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Molluscicidal Activity Of Chrysanthemum Cinerariaefolium Extracts Against Biomphalaria Pfeifferi Snails

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Abstract: *Schistosomiasis remains a public health problem especially to communities living in areas near slow-moving water bodies in many parts of the world. The transmission of schistosomiasis requires fresh water vector snails as obligate intermediate hosts. Control of snails is one of the most effective methods considered in breaking transmission of schistosomiasis. Niclosamide a synthetic chemical molluscicide has been widely used in control programs but currently, it's very expensive for largescale use in control programmes and no longer readily available. Plants are potential alternative sources of molluscides. The present study was done to determine whether extracts of pyrethrum plant (Chrysanthemum cinerariaefolium) exhibit molluscicidal activity against adult and juvenile Biomphalaria pfeifferi .Snails in groups of 10 were treated with various concentrations of water, methanol and Petroleum (Pet) ether crude extracts derived from pyrethrum plant. De-chlorinated tap water was used as negative control and niclosamide was used as positive control. After exposure to the extracts, the snails were left for period of 24 hours in distilled water to recover and number of dead snails recorded in each experimental set up. Data was analysed by ANOVA and Finney probit analysis was used to estimate the lethal dose (LD) required to kill 50 % and 90% of members of tested population. Pet ether extracts showed the highest molluscidal activity with LD_{50} of 34.5 mg/l and 28.0 mg/l for adult and juvenile B. pfeifferi respectively followed by methanolic extracts with LD_{50} of 44.4 mg/l and 38.9 mg/l for adult and juvenile snails respectively. Aqueous extracts exhibited the lowest molluscicidal activity with LD_{50} (686.7 mg/l and 413.5 mg/l for adult and juvenile snails respectively). The LD_{90} for adult B. pfeifferi was 67.4 mg/l, 86.8 mg/l and 1342 mg/l in Pet ether, Methanol and Aqueous extracts respectively. The LD_{90} for juvenile B. pfeifferi was 59.4 mg/l, 82.6 mg/l and 877 mg/l for Pet ether, Methanol and Aqueous extracts respectively. The results indicate that both the methanolic and Pet ether extracts have molluscicidal activity against adult and juvenile B. pfeifferi.*

Keywords: *Biomphalaria pfeifferi, Chrysanthemum cinerariaefolium, Crude extracts, Molluscicidal activity*

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I. Introduction

Human schistosomiasis is one of the parasitic diseases which is caused by trematode species belonging to the genus *Schistosoma* which reside in various parts of the mammalian viscera.[1] Schistosomiasis is found in tropical and subtropical regions of the World including Africa, Caribbean, Middle East, South America and South East Asia and is endemic in 77 Countries of the World. Currently the number of infected individuals worldwide is estimated to be 252 million with another 800 million being at risk of infection of which 90 % are living in Sub Sahara Africa.[2-4] Both the intestinal and urinary forms of the disease, caused by *Schistosoma mansoni* and *S. haematobium* respectively, occur in Kenya affecting over 3.5 million people and 12 million more with risk of infection (Ministry of Health, Kenya, unpublished data).[5] Man is the definitive host acquiring the infection by coming into contact with freshwater harboring the larval stage of schistosome cercariae, which actively penetrate the skin and later develop to adult worms. Snails become infected with miracidia which hatch from schistosome eggs in human faeces which reach freshwater after humans excrete in open areas near water bodies.[6] Control of schistosomiasis involves various approaches, which can be implemented in combination and

include environmental sanitation, health education, mollusciding and chemotherapy.[7] Chemotherapy is key in any control strategy and is currently recommended by World Health Organization (WHO) especially in endemic areas as it significantly reduces morbidity in the short term. The other recommended approach to reduce and consequently eliminate transmission involves eliminating the snail intermediate hosts harboring the infective schistosomal larvae, thus breaking the parasite's life-cycle however at present there are few safe and environment friendly methods for snail population control.[8]

Whereas snail control is regarded as an effective method of controlling schistosomiasis, there has been few snail control initiatives which is largely attributed to the cost of available molluscicides and their safety.[9] Control of snails using molluscicides is an important tool in integrated approach of controlling the disease. Availability of inexpensive and environmentally friendly molluscide would be key in having a complementary tool in addition to chemotherapy in the control of schistosomiasis.

Many plants have been reviewed and evaluated for molluscicidal properties due to the fact that they are advantageous for being more readily available from plant sources and are relatively less expensive, and environmentally friendly as they are readily biodegradable.[10-12] Pyrethrum plant is readily available in Kenya and its use as plant molluscide may serve as an additional tool to the current methods of snail control and development of safe and effective future generation of molluscicides. Pyrethrum is included in most lists of approved insecticides for use as an organic pesticide worldwide and is one of the most common insecticides.[13] The active insecticidal components of pyrethrum are known as "pyrethrins".[14] Chrysanthemic (pyrethrins I,) and pyrethric (pyrethrins II) acid are the esters which make up pyrethrins. "Total pyrethrins" ussualy refers to combination of pyrethrins I and pyrethrins II esters.[15] Among the biological activities reported for the different species of *Chrysanthemum* plants include antibacterial, inhibitory, pharmacological and insecticidal.[16]

In the present study, a laboratory study was undertaken to evaluate pyrethrum plant (*C. cinerarriaefolium*) for molluscicdal properties against *B. pfeifferi* snails involved in the transmission of human schistosomiasis a major parasitic infection in Kenya.

II. Materials and Methods

Plant material and extracts

The pyrethrum plant and flower were sourced from pyrethrum growing regions of Nakuru County Kenya Latitude (0˚41.563S, Longitude 36˚25.196E) in which pyrethrum plant is still cultivated. Voucher specimens were deposited at the Kenya Medical Research Institute (KEMRI), Centre for Traditional Medicine and Drug Research (CTMDR) herbarium with voucher number Tolo/Mwitari/Keter/001. Pyrethrum Board of Kenya (PBK) assisted in the identification of the regions for sample collection. PBK also provided reference standards of the pyrethrins to be used in analytical studies. Flowers were hand harvested when they were at optimum maturity (when daisies were in full bloom) as this is when the concentration of pyrethrins is at its peak. The dried flowers were placed into burlap sacks and transported to CTMDR facilities at KEMRI Nairobi. The flowers were air dried at room temperature in a dark room. The dried flowers were then grounded into fine powder using laboratory mill (Christy & Norris Ltd., Chelmsford, England). The powder was stored in air tight polythene bags to prevent moisture and away from direct sunlight. The powder was used for extracting the total pyrethrin and total yield calculated as outlined below.

Extraction method of total pyrethrin

Method 1 Aqueous extraction

Extraction process was done using the method as described by Awoyinka *et al*[17] with slight modifications. Two hundred grams (200 g) of the dried powdered plant flower were weighed and soaked in 2000 ml of distilled water. The mixture was then placed in a water bath set at temperature of 60°C for a period of 1 hour and then decanted into clean, dry 2000 ml conical flask. Filtration was done by filtering the mixture by passing it through 2 layers of sterile gauze. The filtrate was freeze dried in portions of 200 ml using a Freeze Dryer Edwards freeze dryer Modulyo (Edwards high vacuum, Crawey England, Britain, Serial No. 2261). The powder extract after freezing was weighed and put in a labeled air tight 100 ml centrifuge tube and stored at 4°C until use.

Method 2 Methanol extraction

Extraction process was carried out as per the methods described by Parekh *et al*[18] with slight modifications. Briefly, 500g of the dried powder plant material was weighed and then soaked in 1700 ml methanol in a flat-bottomed 3 litre conical flask and covered with gauze and left for 3 days at room temperature in a dark room. After three days the mixture was filtered using sterile cotton gauze and concentrated using a rotary evaporator (Büchi Rota vapor R-114) at 70°C. The gummy extract was then weighed and put in air tight capped round bottomed flask at 4°C until use.

Method 3 Pet Ether extraction

Extraction process was carried out based on the methods as described by Shawkat *et al*[19] with slight modifications. 1000 grams of dried plant material was soaked in 3 litres Petroleum ether for 3 days at room temperature under indirect sunlight. The mixture of the solvent and flowers was filtered by using filter paper (whatman filter paper No 1). The filtrate was mixed with methanol 80% at a ratio of 20:80 (extract: alcohol). It was then shaken vigorously and left to settle in order to separate into two layers. The layer that tended to turn into yellow, indicative of pyrethrin presence was isolated. The solution was then concentrated by using Rotary Evaporator (Büchi Rota vapor R-114) at 70°C. The gummy extract obtained was weighed and transferred to a tight capped round bottomed flask. The extract was stored at 4°C until use.

Qualitative Phytochemical Screening

Phytochemical screening to check for presence of tannins, alkaloids, phenols, saponins, flavonoids, terpenoids and glycosides was carried out as per procedures described below;

Alkaloids: Three drops of Mayer's reagent were added to 2ml of the extract. Formation of a yellow colored precipitate indicated the presence of alkaloids

Phenols: Four drops of ferric chloride solution were added to the extract. Formation of a blue-black color indicated the presence of phenols.

Saponins: 5ml of the extract was diluted with distilled water to 10ml in a graduated cylinder and shaken for 10minutes. Formation of a persistent layer of foam indicated the presence of saponins.

Flavonoids: 2ml of dilute ammonia and 2ml of concentrated sulphuric acid was added to the extract. Formation of intense yellow color indicated the presence of flavonoids.

Glycosides: 1ml of glacial acetic acid was added to the 0.5ml of the extract. One drop of iron chloride was added and the mixture shaken. 1ml of concentrated sulphuric acid was then added to the mixture. Formation of a brown ring indicated the presence of glycosides.

Terpenoids: 2ml of chloroform was added to 1ml of the plant extract and shaken vigorously. 2ml of concentrated sulphuric acid was then added and heated for 2minutes. Formation of grey color indicated the presence of terpenoids.

Tannins: 5ml of distilled water was added to 2ml of the plant extract and heated to boil. 2% of iron chloride was then added. A blue-black color formation indicated the presence of tannins

Snail collection and maintenance

Biomphalaria pfeifferi snails were collected from Mwea irrigation scheme in Kirinyaga County Kenya. The snails were transported to KEMRI, Centre for Biotechnology Research and Development (CBRD) Laboratories, for identification using identification keys. The collected and identified snails were screened if they were shedding cerceria by placing the snails in 24 well culture plates and exposing them to strong light (70 watts) for one hour daily for a month. Snails that were not shedding cerceria were used for breeding to obtain first generation snails used in the experiment. The snails were transferred to plastic trays layered with sterilized sand and gravel from Mwea. The trays were then filled with water and water aerated using aeration pumps. The plastic trays were housed at KEMRI snail laboratory with controlled temperatures of 25°C to 28°C. The snails were fed on boiled dried lettuce.

Breeding of juvenile snails

Plastic trays were prepared and layered with sterilized sand and gravel collected from habitats where the snails were collected. The trays were then filled with de-chlorinated tap water. Ten adult snails were put into each tray. Once the snails laid the egg masses on the edges of the trays with water, the eggs were allowed to hatch and the young snails allowed to grow in the same trays with their mothers.

Testing for molluscicidal activity

The evaluation of molluscicidal activity against adult and juvenile snails was performed as per WHO guidelines for testing plant molluscicides.[20] Uninfected laboratory bred first generation *B. pfeifferi* adult snails measuring 6-14 mm in diameter in groups of 10 of were transferred to plastic containers containing distilled water and left overnight. The distilled water in the containers was substituted with known prepared concentrations of the extracts (1 mg/l, 5mg/l, 10mg/l, 25 mg/l, 50 mg/l, 100mg/l, and 250mg/l, 500mg/l and 1000 mg/l) of the plant extracts. Duplicates were set for each concentration. Positive and negative controls were also set with niclosamide (1mg/l) used as the positive control and de-chlorinated tap water used as negative control. Duplicates were also set for the controls. The snails were left in the prepared concentrations of the extracts for a period of 24 hours after which they were removed and put in respective labeled plastic containers with de-chlorinated tap water and left for 24 hours to recover. After 24 hour recovery period dead snails from each experimental set up were counted. Death of the snails was checked by two ways; those that remained

retracted inside their shells at the bottom of the plastic container and did not show any movement after mechanical prodding with wooden spatula. Death was confirmed by lack of any heart beat after examining with dissecting microscope. Live and dead snails in each setup were counted and recorded.

Molluscicidal activity of juvenile snails

One month old juvenile snails measuring 3-5 mm in diameter were placed in groups of 10 in plastic containers holding 500 ml of distilled water. The same procedure which was used for testing molluscicidal activity on adult snails was repeated for juveniles.

Molluscicidal activity of infected and non-infected snails

Uninfected laboratory bred first generation *B. pfeifferi* adult snails measuring 6-14 mm in diameter in groups of 10 of were transferred to plastic containers containing distilled water and left overnight.

Groups of ten uninfected *B. pfeifferi* adult snails and ten infected *B. pfeifferi* were put in respective plastic containers containing distilled water and left overnight. The distilled water in the containers was substituted with known prepared concentrations of the extracts (1 mg/l, 5mg/l, 10mg/l, 25 mg/l, 50 mg/l, 100mg/l, and 250mg/l, 500mg/l and 1000 mg/l) of the plant extracts. Duplicates were set for each concentration. Positive and negative controls were also set with niclosamide (1mg/l) used as the positive control and de-chlorinated tap water used as negative control. Duplicates were also set for the controls. The snails were left in the prepared concentrations of the extracts for a period of 24 hours after which they were removed and put in respective labeled plastic containers with de-chlorinated tap water and left for 24 hours to recover. After 24 hour recovery period dead snails from each experimental set up were counted. Death of the snails was checked by two ways; those that remained retracted inside their shells at the bottom of the plastic container and did not show any movement after mechanical stimulation with wooden spatula. Death was confirmed by lack of heart beat

Data analysis

Statistical analysis was done using IBM Statistical Package for Social Sciences (SPSS) Statistics) 22. One way analysis of variance (ANOVA) was used to determine whether there were significant differences in the molluscicidal activities of the various extracts. Once significant differences were identified, post hoc analyses were done using Dunnett's test. $P<0.05$ was considered statistically significant

Finney probit analysis was also done to estimate LD_{50} and LD_{90} (concentration required to kill 50% and 90% of the juvenile and adult snails respectively).

Ethical Approval

This study was approved by KEMRI's Scientific and Ethics Review Unit (SERU) of the and Animal Care and Use Committee (ACUC), (KEMRI/SERU/CBRD/140/3146).

III. Results

Percentage yield of aqueous methanol and Pet ether extract

The different solvent extracts yielded different yields with methanolic extracts having highest percent yield of 13% as shown in Table 1.

Table 1: Percentage yield of the different extracts

Solvent used	Dry weight (g)	Extraction type	Weight after extraction	Percentage yield (%)
Aqueous	200	Freeze drying	13	6.5
Pet ether	1000	Rotary-evaporation	21.4	2.14
Methanol	500	Rotary-evaporation	65	13

Phytochemical screening of aqueous, methanol and Pet Ether extract

Phytochemical screening of the different extracts revealed the extracts have different constituents in different degrees as tabulated in Table 2. Alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids were detected in the aqueous, methanol and pet ether crude extracts of *C. cinerariaefolium* flower.

Table 2 Phytochemical constituents present in the crude extracts

Classes of phytochemical constituents	Aqueous extract	Methanol extract	Pet ether extract
Alkaloids	-	+++	+++
Flavonoids	+	+++	++
Phenols	++	+++	++
Saponins	++++	+++	++++
Tannins	++	+++ +	++++
Terpenoids	+++	+ +++	++++

Key: +++: strong presence, ++: moderate presence, +weak presence, -: not detected

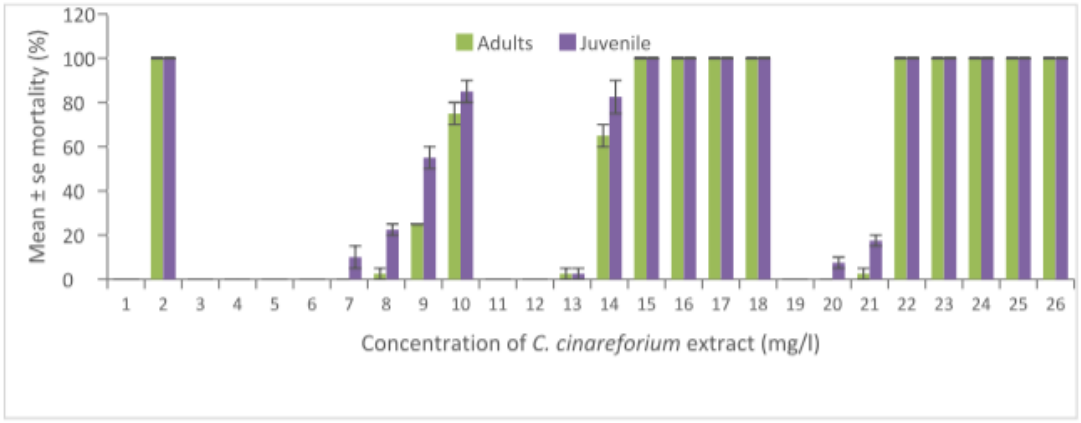
Bench side observation of snails

This study revealed that when the snails were exposed to the Pet ether extracts and methanolic extracts from concentrations of 25 mg/l the toxicity of the crude active plant extracts became manifested. The snails started to immediately move out of the test solution and attached themselves on the side of the container and the snails had to be constantly returned back to the test solution. This behavior of crawling out of test solution can be attributed to behavior of snails to move away from conditions detrimental to their survival. The snails were thus covered with netting material to prevent them from completely crawling out of the containers overnight. On examination under dissecting microscope after 5 hours post exposure the snails showed either a partial retraction in the shell, after prodding of the foot sole with blunt object and visible secretion of mucous over most part of the foot. After 24 hours the snails moved to the bottom of the container and showed no retraction at all after prodding of the foot-sole with a blunt needle while others retracted to their shells. Death was confirmed by lack of heart movements on examination under dissecting microscope. When the snails were introduced to distilled water (negative control) they first retracted into their shells resuming normal activity after about 20 minutes with the snails moving around the container. The snails immediately retracted to their shells when the extended footsole was prodded with wooden spatula. The snails remained active even after 24 hours and no mortality was recorded even after 48 hours.

Mortality of adult and juvenile snails

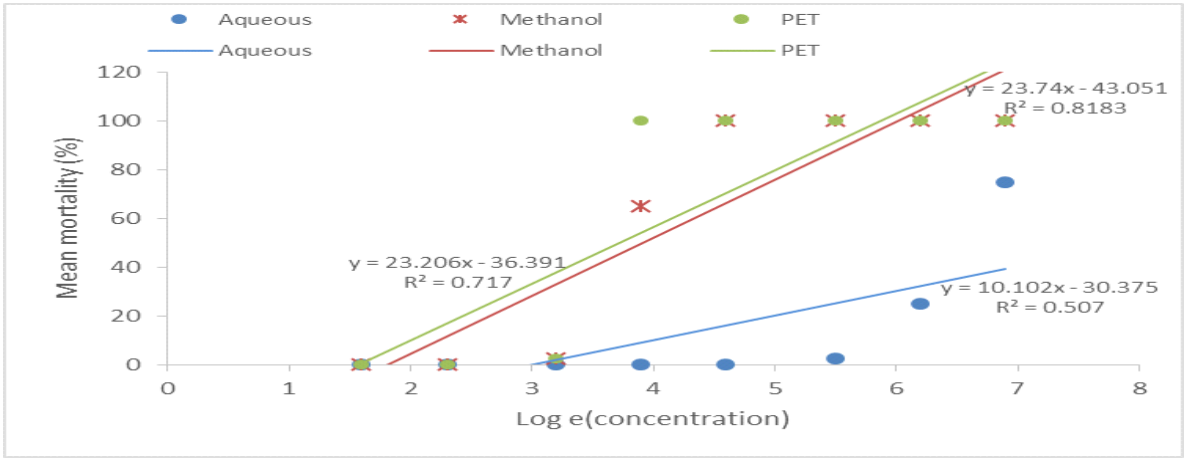
In the aqueous extracts there were statistically significant differences in mean mortalities between adult and juvenile snails at 500 mg/l as shown in the error bars in Figure 1 also significant differences in mean mortality was seen at 50 mg/l and 25 mg/l in methanolic and pet ether extracts respectively (Figure 1).

Fig 1: Graph showing mean mortalities of adult and juvenile snails following 24 hr exposure to different extracts



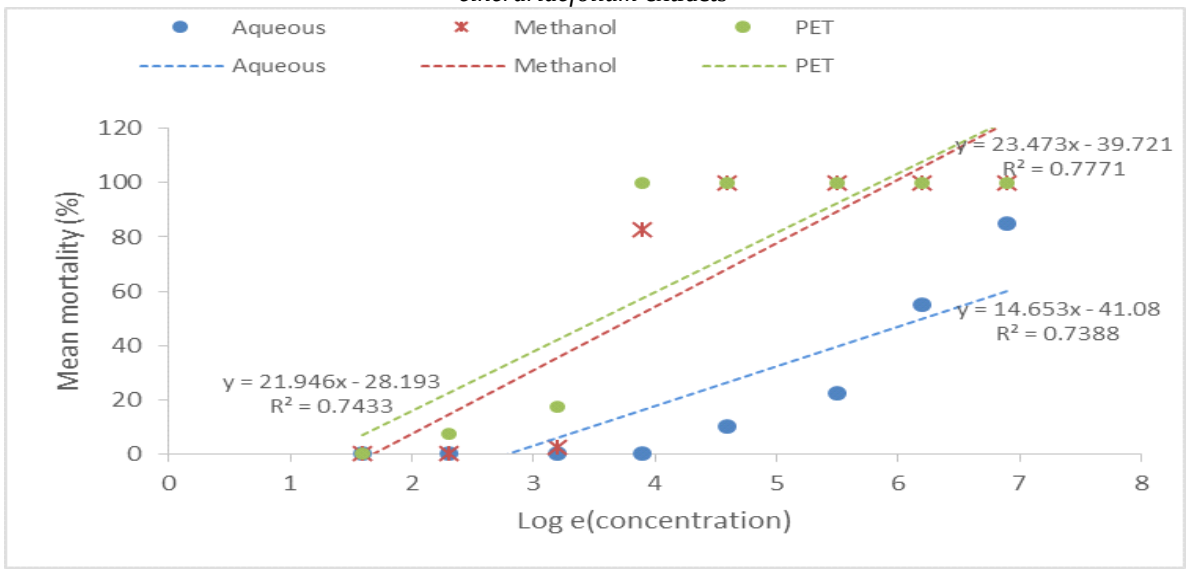
Both the methanol and Pet ether extracts of *C. cinerariaefolium* showed molluscidal activities against juvenile and adult *B. pfeifferi* snails at different concentrations. The extracts showed dose dependent mortality against adult snails as shown in Figure 2. The correlation coefficient R^2 for the adult stage snails was 0.51, 0.82 and 0.72 for aqueous, methanolic and Pet ether extract respectively (Figure 2).

Fig. 2 – Susceptibility of the adult stage of *Biomphalaria pfeifferi* after 24 hours exposure to *C. cinerariaefolium* extracts



In the juvenile stage the R^2 was 0.74, 0.78 and 0.74 for aqueous, methanolic and Pet ether extract respectively (Figure 3). There was strong positive correlation between mean mortalities and the concentrations of the different extract concentrations as shown in Figure 3.

Fig. 3 – Susceptibility of the juvenile stage of *Biomphalaria pfeifferi* after 24 hours exposure to *C. cinerariaefolium* extracts



Aqueous extracts showed average mortality of 2.5%, 25%, and 75% on adult *B. pfeifferi* snails at concentrations of 250 mg/l, 500 mg/l and 1000 mg/l respectively. Pet ether extracts exhibited high molluscicidal activity on adult *B. pfeifferi* snails achieving 100% mortality at 50mg/l (Table 3).

Table 3: Mean % mortality of adult and juvenile *B. pfeifferi* lab bred snails following 24 hr exposure to different extracts

Solvent	Aqueous		Methanol		Pet Ether	
Conc (mg/l)	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles
5	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	2.5	0.0	2.5	7.5
50	0.0	0.0	65.0	82.5	100	17.5
100	0.0	10.0	100	100	100	100
250	2.5	22.5	100	100	100	100
500	25	55.0	100	100	100	100
1000	75	85.0	100	100	100	100
Pos Con (Niclosamide 1mg/l)	100	100	100	100	100	100
Neg Con (Distilled water)	0	0	0	0	0	0

Methanol extracts also showed molluscicidal activities achieving 100% mortality at 100 mg/l. The adult snail mortality rates in methanolic and Pet ether extracts were not significantly different from those observed in the positive control (niclosamide 1 mg/l) (mean±standard error mortality: 100.0%±0.00 for the positive control compared to 59.5%±8.33 for methanolic extracts (p=0.213) and 64.2%±8.33 for the Pet ether extracts (p=0.294). Significantly lower mortality rates were observed in crude aqueous extracts (17.2%±5.02) when compared to the positive control (100.0%±0.00) (p=0.003) (Table 4).

Table 4: Analysis of variance: Effects of solvents on mortality rates of *B. pfeifferi* adult lab bred snails following 24 hours' exposure to the extracts

Solvent	Mean	Std. Error	Mean Difference	Std. Error	P-value
Neg control	0.0	0.00	-100.0	33.44	0.009
Aqueous	17.2	5.02	-82.8	24.73	0.003
Methanol	59.5	8.33	-40.5	24.73	0.213
Pet Ether	64.2	8.33	-35.8	24.73	0.294
Pos control	100.0	0.00			

Analysis of variance indicated there was no statistically significant difference between the mean mortality of the adult and juvenile snails (p>0.05) (Table 5).

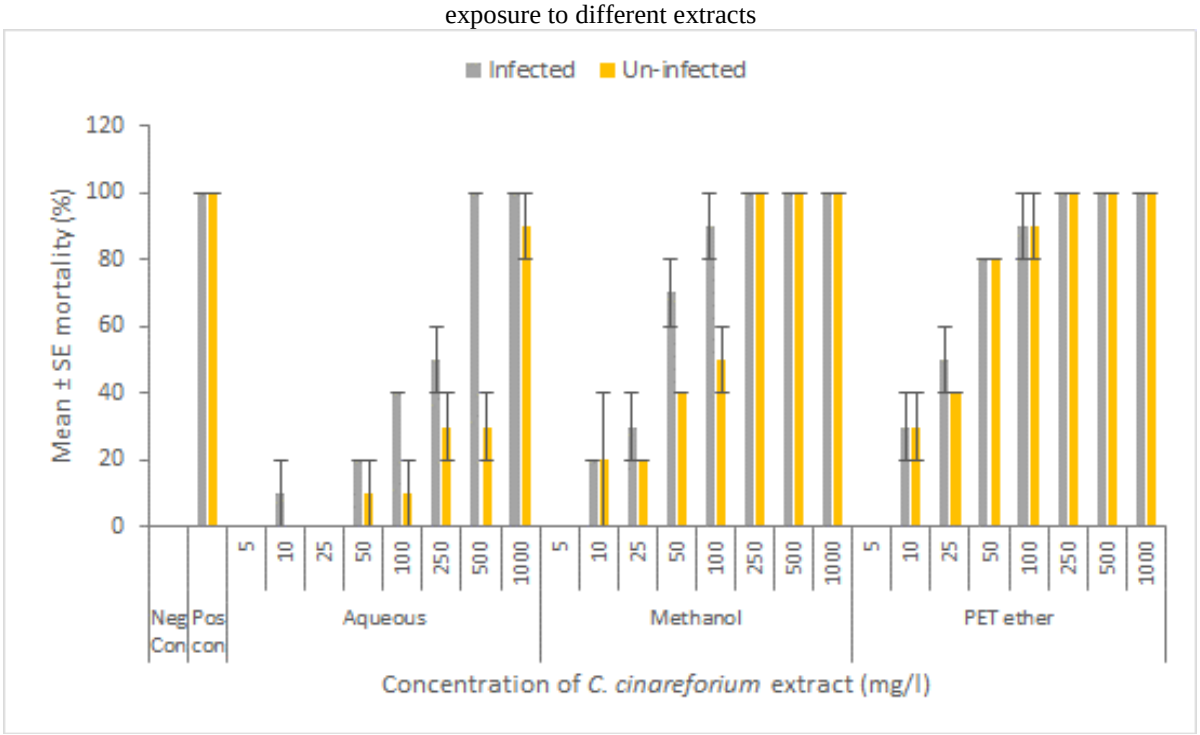
Table 5: Susceptibility of the different *B. pfeifferi* lab bred adult and juvenile snails

Solvent	Mortality (%)						P-value
	Adults			**Juveniles**			
	N	**Mean**	**se**	**N**	**Mean**	**se**	
Aqueous	16	8.8	2.72	16	15.9	4.55	0.185
Methanol	16	71.6	9.98	16	72.8	9.83	0.929
Pet Ether	16	82.2	6.92	16	86.6	5.96	0.636

Mean mortality of infected and none-infected snails

The extracts were evaluated for activity against infected and none infected snails and the results are as shown in Figure 4. The results indicate there were no statistically significant differences in mean mortalities between infected and none-infected snails as shown in Figure 4 in most concentrations. Significant differences in mean mortality was noted at 50 mg/l and 100 mg/l in methanolic extract Figure 4.

Fig 4: Graph showing mean mortality of infected snails and uninfected field collected snails following 24 hr exposure to different extracts



There was no statistically significant difference between the mean mortality of the infected and none-infected field collected snails (p>0.05) (Table 6).

Table 6: Susceptibility by infection status, i.e., infected vs. non infected snails

Solvent	Mortality (%)						
	Infected			**Non-infected**			
	N	**Mean**	**se**	**N**	**Mean**	**se**	P-value
Aqueous	16	40.0	10.00	16	21.3	7.63	0.147
Methanol	16	63.8	10.04	16	53.8	10.12	0.488
Pet Ether	16	68.8	9.30	16	67.5	9.46	0.926

Acute toxicity of the extracts

Pet ether extracts had the highest molluscidal activity with LD_{50} and LD_{90} of 34.5 mg/l and 67.4 mg/l respectively for adult *B. pfeifferi* snails as shown in Table 7. Methanolic extracts also exhibited activity against the adult snails with LD_{50} and LD_{90} of 44.4 mg/l and 86.8 mg/l respectively (Table 7).
Aqueous extracts exhibited the lowest molluscicidal activity against adult *B. pfeifferi* snails with LD_{50} and LD_{90} of 686.7 mg/l and 1342.0 mg/l respectively (Table 7).

Table 7: Acute toxicity of *C. cinerariaefolium* extracts against adult *B. pfeifferi* after 24 hours at 95% Confidence Interval

Type of extract Control	**Lethal Dose (mg/l)**		**Negative control**
	LD_{50}	**LD_{90}**	
Water extract	686.7 CI (593.1-799.6)	1342.0 CI(1124.2-1675.4)	0
Methanol extract	44.4 CI(38.2-51.6)	86.8 CI(73.4-106.7)	0
Pet ether extract	34.5 CI(29.5-40.3)	67.4 CI(56.8-83.0)	0

Key- CI Confidence Interval

Pet ether extracts had also the highest molluscidal activity against juvenile snails with LD_{50} and LD_{90} of 28.0 mg/l and 59.4 mg/l respectively for adult *B. pfeifferi* snails shown in Table 8.
Similarly, aqueous extracts exhibited the lowest molluscicidal activity against juvenile *B. pfeifferi* snails with LD_{50} and LD_{90} of 413 mg/l and 877.4 mg/l respectively (Table 8).

Table 8: Acute toxicity of *C. cinerariaefolium* extracts against Juveniles *B. pfeifferi* after 24 hours at 95% Confidence Interval

Type of extract Control	**Lethal Dose (mg/l)**		**Negative control**
	LD_{50}	**LD_{90}**	
Water extract	413.5 CI (327.9-523.0)	877.4 CI(679.5-1226.2)	0
Methanol extract	38.9 CI(30.0-50.3)	82.6 CI(63.1-116.3)	0
Pet ether extract	28.0 CI(21.6-36.2)	59.4 CI(45.3-84.1)	0

Key- CI Confidence Interval

All the extracts also exhibited molluscidal activity against schistosome infected snails at lower doses compared to the none-infected snails as shown in Table 9. The LD_{50} of aqueous, methanol and Pet ether extracts was 128.2, 31.1 and 23.1 mg/l respectively while the LD_{90} of aqueous, methanol and Pet ether extracts was 479.1, 73.5 and 54.9 mg/l respectively.

Table 9: Acute toxicity of *C. cinerariaefolium* extracts against infected *B. pfeifferi* field snails after 24 hours at 95% Confidence interval

Type of extract Control	**Lethal Dose (mg/l)**		**Negative control**
	LD_{50}	**LD_{90}**	
Water extract	128.2 CI (84.8-196.1)	479.1 CI(301.8-885.7)	0
Methanol extract	31.1 CI(20.1-47.6)	73.5 CI(73.5-209.7)	0
Pet ether extract	23.1 CI(14.9-35.4)	54.9 CI(56.8-83.0)	0

Key- CI Confidence Interval

IV. Discussion

Following exposure to the methanolic and pet ether crude extracts the snails showed several behavioral responses such as crawling out of test solution and retracting into their shells. These behaviours can be described as "distress syndrome" as was evidenced in similar studies conducted for other planorbid species of snails. Different plants possess different chemicals which have different modes of action which can be divided into different categories such as contact poisons, growth inhibitors, neurotoxins, enzyme inhibitors, stomach poisons and respiratory poisons.[21-25] These observations are also supported by similar studies done by Brackenbury[26] in which they studied extracts of *Apodytes dimidiate* and the structural damage they cause to the foot-sole epithelium of *B. africanus.* In another study of effects of methanolic extracts of *zingiber officinale,* on *B. globusus* snails where the snails developed hemorrhagic blisters on the foot sole and visible swelling of the cephalopodal mass. The swelling could be attributed to the toxic effects of the extracts which resulted to damage of the epithelial surface thus increased permeability of epithelial membranes and accumulation of water in tissues and haemorrhage leading to loss of normal osmoregulatory function.[25] The visible secretion of mucous from the snails exposed to toxic concentrations of the extracts could be explained as snails trying to detoxify themselves upon encounter with toxic substances. The mucous secretion is thought to be snails effort to detoxify its body by excreting the toxic substance via mucous secretion.[27]

In the past decade experiments involving extracts of plant origin have been carried out against parasitic infections in which snails act as vectors. The studies showed that molluscicidal activities vary greatly from different plant species and between different plant parts of the same plant.[28] The results obtained from the studies are vital in helping scientists work on improving molluscicidal properties of known natural compounds and discover new chemicals with similar properties and with better activities. Various defensive chemicals in various categories have been isolated from plants and include terpenoids, alkaloids, glycosides, phenols and tannins which possesses an array of effects both behavioral and physiological on pests.[29] Phytochemical analysis of the *C. cineraliaefolium* extracts showed that the different solvent extracts contained the following phytochemicals; alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. The results of this study showed that both methanol and Pet ether extracts exhibited strong molluscicidal activity and the results were not statistically significant different from the positive control ($p>0.05$). The results also indicated that infected snails were more susceptible to the extracts compared to none -infected snails. According to WHO[30], a potential candidate for plant moluscide the crude extracts of its parts should be lethal at concentration of $\leq$100 mg/l and kill 90% of snails exposed after 24 hours. Therefore, from the results obtained, *C. cinerariaefolium* extracts are potential candidates for a molluscide. The molluscicidal properties could be attributed to presence of tannins, terpenoids and saponins which were shown to be strongly present after phytochemical analysis of the extracts. Saponins are plant glycosides which occur naturally and consist of a sugar moiety and an aglycone unit which are toxic as they inhibit the activity of Acetylcholinesterase enzyme in the internal tissue of snails.[31] *Phytolacca dodecandra* berries have been shown to have strong molluscicidal activity in Ethiopia. The compounds identified for the molluscicidal properties were triterpenoid saponins, with LD_{100} values as low as 2 ppm.[32] Molluscicidal effects of *Euphorbia conspicua* and *Euphorbia splendedens* which contain tannins have been studied and shown that the LC_{50} is 40 ppm for the cold water extracts of dry leaves.[33] These results also show that extracting plants with various organic solvents helps identify which solvents are good for extracting some of the active compounds with the molluscicidal activity. The results collaborate with other studies done where extracts of leaves of *S. nigrum* showed the Pet ether extracts had higher molluscidal activity where LD_{50} and LD_{90} was 4.2 mg/l and 8.62 mg/l respectively compared with Methanol extracts LD_{50} and LD_{90} was 6.9 and 16.2 respectively.[34] According to Ngule *et al* [35] crude plant extract with a LD_{50} ranging from 0 -500 mg/l is considered that the plant extract is very toxic; whereas crude plant extract with a LD_{50} of between 500-1000 mg/l indicates that the plant extract is not highly toxic while crude plant extract with a LD_{50} which is > 1000 mg/l shows that the plant is not toxic.

Pyrethrum plant is readily available in Kenya and is grown in the following areas; Nyandarua, Kiambu, Kisii, Nyamira, Nakuru, Bomet, Kericho, Koibatek, Nandi, Elgeyo Marakwet, Nyeri, Laikipia, Murang'a and Mt Elgon thus its use as plant molluscide may be additional contribution to current methods of snail control and development of safe and effective future generation of molluscicides. Pyrethrum is already in most lists of approved organic insecticides for use in organic production throughout the world and one of most common pesticide.[36]

V. Conclusion

The results obtained from the study indicate that both the methanolic and Pet ether extracts possess molluscicidal properties against adult and juvenile *B. pfeifferi* snails. The adult and juvenile snails were more susceptible to the Pet ether extract compared to the methanol extracts. Aqueous extracts showed very low molluscicidal activity when tested against laboratory bred snails and none infected snails as opposed to infected snails. The results therefore indicate the potential new use of *C. cinerariaefolium* as molluscicide after industrial

isolation of pure pyrethrins and being synergized with piperonyl butoxide and thus may be even more toxic to snails at even lower concentrations. Pyrethrum plant is readily available in Kenya and use as plant molluscide may be additional contribution to current methods of snail control and may be developed as a safe and effective future generation of molluscicides. The discovery of *C. cinerariaefolium* plant as potential new molluscicides will offer a cheap alternative to the synthetic molluscicides which are expensive to import and very toxic to non-target macrofauna.

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