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Molluscicidal Activity of Selected Plant Extracts against Adult and Juvenile Biomphalaria Pfeifferi

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Abstract
Human Schistosomiasis is a debilitating infection whose transmission depends solely on the presence of snail intermediate hosts. It is a major public health problem second only to malaria in terms of morbidity and mortality and predominant in tropical and subtropical countries. Globally, over 240 million people are infected and close to 600milion are at risk.Strategies for Control of Schistosomiasis include; Chemotherapy with praziquantel, molluscicide with Niclosamide, health education, community participation, provision of clean water and improved sanitation. Chemotherapy and use of synthetic molluscicides faces serious drawbacks of being costly, poses negative impact on environment and development of resistant strains of intermediate host snails and parasites. Plant extracts that are effective against host snails and non-toxic to non-target organisms could provide an alternative molluscicide to the current Niclosamide.This study evaluated molluscicidal activity of aqueous and methanol extracts against juvenile and adult *Biomphalaria pfeifferi in vitro.*Assesment of snail susceptibility to extracts was done by immersion method in accordance to WHO protocal.10 adult and juvenile snails were exposed separately to serial dilutions of 10, 20, 50, 100 and 150ppm (mg/l) of both aqueous and methanol extracts for 48 hours. Mean mortality of snails was subjected to probit analysis to determine the lethal dose (LD_{50}) that killed 50% of the snails. The most active extracts on both juvenile and adults were *Phytolacca dodecandra* (LD_{50} 8.78 and 12.58ppm) and *Solannum linaeanum* (LD_{50} 16.81 and 23.25ppm) respectively.This was followed by *Solanum americanum* (LD_{50} 36.17 and 38.00ppm) and *Annona squamosa* (LD_{50} 51.96 and 59.00ppm) respectively. The least active extracts were *Piper nigrum* (LD_{50} 74.13 and 89.95ppm) and *Rhizophora mucranata* LD_{50} 94.56 and 109.82 ppm) respectively. Average mortality of *Phytolacca dodecandra* and *solanum linaeanum* was significantly similar ($p > 0.05$) to that of niclosamide the currently used molluscicde.Since a good molluscicide should kill snails at a concentration of 100ppm or lower, this study demonstrated potency of four plant species from Kenya with molluscicidal activity against the intermediate hosts of *Schistosoma mansoni*.
Keywords: Schistosomiasis, Biomphalaria pfeifferi, Schistosoma mansoni, Molluscicide, Phytolacca dodecandra, Solanum linaeanum, Solanum americanum, Annona squamosa.

1.0 INTRODUCTION

Schistosomiasis, transmitted by water snails, is the most widespread of all vector-borne diseases, affecting almost 240 million people worldwide.It is a parasitic disease caused by trematodes belonging to genus *Schistosoma*. It is an important public health problem second only to malaria in terms of morbidity and mortality (WHO, 2002; Domo *et al.,* 2009; Mohammed, 2009).Two forms of human schistosomiasis endemic in Kenya are Intestinal schistosomiasis caused by *Schistosoma mansoni* and transmitted mainly by *Biomphalaria pfeifferi* snails and urinary schistosomiasis caused by *Schistosoma haematobium* which is transmitted by *Bulinus* species (Kloos *et al.,*1988).In Kenya, 6 million people are infected and an estimated 15 million are at risk (Hotez and Kamath, 2009).This disease poses serious socio-economic and health effects since it is endemic in developing countries.Infection in school children has been reported to lead to stunted growth and poor academic performance. Productivity of the infected community is severely reduced due to weakness and lethargy (Kloos and McCollough,1987). People acquire schistosomiasis through repeated contact with fresh water during fishing, farming, swimming, washing, vehicles, motorbikes, clothes, bathing and recreational activities.

Sustainable control of schistosomiasis requires integrated approach including repeated mass chemotherapy using Praziquantel, public health education focusing on behavior changes towards risk factors, improving sanitation, provision of clean water supply, and snail control (Mazigo *et al.,* 2012). Since there is a possibility of re-infection even after repeated mass chemotherapy with praziquantel (Hassan *et al.,* 2011; complementing chemotherapy with measures such as snail control is necessary to prevent re-infection of people in endemic areas. The use of Praziquantel, the current drug of choice for treatment of all kinds of schistosomiasis, in developing countries is limited by its high costs. Its long-term worldwide application coupled with the recent discovery of Praziquantel-tolerant schistosomes has generated concern over the development of drugs-resistant

Schistosoma strains. There is no vaccine developed to date against Schistosomiasis, aggravating the problem even further, hence the need for cheap, safe and effective biorationals.Snail control through the use of synthetic molluscicides forms an important part in the integrated schistosomiasis control programme (Adetunji *et al.,*2010) However, the high cost of synthetic compounds such as Bayluscide® (Bayer, Leverkusen, Bayerwerk,Germany) and their negative impact on the environment have stimulated interest in search for alternative molluscicides of plant origin (Ontarigho *et al.,* 2012).

2.0 MATERIALS AND METHODS

2.1.Collection of plant specimen

Six plant species used in this study were collected from their natural habitats on the basis of ethnobotanical information and with bio-conservation aspects in mind. They were from Mavoko location, Machakos County. Taxonomist from the National Museums of Kenya (NMK) authenticated the botanical identity of the plant materials and voucher specimens were preserved in the herbarium. Plant parts collected for use in this study were: leaves of *Annona squamosa* (AS), seeds of *Solanum americanum* (SA), berries of *Solanum linaeanum* (Sl), berries of *Phytolacca dodecandra* (PD),seeds of *Piper nigrum* (PN),and leaves of *Rhizophora mucranata* (Rm) from Kenyan coastal area.

2.2 Preparation of plant extracts for molluscicidal bioassay

The six plant materials used in this study were collected as described earlie in 3.2.1.
Solvent extract was made by dissolving 25g of each of the fine powdered plant materials in 250 ml of analytical grade methanol overnight with agitation then filtered using muslin cloth and watsman filter paper. Filtrate was extracted in a soxhlet apparatus for 10 cycles at 40 ^{0}C.The extracted material was concentrated to dryness under reduced pressure using rotary evaporator at 45°C to remove methanol.The final extract of each plant was label as PdME,SlME,SaME,AsME,PnME and RmME (i.e. plants initials and ME for methanol extract) then refrigerated till needed for bioassays. Aqueous extract was prepared by soaking 100 g of each dried plant powder in 1000 ml of distilled water for 24 hours at room temperature (25°C) with shaking. The crude extract was filtered using muslin cloth, and then filtrate frozen and later freeze dried. Yield of each extract recorded after weighing and finally labeled as PdAE, SlAE, SaAE, AsAE, PnAE and RmAE (i.e. AE for aqueous / water extract) then refrigerated till needed for bioassays (Molla ,2011; Salawu *et al.,*2011).

2.3 Collection, screening and maintenance of the snail intermediate host

Biomphalaria pfeifferi snails were collected from canals in Mwea Irrigation Scheme in Mbeere South sub county, Embu county of Kenya. Snails were scooped out of the water using dip-net scoop, cleaned from debris attached to them and placed in open plastic buckets containing some sand, vegetation and water.The snails were packed in plastic containers which had holes for aeration in layers separated by wet cotton wool.They were transported to Institute of Primate Research (IPR) malacony laboratory. Snails were screened for schistosomes according to Christensen and Frandsen (1985) under strong light (100 watts) for two hours for six consecutive weeks to exclude infections from the wild. Negative snails were housed in temperature controlled (25-27ºC) and light-controlled (12h light /12h dark) snail room at the Institute of Primate Research (IPR). They were placed in water tanks containing non -chlorinated tap water from IPR wells which was changed twice a week. Soft lettuce (steamed and gently dried in oven) was added to feed the snails (Syombua *et al.,* 2013).To obtain juvenile snails, trays holding snail water, lettuce and daphnia were prepared. Into each of the trays, 10 snails were placed.

The snails were allowed time to lay eggs. Juvenile snails that hatched from the egg masses in the water were allowed to grow in the same trays with their mothers (Syombua *et al.,* 2013).

2.4 Molluscicidal activity of extracts on adult and juvenile snails

The snail species used in this study was *Biomphalaria pfeifferi* and the molluscicidal potency test was performed according to established procedure (WHO, 1983).The plant extracts were evaluated for molluscicidal activity on adult and juvenile snails as follows: Groups of 10 snails were placed in plastic containers holding 500ml of distilled water.The set ups were left for 24h and snails were fed on dried lettuce. Different concentrations; 10 mg/litre, 20 mg/litre and 50 mg/litre,100mg/L and 150 mg / L were prepared by weighing 0.01g,0.02g,0.05g, 0.1g and 0.15g then dissolving each in one litre of distilled water.Positive control was prepared as 1 mg/litre of niclosamide (McCullough, 1992), and the negative control was 500 ml of distilled water only.After 24 h,distilled water was discarded from each container holding 10 snails and replaced with 500 ml of the different extract concentrations, positive control and a negative control. For each dosage, a duplicate set up was made for both adult and juvenile snails.

The snails were not fed and after 48h the extracts were discarded and replaced with distilled water for recovery. After 24 h of recovery period, snails were observed and mortality recorded.Healthy snails live up to 5 days or more without food provided other environmental conditions are constant (Adetunji *et al.,* 2010). Dead

snails remained retracted inside their shells with discolored body at the bottom of the plastic container. Mortality was also assessed by probing the snails with a blunt wooden probe to elicit typical withdrawal movements. Confirmation of death was done by locating the heart and observing the heart beat under the dissecting microscope.Lack of heart beat signified death of the snail (WHO 1985; Syombua *et al.,* 2013). Toxicity of extracts was expressed as LC_{50} corresponding to concentrations that killed 50% of the tested snails after being exposed to extracts for 48 hours (Syombua *et al.,* 2013; Chagbunjong *et al.,*2010 and Eissa *et al.,*2011).

3.0 DATA ANALYSIS

Data on molluscicidal activity was analyzed by Finney's probit analysis using BIOSTAT Pro 2009 Version 5.8.4 to estimate LC_{50} (concentration required to kill 50% of the test organisms) values of the various extracts on adults and juvenile snails. ANOVA (Analysis of variance) was used to determine whether there were any significant differences between the extracts. Once significant differences were identified, post hoc ANOVA was done with Turkey/Dunnet Test to compare each treatment with the positive control. In all the analysis, the probability level /significance level used was $p < 0.05$ which signifies 95% confidence level.

4.0 RESULTS

4.1 Mortality of juvenile and adult snails

The data collected on molluscicidal activity of both aqueous and methanol plant extracts against test snails was organized as shown in Table1 below. The average mortality of both adult and juvenile snails at different extract concentrations was expressed as percentages and shown in Table 5 below. Efficacy of aqueous and methanol plant extracts against juvenile and adult snails are illustrated in Figure 1 & 2 below. The most active aqueous extracts were *Phytolacca dodecandra* registering mortality of 70% and 100% ;60% and 100% on juveniles and adult snails at extract concentrations of 20ppm and 100ppm respectively followed by *Solannum linaeanum* which killed 60% and 100 %;50% and 90% at 20ppm and 100ppm respectively.

Average mortality of these two extracts were not significantly different from that of Niclosamide ($p< 0.05$). Moderate molluscicidal activity was observed in *Solannum americanum* (40% and 80% for juveniles; 30% and 80% for adults at 20ppm and 100ppm respectively and *Annona squamosa* (30% and 70% for juveniles; 10% and 70%). The least active extracts were *Piper nigrum* (20% and 60% of juveniles; 0% and 60% of adults) followed by *Rhizophora mucranata* with 10% and 50% juveniles;0% and 40% adults at 20ppm and 100ppm respectively. There was no significant difference in snail mortality exposed to methanol and aqueous extracts ($p<0.05$). *P.dodeccandra* and *S.linaeannum* methanol extracts caused highest snail mortality followed by *S.americanum* and *A.squamosa*.*P.nigrum* and *R.mucranata* were the least effective killing only 60% and 30% at 150ppm respectively. All the extracts tested had very low percentage mortalities at 10ppm with only *P.dodeccandra* extract killing 40% and 30% on juvenile and adult snails respectively. There was no typical withdrawal movement elicited when dead snails were probed at foot with a blunt wooden splint. Niclosmide, the molluscicide of choice was used as positive control and killed 100% of test snails at 1ppm.

In Figure 1 below,efficacy of plant extracts were compared at each dosage and the most active extract is that of the kenyan *Phytolacca dodecandra* followed by *Solanum linaeanum*.Mortality increased with increase in concentration and at 20ppm both the plants had killed 70% of adult snails while *Solanum americanum* and *Annona squamosa* killed only 30%.At 100ppm four plant species had registered mortality in the range of 70%-90% while two were below 40%.It was shown that molluscicidal activity of *Solanum linaeanum* was higher than that of *Phytolacca dodecandra* at 50ppm and 100ppm ;90% and 100% vs 80% and 90% respectively on juvenile snails.At 10ppm and 20ppm *Rhizophora mucranata* was inactive registering 0% mortality and managed to kill only 30% of juvenile snails at 150ppm.

None of the plant extracts was able to kill 50% of juvenile or adult snails at 10ppm. At 150ppm all extracts except *Rhizophora mucranata* registered 100% mortality. However, Efficacy of *Solannum linaeanum* and *Phytolacca dodecandra* were exceptional as they killed over 60% of snails at 20ppm.There was an increase in mortality with increase of extract concentration suggesting they were dose-dependent.

Figure 1: Dose response relationship of extracts on *Biomphalaria pfeifferi* snails

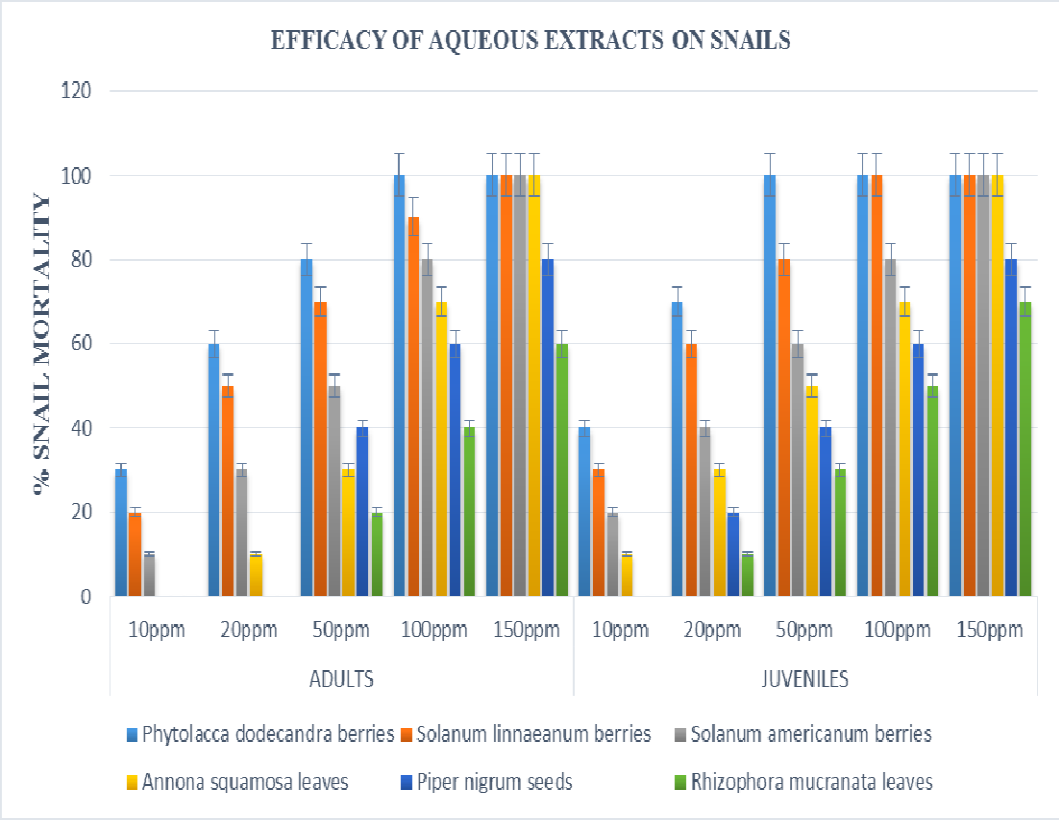

Figure 2: Dose-response relationship of methanol extracts against *Biomphalaria pfeifferi* snails

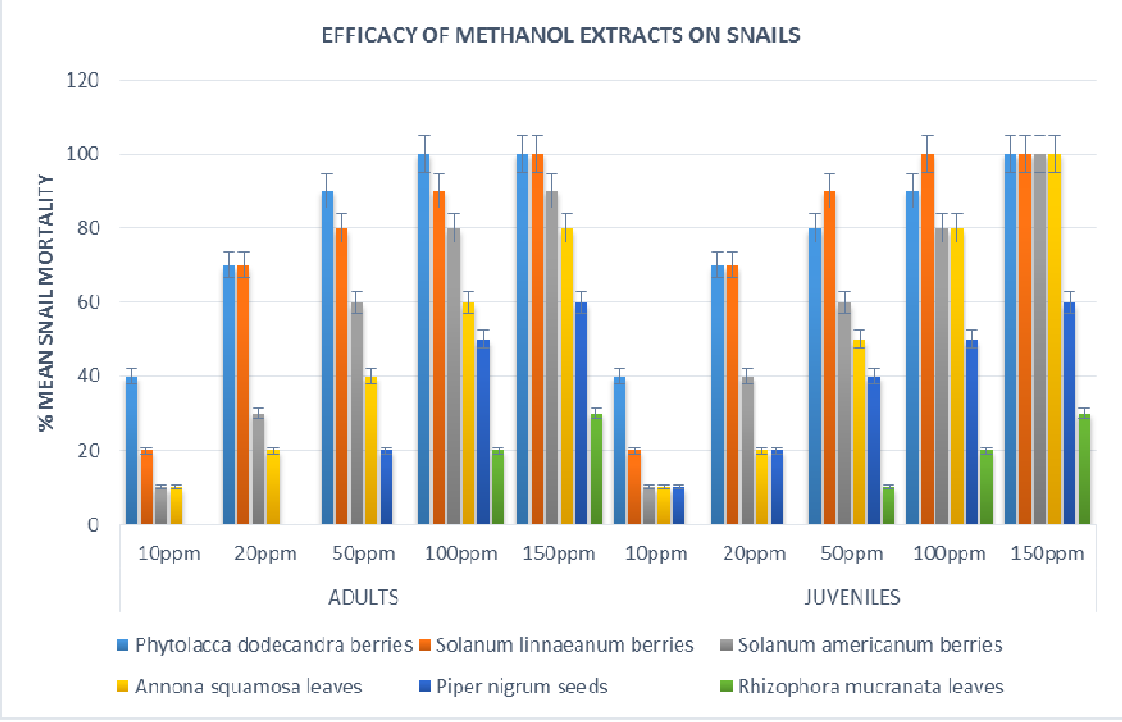

Table 1: Percentage average mortality of *Biomphalaria pfeifferi* snails

	% AVERAGE MORTALITY									
	ADULTS					JUVENILES				
Aqueous plant extract	**10ppm**	**20ppm**	**50ppm**	**100ppm**	**150ppm**	**10ppm**	**20ppm**	**50ppm**	**100ppm**	**150ppm**
Phytolacca dodecandra berries	30	60	80	100	100	40	70	100	100	100
Solanum linnaeanum berries	20	50	70	90	100	30	60	80	100	100
Solanum americanum berries	10	30	50	80	100	20	40	60	80	100
Annona squamosa leaves	0	10	30	70	100	10	30	50	70	100
Piper nigrum seeds	0	0	40	60	80	0	20	40	60	80
Rhizophora mucranata leaves	0	0	20	40	60	0	10	30	50	70
CONTROLS			ADULTS					JUVENILES		
Positive control-Niclosamide (**1ppm**)			100					100		
Negative control-Distilled water (**0 ppm**)			0					0		
Methanol plant extracts										
Phytolacca dodecandra berries	40	70	90	100	100	40	70	80	90	100
Solanum linnaeanum berries	20	70	80	90	100	20	70	90	100	100
Solanum americanum berries	10	30	60	80	90	10	40	60	80	100
Annona squamosa leaves	10	20	40	60	80	10	20	50	80	100
Piper nigrum seeds	0	0	20	50	60	10	20	40	50	60
Rhizophora mucranata leaves	0	0	0	20	30	0	0	10	20	30
CONTROLS			ADULTS					JUVENILES		
Positive control-Niclosamide (**1ppm**)			100					100		
Negative control-Distilled water (**0 ppm**)			0					0		

Ppm=parts per million=mg/L

4.2 Dose –response of snails to plant extracts

Results of Probit analysis using BIOSTAT Pro 2009 to determine the lethal dose (LC_{50}) which is the concentration that killed 50% of test organisms were expressed as plus or minus their standard error (SE) and shown in Table 6 : below. According to WHOPES (2005) and Nguta *et al.,* (2011) a plant extract with a LC_{50} within the range of 0-500ppm is highly toxic ; LC_{50} between 500ppm-1000ppm shows moderate toxicity while LC_{50} which is over 1000ppm shows the extract is nontoxic to target organisms.

The most active aqueous extracts on both juvenile and adults were *Phytolacca dodecandra* (LC_{50} 10.69 and 11.80ppm) and *Solannum linaeanum* (LC_{50} 14.39 and 15.18 ppm) respectively.Average mortality of these two extracts were not significantly different from that of Niclosamide ($p<0.05$).Moderate molluscicidal activity was observed in *Solanum americanum* (LC_{50} 36.17 and 37.92 ppm) and *Annona squamosa* (LC_{50} 51.96 and 58.86ppm).The least active extracts were *Piper nigrum* (LC_{50} 109.82 and 91.14) and *Rhizophora mucranata* LC_{50} 193.07 and 146.21ppm) respectively. There was no significant difference in snail mortality exposed to methanol and aqueous extracts ($p>0.05$).

Table 2: Toxicity of plant extracts on juvenile and adult snails

Extract	Plant species	Adult snails (LC_{50}±SE)	Juvenile snails (LC_{50}±SE)
Aqueous	*Phytolacca dodecandra*	11.80±10.96	10.69±7.13
	Solannum linaeanum	15.18±9.24	14.39±7.36
	Solannum americanum	37.92±9.92	36.17±7.87
	Annona squamosa	58.86±15.48	51.96±13.27
	Piper nigrum	109.82±17.55	91.14±18.25
	Rhizophora mucranata	193.07±18.18	146.21±12.35
Methanol	*Phytolacca dodecandra*	13.80±10.96	11.69±7.15
	Solannum linaeanum	15.21±9.24	13.40±7.36
	Solannum americanum	38.92±9.92	35.17±7.89
	Annona squamosa	59.86 ±15.48	51.96±13.27
	Piper nigrum	110±16.55	95.14±18.35
	Rhizophora mucranata	199.07±19.18	156.21±13.45

Alpha ($p< 0.05$), SE=Standard error, LC=lethal Concentration

4.3 Comparisons of efficacy of plant extracts and controls.

The experimental data was analyzed using ANOVA post – hoc to determine whether there were any significant differences between the different treatment groups and control groups. A value $p<0.05$ was considered significant. Molluscicidal activity of *Phytolacca dodecandra* and *Solanum linaeanum* were significantly similar to niclosamide ($p > 0.05$) while the rest of the extracts were significantly different ($p<0.05$).ie *P.dodeccandra* had F=1.75,p=0.228; *S.linaeanum* F=3.84,p = 0.0854; *S.americanum* F=8.0,p = 0.022; *A.squamosa* F= 28,p = 0.001;*P.nigrum* F=22.022,p= 0.002 and *R.mucranata* F= 49.23,p = 0.0001at 95% confidence level. Efficacy of *Phytolacca dodecandra* which had the highest molluscicidal activity was compared with those of other extracts by Post-Hoc ANOVA using Turkey's test. Results showed that molluscicidal activity of *Solanum linaeanum, S.americanum* and *Annona squamosa* were not significantly different from that of *P.dodeccandra* ($p > 0.05$) i.e S.*linnaeanum*; F= 0.11, $p = 0.75$, *S.americanum*; F = 0.876, $p = 0.377$;*A.squamosa* ; F=1.09,$p = 0.327$;*P.nigrum* F=5.26,$p = 0.051$ and *R.mucranata* F=14.029, $p = 0.0057$. Molluscicidal activity of extracts against juvenile and adult snails was not significantly different $p > 0.05$; $p = 0.864$, F = 0.0318. The results on comparison of aqueous extracts with methanol extracts showed that molluscicidal activities of aqueous extracts against test snails was not significantly different from activity of methanol extracts (F= 69.25; p> 0.05).

5.0 DISCUSSIONS

Control of schistosomiasis could be achieved through intermediate host snail control using molluscicides from plant origin. Phytochemicals have been shown to have mollscicidal, larvicidal, pupicidal, antimicrobial, antiparasitic and ovicicidial activities among others. Bioactivity is influenced greatly by abiotic factors like geographical and climatic factors. Bioactive principles are concentrated in various parts of the plant while others are equally distributed in all parts (Syombua *et al.,* 2013). The aim of the current study was to evaluate molluscicidal activity of six plant species from different parts of Kenya, against juvenile and adult *Biomphalaria pfeifferi* snails.*P.dodeccandra* and *Solanum linaeanum* had the highest molluscicidal activity against juvenile and adult snails and this activity was not significantly different from that of Niclosamide $p > 0.05$ both of which killed 50% of snails at extract concentration less than 20ppm.

Phytolacca dodeccandra was the most active, killing 50% of juvenile and adult snails at 10.69 and 11.80 ppm respectively followed by *Solannum linnaeanum* at 15.18 and 14.39 ppm. Comparison of activity of these extracts to Niclosamide gave $p > 0.05$ meaning that there was no significant difference in molluscicidal activity, therefore *P.dodeccandra* and *S.linaeannum* contain potent molluscicides effective against both juvenile and adult *B.pfeifferi* snails, the intermediate hosts of *Schistosoma mansoni.* The rest of the extracts had $p< 0.05$ meaning that their molluscicidal activity was significantly different from that of Niclosamide. However, since a good plant molluscicide should kill snails at 100ppm or less, *Solanum americanum* and *Annona squamosa* recorded moderate activity killing 50% of adults and juvenile snails at 37.92, 36.17; 58.96 and 51.96 ppm respectively.Therefore the least effective extracts were *Piper nigrum* and *Rhizophora mucranata* that killed 50% of adult and juvenile snails at 109.82, 91.14; 193.07, 146.21ppm respectively. Comparison of susceptibility of juvenile and adult snails to extracts revealed that there was a significant difference ($p< 0.05$), juveniles being more susceptible to aqueous extracts than adult snails. This could be attributed to the fact that adult snails have protective shell that limits exposure to toxic effects of extracts, a structure lacking in juveniles hence the higher mortality. Syombua *et al.,*2013, when investigating the molluscicidal activity of *Entada leptostchya methanolic extract on B.pfeifferi* snails found that the extract killed 50% of juveniles at 30.21ppm and adults at 40.93ppm hence juveniles were more susceptible to toxic effects of extract than adults supporting the results obtained in this study. Molluscicidal activity was dose-dependent meaning that in all extracts, snail mortality increased with increase in concentration of extract used.

Various aqueous and methanol extracts had molluscicidal activity which was significantly different from the different treatments used ($p< 0.05$). This means that the active principles are responsible for snail mortality were not equally distributed in the plant species tested. Comparison of efficacy of methanol and aqueous extracts on test snails was not significantly different $p > 0.05$ meaning that they were extracting similar compounds. This is advantageous because the ultimate goal is to get a biorational that can be used at the most convenient and cheapest way by the communities.

According to Akillu Lemma (1970), dried berries of endod (*Phytolacca dodecandra*) also known as soapberry are widely used in Ethiopia instead of soap for laundering clothes. It was observed that in natural bodies of water where endod had been used there was a high mortality of snails.

Subsequently, the molluscicidal potencies of various parts of endod plants were determined and the berries were found to be the most potent.This is in line with this study that used berries of *P.dodeccandra* found in Kenya.

6.0 CONCLUSIONS

The present study has demonstrated that four plant species: *Phytolacca dodecandra, (Phytolaccaceae) Solanum*

linaeanum, Solanum americanum (Sollanaceae) and *Anonna squamosa (Annoaceae)* killed 90% of snails at 100ppm or lower, hence could be considered for development of molluscicides. Since development of resistance of snails to current synthetic molluscicide -Niclosamide, its high costs and toxicity to non-target organisms/negative impact on the environment has been reported, these plant extracts may offer a cost effective, safe and environmentally friendly alternative for Schistosomiasis control in endemic Africa.

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COMPETING INTERESTS
The Authors declare that they have no competing interests.

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