50µg/ml, 150µg/ml and 300µg/ml) caused mortality of snails. Highest mortality of 100% was observed after exposing the snails to 150µg/ml and 300µg/ml concentrations of the extract while 50µg/ml concentration of the extract caused 85% mortality. When mortality levels due to the different concentrations (50µg/ml, 150µg/ml and 300µg/ml) of *B. micrantha* were compared, there was significance difference. The significance level associated with concentration was 0.000 which is ≤0.05. 50µg/ml, 150µg/ml and 300µg/ml concentrations of *B. micrantha* were not significantly different from Niclosamide in terms of molluscicidal activity. The significance levels obtained was (p≥0.05).

All the three concentrations of *O. americanum* (50µg/ml, 150µg/ml and 300µg/ml) caused high mortality of snails. 100% mortality was observed after exposing the snails to 300µg/ml of the extract. 90% mortality was observed after exposing the snails to 150µg/ml concentration whereas 50µg/ml concentration caused 70% mortality. There was significant difference in terms of mortality (molluscicidal activity) among the three concentrations. The significance level corresponding to concentration was (p≤0.001). 50µg/ml, 150µg/ml and 300µg/ml concentrations of *O. americanum* were not significantly different from Niclosamide in terms of molluscicidal activity. Significance levels of their comparisons with Niclosamide were ≥0.05.

50µg/ml and 150µg/ml concentrations of *C. ambrosoides* extract caused low mortality of snails. 50µg/ml concentration caused a mortality of 2.5% whereas 150µg/ml concentration caused a mortality of 10%. 300µg/ml concentration caused a mortality of 0%. None of the concentrations of *C. ambrosoides* had significantly similar molluscicidal activity to that of Niclosamide. Significant levels obtained after the comparisons were ≤ 0.05 implying that the mortality due to *C. ambrosoides* was statistically different from the mortality due to Niclosamide.

When effect of the three plant extracts on *B. pfeifferi* snails was jointly compared, all the significance levels obtained were 0.000, (p≤0.05). This implies that at each concentration, the activity of the extracts among the three plants was significantly different.

3.1.2 Lethal dose 50 (LD_{50}) values of the plant extracts

Lethal dose 50 (LD_{50}) is the dosage that kills 50% of the entire defined experimental animal population. In our case, it refers to the plant extract concentration that kills 50% of *B. pfeifferi* snails. *B. micrantha* had highest molluscicidal activity followed by *O. americanum*; *C. ambrosoides* had lowest molluscicidal activity. *B. micrantha* had a LD_{50} value of 29.775 µg/ml, *O. americanum* had a LD_{50} value of 37.5920 µg/ml and *C. ambrosoides* had a LD_{50} value of 1909.13 µg/ml. This is as shown in Table 1.

Table 1: Lethal Dose 50 (LD_{50}) values of the plant extracts

Plant	Average mortality at various concentrations			LD_{50} (µg/ml)	±SD
	50 (µg/ml)	150 (µg/ml)	300 (µg/ml)		
B. micrantha	8.5	10	10	29.775	±36.46
O. americanum	7	9	10	37.5920	±20.54
C. ambrosoides	0.25	1	1	1909.13	±109.81

3.2 Miracicidal Lethal time 50 (LT_{50}) results

Lethal time 50 (LT_{50}) refers to the time it takes for a dose to kill 50% of the entire defined animal population, in

our case miracidia. The time needed to kill 50% of miracidia decreased with increase in concentrations as shown in Table 2 for all the three extracts.

All the three plant extracts exhibited miracicidal activity. *O. americanum* had highest miracicidal activity, followed by *B. micrantha* and lastly *C. ambrosoides*. At 30 µg/ml concentration of the plant extracts, *B. micrantha* had a LT_{50} value of 56.27 minutes, *O. americanum* had a LT_{50} value of 50.08 minutes and *C. ambrosoides* had a LT_{50} value of 71.45 minutes. Both *B. micrantha* and *O. americanum* had a shorter cercaricidal LT_{50} compared to *C. ambrosoides* which had a longer LT_{50}.

Table 2: Miracicidal Lethal time 50 (LT_{50}) results

***O.americanum* extract Concentration**	**Number of dead miracidia at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	1	3	5	6	**87.38**	±22.51
15 µg/ml	0	0	4	5	7	8	**63.01**	±17.36
30 µg/ml	0	4	6	8	9	10	**50.08**	±15.36
***B. micrantha* extract concentration**	**Number of dead miracidia at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	0	1	2	3	**101.05**	±39.06
15 µg/ml	0	0	0	3	6	7	**69.86**	±19.12
30 µg/ml	0	3	7	5	8	10	**56.27**	±17.87
***C. ambrosoides* extract Concentration**	**Number of dead miracidia at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	0	1	2	3	**131.08**	±24.47
15 µg/ml	0	0	0	2	4	5	**90.05**	±15.13
30 µg/ml	0	0	0	2	5	7	**71.45**	±12.35

3.3 Cercaricidal Lethal time 50 (LT_{50}) results

The time needed to kill 50% of cercariae decreased with time as shown in Table 3 for all the three extracts. All the three plant extracts exhibited cercaricidal activity. *O. americanum* had highest cercaricidal activity, followed by *B. micrantha* and lastly *C. ambrosoides*. At 30 µg/ml concentration of the plant extracts, *B. micrantha* had a LT_{50} value of 55.21 minutes, *O. americanum* had a LT_{50} value of 53.85 minutes and *C. ambrosoides* had a LT_{50} value of 79.14 minutes. Both *B. micrantha* and *O. americanum* had a shorter cercaricidal LT_{50} compared to *C. ambrosoides* which had a longer LT_{50}.

Table 3: Cercaricidal Lethal time 50 (LT_{50}) results

***O.americanum* extract concentration**	**Number of dead cercariae at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	3	5	7	9	**62.15**	±15.58
15 µg/ml	0	2	4	7	9	10	**53.12**	±13.34
30 µg/ml	1	3	5	8	9	10	**53.85**	±18.95
***B. micrantha* extract concentration**	**Number of dead cercariae at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	0	2	4	8	**69.02**	±17.22
15 µg/ml	0	0	0	3	5	9	**63.75**	±12.80
30 µg/ml	0	0	0	4	8	10	**55.21**	±7.84
***C. ambrosoides* extract concentration**	**Number of dead cercariae at various time intervals (min)**						**LT_{50} (min)**	**±SD**
	5	10	20	30	45	60		
5 µg/ml	0	0	1	2	4	4	**105.49**	±28.22
15 µg/ml	0	0	0	1	2	3	**90.97**	±25.89
30 µg/ml	0	0	0	1	3	6	**79.14**	±14.63

4.0 DISCUSSION

4.1 Molluscicidal effect of plant extracts

Snail control is an important practice in controlling transmission of schistosomiasis. Effective control of snails which are intermediate hosts of *Schistosoma* parasites require an approach involving environmental modification like clearing vegetation in canals and frequent flashing of water in canals; use of molluscicides and biological

control of snails which involves use of predator snails, decoy snails and even plants with molluscicidal activities (Chitsulo *et al*., 2000). The current study was done to investigate molluscicidal, miracicidal and cercaricidal activities of extracts of *Bridelia micrantha, Ocimum americanum* and *Chenopodium ambrosoides.*

Methanol extract of *B. micrantha* had highest molluscicidal activity against adults *B. pfeifferi* snails. At 50 µg/ml, 150µg/ml and at 300 µg/ml concentrations of the extract, there was 85%, 100% and 100% mortality of adult snails observed respectively. Comparison of this mortality with the mortality of Niclosamide using Dunnett test showed that the mortality was not significantly different from the mortality caused by Niclosamide which was 100% ($p \geq 0.05$). The LD_{50} value of methanol extract of *B. micrantha* was 29.775µg/ml. This means that the extract was toxic and had molluscicidal activity against *B. pfeifferi* adult snails. Similar results have been reported on molluscicidal activity of *Bridelia atroviridis* which was shown to have activity against *Bulinus globosus* (Adewunmi *et al*., 1982).

Similarly *O. americanum* hexane extract was highly active against *B. pfeifferi* snails. At 50 µg/ml, 150 µg/ml and 300 µg/ml concentrations of the extract, the mortality was 70 %, 90 % and 100 % respectively. The LD_{50} value of *O. americanum* hexane extract was 37.59 µg/ml. This means that the extract had molluscicidal activity and was highly toxic to snails. Mortality was likewise compared with the mortality caused by Niclosamide using Dunnett test. The results of the comparison showed that the mortality caused by hexane extract of *O. americanum* was not significantly different from the mortality caused by Niclosamide ($p \leq 0.05$). Similar results have been reported by (Ndamukong *et al.,* 2006) who showed that a related species, *Ocimum basilicum* possesses molluscicidal activity against *Bulinus camerunensis* and *Bulinus trancatus* (*Ocimum basilicum* killed 90% of the snails). Elsewhere aqueous extracts of *Ocimum canum* have been reported to have molluscicidal activity against *B. globosus* (Singh *et al*., 2006) and this is in line with the results obtained in the current study of *O. americanum*. Molluscicidal activity of *O. canum* has made the plant to be classified as an eco-friendly molluscicide (Singh *et al*., 2006).

On the other hand no molluscicidal activity was observed in aqueous extract of *C. ambrosoides*. All the three concentrations used in the experiment caused very low mortality of *B. pfeifferi* snails. LD_{50} of aqueous extract of *C. ambrosoides* was more than 1000 µg/ml implying that the extract was non toxic to snails and consequently had no molluscicidal activity. On comparison with Niclosamide using Dunnett test, the mortality of snail caused by the aqueous extract of *C. ambrosoides* was significantly different from the mortality caused by Niclosamide ($p \leq 0.05$).

O. americanum and *B. micrantha* are similar to Niclosamide in terms of molluscicidal activity with *B. micrantha* being better. Plants have various bioactivities which include molluscicidal, antifungal, antibacterial, larvicidal, cytotoxic and insecticidal among others (Norman, 1966). Biological activities are attributed to presence of various secondary metabolites present in the plants known as phytochemicals (Norman, 1966). The phytochemicals are concentrated in various parts of plants while others are equally distributed in all parts of plants. Hence phytochemicals present in the bark/leaves of *B. micrantha* and in *O. americanum* could have been responsible for the molluscicidal activity observed in the two plants. Some phytoconstituents are known to be polar and are extracted from the various plant parts using polar solvents. Other phytoconsituents are non-polar and are extracted using non polar solvents. Aqueous extract of *C. ambroisoides*, methanol extract of *B. micrantha* and hexane extract of *O. americanum* were used in the experiment. Water extracts only contain phytoconstituents which are able to dissolve in water while use of organic solvents like methanol and hexane can dissolve other phytoconstituents which may not dissolve in water. This means that the phytoconsituents responsible for molluscicidal activity were found in the methanol extract of *B. micrantha* and hexane extract of *O. americanum*; the phytoconstituents responsible for molluscicidal activity were lacking in aqueous extract of *C. ambrosoides.*

4.2 Cercaricidal and miracicidal effect of plant extracts

Methanol extract of *B. micrantha, O. americanum* hexane extract and aqueous extract of *C. ambrosoides* had cercaricidal activities at high concentrations. The longer cercariae were exposed to the various concentrations of plant extracts the more they died. The exposure time required to obtain 50% cercariae mortality was less for *O. americanum*, followed by cercaricidal LT_{50} for *B. micrantha* which was longer compared to the cercaricidal LT_{50} for *O. americanum*; *C. ambrosoides* had the longest cercaricidal LT_{50}. At 30 µg/ml, the cercaricidal LT_{50} for *O. americanum*, *B. micrantha* and *C. ambrosoides* was 53.85 min, 55.21 min and 79.14 min respectively. These results show that *O. americanum* hexane extract was the most active extract against cercariae, followed by *B. micrantha* methanol extract and lastly *C. ambrosoides* aqueous extract.

Miracicidal activity of the three plants was similar to the cercaricidal activity of the plants. The only difference was that the miracicidal LT_{50} was longer compared to the cercaricidal LT_{50} in all the concentrations. Methanol extract of *B. micrantha, O. americanum* hexane extract and aqueous extract of *C. ambrosoides* had miracicidal activities at high concentrations. The longer miracidia were exposed to the various concentrations the more they died. The exposure time required to obtain 50% miracicidal mortality was less for *O. americanum*,

followed by miracicidal LT_{50} for *B. micrantha* which was longer compared to miracicidal LT_{50} for *O. americanum*; *C.ambrosoides* had the longest miracicidal LT_{50}. At 15 µg/ml, miracicidial LT_{50} values for *O. americanum*, *B. micrantha* and *C. ambrosoides* was 63.01 minutes, 69.86 minutes and 90.05 minutes respectively. These results show that *O. americanum* hexane extract was the most active extract against miracidia, followed by *B. micrantha* methanolic extract and lastly *C. ambrosoides* aqueous extract.

Miracicidal and cercaricidal activity of the three plant extracts is due to phytoconstituents present in the extracts. The fact that aqueous extracts of *C. ambrosoides* had both cercaricidal activity and miracicidal activity but lacked molluscicidal activity can be explained by the fact that snails are stronger and possess protective structures such as calcium shells and probably were able to resist toxic effects of the phytoconstituents present in the aqueous extract of *C. ambrosoides*. On the contrary, cercariae and miracidia are developmental stages of schistosomes, they posses less protective structures and are more active compared to snails which probably exposed them to more of the extracts and hence they died due to toxic effects. On the same note, cercaricidal LT_{50} is shorter compared to miracicidal LT_{50} implying that the extracts were able to kill cercariae faster than miracidia.

5.0 CONCLUSION

B. micrantha methanol extract and *O. americanum* hexane extract were shown to have high molluscicidal activity against *B. pfeifferi* snails which is not significantly different from the activity of Niclosamide. Similarly hexane extract of *O. americanum*, methanol extract of *B. micrantha* and aqueous extract of *C. ambrosoides* were shown to possess cercaricidal and micaricidal activities. The results showed that cercariae were more susceptible to the extracts compared to miracidia. Hence both *B. micrantha* methanol extract and *O. americanum* hexane extract can be considered for developing molluscicides which have added advantages of killing both miracidia and cerceriae.

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