MOLLUSCICIDAL ACTIVITY OF SELECTED PLANT EXTRACTS

BY

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DECLARATION

This thesis is my original work and has not been presented for any academic award in any other university am aware of.

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DEDICATION

This thesis is dedicated to my husband and children. Many thanks and may God bless you all.

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ABBREVIATIONS AND ACRONYMS

+ ....Present

- Absent;

AAE *Aloe secundiflora* aqueous extract

AEE *Aloe secundiflora* ethanolic extract

BAE *Bridelia micrantha* aqueous extract

BEE *Bridelia micrantha* ethanolic extract

CAE *Croton megalocarpus* aqueous extract

CEE *Croton megalocarpus* ethanolic extract

OAE *Ocimum americanum* aqueous extract

OEE *Ocimum americanum* ethanolic extract

SAE *Sonchus luxurians* aqueous extract

SEE *Sonchus luxurians* ethanolic extract

µm Micrometre

ABSTRACT

Schistosomiasis is one of the most widespread parasitic infections and the third most prevalent parasitic disease in the world in terms of overall morbidity, socioeconomic and public health importance. It is the most prevalent of water borne and parasitic diseases and it affects over 240 million people worldwide. One approach to the control of the disease is the elimination of the intermediate host responsible for its transmission. The use of synthetic molluscicides is becoming unpopular owing to their high cost and enviromental pollution. As a result, plant molluscicides have received considerable attention in the search for cheaper alternatives to chemotherapy and synthetic molluscicides in schistosomiasis control. The aim of this study was to determine the molluscicidal activity of aqueous and ethanol extracts from five plant species; *Ocimum americanum, Sonchus luxurians, Aloe secundiflora, Bridelia micrantha* and *Croton megalocarpus* against *Biomphalaria pfeifferi* adult and juvenile snails. Dried plant materials were ground into powder. They were extracted using ethanol and water. Phytochemicals were detected which include; flavonoids, saponins, tannins, alkaloids, glycosides, steroids and triterpenes.

Adult and juvenile snails were subjected to concentrations of 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l of the aqueous and ethanol plant extracts. Generally, only *B. micrantha* which had molluscicidal effect against adult and juvenile snails. *B. micrantha,*extracts that were found to have molluscicidal effects, were screened for their miracicidal and cercaricidal activity

against *Schistosoma mansoni* miracidia and cercariae. Concentrations of 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l were used. The ability of *B.micrantha* aqueous extract to immobilise miracidia and cercariae was greater than that of the ethanol extract. From this study, *B.micrantha* demonstrated molluscicidal, miracicidal and cercaricidal effects.

CHAPTER ONE

1.0: INTRODUCTION AND LITERATURE REVIEW

1.1: INTRODUCTION

Schistosomiasis is one of the most important public health problems after malaria (Mohammed, 2009). It's a widespread disease infecting over 240 million people (WHO, 2013). It is found in tropical countries in Africa, Caribbean, Middle East, South America and South East Asia. However an estimated 85% of the world's cases of schistosomiasis are in Africa, where prevalent rates can exceed 50% in local populations (Fenwick *et al*., 2006). Out of the infected, over 120 million are symptomatic while over 20 million have severe disease (Hamed, 2010). A further 779 million are at risk of infection in tropical and subtropical areas of the world (Steinmann *et al*., 2006). In Kenya, it is estimated that over one million people are infected with the disease (WHO, 2013).

Schistosomiasis is one of the most widespread parasitic infections in tropical and subtropical areas second to malaria in public health importance and human impact (Engels *et al.,* 2002). It's also the most prevalent of the waterborne diseases and one of the greatest risks to health in rural areas of developing countries (José *et al*., 2003). It is a debilitating disease that affects people who have contact with water harbouring infected snails. The eggs of the parasite are generally responsible for the pathological effects of the disease, the symptoms of which depend upon the intensity of infection (Hirata *et al*., 1993). Hypersensitivity reactions against worms' eggs trapped in the venules of various body organs

(Hirata *et al*., 1993) produce varying degree of tissue lesions, anaemia, diarrhoea, abdominal pain, culminating in irreversible effects if not treated and, sometimes death (Rabello *et al*., 1994). Among the affected organs, the liver and the intestines are the major sites of egg depositions in human schistosomiasis (Hirata *et al*., 1993).

Various factors are responsible for the increasing importance of schisto-somiasis. The most significant factors are the expanding use of fresh water in the tropics to meet the increase in demand for food production, construction of irrigation system and dams leading to creation of new and highly suitable habitats for the fresh water snails. This plays a vital role in the transmission of schistosomiasis. Other factors include: lack of sufficiently trained health workers, inadequate safe water, high cost of chemicals for snail control and therapeutic drugs (Madsen *et al.*, 1989).

Schistosomes, the causative agents of the disease have an indirect life cycle requiring aquatic snails, as intermediate host, for their transmission to man. Thus reduction of the parasite transmitting snails plays an important role among the strategies to control the disease (Liu *et al*., 1997). It is generally considered that snail control is one of the most rapid and effective means of reducing transmission of schistosomiasis (McCullough *et al*., 1980; Fenwick *et al.*, 1982; Klump & Chu, 1987). Snails of the genus *Biomphalaria* are the intermediate host for *Schisto-soma mansoni* with the group *pfeifferi* being the most common in Kenya. Currently there is no vaccine against schistosomiasis (Fenwick *et al.*, 2006) and the drug of choice, Praziquantel faces the problem of resistance;

in addition, re-infection occurs when treated people have repeated contact with infested water (John, 2004; WHO, 1993; WHO, 1985). Sanitation as a means of control against schistosomiasis is expensive and involves change of people's culture thus posing challenge in schistosomiasis control. The molluscicide of choice is niclosamide (WHO, 1985). However commercial molluscicides are not frequently used as a major control mechanism due to cost implications and toxicity effects on non target organisms such as fish (John, 2004; WHO, 1985), therefore a need for search of a cheap, non-toxic and safer molluscicide (Clark *et al*., 1997; Massoud *et al.,* 2003). Although the snail does not play an active role in transmission of the parasite from one host to another, as do insect vectors, it is an indispensable intermediate host for the development of the parasite.

The transmission of the infective stage of the parasite is accentuated through shedding of the cercariae by the snail host and the various human water contact activities (Dalton & Pole, 1978). Schistosomiasis can be controlled by destroying the carrier snail and thus breaking the life cycles of the parasite (Amrita & Singh, 2001). Consideration of cost and environmental effect of most molluscicides in current use for the control of schistosomiasis has generated the search for cheaper and less polluting molluscicides, especially those of plant origin. Molgaard *et al*. (1999) expressed hope that plants showing molluscicidal properties could be used on self-help basis to control diseases in rural areas.

1.1.2: Different types of Schistosomes

Five most common species of schistosomes infect man and cause human schistomiasis. These are; *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi* and *Schistosoma intercalatum.* Other species of *Schistosoma* which parasitize man are *Schistosoma matthei* and *Schistosoma bovis* (Hamed, 2010). Each causes a different clinical presentation of the disease. *Schistosoma haematobium* is the most prevalent and widespread species in Africa, Eastern Mediterranean and the Middle East. *Schistosoma mansoni* is found in over 52 countries in Africa, Caribbean, eastern Mediterranean, and Latin America. *Schistosoma intercalatum* is found in 10 countries in the rain forest belt of Africa in parts of Central and West Africa while *Schistosoma japonicum* is found in Indonesia parts of China and Southeast Asia. *Schistosoma mekongi* is found in Cambodia and Laos, (Utzinger *et al.*, 2001).

1.1.3: Schistosomiasis

Schistomiasis has several presentations depending on duration of infection and the particular parasite.

1.1.3.1: Acute Schistomiasis

Onset of egg laying in humans is sometimes associated with an onset of fever (Katayama fever). Acute schistosomiasis is a systemic, serum-sickness-like illness that develops after several weeks in some but not most individuals with new schistosomal infection. It may correspond to the 1st cycle of egg deposition and is associated with marked peripheral eosinophilia and circulating immune complexes. It is characterized by

fever, headache, lethargy, eruption of pale temporary bumps associated with severe itching (urticarial), rash, often painful hepatomegaly and/or spenomegaly and bronchospasm (Ross *et al.,* 2007).

1.1.3.2: Chronic Schistosomiasis

The pathology of chronic schistosomiasis results from egg induced immune response, granuloma formation and associated fibrotic changes. Schistosome eggs are highly immunogenic and induce vigorous circulating and local immune responses. The immune responses can lead to obstruction of the colon and blood loss. The eggs enter the circulation and lodge in organs causing granulomatous reactions. Eggs lodged in the liver can lead to blood pressure (Aly & Hamed, 2006; Hamed, 2006), spleenomegaly, buildup of fluid in the abdomen, and potentially life-threatening dilations or swollen areas in the esophagus or gastrointestinal tract that can tear and bleed profusely (esophageal varices). Rarely, central nervous system schistosomiasis may develop; this form is thought to result from aberrant migration of adult worms or eggs depositing in the spinal cord or brain. Signs and symptoms are related to the site of the granulomas in the central nervous system and can present as transverse myelitis. Eosinophilia may be present (Bierman *et al*., 2005). *Schistosoma mansoni* and *Schistosoma japonicum* eggs most commonly lodge in the blood vessels of the liver or intestine and can cause diarrhoea, constipation, and blood in the stool. Chronic inflammation can lead to bowel wall ulceration, hyperplasia, polyposis and, with heavy infections, to periportal liver fibrosis. *Schistosoma haematobium* eggs typically lodge

in the urinary tract and can cause dysuria and hematuria. Calcifications in the bladder may appear late in the disease (Smith *et al*., 1975). Schistosomiasis may localize in different parts of the body, and its localization determines its particular clinical profile (Gryseels, 1989). *Schistosoma haematobium* causes urogenital schistosomiasis, while *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi* and *Schistosoma intercalatum* causes intestinal schistosomiasis (Hamed, 2010).

1.1.4: Hosts of Schistosomes

Shistosomiasis is a snail-borne trematode infection of humans, domestic and wild animals (Gryseels, 1989; Hamed, 2010). Schistosomiasis is transmitted by species of freshwater snails belonging to the family Planorbidae (Gryseels, 1989). Strains of *Schistosoma* species which infect man vary widely in their ability to infect passive intermediate snail hosts (Hamed, 2010). Snails are indispensable intermediate hosts for the development of schistosomes. The transmission of the infective stage of the schistosomes is accentuated through shedding of the cercariae by the snail host and the various human water contact activities (Dalton & Pole, 1978). Snails of the genus *Biomphalaria* serve as intermediate hosts of *Schistosoma mansoni*. 1t has been observed in Venezuela that, a land snail *Achatina fulica* can also serve as a host of *Schistosoma mansoni* (Hamed, 2010). Snails of the genus *Bulinus* serve as the intermediate hosts of *Schistosoma haematobium* and *Schistosoma intercalatum*, *Oncomelania* species serve as the intermediate host of *Schistosoma japonicum*, and

snails of the genus *Lythoglyphopsis* and genus *Tricula* serve as the intermediate host of *Schistosoma mekongi* (Gehad *et al*., 2009).

1.1.5: Life cycle of fresh water snails

All species of *Biomphalaria* and *Bulinus* are hermaphrodite, possessing both male and female organs and are capable of self- or cross-fertilization. The eggs are laid at intervals in batches of 5–40, each batch being enclosed in a mass of jelly-like material. The amphibious *Oncomelania* snails, which may live for several years, have separate sexes. The female lays its eggs singly near the water margin (Gryseels, 1989).The young snails hatch after 6–8 days and reach maturity in 4–7 weeks, depending on the species and environmental conditions. A snail lays up to 1000 eggs during its life, which may last more than a year. Temperature and food availability are among the most important limiting factors. For reproduction, temperatures between 22°C and 26°C are usually optimal. A single specimen can invade and populate a new habitat.

1.1.6: Life cycle of *Schistosoma mansoni*

Schistosoma mansoni has a typical trematode vertebrate – invertebrate life cycle. The parasite passes its life cycle in two different hosts, the definitive host and a fresh water snail, the intermediate host (Engels *et al*., 2002). *Schistosoma mansoni* male/female reproductive pair (Fig. 1 (a)) is found predominantly in the mesenteric blood vessels surrounding the large intestine and ceacal region of the host. The adult female worm resides within the adult male worm's gynaecophoric canal which is a modification of the ventral surface of the male forming a groove (Ojewele, 2004).

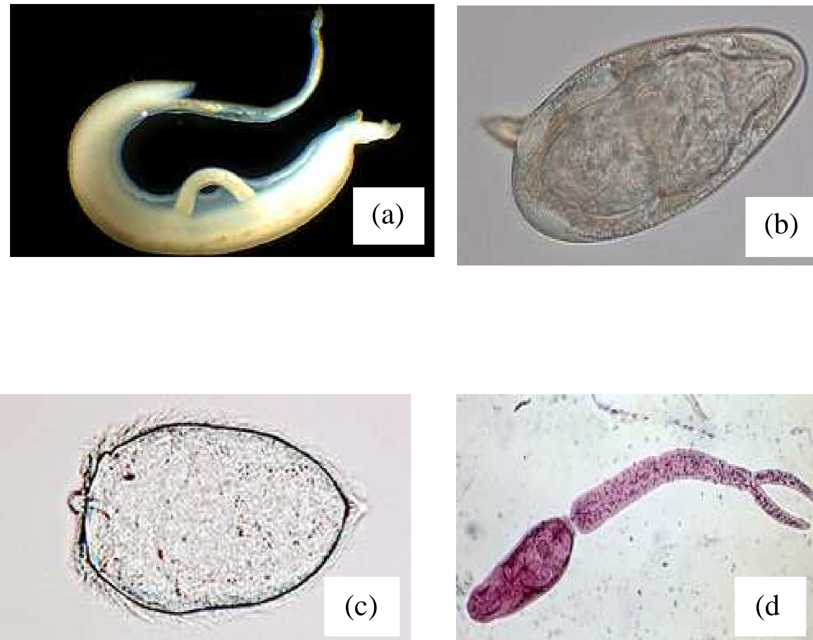

Figure 1: developmemtal stages *Schistosoma mansoni*
(a): *S. mansoni* male/female reproductive pair. (b): *S. mansoni* egg. (c): The miracidium stage of *S. mansoni*. (d): The cercaria stage of *S. mansoni*

Females release their eggs into the blood vessels. The eggs have characteristic lateral spine (Fig. 1 (b)). The eggs move into the lumen of the host's intestines and are emitted to the environment with the feaces. The miracidium stage can be seen within the egg. The mature miracidium hatches out of the eggs in response to temperature, light and dilution of feaces with water. The miracidium is a ciliated larva which is a free-living life-cycle stage infective only to molluscs (Crewe, 1997) (Fig. 1 (c)).

The miracidium never feed and lives for about a day. The miracidium searches for a suitable water snail (*Biomphalaria pfeifferi*) to act as intermediate host. Once in contact with the snail host, the miracidium

undergoes exploratory movement over the snail surface until it reaches its preferred site of penetration, usually the anterior portion of the lateral edge of the foot (Stewart, 1998) and penetrates it. Following penetration, the miracidium transforms near the site of penetration and develops within 48 hours into saccular mother sporocyst located in the region of the host's head-foot or in the tentacles and mantle (Faust *et al*., 1968; Bloch, 1980). Germ cells within the mother sporocyst then divide to produce daughter sporocysts. The embryos of daughter sporocyst soon develop in the body of the mother sporocyst, make their way to the digestive glands, grow and give rise to next stage in the life cycle, the cercaria stage (Faust *et al*., 1968). The sporocyst produces cercariae through asexual reproduction and the snail begins to shed cercariae into the water around 4 weeks after being infected (Bloch, 1980). The cercariae emerges from the snail in a circadian rhythm dependent on ambient temperature and light. The ceracariae never feed and live for about a day. From a single miracidium, a few thousand cercariae results. The cercaria is the final stage of the larval development in the mollusc and has a bifurcated tail (Fig. 1 (d)). A single miracidium produce cercariae of the same sex.

The cercaria propels itself by the aid of the bifurcated tail, swimming tail -first through the water after leaving the snail (Crewe, 1997). The cercaria exhibit a number of features that enable it to locate man. They show bursts of upward swimming to bring them to the surface of the water, followed by periods of passive sinking. They are also affected by other stimuli such

as shadows on the water, turbulence and chemicals secreted from the human skin such as fatty acids and amino acids (Stewart, 1998).

Human skin penetration occurs in three stages, an initial attachment to the skin, creeping over the skin searching for a suitable penetration site, often a hair follicle, and finally penetration of the skin into the epidermis using proteolytic secretions from the cercarial post-acetabular, then pre-acetabular glands. The cercariae lose tails during penetration and the head transforms into an endoparasitic larva, the schistosomule. Each schistosomule spends a few days in the skin and then enters the circulation starting at the dermal lymphatics and venules. Here they feed on blood, regurgitating the haem as haemozoin (Oliveira *et al.,* 2000). The schistosomule migrates to the lungs within 5-7 days post- penetration (Beltran & Boissier, 2008), moves via circulation and reach the liver which is the site of maturation and sexual differentiation (Amer, 1994). In the liver, if it meets a partner of the opposite sex, it develops into adult and the pair migrates to the mesenteric veins (Beltran & Boissier, 2008).

Male schistosomes undergo normal maturation and morphological development in the presence or absence of a female but, the female schistosomes do not mature without a male. Female schistosomes from single sex infections are under developed and exhibit an immature reproductive system. Although the maturation of the female worm seems to be dependent on the presence of the mature male, the stimuli for female growth and for reproductive development seem to be independent from each other.

The paired worms move against the flow of blood to their final niche in the mesenteric circulation where they begin egg production. Each female lays approximately 300 eggs a day, which are deposited on the endothelial lining of the venous capillary walls (Loverde & Chan 1991). In about a month, the cercariae have developed into mature schistosomes that have paired, migrated to the blood vessels and have begun to produce eggs. Some of the eggs produced reach the outside environment by passing through the wall of the intestine. The eggs move into the lumen of the host intestines and are released into the environment with feaces (Beltran & Boissier, 2008). The rest are swept into the circulation and become lodged within the host's tissues. The eggs, which become lodged within the host's tissues, are the major cause of pathology in schistosomiasis (Bierman *et al*., 2005).

1.1.7: Control of Schistosomiasis

Control falls into two parallel interdependent strategies; Control of the disease in the human population and control of transmission of the disesase (Chen *et al*., 2005).

1.1.7.1: Chemotherapy

Treatment of schistosomiasis is based on chemotherapy with praziquantel, which is currently the available drug of choice for all forms of the disease (Fenwick *et al.,* 2006). However resistance to praziquantel has been demonstrated in many schistosomiasis endemic areas of the world (John, 2004; Fenwick *et al.,* 2006).

1.1.7.2: Vacination

It is precisely the host immune response that gives rise to the granulomas responsible for the morbidity of schistosomiasis. Many trials of vaccinations are based on homologous or heterologous antigens. Bashtar *et al*., (2006) found that schistosomal worm and egg antigen had a potency role in protection against schistosomiasis, while Hamed (2006) postulated the excretory-secretory product of *Fasciola hepatica* worms, can be used for production of a vaccine for immunization against schistosomiasis. However, generating immunity through the use of vaccines is complex and has not been successful so far.

1.1.7.3: Health Education

An improvement in hygiene is key to long term control of schistosomiasis. By eliminating human wastes in fresh water bodies, part of the complex life cycle of the schistosome can be eliminated (Brinkmann & Steingruber, 1986). Defecation or urination in or near open waters should be avoided so that snails have less chance of becoming infected. Latrines or toilets should be constructed (Gryseels, 1989). A high rate of reinfection demonstrates the need for health education and it's essential to ensure community involvement in the construction and use of facilities (Gryseels, 1989).

1.1.7.4: Sanitation

The installation of a safe water supply is, in most areas, the most cost-effective control measure. It is important to provide water for drinking,

bathing and washing clothes as this encourage people to stay away from streams and ponds that are infested. People should avoid swimming, wading, washing or bathing in water suspected of infestation. However, sanitation as a means of control against schistosomiasis is expensive and involves change of people's culture (Fenwick *et al.,* 2006).

1.1.7.5: Environmental Management

Environmental management methods include drainage, filling in, and the lining of canals with concrete. These methods are generally expensive but long-lasting (Gryseels, 1989).

1.1.7.6: Reduction of snail habitats

Association of snail with vegetation is attributed to feeding, protection or reproduction as plants may provide surfaces for egg laying. The removal of vegetation in irrigation ditches and canals therefore reduces the number of snails (Ferguson, 1978). Where sufficient resources are available, canals can be lined with cement to prevent or reduce the growth of vegetation. People can also remove plants from places where children swim or where clothes or dishes are washed. Where water quantity is not a limiting factor, raising and lowering water levels and increasing flow rates can disturb snail habitats and their food sources. Rapid complete drainage reduces the amount of vegetation and kills the snails by desiccation (Gryseels, 1989).

1.1.7.7: Removal and destruction of snails

Snails can be removed from canals and watercourses with dredges and crushed or left to die of desiccation. This happens in irrigated areas of Egypt and Sudan as a beneficial side-effect of efforts to improve the flow of water by removing mud from canal bottoms (Gryseels, 1989).

1.1.7.8: Biological Control of snails

Fishes such as *Trematocranus placodon* are employed in the control of snails where the fish consider the snail as its preferred food. Tilapia fish can be used for the biological control of *Biomphalaria* in fish ponds (El-Mannan, 2001). Investigations have shown that there is a possibility of using the Ghanaian strain of an ampullariid snail, *Lanistes varicus*, for the biological control of *Biomphalaria pfeifferi*. Adult and 2-week-old *L. varicus* were found to feed voraciously on the egg masses and juveniles of *Biomphalaria pfeifferi* (Anto *et al*., 2005)

1.1.7.9: Snail control by use of molluscicides

The main objectives of mollusciciding are to decrease snail host population density, reduce snail/human infection potential and eliminate infected snails (WHO, 1980), thus breaking the life cycle of the parasite (Amrita & Singh, 2001). It is an important strategy for schistosomiasis control (José *et al*., 2003) and at present, it’s the most reliable method of achieving drastic reductions in snail population density in short term and in elimination of schistosomiasis (WHO, 2013).

1.2: Literature Review

1.2.1: Chemical molluscicides

Since 1911 about 700 chemicals have been screened for molluscicidal activity; most have proved deleterious to the environment and currently, only one chemical molluscicide namely, niclosamide (Bayluscide) is available for field snail control. For practical use a concentration of 0.6 – 1 mg/l is recommended with an exposure time of eight hours (McCullough, 1992). Molluscicides affect the metabolic activities of the snail (Rawi *et al*., 1995). They act on different enzymes chiefly those of respiration and carbohydrate metabolism (Sakran & Bakry, 2005; Sharaf El-Din, 2006). This in turn affects energy production since gastropods metabolise primarily carbohydrates in order to promote energy production (Livingstone & deZwaan, 1983). The wettable powder of niclosamide is costly, toxic, has a lower dispersibility and precipitates rapidly (Jose' 2003). To ensure rapid dispersion in water and to reduce the surface tension involved in transition from the solid to the liquid phase, easily absorbed surfactants are added to niclosamide preparation resulting in very small suspended particles (Zhang *et al*., 1996; Guido & Tadros, 2000). However, since the surfactants readily absorb water, it can be damaged during transport and storage with small clumps forming that do not disperse evenly in the water (Liu *et al.*, 1998; Huang *et al*., 2003). The high costs of Bayluscide, its impact on the community and its environmental effects have stimulated interest in search for alternative molluscicides of plant origin (McCullough *et al*., 1980).

1.2.2: Plant Molluscicides

Plant molluscides can provide an ideal source of low cost, locally produced, safe and effective molluscicide (Sigh *et al.,* 1996). The plants can be cultivated locally instead of importing synthetic compounds (Ndamba *et al*., 1994) and may provide an opportunity of incorporating snail control into the community - based Primary Health Care (PHC) approach of schistosomiasis control (Fenwick *et al*., 2006). The use of plant products and extracts is also environmentally acceptable as an alternative for effective control of aquatic snail populations (Adewunmi & Sofowora, 1980; El-Sawy *et al*., 1981). Among the plants of great interest are those which contain large quantities of saponins. Saponins possess high toxicity against cold blood organisms including snails (Hostettmann *et al.,* 2000).

For a plant to be considered as molluscicide, it should be registered in concentrations up to 100 mg/l and be able to kill 90% of the snails 24 hours after contact (Mott, 1987).

Mott (1987) reported the following criteria for an ideal plant molluscicide:

- High toxicity against target organisms and lower toxicity against non-target organisms at molluscicidal concentrations.
- Locally available, with high yield of molluscicidal material per plant and per unit area of cultivated plants.
- The plant type is perennial rather than annual, reproduce by seeds rather than tubers, drought resistant; semi aquatic or aquatic for use

directly, with high propagation and rapid growth rates, high adaptability to differing local environmental conditions, high resistance to pests, weeds, and grazing livestock.

- Plant parts have a localization of high potency levels in regenerating parts (berries, fruits, flowers and leaves) or vegetative plant tubers.
- Molluscicidal material of seasonally producing plants should not lose potency during storage for at least one year.
- Active ingredients should be extractable by simple apparatus and commonly available solvents, preferably water.
- Retention of molluscicidal potency under physiochemical influence (pH, sunlight, temperature, silt, organic matter and water pollution) normally found in the endemic area during the annual cycle (physiochemical stability).
- A good knowledge of growing habits and requirements, toxicity and any medicinal properties of plant by local people is an asset.
- Cultural acceptability by means of absence of spiritual and ceremonial uses of plants and aversions on folklore and magic, which might interfere with their use for snail control, is desirable.
- Suitability of the same plant parts for other public health, local, and industrial uses.

Endod (P*hytolacca dodecandra*, (Phytolaccaceae)) in Ethiopia was one of the first plants that have been extensively studied and found to be molluscicidal (Strikland & Abdel-Wahab, 1991, Lemma *et al*., 1984). Unfortunately; this plant has a high toxicity to non target organisms (Madsen, 1985). Extracts of the fruits of *Tetrpleura tetrptera* (Leguminosae), have molluscicidal properties comparable to *Phytolacca dodecandra* (Hostettmann *et al*., 2000). A field control project in Nigeria produced encouraging results with a large decrease in the number of *Bulinus globosus* snails at sites treated with concentrations of 10 mg of methanol extract (Adewunmi, 1984; Hostettmann *et al*., 2000).

In Tanzania, *Bobgunnia madagascariensis* is one of the most promising plants for the control of schistosomoasis. It bears large fruits which were shown to be toxic to snails and each tree can carry up to 30-40 kg of pods (Mozley, 1939; Hostettmann *et al*., 2002). Water extracts exerted significant molluscicidal activity against *Biomphalaria glabrata* and *B. globosus* snails up to dilutions of 100 mg of ground pods per litre (Hostettmann *et al.*, 2000). Extracts from the leaves of *Sesbania sesbanan* (Leguminosae) have molluscicidal activity and a phytochemical investigation of the aerial parts revealed the presence of four saponins (Hostettmann *et al.*, 2000). The root of *Dolichos kilimandscharicus* (Leguminosae) from Kenya was both molluscicidal and fungicidal, after extraction. Thin Layer Chromatography (TLC) analysis indicated the presence of saponins which were molluscicidal against *Biomphalaria glabrata* snails (Marston *et al.,* 1988). An active water extract of the stem

bark of *Cussonia spicata* (Aratiaceae) from Malawi, fractionated by Medium Performance Liquid Chromatography (MPLC) on silica gel, after preliminary partitioning with n-butanol, was active against *Biomphalaria glabrata* snails at 12.5 ppm (Hostettmann *et al*, 2000). *Diospyros usambarensis* (Ebenaceae) is also a plant with a strong molluscicidal activity (4ppm), required to kill 100% of snails after 24h (Marston *et al.,* 1988).

In Egypt, *Ambrosia maritima* was found to have molluscicidal and ovicidal activities and harmless to fish at 100 ppm (Sherif & Elsawy, 1982). At 70 ppm, *Bulinus truncatus* and *Biomphalaria alexandrina* stopped feeding, drifted downstream, and died (El- Sawy *et al*., 1981). The survival rate of *Biomphalaria alexandrina* maintained in 120 ppm aqueous solutions of *Calendula micrantha* and 82 ppm of *Anagillis arvensis*, decreased gradually with time until the 9th and 10th week respectively, when survival was zero. The comparable survival rate of non-treated control was 20%. The two plant extracts also reduced the hatchability of snail egg – masses, the percentage of hatching following exposure to *Anagillis arvensis* (114 ppm) and *Calendula micrantha* (150 ppm) was 46% and 72% after 7 and 5 days exposure respectively, compared with non - treated controls (97.29%); (Mostafa & Tantawy, 2000).

In Sudan, El –Kheir and El-Tohami (1979) screened 78 plants used in folk –medicine for molluscicidal activity. Eighteen plants belonging to 8 species, 6 genera and 5 families proved to possess molluscicidal activity.

They were active against *Bulinus truncatus* and 7 of them were also found to be active against *Biomphalaria pfeifferi*. The most potent plants parts were those of *Gnidia kraussiana* root and stem and *Gardenia vogelii* fruit pulp. These were followed in activity by *Dipcadi fesoghlense* bulb, *Randia nilotica* root, bark and fruit and *Jatropha aceroids* seeds. The least active samples were root and stem of *Jatropha aethiopica*.

In another survey El- Kheir and El-Tohami (1979) treated different parts of *Gnidia kraussiana* against *Bulinus truncatus* using aqueous screening extracts and successive extraction of the powdered plant parts with different solvents of increasing polarity; petroleum ether, cyclohexane, benzene, chloroform, acetone and ethanol. Each extract fraction was tested for its molluscicidal activity. The results showed that the boiled extracts were more active than the cold water extracts of root, stem and leaf. The petroleum ether extracts of the roots and stem were the most potent, revealing LC50 (0.02 ppm), LC100 (0.07ppm) and LC50 (0.05ppm), LC100 (0.3 ppm), respectively. For the leaf, the ethanol extract was the most potent, revealing LC 50 (3ppm), LC 100 (5ppm).

Elamin *et al*., (2005) evaluated the toxic dose of *Balanites aegyptica* fruit extract when applied naturally against *Bulinus truncatus* and *Biomphalaria pfeifferi*. The primary study showed that, the spraying of eight kg of *Balanites aegyptica* fruit extract in canals (width 5 meters) caused 100% mortality of the targeted snails, while the LC90 of both types of snails was 9ppm. Methanolic extract of *Jatropha curcas* showed high

toxicity with LC100-values of 25 ppm for *Biomphalaria glabrata* and 1 ppm for *Bulinus truncatus*. *Dodonaea viscosa* and *Haplophyllum tuberculatum* herbs also showed moluscicidal potency (Rug & Ruppel, 2000).

The latex of *Euphorbia conspicua* (Euphorbiaceae) has molluscicidal and cercaricidal activities. It exhibited high activities against adult snails with LC_{90} values of 4.87 µg mL^{-1} and showed a lethal effect to the cercaria of *Schistosoma mansoni* at concentrations of 100 µg mL^{-1} (Dos Santos *et al.*, 2007). Howida (2005) screened seeds, stems, and leaves of *Jatropha aceroides* (Euphorbiaceae) plant for their molluscicidal activity against *Bulinus truncatus*. The aqueous screening revealed that the seeds have an LC100 of 100 ppm, while the same concentration of the leaf killed 80% of the snails and the stem extract showed LC50 of 100ppm (24h exposure). In the Successive Organic Solvent Extraction (SOSE), the chloroform extract was superior in activity to the other extracts.

A water extract of the seed of *Corton macrostachys* was nearly as effect tive as the major synthetic molluscicides against *Bulinus truncatus* in laboratory and field trials. The extract has ovicidal potency against the late stages of *Biomphalaria pfeifferi* eggs, but it's nontoxic to *Tilapia nilotica* at the lethal concentration, LC90 for *Biomphalaria pfeifferi* (20ppm). However the seeds were reported to have high toxicity to humans (Daffalla & Amin, 1976). Ali (1997) investigated the potency of aqueous, acetone, and ethanol extracts of leaves, stem, and flowers of *Pulicaria crispa* (Asteraceae) against *B. pfeifferi* snails in Sudan. The

aqueous extracts of the leaves were most potent and the aqueous and acetone extracts did not lose activity against snails after two months of storage at -20°C. Somia (1999) reported activity of *P. crispa* leaves aqueous extracts on *Lymnaea cailludi* snails and anophline larvae with LC50 and LC100 on concentrations of 1086.4 ppm and 1500 ppm, respectively. However those extracts were not toxic to non target organisms such as fish and tadpoles. Somia (2003) also found that juveniles of *B. pfeifferi* snails were more susceptible to the *P. crispa* leaves aqueous extract than adult snails. Atta El Mannan (2001) showed molluscicidal activity of *P. crispa* whole plant against *L. natalensis* in the field.

Allium cepa (onion), *Allium sativum* (garlic) and dry powder of *Capparis spinosa* as well as that of *Acacia arabica* leaves have molluscicidal effect on *Biomphalaria alexandrina* snails. The aerial parts of *Melochia arenosa* plant was found to be 100% lethal to *Biomphalaria glabrata* snails at 200 $\mu g\ mL^{-1}$ and showed LD_{50} of 143 $\mu g\ mL^{-1}$ (Mantawy & Mahmoud, 2002; Mantawy *et al*., 2004; Aly *et al*., 2004). *Commiphora molmol* (Myrrh) has molluscicidal effect on *Biomphalaria* snails, where the number of dead-snails increased with increasing time of exposure. One day-old egg masses were more susceptible to the ovicidal effect of *Commiphora molmol* than five-day old ones. Based on safety to man and animals, *Commiphora molmol* was recommended as a safe molluscicide (Massoud *et al.,* 2004; Al-Mathal & Fouad, 2006). The latex of *Euphorbia splendens* var. *hislopii* is promising plant molluscicides because it meets the recom-

mendations of the World Health Organization (WHO, 2002). The researchers found that 0.6 mg L^{-1} of the latex of *Euphorbia splendens* var. *hislopii* causes a sharp reduction in the reserves of glycogen in the digestive gland and elevation of the protein content in the hemolymph of *B. glabrata* (Mello-Silva *et al.,* 2006).

1.2.3: Molluscicidal Plants

1.2.3.1: *Bridelia micrantha*

Uses

A bark decoction is taken as a remedy for stomach-ache and tapeworm. The bark is also boiled to make a soup for treating diarrhoea in children, or is mixed with milk and drunk as a tonic. A decoction of roots is drunk to cure aching joints. The leaf sap is used as an application to sore eyes and, in a decoction with a number of other plants, for the treatment of conjunctivitis. The root is used as a remedy for severe epigastric pain and is applied to the scalp to relieve headache. A decoction of the root is drunk as a purgative, an anthelmintic or an antidote for poison, as it causes vomiting or diarrhoea that gets rid of the poison. An infusion made from the root is taken orally for coughs. The powdered bark is applied to burns to speed healing. The plant is known to contain saponins (Schmidt *et al.,* 2002). All parts of the plant are used in traditional medicine. Traditionally, it's used in the treatment of stomach ailments and diseases such as gastritis, salmonellosis, gastro-enteritis, diarrhoea and constipation, tapeworms and as an emetic for poisons (causes vomiting). The extracts of the whole stem demonstrate antimicrobial activity by inhibiting the growth of

Helicobacter pylori and *Campylobacter jejuni/coli*. It is used as a treatment for skin problems such as ulcers, boils and rashes; for respiratory problems such as persistent cough, tuberculosis, pneumonia, bronchitis and pleurisy; as an analgesic (pain reliever); as an antimalarial; for toothache and gum diseases; for painful menstruation; to prevent abortion; as a stimulate and restorative tonic (alternative) for fortifying pregnant women; for sickle cell anemia, and anemia in general.

Preliminary research on medical properties of *Bridelia micrantha* has shown this herb to be beneficial in treating HIV/AIDS as it cures diarrhoea and stomach discomfort, which are common illnesses in AIDS and contributes to the well-being of the patient. It has also been shown to be a possible principle inhibitor to HIV-1 reverse transcriptase. Research shows that *Bridelia micrantha* contains the sterol, taraxerol (a phytochemical found in dandelions and seaweed to name a few), which is used in producing anti-cancer drugs. Taraxerol-3B-acetate improves tolerance of exercise, removes fatigue and is an anti-aging, anti-tumour, hepatoprotective, an antineoplastic agent and an immune stimulate. *Bridelia micrantha* is also used traditionally in Africa to treat psychological problems such as neurosis and psychosis (Coates, 2002).

1.2.3.2: *Acacia nilotica*

Uses

It is used as firewood and for fencing posts. The leaves are eaten by most browsers. The bark exudes an edible gum and is used medicinally. The gum can also be used as glue. The Zulus take a decoction of the bark as a

cough remedy. The Voortrekkers made ink and dyes from the pods (red, black and yellow). Other parts of the tree were used to treat eye diseases, or as a tranquillizer and even as an aphrodisiac. A root extract was used in the treatment of tuberculosis, impotence, diarrhoea, haemorrhages, tooth-ache, dysentery and gonorrhoea. Extracts made from the leaves are used in the treatment of menstrual problems, eye infections, sores (specifically those caused by leprosy), ulcers, indigestion and haemorrhage (Van Wyk *et al,*. 2000).

1.2.3.3: *Balanites aegyptiaca*

Uses

Various parts of *Balanites aegyptiaca* tree have been used for medicinal value. Traditionally is used in treatment of various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever .Its fruits and back have lethal properties to properties to snail intermediate hosts, schistosome miracidia and cercariae and the cercariae of other trematode (Chothan & Vaghasiya., 2011).

1.2.3.4: *Allium cepa*

Uses

Commonly used as an ingredient in various warm dishes, and may also be used as a main ingredient. It is believed to lighten the balance of blood and it’s known to facilitate bowel movements, to relieve headaches, coughs, snake bites and hair loss. Theleaves have molluscicidal effect on *Biomphalaria alexandrina* snails. (Mantawy & Mahmoud, 2002;

1.2.3.5: *Phytolacca dodecandra*

Uses

Common medicinal uses include treatment of skin itching (ringworm), abortion, gonorrhea, leeches, intestinal worms, anthrax and rabies. The plant has been extensively studied and found to be molluscicidal (Strikland & Abdel-Wahab, 1991, Lemma *et al*., 1984).

1.2.3.6: *Sesbania sesbanan*

Uses

Dried leaves are used in some countries as a tea which is considered to have antibiotic, anti-helminthic, anti-tumour and contraceptive properties. Extracts from the leaves of *Sesbania sesbanan* (Leguminosae) have molluscicidal activity and a phytochemical investigation of the aerial parts revealed the presence of four saponins (Hostettmann *et al.*, 2000).

1.2.4: Secondary Phytochemicals

Molluscicidal plants are now getting more attention. The molluscidal, miracicidal and cercaricidal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action in the targeted organism (Akinmoladun *et al*., 2007). Some of the most important bioactive phytochemical constituents that produce definite physiological action are alkaloids, flavonoids tannins, terpenoids, saponins and phenolic compounds. Different phytochemicals have been found to posses a wide range of activities.

1.3: Problem Statement

Schistosomiasis is second to malaria in terms of most widespread and important parasitic infections in tropical and subtropical areas in terms of public health importance. It's endemic in many parts of tropical and sub tropical areas of the world infecting over 240 million people residing in rural and agricultural areas (WHO, 2013) and a further 779 million are at risk (Steinmann *et al*., 2006). Snails are intermediate hosts of schistosomes in which the transformation from miracidium to cercariae occurs, therefore a need to attack and break the life cycle of schistosomes through controlling of the snail. Currently there is no vaccine or preventive drug against schistosomiasis (Fenwick *et al*., 2006), and treatment of schistosomiasis is based on chemotherapy with praziquantel, which is currently the only available drug of choice for all forms of the disease (Fenwick *et al*., 2006). However resistance to praziquantel has been demonstrated in some schistosomiasis endemic areas of the world (Ojewele, 2004; Fenwick *et al*., 2006). Sanitation as a means of control against schistosomiasis is expensive and involves change of people's culture (Fenwick *et al*., 2006). Presently, the molluscicide of choice is niclosamide (WHO, 1985). However commercial molluscicides are largely not being used as a major control mechanism due to cost implications, toxicity effects on non target organisms such as fish (WHO, 1985; Ojewele, 2004), increasing concern over the possible built up of snail resistance of these molluscicides, deleterious long-term effects to the environment mainly the aquatic biota such as genotoxicity and

carcinogenic effects. This therefore brings to the fore a need to search for a cheap and safe molluscicide (Clark *et al*., 1997; Massoud *et al*., 2003).

1.4: Justification and Significance of Research

The social-economic and health effects of schistosomiasis cannot be underestimated. Infected children have retarded growth and poor school performance. The work capacity of rural inhabitants is severely reduced due to weakness and lethargy caused by the disease (Kloos & McCollough, 1987). The high cost and environmental effect of molluscicides in current use has generated the idea of screening plants for their intrinsic molluscicidal properties in search of an acceptable and self sustaining substance (El-Sawy *et al*., 1981). This could replace the costly synthetic products (Martson & Hostettmann, 1991) and can improve the accessibility of poor communities to molluscicidal agents to treat their water collections (Mott, 1987). Plant molluscicides are environmentally acceptable (Adewunmi & Sofowora, 1980 & El-Sawy *et al*., 1981) and therefore could be used on self-help basis to control diseases in rural areas. Snail control procedures using plant molluscicides therefore, remain among the methods of choice for the control of schistosomiasis (WHO, 1994).

1.5: Hypothesis

The aqueous and ethanol extracts of *Ocimum americanum, Sonchus luxurians, Aloe secundiflora, Bridelia micrantha,* and *Croton megalocarpus* have no molluscicidal, activity on *Biomphalaria pfeifferi* adult and juvenile snails.

1.6: Objectives

1.6.1: General objective

To determine the molluscicidal activity of aqueous and ethanol extracts of *Ocimum americanum, Sonchus luxurians, Aloe secundiflora, Bridelia micrantha,* and *Croton megalocarpus* on *Biomphalaria pfeifferi* adult and juvenile snails.

1.6.2: Specific objectives

1. To determine the phytochemicals in the selected plant extracts.
2. To determine the molluscicidal activity of the aqueous and ethanol extracts of *Ocimum americanum, Sonchus luxurians, Aloe secundiflora, Bridelia micrantha,* and *Croton megalocarpus on Biomphalaria pfeifferi* adult and juvenile snails.
3. To determine the miracicidal and cercaricidal activity of the aqueous and ethanol extracts of *Bridelia micrantha,*on *Schistosoma mansoni* miracidia and cercariae.

CHAPTER TWO

2.0: MATERIALS AND METHODS

2.1: Plants

The plants used in this study were selected on the basis of information by traditional healers to be useful in the treatment of helminthes. Also the plants of the family euphorbiaceae have previously been used and xhibited high molluscicidal activities against adult snails and showed a lethal effect to the cercariae of *Schistosoma mansoni* (Dos Santos *et al*., 2007).

2.1.1: *Ocimum americanum*

It is an erect, much branched herb. The flowers are white to pale blue (Fig.2 (a)) and the leaves are opposite (Fig.2 (b)).

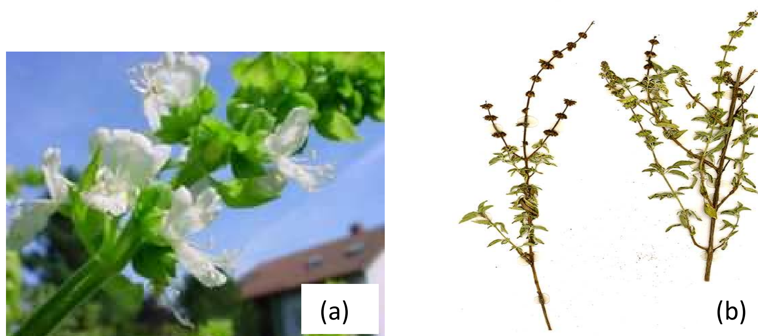

Figure 2: Stalks of *Ocimum americanum*
(a): Flowering stalk of *O. americanum*. (b): A mature stalk of *O. americanum*

2.1.2: *Croton megalocarpus*

Description

Croton megalocarpus grows to 15-35 m; it has distinctive layering of branches and a rather flat crown. Bark dark grey, rough, and crackling. It is hardy and fast growing. Leaves are variable, long, oval and pointed to about 12 cm. The upper surface is dull green contrasting with the pale, silvery underside. Flowers are conspicuous but very short-lived; yellow white, inserted in many-flowered, silver-budded racemes, up to 30 cm long; a few female flowers towards the base, the remainder male. Fruit turns from green to greyish-brown as it matures (Fig 3).

Figure 3: A *Croton megalocarpus* tree from Kagera region

Endocarp is hard and woody. Each fruit contains 3 ellipsoid-ovoid or oblong-ellipsoid seeds, 2.2-2.4 cm long and 1.2-1.4 cm wide. Seeds white when immature, grey-brown when mature, with a minute caruncle (ICRAF, 1992).

2.1.3: *Bridelia micrantha*

Description

Bridelia micrantha is a semi-deciduous to deciduous tree up to 20 m tall with a dense rounded crown and tall, bare stem; bark on young branches is grey-brown and smooth while on older branches and stems it's dark brown and rough, cracking into squares. Leaves have alternate, margins entire or slightly wavy. Inflorescence has flowers in axillary clusters containing male and female flowers. Fruit is black (Coates, 2002). (Fig 4)

Figure 4: Fruiting stalk of *Bridelia micrantha*

2.1.4: *Aloe secundiflora*

Description

Succulent, perennial herb, without stem or with stem up to 30 cm long, usually solitary, sometimes suckering to form small groups. Leaves about 20 in a dense rosette; stipules absent; petiole absent; blade ovate-lanceolate, apex long-acuminate, margin with dark brown, sharp teeth, 3–6 mm long, 1–2 cm apart, surface smooth, dull green, often with horny margin; exudate yellow. Inflorescence consisting of racemes, 15–20 cm long, lax, flowers arranged at one side; peduncle 1–1.5 m long, with up to 12

branches, the lower branches rebranched; bracts ovate-acute, 3–7 mm long, pale brown, papery. Flowers bisexual, regular, 3-merous; pedicel 5–10 mm long; perianth tubular, 2.5–3.5 cm long, inflated around the ovary, lobes 6, 12–17 mm long, rose-pink to dull scarlet, paler at mouth; stamens 6, exserted; ovary superior, 3-celled, style filiform, stigma head-shaped, exserted. Fruit an oblong-ovoid capsule up to 25 mm × 14 mm, pale brown, dehiscing loculicidally and contains many seeds. The Seeds are 8.5 mm long, blackish brown and with speckles (Peter & Mbilu, 2009) (Fig. 5).

Figure 5: ***Aloe secundiflora*** **plant**

2.1.5: ***Sonchus luxurians***

Description

Annual or short-lived perennial herb.The stems, branched from base. Leaves grey-green, sessile, ovate or lanceolate in outline, the lower leaves usually pinnately lobed while the upper leaves usually entire or with few lobes, with a very broad base, sagittate and broadly auriculate, margins dentate, apex acute to attenuate. Capitula several together in dense clus-

ters, mature capitula surrounded by bud capitula, their bases and the upper part of the capitulum stalk with a white tomentum; phyllaries green with red-purple tinge on margins and apex, ovate to lanceolate, 3–15 mm long, obtuse, the outer proximally tomentose, the inner glabrous. Florets many; corolla yellow, the outer orange beneath, tube cylindrical, distally pilose. Achenes pale brown, narrowly ellipsoid, slightly flattened, angular and ribbed, rugulose, glabrous (Fig. 6) (Everitt *et al.,* 2007).

Fig. 6: A stalk of *Sonchus luxurians*

The barks of *Bridelia micrantha* and *Croton megalocarpus* were collected from Kiambu, *Sonchus luxurians* whole plant was collected from Nyandarua while *Ocimum americanum* and *Aloe secundiflora* plants were collected from Machakos, after identification by a taxonomist. Whole *Ocimum americanum, Sonchus luxurians,* and *Aloe secundiflora* plants were uprooted and separately packed in clearly labelled plastic bags. For *Bridelia micrantha* and *Croton megalocarpus*, the bark of the tree were obtained and separately packed in clearly labelled plastic bags.

2.2: Plant identification

Voucher specimens of whole *Sonchus luxurians*, *Ocimum americanum* and *Aloe secundiflora* plants, as well as the back parts of *Bridelia micrantha* and *Croton megalocarpus* were submitted to the National Museums of Kenya Herbarium for identification.

2.3: Plant drying

The three whole plants; *Ocimum americanum, Sonchus luxurians,* and *Aloe secundiflora* and the barks of *Bridelia micrantha* and *Croton megalocarpus* were dried under shade for about two months. When completely dry, they were ground into powder using an electrical grinder. The powder was stored at room temperature.

2.4: Preparation of the plant extracts

2.4.1: Ethanol extraction

For the five plants, 100 g of the powder was soaked in 500 ml of 70% ethanol for one day (24 h). The solutions were filtered using filter paper (Whatman No. 1) and subjected to freeze drying using laboratory freeze drier located in the School of Biological sciences, University of Nairobi. The freeze dried materials constituted the ethanol extract (Das *et al.*, 2010).

2.4.2: Aqueous extraction

For each of the plants, 100 g of the powder was soaked in 500 ml of distilled water for three days (72 h) in order to extract polar materials. The solutions were filtered using filter paper (Whatman No. 1) and allowed to

freeze. The frozen material was then subjected to freeze drying using laboratory freeze drier located in the School of Biological sciences, University of Nairobi. The freeze dried materials constituted the aqueous extract for each of the specific plants (Das *et al*., 2010).

2.5: Phytochemical Screening of crude extracts

Phytochemical screening was performed using standard procedures (Trease & Evans, 1989; Siddiqui & Ali, 1997; Harborne, 1998). The various extracts were tested for triterpenes, sterols, flavonoids, glycosides, saponins, tannins and alkaloids.

2.5.1: Test for flavonoids

Two methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was then added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. Second, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was thereafter filtered and 4 ml of the filtrate shaken with 1 ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

2.5.2: Test for saponins

The extract was diluted with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins. The frothing was mixed with 3 drops of

olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.5.3: Test for tannins

About 0.5 g of each extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.5.4: Test for alkaloids

For each of the plants, 0.5 g of the extract was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was then filtered and 1 ml of the filtrate treated with two drops of Mayer's reagent. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

2.5.5: Test for sterols and triterpenes

For each of the plants, 10 ml of the extract was placed in a small beaker (50 ml) and evaporated to dryness. The residue was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. The solution was transferred into a dry test tube and concentrated solution of sulphuric acid (2 ml) added slowly. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet colouration denoted the presence of sterols and triterpenes.

2.5.6: Test for glycosides

Two separate beakers were prepared. 1 g of each extract was placed into each of the two separate beakers. To one of the beakers 5 ml of dilute

sulphuric acid was added while 5 ml of water was added to the other beaker. The two beakers were heated for 3 – 5 min and the contents filtered into labelled test tubes. The filtrates were made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. Presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate was regarded as positive for glycosides.

2.6: Snail collection and maintainance

Biomphalaria pfeifferi snails were collected from canals in Mwea irrigation scheme. Snails were scooped out of the water using a sieve attached to a long pole. The snails were then placed in plastic containers with holes and lined with wet cotton wool. The packed snails were transported to Institute of Primate Research snail room. Once in the snail room, they were maintained in plastic trays containing snail water and daphnia, under controlled room temperature (25°c - 28 ^{o}c) and constant light for 12 h daily (12 h light and 12 h dark). The snails were fed on dried lettuce (Yole *et al*., 1993).

2.7: Breeding of juvenile snails

Trays were prepared holding snail water, lettuce and daphnia. Into each of the trays, 10 snails were placed. The snails were allowed to lay eggs. Once the snails had laid egg masses in the water, the eggs were allowed to hatch and the young ones allowed to grow in the same trays with their mothers (Yole *et al*., 1993).

2.8: Molluscicidal activity of adult snails

The plants were evaluated for molluscicidal activity on adult snails as follows: Groups of 10 snails were placed in plastic containers holding 500 ml of distilled water. The set ups were left for 24 h and snails were fed on dried lettuce. Different concentrations; 5 mg/litre, 10 mg/litre, 20 mg/litre and 40 mg/litre of both the aqueous and the ethanol extracts of the five plants were made.

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 the containers holding 10 snails each and replaced with 500 ml of the different extracts concentrations, positive control and a negative control preparation.

Duplicate set – ups were set for each of the different concentrations made. The set ups were left for 24 h without feeding the snails. After 24 h the plant extracts were replaced with 500 ml of distilled water, without feeding the snails with dried lettuce. After the 24 h in distilled water, the snails were observed to determine whether they were dead or alive. This was done by; pocking the foot of the snail with a wooden applicator stick where, lack of motion signified death of the snail. Location of the heart and observation of the heart beat was done under the dissecting microscope. Lack of heart beat signified death of the snail (WHO 1985; Yole *et al*., 1993).

2.9: Molluscicidal activity of juvenile snails

The juvenile snails were cleaned and placed in groups of 10 in plastic containers holding 500 ml of distilled water. The same procedure (2.8) which had been used for adult snails was applied to juveniles.

2.10: Activity of *B. micrantha* on *Schistosoma mansoni* miracidia

Bridelia micrantha aqueous and ethanol extracts which were the only ones that were found effective against both adults and juvenile snails were used to test miracicidal activity.

New miracidia were hatched from eggs from faecal samples of baboons with *Schistosoma mansoni* chronic infection. The feacal samples were obtained from the chronically infected baboons and transferred into a plastic jug. Water was added, and the mixture stirred with a rod to mix evenly. The mixture was passed through two successive sieves, a 600 µm sieve and a 250 µm sieve to a collecting tray. The collected sample was transferred to urine jars and put in dark room for 30 min. After the 30 min were over, the supernatant was poured out. More water was added to fill up the urine jars and the jars kept in a dark room for a further 30 min. This process was repeated four times. After the fourth time, the supernatant was poured out and the pellets transferred using a dropper to already prepared petri dishes containing water and placed under light. The set up was left for a period of 30 min for the miracidia to hatch.

Different concentrations : 5 mg/litre, 10 mg/litre, 20 mg/litre and 40 mg/litre of both the aqueous and the ethanol extracts of *Bridelia micrantha*

were made. Ten miracidia were placed in 5 cm petri dishes. Two millilitres of each of the already prepared concentrations of the aqueous and ethanol extracts were added to the 10 miracidia in the 5 cm petri dishes. A duplicate was set up for each concentration. Positive control had 2 ml of 1mg/litre niclosamide and negative control had 2 ml distilled water. Dead and surviving miracidia were counted under the dissecting microscope, at 10, 20, 30 and 60 min interval for a period of one hour. Constant motion signified that the miracidia were alive while no motion signified death (WHO 1985; Yole *et al*., 1993).

2.11: Snail infection with *Schistosoma mansoni*

Miracidia were hatched from eggs from faecal samples of baboon with *Schistosoma mansoni* chronic infection as decribed in (2.10) above. Five to eight miracidia were placed in each well of a 24-well culture plate. One snail was then transferred into each well containing the miracidia and the set up was left for 30 min for the miracidia to penetrate. The infected snails were transferred to clean trays with snail water and they were fed with dry lettuce. The infected snails were maintained for four weeks to allow the development of the miracidia to cercariae (Yole *et al*., 1993).

2.12: Activity of *Bridelia micrantha* on *Schistosoma mansoni* cercariae

The infected snails were placed in a beaker containing water and placed under 200 watts lamp shielded with glass. They were left for a period of one hour to shed the cercariae. The shed cercariae were placed in each well of a 24-well culture plate. Different concentrations: 5 mg, 10 mg, 20 mg and 40 mg of both the aqueous and the ethanol extracts of *Bridelia*

micrantha were made. Ten cercariae were placed in 5 cm petri dishes. Then 2 ml of each of the already prepared concentrations of the aqueous and ethanol extracts were added to the 10 cercariae in the 5 cm petri dishes. A duplicate was set up for each concentration. Positive control had 2 ml of 1 mg/litre niclosamide and negative control had 2 ml distilled water. Dead and surviving cercariae were counted under the dissecting microscope, at 10, 20, 30 and 60 min interval for a period of one hour. Constant motion signified that the cercariae were alive while no motion signified death. (Yole *et al*., 1993).

2.13: Data analysis

Molluscicidal miracicidal and cercaricidal data was analyzed by one way ANOVA with help of SPSS (statistical package for the social sciences) version 16. The Molluscicidal activity data was subjected to ANOVA to determine whether there were significant differences. Once significant differences were identified, post hoc ANOVA was done with Dunnet Test to compare each treatment with the positive control group The data was also analyzed by Finney's Probit analysis to estimate the LD_{50} of the various aqueous and ethanol extracts. In all the analysis, the probability level/significance level used in the analysis was ≤ 0.05.

CHAPTER THREE

3.0: RESULTS

3.1: Yield of the extracts from the plants

Different yields were obtained when extraction was done using either distilled water or 70% ethanol. The results obtained for the aqueous and the ethanol extracts are shown in Table 1.

Table 1: Yield of the aqueous and ethanol extracts

Part of the plant used	**Plant**	**Yield in grams**	
		Distilled water	**Ethanol**
Bark	*Bridelia micrantha*	10.81	6.74
	Croton megalocarpus	6.44	6.05
Whole plant	*Aloe secundiflora*	4.78	3.72
	Sonchus luxurians	8.95	2.17
	Ocimum americanum	3.75	1.78

3.2: Qualitative analysis of the crude extracts

Phytochemical screening of the crude plant extracts revealed differences in the constituents of *S.s luxurians*, *O. americanum*, *A. secundiflora*, *B. micrantha* and *C. megalocarpus* and their medium of extraction; aqueous and ethanol. The phytochemicals tested were flavonoids, saponins, tannins, alkaloids, glycosides, steroids and triterpenes. Tannins, flavonoids and steroids/triterpenes were detected in both ethanol and aqueous crude extracts of all the plants tested. Alkaloids were detected in the ethanol and aqueous crude extracts of *C. megalocarpus* and *A. secundiflora* as well as the ethanol crude extracts of *O. americanum* and *B. micrantha* and aqueous crude extracts of *S. luxurians.* Saponins were detected in the ethanol and aqueous crude extracts of *O.americanum, B. micrantha* and *Sonchus luxurians* as well as the aqueous crude extracts of *C. megalocarpus* and *Aloe secundiflora*. Glycosides were detected in ethanol and aqueous crude extracts of *O. americanum, B. micrantha* and *S. luxurians* as well as the aqueous crude extracts of *C. megalocarpus* and *A. secundiflora*. All the phytochemicals tested were detected in *O. americanum* and *B. micrantha* ethanolic extracts while alkaloids were not detected in their aqueous extracts. All the phytochemicals tested were also detected in aqueous extracts of *C. megalocarpus* and *A. secundiflora* while saponins and glycosides were not detected in their ethanolic extracts. The tested phytochemicals were also detected in aqueous extracts of, *S luxurians* while alkaloids were not detected in its ethanolic extract. In all the ethanolic extracts tested only in *S. luxurians'* ethanolic extract where alkaloids were not detected. The results are shown in Table 2.

Table 2: Phytochemicals in the crude extracts of *the plants*

	Extracts									
Phyto-chemicals	**0EE**	**0AE**	**BEE**	**BAE**	**CEE**	**CAE**	**AEE**	**AAE**	**SEE**	**SAE**
Alkaloids	+	-	+	-	+	+	+	+	-	+
Saponins	+	+	+	+	-	+	-	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Sterols/ Triterpenes	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	-	+	-	+	+	+

KEY:

+ Present.

- Absent.

AAE *Aloe secundiflora* aqueous extract.

AEE *Aloe secundiflora* ethanolic extract.

BAE *Bridelia micrantha* aqueous extract.

BEE *Bridelia micrantha* ethanolic extract.

CAE *Croton megalocarpus* aqueous extract.

CEE *Croton megalocarpus* ethanolic extract.

OAE *Ocimum americanum* aqueous extract.

OEE *Ocimum americanum* ethanolic extract.

SAE *Sonchus luxurians* aqueous extract.

SEE *Sonchus luxurians* ethanolic extract.

3.3: Molluscicidal activity

Molluscicidal activity of the aqueous and the ethanol plant extracts and their different concentrations was observed on both the adult and the juvenile snails. The snails were observed under dissecting microscope to determine if dead or alive. Dead and surviving snails were counted at the end of day three.

The various ethanol extracts had molluscicidal activity which was significantly different among the various treatments ($F_{6,\ 21}$ = 31.636; $p < 0.05$). Dunnent test was used to compare all the treatments groups with the positive control groups. Only the comparison of *B. micrantha* ethanolic extracts with niclosamide gave a sig. level > 0.05 for both juvenile and adult snails at 0.73 and 0.51 respectiveley. The other comparison of the other ethnolic extracts from the other plants with niclosamide gave a sig. level< 0.05. Hence, only the ethanolic extracts of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide.

Molluscicidal activity due to aqueous extracts was significantly different among all the treatments ($F_{6,\ 21}$ = 20.970; $p < 0.05$). Dunnet test showed that only the comparison of *B. micrantha* aqueous gave a sig. level > 0.05 for both juvenile and adult snails at 0.62 and 0.53 respectively. Hence, only the aqueous extracts of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide.

3.4: Miracicidal activity

The various concentrations of the aqueous and ethanol extracts of *B.micrantha* had significantly different miracicidal activity within 60 minutes ($F_{4, 15} = 4.579$; $p < 0.05$). Under multiple comparisons, P values obtained from the comparison of concentrations of the aqueous extracts with niclosamide were > 0.05. In the ethanol extracts comparison of 20mg/l and 40 mg/l gave p. Values > 0.05 i.e 0.921. Hence, all the concentrations of the aqueous extracts of *B.micrantha* (5, 10, 20 and 40 mg/l) and the 20 and 40 mg/l of the ethanol extract of *B.micrantha* had a miracicidal activity similar to that of niclosamide.

3.5: Cercaricidal activity

The various concentrations of the aqueous and ethanol extracts of *B.micrantha* had significantly different cercaricidal activity within 60 minutes ($F_{4, 15} = 3.949$; $p < 0.05$).. Dunnet test showed that P.values obtained after comparison of all the concentrations of the aqueous extract of *B.micrantha* with niclosamide were > 0.05. Only comparison of 40mg/l of ethanol extract of *B.micrantha* gave a p. value > 0.05 i.e 0.407. Hence 5, 10, 20 and 40 mg/l of aqueous extracts of *B.micrantha* together with 40 mg/l of the ethanolic extract of *B.micrantha* had cercaricidal activity which was not significantly different from that of

3.6: LD_{50} Determination

LD_{50} was calculated using Finney Probit analysis method to measure the dose that killed or immobilised 50% of the target organisms within the exposure period. Probit analysis is a method of survival analysis which

involves conversion of the percentage mortalities into probits and also conversion of the concentrations into logarithims. Plotting the logarithims against the probits gives a straight line which can be used to estimate the LD_{50} value.

3.6.1: LD_{50} Determination of the extracts on adult snails

The aqueous extract of *Bridelia micrantha* was found to have a LD_{50} value of 24.98 mg/l and the ethanol extract LD_{50} value 19.01 mg/l. The other extracts were found to have a LD_{50} value of above 100 mg/l. The results are shown in Table 3.

Table 3: LD_{50} values of the extracts on adult snails**.**

	Mean ± SE	
Plant species	**Aqueous**	**Ethanol**
S. luxurians	100.94 ± 20.63	1422.69±329.90
B. micrantha	24.98 ± 3.35	19.01 ± 3.61
O. americanum	100.94 ± 20.63	100.93 ± 20.67
A. secundiflora	100.94 ± 20.63	106.83 ± 17.82
C. megalocarpus	106.83 ± 17.83	100.93 ± 20.67
Niclosamide	6.37 ± 2.72	6.37 ± 2.72
Distilled water	1422.69±329.90	1422.69±329.90

3.6.2: LD_{50} Determination of the extracts on juvenile snails

The aqueous extracts of *Bridelia micrantha* was found to have a LD_{50} value of 22.860 mg/l, while the ethanol extracts was found to have a LD_{50} value of 26.30 mg/l The other extracts from the other plants were found to have a LD_{50} value of above 100 mg/l.The results are shown in Table 4.

Table 4: LD_{50} values of the extracts on juvenile snails

	Mean ± SE	
Plant species	**Aqueous**	**Ethanol**
S. luxurians	1422.69±329.90	1422.69 ± 329.90
B. micrantha	22.86 ± 4.69	26.30 ± 4.58
O. americanum	100.93 ± 20.67	1422.69 ±329.90
A. secundiflora	1422.69 ± 329.90	100.93 ± 20.67
C. megalocarpus	1422.69 ± 329.90	1422.69 ± 329.90
Niclosamide	6.37 ± 2.72	6.37 ± 2.72
Distilled water	1422.69 ± 329.90	1422.69 ± 329.90

3.6.3: LD_{50} Determination for Miracicidal and Cercaricidal activity

Bridelia micrantha aqueous extract had a LD_{50} value of 11.108 mg/l on the *S. mansoni* miracidia and a LD_{50} value of 4.465 mg/l on the cercariae. The ethanol extract was found to have a LD_{50} value of above 40 mg/l for the miracidia and the cercariae. The results are shown on Table 5.

Table 5: LD_{50} values of aqueous and ethanol extracts of *B. micrantha* on *Schistosoma mansoni* miracidia and cercariae

Extract	**LD_{50} value (mg/l)**	
	Miracidia	**Cercariae**
Aqueous extract	11.108	4.465
Ethanol extract	67.011	44.821

CHAPTER FOUR

4.0: DISCUSSION

4.1: Yield of the extracts from the plants

Some of the properties affecting the choice of a solvent are the quantity of phytochemicals to be extracted and, promotion of rapid physiologic absorption of the extract (Eloff, 1998). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Eloff, 1998). Therefore the quantity of phytochemicals extracted could be due to the type of solvent used. In both the aqueous and the ethanol extracts, *Aloe secundiflora* had the highest yield. When water was used as the medium of extraction, the extract yield was higher compared to when ethanol was used as the solvent in all the plants used.The results showed that distilled water is a better extractor than ethanol, yielding higher quantities of the extract than ethanol. This could be because water is a universal solvent, used to extract plant products (Das, *et al.*, 2010).

4.2: Qualitative analysis of the crude extracts

Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. Presence of phytochemical components in these extracts illustrate that there are bioactive components in these extracts. Phytochemical screening of the crude plant extracts revealed some differences in the constituents of *Sonchus luxurians*, *Ocimum*

americanum, *Aloe secundiflora*, *Bridelia micrantha* and *Croton megalocarpus* and depending on the medium of extraction.

Tannins, flavonoids and steroids/triterpenes were detected in both ethanol and aqueous crude extracts of all the plants tested. Alkaloids were detected in both the ethanol and aqueous crude extracts of *Croton megalocarpus* and *Aloe secundiflora.* Alkaloids were also detected in the ethanol crude extracts of *Ocimum americanum* and *Bridelia micrantha* and in aqueous crude extracts of *Sonchus luxurians.* Alkaloids are intermediate polar compounds and as such would be extracted by intermediate polar compounds like ethanol (Kotze & Eloff, 2002). However, because of the structural diversity of alkaloids, there is no single method of their extraction from natural raw materials. Most methods exploit the property of most alkaloids to be soluble in organic solvents but not in water, and the opposite tendency of their salts (Manske, 1965). For this reason, the absence of alkaloids in the aqueous extracts of *Bridelia micrantha* and *Ocimum americanum* and yet they were present in their ethanol extracts may indicate that their occurrence in these plants is not in form of salts of organic solvents. The absence of alkaloids in the ethanol crude extract of *Sonchus luxurians* and yet they were present in the aqueous extract may indicate that their occurrence in these plants is in form of salts of organic solvents. However, most alkaloids are present in the raw plants in the form of salts of organic acids (Manske, 1965).

Saponins were detected in the ethanol and aqueous crude extracts of *Ocimum americanum, Bridelia micrantha* and *Sonchus luxurians* as well as the aqueous crude extracts of *Croton megalocarpus* and *Aloe secundiflora*. Saponins frequently are isolated by boiling in methanol (Oleszek *et al*., 1992). However, saponins have special structural features and in general it is difficult to use a single technique for extraction of saponins. For this reason, the absence of saponins in the ethanol extracts of *C. megalocarpus* and *A. secundiflora* and yet they were present in their aqueous extracts may indicate that saponins from various sources have different extraction procedures (Yadav & Munin, 2011)

Glycosides were detected in ethanol and aqueous crude extracts of *O. americanum, B. micrantha* and *S. luxurians* as well as the aqueous crude extracts of *C.megalocarpus* and *A. secundiflora.* Glycosides are water so-luble compounds. However, some glycosides are soluble in alcohol. The absence of glycosides in the ethanol extracts of *C. megalocarpus* and *A. secundiflora* may indicate that the glycosides in these plants are insoluble in alcohol.

4.3: Activity of the plant extracts on the adult and juvenile snails

In both the aqueous and ethanol extracts, *Bridelia micrantha* had the highest effect on the juvenile and adult snails. The significant value obtained after the analysis is 0.000. This significant value is < 0.05. Hence, we conclude that the various ethanol and aqueous extracts had molluscicidal activity which was significantly different among the various

treatments. On comparison to niclosamide, *B. micrantha* ethanolic extract gave a significant level > 0.05 for both juvenile and adult snails of 0.73 and 0.51 respectiveley. Hence only the ethanolic extract of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide. For the aqueous extracts, comparison of *B. micrantha* aqueous extracts with niclosamide gave a significant level > 0.05 for both juvenile and adult snails i.e 0.62 and 0.53 respectively. Hence only the aqueous extracts of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide. Comparison of the other ethnolic and aqueous extracts from the other plants with niclosamide gave a significant level < 0.05.

The aqueous extract of *B. micrantha* was more effective (significant value 0.53) than the ethanol extract (significant value 0.51) The adult snails were more susceptible to *B. micrantha* extracts than the juvenile snails. The death of the snails in *B. micrantha* aqueous and ethanol extracts could be due to the findings that the plant is known to contain saponins (Schmidt *et al.,* 2002). The molluscicidal activity of the saponins may be due to their characteristic detergent effect on the soft body membranes of the molluscs. However, saponins are structurally diverse and have multiple effects in animal cells (Francis *et al*., 2002). A large number of saponins have an effect on the cell membrane's permeability by either forming pores in the membranes altering sodium- patassium and calcium magnesium ATPase activity or insertion of the hydrophobic saponin nucleus into the lipid bilayer. Others have the ability to lower serum cholesterol land increase cholinesterase activity (Edoga *et al*., 2005).

It is difficult to establish clear functionality and structure - activity relationships regarding the effects of saponins in biological systems due to the complexity of their cellular reactions (Francis *et al*., 2002). Thus there is need for further reseach for a clear explanation on the molluscicidal activity of *B. micrantha* aqueous and ethanol extracts while all the extracts from the other four plants showed no molluscicidal activity yet they were found to have saponins.*B. micrantha* has prospects to warrant further research towards development of a molluscicide which may give solution to the control of schistosomiasis transmission by snails.

4.4: Miracicidal activity of *Bridelia micrantha*

Analysis of significant value obtained is 0.13 which is < 0.05. The various concentrations of the aqueous and ethanol extracts of *B.micrantha* had significantly different miracicidal activity within 60 minutes. Significant values obtained from the comparison of concentrations of the aqueous extracts with niclosamide were > 0.05. (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l gave p values of 0.744, 0 .949, 0 .981 and 1.000 respectively. In the ethanol extracts comparison of 20mg/l and 40 mg/l gave p. values of 0.921 ($P > 0.05$). Hence all the concentrations of the aqueous extracts of *B.micrantha* (5, 10, 20 and 40 mg/l), 20 and 40 mg/l of the ethanol extract of *B.micrantha* had a miracicidal activity similar to that of niclosamide.

4.5: Cercaricidal activity of *Bridelia micrantha*

Various concentrations of the aqueous and ethanol extracts of *B.micrantha* had significantly different cercaricidal activity within 60 minutes. A p value of 0.22 which is < 0.05 was obtained.

After comparison of all the concentrations of the aqueous extract of *B.micrantha* with niclosamide p value > 0.05 were obtained. The p values were 0.058, 0.298, 0.058 and 0 .298 for 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l respectively. Only comparison of 40mg/l of ethanol extract of *B.micrantha* gave a p. value > 0.05 0.407. Hence the 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l concentrations of aqueous extracts and the 40 mg/l of the ethanol extract of *B.micrantha* had cercaricidal activity which was not significantly different from that of niclosamide within the 60 minutes period. The number of dead cercariae in the ethanol extracts remained fairly constant with varying time of exposure.

4.6: LD_{50} Determination

LD_{50} is the dose required to kill half the members of a tested population after specified test duration. LD_{50} of the extracts was determined to measure the dose that killed or immobilised 50% of the target organisms within the treatment period. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity (Fleming & Hunt, 2000). A large LD_{50} means it takes a large quantity of the material to cause a toxic response (Walker & Lupien, 2000). LD_{50} < 100 mg/l indicates that the the substance is highly toxic, LD_{50} > 100 < 500 mg/l indicates that the the substance is moderately toxic, LD_{50} > 500 < 1000 mg/l indicates that the the substance is weakly toxic while LD_{50} > 1000 mg/l indicates that the the substance is non toxic (Nguta *et al.*, 2011).

Among the extracts used on adult snails, *B. micrantha* aqueous extract was highly toxic on adult snails; LD_{50}<100mg/l while *S. luxurians,*

O. americanum, A. secundiflora and *C. megalocarpus* aqueous extracts were moderately toxic; LD_{50}>100< 500mg/l. *B.micrantha* ethanol extract was highly toxic; LD_{50}<100mg/l, *O. americanum, A. secundiflora* and *C. megalocarpus* were moderately toxic; LD_{50}>100< 500mg/l while *S. luxurians* was non toxic on the adult snails; LD_{50}>1000mg/l. The aqueous extract of *Bridelia micrantha* was found to have a LD_{50} value of 24.98 mg/l, while its ethanol extract was found to have a LD_{50} value 19.01 mg/l.

When these extracts were tested on the juvenile snails, *B.micrantha* aqueous extract was highly toxic; LD_{50}<100mg/l, *O.americanum* was moderately toxic; LD_{50}>100< 500mg/l while *S. luxurians, A. secundiflora,* and *C. megalocarpu*s were non toxic on juvenile snails; LD $_{50}$>1000mg/l. For the ethanol extracts, *B.micrantha* was highly toxic; LD_{50}<100mg/l, *A. secundiflora* was moderately toxic; LD_{50}>100< 500mg/l, *S. luxurians, O. americanum,* and *C. megalocarpus*, were non toxic on juvenile snails; LD_{50}>1000mg/l. The aqueous extract of *B. micrantha* was found to have a LD_{50} value of 22.86 mg/l, while the ethanol extracts of *B. micrantha* was found to have a LD_{50} value of 26.30 mg/l. For the juvenile snails the aqueous extract of *B. micrantha* was more efficacious with a LD_{50} value of 22.86 mg/l than the ethanol extracts, with a LD_{50} value of 26.30 mg/l. For plant to be considered to be a molluscicide, it should be registered in concentrations of up to 100 mg/l (Mott, 1987). The concentrations of *B. micrantha* were therefore within the acceptable concentration.

On testing the efficacious *Bridelia micrantha* on the *Schistosoma mansoni* miracidia, the aqueous extract had a LD_{50} value of 11.108 mg/l, while the

ethanol extract was found to have a LD_{50} value of above 40 mg/l. Therefore the other extracts required a higher dose than 40 mg/l to kill the miracidia.The miracidia were more susceptible to the aqueous extract than to the ethanol extract. On testing the efficacious *Bridelia micrantha* on the *Schistosoma mansoni* cercariae, the aqueous extract had a LD_{50} value of 4.465 mg/l, while the ethanol extract was found to have a LD_{50} value of above 40 mg/l. For the cercariae, the aqueous extract of *B. micrantha* was more efficacious with a LD_{50} value of 4.465 mg/l, than the ethanol extract, with a LD_{50} value of above 40 mg/l. Therefore the other extracts required a dose higher than 40 mg/l to kill the cercariae.

Water is a universal extracter. The higher susceptibility of the miracidia and the cercariae to the aqueous extract than to the ethanol extract may be due to presence of some phytochemicals especially those extracted by water which have different structures form those extracted by ethanol (Sen *et al*., 1998b)

4.7: Conclusion

In this study distilled water and 70% ethanol were used for extraction of crude plant extracts from five plants. Distilled water proved to be a better extractor. There are appreciable numbers of compounds that are present in the ten extracts. They include alkaloids, saponins, tannins, flavonoids sterols/triterpenes and glycosides**.** Out of the tested plant extract samples *B. micrantha* has proven to be the best plant extract in molluscicidal effects.The plant extract also had ability to destroy cercariae and to a less extent the miracidia. Ethanol extracts, of *B. micrantha* was more

efficacious on the adult snails with a lower LD_{50} value than the aqueous extract. For the juvenile snails, the miracidia and the cercariae, aqueous extract of *B. micrantha* was more efficacious with a lower LD_{50} value than the ethanol extract.

Generally the adult snails were found to be more susceptible to the lethal effects of the plant extracts than the juvenile snails. The cercariae were found to be more susceptible to the lethal effects of the plant extracts than miracidia and there are no obvious explanations for these differences.

This study has been able to demonstrate significant molluscicidal, miracicidal and cercaricidal activity in *B.micrantha* which could be used in control of schistosomiasis. The results suggest that *B.micrantha* extract has highly promising properties.

4.8: Recommendations

1. The extracts of *B.micrantha* should be further analyzed to isolate the probable molluscicidal, miracicidal and cercaricidal principles in them.
2. Toxicity studies of the effective plants should also be done to determine the safety indices of the extracts.
3. In this study, the *B. micrantha* extracts were made from only one part of the plant. It is suggested that many plant parts and also of varied ages be used since there are variations in the accumulation of the chemical compounds in various plant parts with time.

REFERENCES

Abd El-Aziz, E. Shadia and Nofal, M.A. (1998). The efficiency of Bacteria, fungi and natural products in baits against the greasy cutworm, *Agrotis ipsilon* in Egypt. J Egypt Ger Soc Zool 27, Entomology: 129-139.

Abd El-Aziz, Shadia E and F.M.A. Fatma El-Hawary, (1997). Inhibitory effects of some essential oils on the development of the cotton leaf worm, *S. littoralis* (Boisd.). *Entomology*: 117-130.

Adewunmi, C.O. and Sofowora, E.A. (1980). Preliminary screening of some plant extracts for molluscicidal activity. *Planta Medica* **39,** 57-65.

Akinmoladun, A.C, Ibukun E.O, Afor, E, Oboutor, E.M and Farombi, E.O (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum.* Science Research Essay. 2: 163-166.

Al-Mathal, E.M. and Fouad, M.A. (2006). Effect of *Commiphora molmol* on adults, egg masses and egg-deposition of *Biomphalaria ara-bica* under laboratory conditions.*Parasitology* **36,** 305-314.

Aly, S.A. Aly, H.F. Saba-el-Rigal, N. and Sammour, E.M. (2004). Induced changes in biochemical parameters of the molluscan tissue non-infected using two potent plants molluscicidies. *Parasitology* **45,** 527-542.

Aly, S.A. and Hamed, M.H. (2006). Effect of Ailanthus altissima and *Zizyphus spina christi* on bilharzial infestation in mice: Histological and histopathological studies. *Applied Science* **29,** 1437-1446.

Amer, M. (1994). Cutaneous Schistosomiasis. *Contemporary Tropical Dermatology* **12**, 713-717.

Amrita, S. and Singh, N.K. (2001). Molluscicidal activities of the custard apple (Annona sqaunwsa l) alone and in combination with other plant derive molluscicide. *Herbs, spices and medicinal plants* **8**, 31.

Anto, F. Bosompem, K. Kpikpi, J. Adjuik, M. and Edoh, D. (2005). Experimental control of *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni*, by the ampullariid snail *Lanistes varicus*. Tropical Medicine and Parasitology **99, (2):** 203-209.

Atta El-Mannan, A. M. (2001). Studies on biological control of *Lymnaea natalensis* snails using a molluscicidal indigenous plant *Pulicaria crispa* (Forssk.) Oliv. (Compositae). Ph. D. Thesis. Faculty of Veterinary Sciences, University of Khartoum, Sudan.

Beltran, S. and Boissier, J. (2008). Schistosome monogamy: who, how and why? *Parasitology* **24,** 386-391.

Bierman, W. Wetsteyn, J.C. and van Gool, T. (2005). Presentation and diagnosis of imported schistosomiasis: relevance of eosinophilia, microscopy for ova, and serology. *Travel Medicine* **12,** 9–13.

Bloch, E.H. (1980). In vivo microscopy of schistosomiasis. Migration of *Schistosoma mansoni* in the lungs liver and intestines. *Tropical Medicine and Hygiene* **29,** 62-70.

Brinkmann, A. and Steingruber, R. (1986). Possible modification in the construction of snail dams to prevent the spread of schistosomiasis. *Parasitology* **37,** 199-201.

Cavalcanti, E.S.B. Morais, S.M. Lima M.A.A and Santana, E.W.P. (2004). Larvicidal Activity of Essential Oils from Brazilian Plants against *Aedes aegypti* L. Mem Inst Oswaldo Cruz, Rio de Janeiro, 99: 541-544.

Chen, X.Y, Wang, L.Y. Cai, J.M. Zhou, X.N. Zheng, J. Guo, J.G. Wu, X.H. Engels, D. and Chen, M.G. (2005). Schistosomiasis control in China: the impact of a 10-year World Bank Loan Project (1992–2001). WHO 83: 43–48.

Chothani D.L. and Vaghasiya H.U. (2011). A review of *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses and pharmacological activity. *Pharmacogn Rev* **5**: 55-62.)

Clark, T.E. Appleton, C.C. and Drewes, S.E. (1997). A semi quantitative approach to the selection of appropriate candidate plant molluscicides - a South African application. *Ethnopharmacology* **56,** 1-13.

Coates Palgrave, M. (2002). *Keith Coates Palgrave Trees of southern Africa,* 3rd edition. Struik. Cape Town.

Crewe, W. (1977). A guide to human parasitology. The English Language Book Society and H.K Lewis & Company, London.

Daffalla, A. A. and Amin, M. A. (1976). Laboratory and field evaluation of the molluscicidal properties of habit- el- mollok (*Corton spp*.). *Medical Research* **16,** 185-195.

Dalton, P.R. and Pole, D. (1978). Water- contact patterns in relation to *Schistosoma haematobuim* infection. World health Organization **56**, 417–426.

Das, K. Tiwari, R.K.S. and Shrisvastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methodsand future trends. *Medicinal Plants Research* **4(2)**, 104-111.

Dos Santos, A.F. de Azevedo, D.P. dos Santos Mata Rda, C. de Mendonca, D.L. and SantAna, A.E. (2007). The lethality of *Euphorbia*

conspicua to adults of *Biomphalaria glabrata*, cercaria of *Schistosoma mansoni* and larvae of *Artemia salina*. Bioresour. *Technolology* **98,** 135-139.

Edoga, H.O. Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4 (7)** 685-688.

El- Khier, Y. M. and El- Tohami, M. S. (1979). Investigation of Mollus-cicidal Activity of Certain Sudanese plants Used in Folk-Medicine. *Tropi-cal Medicine* **82,** 237-241

Elamin, E. Subki, H. and Koko, W. S. (2005). Utilization of Balanites aegyptiaca fruit extract in the eradication of schistosomiasis transmitting snails in Sudan (Primary investigation*).Albuhuth Sudan Journal of Scientific Research* **8 (2):** 112-119.

Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethnopharmacology* **60**., 1–8.

El- Sawy, M. F. Bassuony, K. and Magdoub, A. I. (1981). Biological Combact of Schistosomiasis: *Ambrosia maritima* (damsisa) for Snail Control. *Egyptian Society Parasitology* **11,** 99-117.

Engels, D.L. Chitsulo, A. Montresor, and Savioli, L. (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica* **82,** 139-146.

Everitt, J.H. Lonard, R.L. and Little, C.R. (2007). *Weeds in South Texas and Northern Mexico*. Lubbock: Texas Tech University Press.

Faust, E.C. Beaver, P.C. and Jung, R.C. (1968). Animal Agents and Vectors of Human Diseases. Lea and Febiger, Philadelphia.

Fenwick, A. Cheesmond, A. K. Kardaman, M. Amin, M. A. and Manjing, B. K. (1982). Schistosomiasis among the labouring communities in the Gezira irrigated area, Sudan. *Tropical Medicine and Hygiene* **88,** 3-11.

Fenwick, A. Keiser, J. and Utzinger, J. (2006). Epidemiology, burden and control of schistosomiasis with particular consideration to past and current treatment trends. *Drugs Future* **31**, 413-425.

Ferguson, F. F. (1978). The role of the biological agents in the control of Schistosomiaisis bearing snails, U.S. Dept. of Health Education and Welfare, Centre for Disease Control. Atlanta, Georgia.

Fleming, D. O. and Hunt, D. L. (2000). Biological Safety: principles and practices. Washington, DC: ASM Press. p. 267.

Gehad, T. El-Sherbini, Rawia A. Zayed, and Eman T. El-Sherbini (2009). Molluscicidal Activity of some *Solanum* species extracts against the snail *Biomphalaria alexandrina. Parasitology Research* Volume 2009, Article ID 474360.

Gryseels B. (1989). The relevance of schistosomiasis for public health. *Parasitology* **40**, 134–142.

Guido, B. and Tadros, T.F. (2000). Fundamental aspects of suspension stabilization and some of their applications. *Ethnopharmacology* **82**, 21–28.

Hamed, M.A. (2006). Excretory secretory product of *Fasciola hepatica* worm protects against *Schistosoma mansoni* infection in mice. *Biology* **44,** 554-561.

Hamed, M.A. (2010). Strategic control of schistosome intermediate host. *Epidemiolology* **3,** 123-140.

Harbone, J.B. (1998). Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. 3rd edition.Chapman and Hall Ltd, London pp 279.

Hirata, M. Takushima, M. Kage, M. and Fakuma, T. (1993). Comparative analysis of hepatic, pulmonary, and intestinal granuloma formation around freshly laid *Schistosoma japonicum* eggs in mice. *Parasitology research* **79,** 316-321.

Hong, T.D. Linington, S. and Ellis R.H. (1996). Seed storage behaviour: a compendium. Handbooks for Genebanks: No. 4. IPGRI.

Hostettmann, K. Mariston, A. Ndjoko, K. and Jean- Luc, W. (2000). The Potential African Plants as a Source of Drugs. *Current Organic Chemistry, Bentham Science Publisher Ltd.*, **4:** 973-1010.

Huang, Y.X. Xi, W.P. Zhang, Y.P. Wu, F. and Hong, Q.B. (2003). Evaluation on molluscicidal effectiveness of niclosamide ethanolamine salt with different methods. Schistosomiasis Control. *Ethnopharmacology* **15**, 124–128.

ICRAF. (1992). A selection of useful trees and shrubs for Kenya: Notes on their identification, propagation and management for use by farming and pastoral communities. ICRAF.

John Ojewele, (2004). Indigenous plants and schistosomiasis control in South Africa. *Boletin Latinoamericano y del Caribe de Plantas Medicina-les y Aromaticas* **3,** 8-22.

José Augusto A dos Santos, Therezinha Coelho B Tomassini, Deise Cristina Drummond Xavier, Ivone Maria Ribeiro, Melissa Teixeira G da Silva, Zenildo Buarque de Morais Filho, (2003). Molluscicidal Activity of *Physalis angulata* Extracts and Fractions on *Biomphalaria tenagophila* under Laboratory Conditions. *Mem Institute Oswaldo Cruz,* Rio de Janeiro, Volume **98,** 425-428.

Kloos, H. and McCollough, F.S. (1987). Plants with recognized mollus-cicidal activity. *Planta Medica* **57**, 190-199.

Kloos, H. and McCullough, F.S. (1982). Plant molluscicides. *Planta Medica* **46,** 195-209.

Klump, H. and Chu, K. Y. (1987). Focal mollusciciding: an effective way aug-ments chemotherapy of Schistosomiasis. *Parasitology Today* **3,** 74-76.

Kotze, M. and Eloff, J.N. (2002). Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae) *South African Journal Biotechnology* **68,** 62–67.

Lemma, A. Heyneman, D. and Silangwa, S. M. (1984). *Pytolacca dode-candra* (Endod). *Tycooly International*, Dublin.

Libora, M. Morales, G. Carmen, S. Isbelia, S. and Luz, A. P. (2010). First finding in Venezuela of *Schistosoma mansoni* eggs and other helminths of interest in public health found in faeces and mucous secretion of the mollusc *Achatina fulica*. *Zootecnia Tropical* **28**, 383-394.

Liu, S.Y. Sporer, F. Wink, M. Jourdane, J. Henning, R. Li, Y.L. and Ruppel, A. (1997). Anthraquinones in *Rheum palmatum* and *Rumex dentatus* (Polygonaceae), and phorbol esters in *Jatropha curcas* (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails *Oncomelania*, *Biomphalaria* and *Bulinus*. *Tropical Medicine and International Health* **2,** 179-188.

Liu, H.C. Pen, D.Y. Fu, Y. Huang, X.B. Li, S.S. Qin, Z.H. and Yu, B.X. (1998). An analysis on cost-effectiveness of snail-eradication with niclosamide in the field. Schistosomiasis Control. *Ethnopharmacology* **10**,129–132.

Livingstone, D.R. and deZwaan, (1983). Carbohydrate metabolism of gastropods. In: The Mollusca. Academic Press, New York.

Loverde, P.T. and Chen, L. (1991). Schistosome female reproductive development. *Parasitology* **7,** 303-308.

Madsen, H. (1985). Ecology and Control of African Freshwater pulmonate snails. Danish Bilharziasis Laboratory. WHO Collaborating Centre for Applied Medical Malacology and Schitosomiasis Control, Charlottenlund, Denmark.

Madsen, H. Simonsen, P. E. and Christensen, N.Q. (1989). A basic field guide to the epidemiology of African human Schistosomiasis II. Danish Bilharziasis Laboratory. *WHO Collaborating Center for Applied Malacology and Schistosomiasis Control, Charlottenlund, Denmark.*

Manske, R. H. F. (1965). The Alkaloids Chemistry and Physiology. Volume VIII. – New York: Academic Press, pp. 673

Mantawy, M.M. and Mahmoud, A.H. (2002). Effect of *Allium cepa* and *Allium sativum* feeding on glucose, glycogen, protein bands profile and phenol oxidase activity in *Biomphalaria alexandrina*. *Parasitology* **32,** 271-283.

Mantawy, M.M. Hamed, M.A. Sammour, E.M. and Sanad, M. (2004). Influence of *Capparis spinosa* and *Acacia arabica* on certain biochemical haemolymph parameters of *Biomphalaria alexandrina*. *Parasitology* **34,** 659-677.

Marston, A. Gafner, F. Dossaji, S. F. and Hostettmann, K. (1988). Plant molluscicides. *Phytochemistry* **27**, 1325.

Marston, A. and Hostettmann, K. (1991). Assays for Molluscicidal, Cercaricidal, Schistosomicidal and Piscicidal Activities. *Methods in plant biochemistry* **6**, 153-176.

Mascolo, N. Izzo, A.A. Borrelli, F. Capasso, R. Di Carlo, G. Sautebin, L. and Capasso, F. (2004). Healing powers of aloes. In: Reynolds, T. (Editor). Aloes: the genus Aloe. CRC Press, Boca Raton, Florida, United States. pp. 209–238.

Massoud, A.M. and Habib F.S. (2003). The effects of Myrrh *Commiphora molmol* on the infected snails of *Schistosoma* species and their egg masses: effect on shedding of cercariae and on snail fecundity. *Parasitology* **33,** 585–596.

Massoud, A. Metwally, D.M. Khalifa K.E. and Habib, F.S. (2004). Compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* after exposure to sublethal concentrations of Myrrh. *Parasitology* **34,** 995-1008.

McCullough, F. S. Gayral, P. Duncan, J. and Christie, J. D. (1980). Molluscicides in Schistosomiasis control. Bulletin of the World Health Organization **58**, 681-689.

McCullough, F.S. (1992). The role of mollusciciding in schistosomiasis control. Geneva, World Health Organization.

McIndoo, I. (1983). USDA. Bur. Entomol and Pl. Quar. Pers. Commn. In Michael *et al*., (1985). Plant species reportedly possessing pest control properties. An.EWC/UH DATA BASE. Univ.of Hawaii, pp: 249. 14.

Mello-Silva, C. C. Vasconcellos, M. C. Pinheiro, J. and Rodrigus, M. L. A. (2006). Pysiologival changes in *Biomphalaria glabrata* (Say), (Pu-lonata: Planorbidae) caused by sub-lethal concentrations of the latex of *Euphorbia splendens var. hisloppii* N. E.B. (Euphorbiaceae). *Memorias-Instituto Oswaldo Cruz. Rio de Janeiro*, **101 (1):** 3-8.

Mohammed, Y. A. I. (2009). Studies on Molluscicidal Activity of Some Plants from Darfur against *B. truncatus* with Emphasis on *Alternanthera nodiflora* (Amaranthaceae) Phd thesis, University of Khartoum pp 86.

Molgaard, P. Chihaka, A. Furu, P. Ndekha, A. and Nyazema, N. (1999). Community based schistosomiasis control in Zimbabwe using the molluscicidal berries of *Phytolacca dodencadra* AETFAT conference in Harare, Zimbabwe.

Mostafa, B. B. and Tantawy, A. A. (2000). Bioactivity of *Anagllis ar-vensis* and *Calendula micrantha* plants, treated with ammonium nitrate, super phosphate and potassium sulphate fertilizers, on *Biomphalaria alexandrina. Cab Abstract*, **30:** 929-942.

Mott, K.E. (1987). Plant Molluscicides, UNDP/World Bank/WHO, John Wiley & Sons Ltd., New York, 326.

Mozley, A. (1939). Freshwater mollusca of the Tanganyika Territory and the Zanzibar Protectorate, and their relation to human schistosomiasis. *African Region Society* **59,** 87-730.

Ndamba, J. Nyazema, J. Makaza, N. Anderson C. and Kaondera, K. (1994). Traditional herbal remediesused for the treatment of urinary schistosomiasis in Zimbabwe. *Ethnopharmacology* **42,** 125-132.

Newton, L.E., (2006). *Aloe secundiflora* Engl. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médici-nales 1. [CD-Rom]. PROTA,

Nguta, J. M., Mbaria, J. M., Gakuya, D. W., and Gathumbi, P. K. (2013). Biological Scree Ig Of Ke Ya Medici Al Pla Ts Usi G Artemia Salina L.(Artemiidae).

Ntezurubanza, L, J.J.C. Scheffer and A. Looman, (1986). Composition of the essential oil of *Ocimum canum* grown in Rawanda. Pharama-ceutisch Weekblad. 7: 273-276.

Oliveira, M.F. d'Avila, J.C. and Tores, C.R. (2000). Haemozoin in *Schistosoma mansoni. Parasitology* **111,** 217-221.

Oleszek, W. and M. Jurzysta (1992). New saponins from alfalfa tops. Pages 315-316 in The Structure and Biological Activity, Proc. X Int. Conf. EUCARPIA *Medicago spp.* Group.

Peter, L.M. and Mbilu, Z.M. (2009). The efficacy of crude extract of *Aloe secundiflora* on *Candida albicans*. *Traditional, Complimentary and Alternative Medicines* **6,** 592-595.

Rabello, A.L. Dasilva, R.A.P. Rocha, R.S. and Katz, N. (1994). Recent molecular approaches to the study of Schistosome Genetics. *Tropical Medicines and Hygiene* **50,** 748-752.

Rawi, S.M. El-Gindy, H. Haggag, A.M. Abou El Hassan, A. and Abdel Kadel, A. (1995). New possible molluscicides from *Calendula micrantha officinalis* and Ammi majus plants. Physiological effect on *B. alexandrina* and *B. truncatus*. *Egyptian German Society of Zoology* **16,** 69-75.

Ross, A. Vickers, D. Olds, G. (2007). Katayama syndrome. *Lancet* **7,** 18–24.

Rug, M. Sporer, F. Wink, M. Liu, S.Y. Henning, R. and Ruppel, A. (1996) Molluscicidal activity of *Jatropha curcas* seeds against *Schisto-soma mansoni* and *S. japonicum* vector snails. *Deutsche Gesellschaft für Parasitologie* (17th Symposium, München) 27-29 March.

Rug, M. and Ruppel, A. (2000). Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of schistosomes. *Tropical Medicine and International Health*. **5,** 423-430.

Sakran, A.M.A. and Bakry, F.A. (2005). Biological and physiological studies on *Biomphalaria alexandrina* snails exposed to different plant molluscicides. *Egyptian German Society of Zoology* **48,** 237-256.

Schmidt, E. Lötter, M. and McCleland, W. (2002). Trees and shrubs of Mpumalanga and Kruger National Park. Jacana, Johannesburg.

Sen, S. H. P. S. Makkar, S. Muetzel, and K. Becker. (1998b). Effect of *Quillaja saponaria* saponins and *Yucca schidigera* plant extract on growth of *Escherichia coli*. *Microbiology*. **27**:35-38.

Sharaf El-Din, A.T. (2006). Molluscidal effects of three monoterpenes oils on schistosomiasis and fascioliasis vector snails in Egypt. *Egyptian Society of*

Parasitology **36,** 599-612.

Sherif, A. P. and El- Sawy, M. F. (1982). Molluscicidal action of Egyptian herbs laboratory experimentation. *Medical parasitology* **8,** 139-148.

Siddiqui, A.A.and Ali, M.(1997). Practical Pharmaceutical chemistry.1st ed., CBS Publishers and Distributors, New Delhi, pp 126-131.

Sigh, K.Sigh, D.K. and Sigh D.K. (1996). Molluscicidal activity of Neem (*Azadirachta indica*). *Ethnopharmacology* **52,** 35-40.

Smith, J.H.M.D. Elwi, A. and Kamel, I.A. (1975). A quantitative postmoterm analysis of urinary schistosomiasis in Egypt. Evaluation and epidemiology. *Tropical Medicine and Hygiene* **24,** 806-822.

Somia, A. M. (1999). The effect of *Pulicaria crispa*. Oliv.(Compositae) on *Lymnaea caillud*i snails and other aquatic lives. M. Sc. Thesis. Faculty of Education, University of Khartoum, Sudan.

Somia, A. M. (2003). Ovicidal and Molluscicidal Potency of *Pulicaria crispa* (Forssk). Oliv. on *Biomphalaria pfeifferi* egg-masses, juveniles and adults. Ph. D. Thesis. Faculty of Education, University of Khartoum, Sudan.

Steinmann, P. Keiser, J. Bos, R, Tanner, M. Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review,meta-analysis, and estimates of people at risk. *Infectious Diseases* **6**, 411– 425.

Stewart, T. (1998). The Schistosome Miracidium. *Schistosome Life cycle Pages. Cam.University Schistosomiasis Research Group. httpGroup.*http://www.path.cam.ac.uk/~schisto/schistolife/miracidium.html.

Strickland, G.T. and Abdel-Wahab, M. F. (1991). Schistosomiasis. *Tropical Medicine*. Saunders Company.

Tanzubil, P.B. and McCaffery, A.R.(1990). Effects of azadrachtin and aqueous neem seed extracts on survival, growth and development of the African armyworm, *Spodoptera exempta*. Crop Protection, 9: 383- 386.

Thompson, D.P. and Geary, T.G. (1995). The structure and function of helminths new Surfaces. In: Marr, J. J, Editor. Biochemistry band Molecular Biololgy of parasites. 1st York: Academic Press; p.203 - 232.

Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 13th edition.Bailliere Tindall, London, pp 176-180.

Utzinger, J. Xiao, S.H. Keiser, J. Chen, Z.J. and Tanner, M. (2001). Current progress in development and use of artemeter for chemoprophylaxis of human major schistosomal parasites. *Tropical medicine* 8**,** 1841-1860.

Van Wyk, B. Van Wyk, P. and Van Wyk, B.E. (2000. *Photographic guide to trees of southern Africa* . Briza Publications, Pretoria .

Walker R. and Lupien J.R. (2000). "The safety evaluation of monosodium glutamate". *Nutrition* **130** (49–52).

WHO, (1980). Organization of Schistosomiasis Control in the African Region. *WHO/SCHISTO/* 80, 48.

WHO, (1985). "The control of Schistosomiasis," *Techinical Report, series No. 728, Geneva, Switzerland*.

WHO, (1985). The control of schistosomiasis.Second Report of a WHO Expert Committee. *Technical Report, Series 830, Geneva.*

WHO, (1993). "The control of Schistosomiasis," *Techinical Report, Series 830, Geneva,Switzerland*.

WHO, (1994). Statistics Quarterly Report 37: 186.

WHO, (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Technical Report. Serial No: 912 1-57.

WHO, (2013). "Epidemiological Record No: 8 81-88.

Yadav, R.N.S. and Munin, A. (2011). Phytochemical analysis of some medicinal plants . *Journal of Phytology* 3(12) 10-14.

Zhang, Z.M. Lui, X.W. and Qian, C.Y. (1996). 50% wettable powder of niclosamide ethanolamine salt. *Ethnopharmacology* **84,** 40–42.

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Appendix I: Multiple comparisons-Molluscicidal activity

Ethanol extract vs Juvenile snails

Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Distilled water	Niclosamide	-10.00000*	.000	-12.6448	-7.3552
S.luxurians	Niclosamide	-10.00000*	.000	-12.6448	-7.3552
B.micrantha	**Niclosamide**	**-6.50000***	**.073**	**-9.1448**	**-3.8552**
O.americanum	Niclosamide	-10.00000*	.000	-12.6448	-7.3552
A.secundiflora	Niclosamide	-9.75000*	.000	-12.3948	-7.1052
C.megalocarpus	Niclosamide	-10.00000*	.000	-12.6448	-7.3552

Ethanol extract vs adult snails

Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Distilled water	Niclosamide	-10.00000*	.000	-13.2783	-6.7217
S.luxurians	Niclosamide	-9.75000*	.000	-13.0283	-6.4717
B.micrantha	**Niclosamide**	**-6.75000***	**.051**	**-10.0283**	**-3.4717**
O.americanum	Niclosamide	-9.75000*	.000	-13.0283	-6.4717
A.secundiflora	Niclosamide	-9.75000*	.000	-13.0283	-6.4717
C.megalocarpus	Niclosamide	-9.50000*	.000	-12.7783	-6.2217

Appendix II: Multiple Comparisons- Molluscicidal activity

Aqueous extracts vs Juvenile snails

Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Distilled water	Niclosamide	-10.00000*	1.17006	.000	-13.2642	-6.7358
S.luxurians	Niclosamide	-10.00000*	1.17006	.000	-13.2642	-6.7358
B.micrantha	**Niclosamide**	**-6.25000***	**1.17006**	**.062**	**-9.5142**	**-2.9858**
O.americanum	Niclosamide	-9.75000*	1.17006	.000	-13.0142	-6.4858
A.secundiflora	Niclosamide	-10.00000*	1.17006	.000	-13.2642	-6.7358
C.megalocarpus	Niclosamide	-10.00000*	1.17006	.000	-13.2642	-6.7358

Aqueous extract vs adult snails

Dunnett t (2-sided)

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Distilled water	Niclosamide	-10.00000*	1.37148	.000	-13.8260	-6.1740
S.luxurians	Niclosamide	-10.00000*	1.37148	.000	-13.8260	-6.1740
B.micrantha	**Niclosamide**	**-5.75000***	**1.37148**	**.053**	**-9.5760**	**-1.9240**
O.americanum	Niclosamide	-9.75000*	1.37148	.000	-13.5760	-5.9240

A.secundiflora	Niclosamide	-9.75000*	1.37148	.000	-13.5760	-5.9240
C.megalocarpus	Niclosamide	-9.75000*	1.37148	.000	-13.5760	-5.9240

*. The mean difference is significant at the 0.05 level.

Appendix III: Multiple Comparisons -Miracicidal activity

Multiple Comparisons using Dunnet test-Miracicidal activity

Miracicidal activity of the Aqueous extracts of *B.micrantha*

Dunnett t (2-sided)

(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-1.75000	1.82802	**.744**	-6.7356	3.2356
10 mg/l	Niclosamide	-1.00000	1.82802	**.949**	-5.9856	3.9856
20 mg/l	Niclosamide	-.75000	1.82802	**.981**	-5.7356	4.2356
40 mg/l	Niclosamide	.00000	1.82802	**1.000**	-4.9856	4.9856

Miracicidal activity of the Ethanol extracts of *B.micrantha*

Dunnett t (2-sided)

(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-3.75000*	1.19373	.022	-7.0057	-.4943
10 mg/l	Niclosamide	-3.75000*	1.19373	.022	-7.0057	-.4943
20 mg/l	Niclosamide	-.75000	1.19373	**.921**	-4.0057	2.5057
40 mg/l	Niclosamide	-.75000	1.19373	**.921**	-4.0057	2.5057

*. The mean difference is significant at the 0.05 level.

Appendix IV: Multiple Comparisons -Cercaricidal activity

Cercaricidal activity of the Aqueous extracts of
B.micrantha

Dunnett t (2-sided)

(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-3.50000	1.31972	**.058**	-7.0993	.0993
10 mg/l	Niclosamide	-2.25000	1.31972	**.298**	-5.8493	1.3493
20 mg/l	Niclosamide	-4.00000*	1.31972	**.058**	-7.5993	-.4007
40 mg/l	Niclosamide	-2.25000	1.31972	**.298**	-5.8493	1.3493

Cercaricidal activity of the Ethanol extracts of
B.micrantha

Dunnett t (2-sided)

(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-3.75000*	1.17615	.020	-6.9577	-.5423
10 mg/l	Niclosamide	-3.50000*	1.17615	.031	-6.7077	-.2923
20 mg/l	Niclosamide	-3.75000*	1.17615	.020	-6.9577	-.5423
40 mg/l	Niclosamide	-1.75000	1.17615	**.407**	-4.9577	1.4577

Cercaricidal activity of the Aqueous extracts of *B.micrantha*

Dunnett t (2-sided)

(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-3.50000	1.31972	**.058**	-7.0993	.0993
10 mg/l	Niclosamide	-2.25000	1.31972	**.298**	-5.8493	1.3493
20 mg/l	Niclosamide	-4.00000*	1.31972	**.058**	-7.5993	-.4007
40 mg/l	Niclosamide	-2.25000	1.31972	**.298**	-5.8493	1.3493

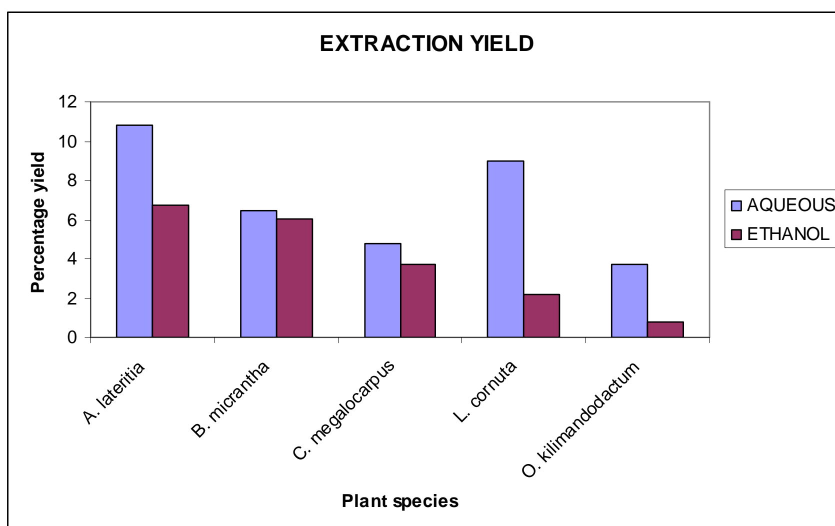
Cercaricidal activity of the Ethanol extracts of *B.micrantha*

Dunnett t (2-sided)

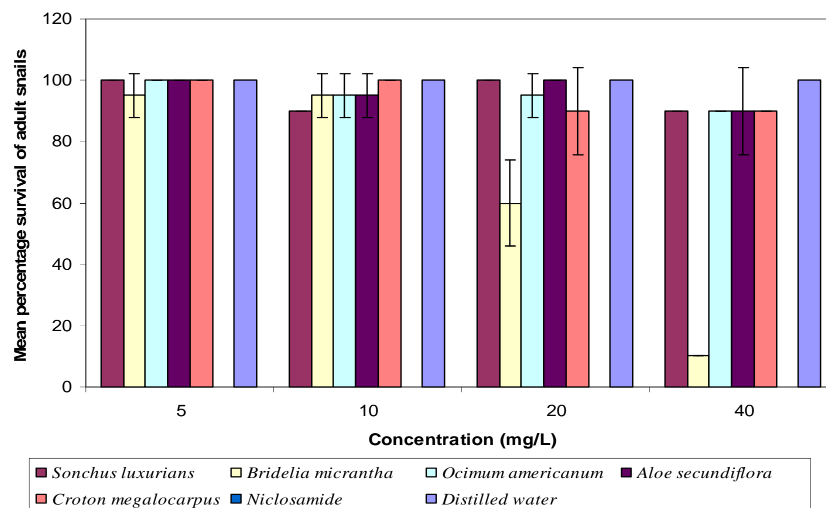
(I) Concentration of *B.micrantha* extracts	(J) Concentration of *B.micrantha* extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5mg/l	Niclosamide	-3.75000*	1.17615	.020	-6.9577	-.5423
10 mg/l	Niclosamide	-3.50000*	1.17615	.031	-6.7077	-.2923
20 mg/l	Niclosamide	-3.75000*	1.17615	.020	-6.9577	-.5423
40 mg/l	Niclosamide	-1.75000	1.17615	**.407**	-4.9577	1.4577

*. The mean difference is significant at the 0.05 level.

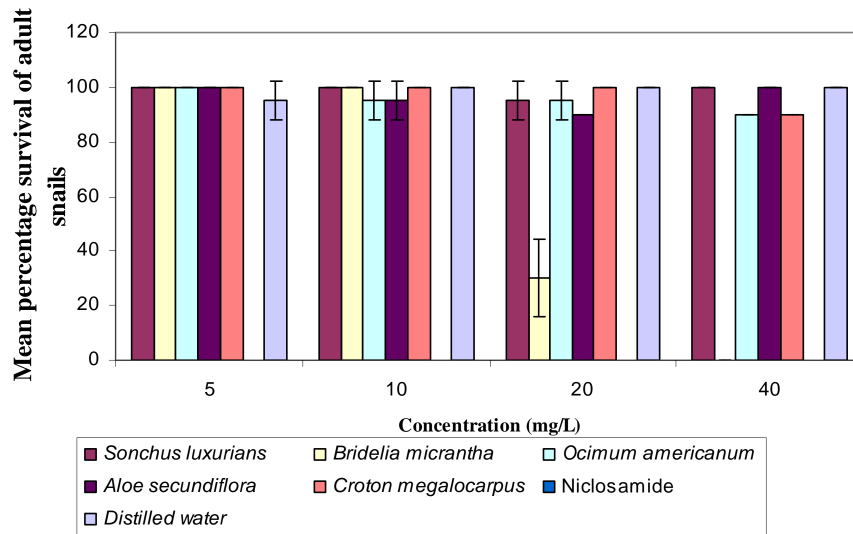
Appendix V: Graph showing yield of the extracts of the five plants.



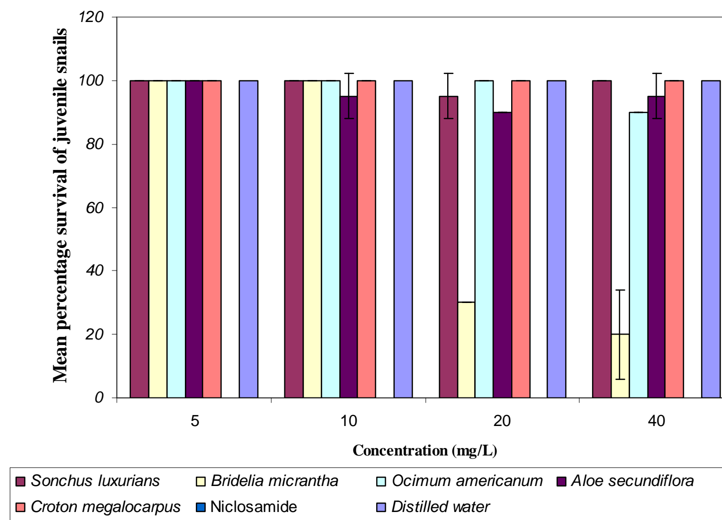
Appendix VI: Graph showing activity of aqueous extracts on adult snails



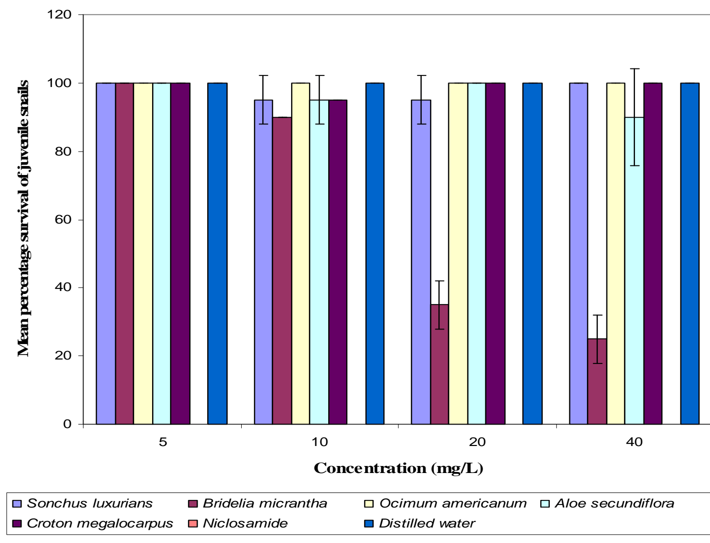
Appendix VII: Graph showing activity of ethanol extracts on adult snails



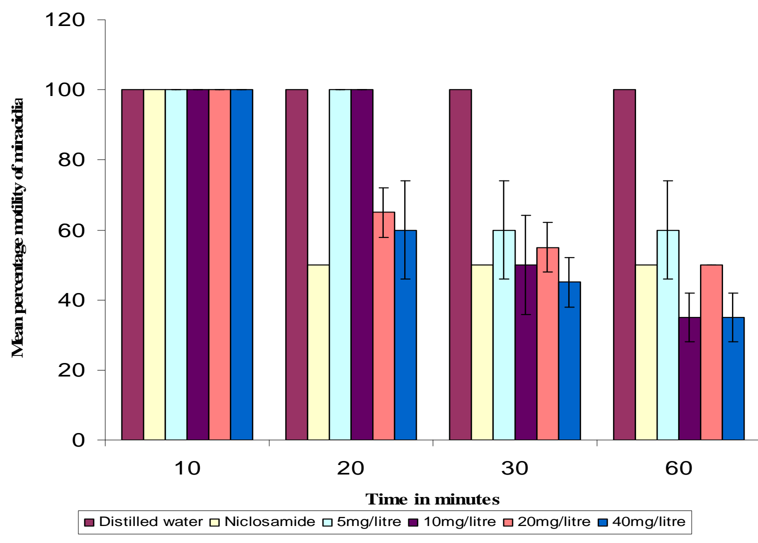
Appendix VIII: Graph showing activity of aqueous extracts on juvenile snails



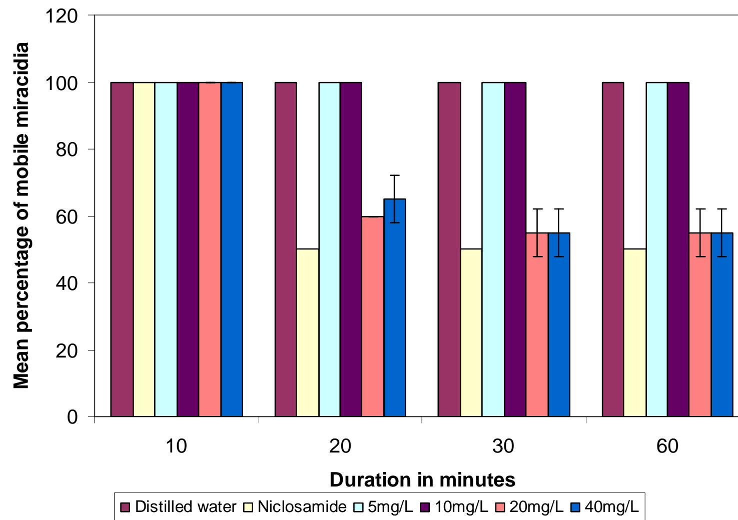
Appendix IX: Graph showing activity of ethanol extracts on juvenile snails



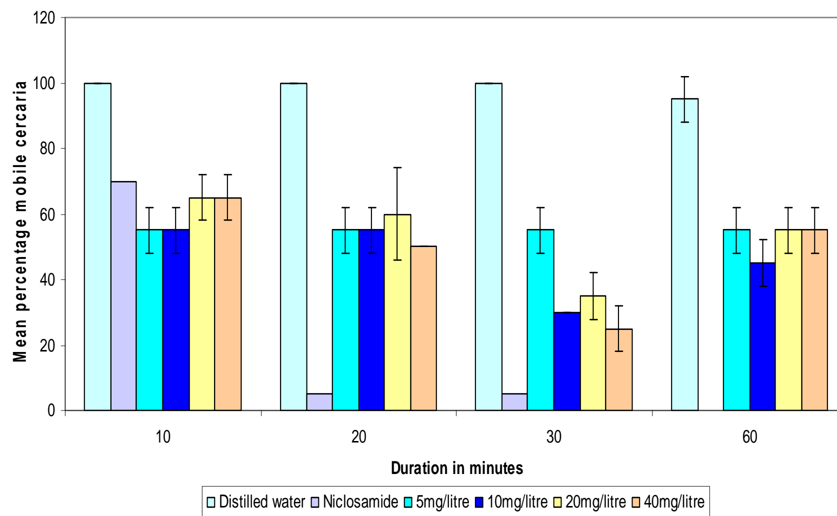
Appendix X: Graph showing miracidal activity of aqueous extract of *Bridelia Micrantha*



Appendix XI: Graph showing miracidal activity of ethanol extract of *Bridelia micrantha*



Appendix XII: Graph showing cercaricidal activity of aqueous extract of *Bridelia micrantha*



AppendixXIII: Graph showing cercaricidal activity of ethanol extract of *Bridelia micrantha*