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 molluscidal, miracidal 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 schistosomiasis. 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 *Schistosoma 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 schistosomiasis. 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

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

1.1.3.1: Acute Schistosomiasis

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 splenomegaly 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

Schistosomiasis 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*. It 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 caecal 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).
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 cercariae 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 disease (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: Vaccination

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 molluscidal 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 molluscicides 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 molluscidal concentrations.
- Locally available, with high yield of molluscidal 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 (*Phytolacca 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 *Tetrapleura tetraptera* (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 schistosomiasis. 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 molluscicidal 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 100 ppm (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 effective 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 caulludi* 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 µg mL⁻¹ and showed LD₅₀ of 143 µg 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. The leaves 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, miracidal 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 subtropical 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 exhibited 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).

![Croton megalocarpus tree](image)

**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)

![Fruiting stalk of Bridelia micrantha](image)

**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).

![Aloe secundiflora plant](image)

**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 maintenance**

*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°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 miracidicidal activity.

New miracidia were hatched from eggs from faecal samples of baboons with *Schistosoma mansoni* chronic infection. The faecal 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*...
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 \leq 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

<table>
<thead>
<tr>
<th>Part of the plant used</th>
<th>Plant</th>
<th>Yield in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Distilled water</td>
</tr>
<tr>
<td>Bark</td>
<td><em>Bridelia micrantha</em></td>
<td>10.81</td>
</tr>
<tr>
<td></td>
<td><em>Croton megalocarpus</em></td>
<td>6.44</td>
</tr>
<tr>
<td>Whole plant</td>
<td><em>Aloe secundiflora</em></td>
<td>4.78</td>
</tr>
<tr>
<td></td>
<td><em>Sonchus luxurians</em></td>
<td>8.95</td>
</tr>
<tr>
<td></td>
<td><em>Ocimum americanum</em></td>
<td>3.75</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>0E</th>
<th>0A</th>
<th>BE</th>
<th>BA</th>
<th>CE</th>
<th>CA</th>
<th>AE</th>
<th>AA</th>
<th>SE</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sterols/Triterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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 ($F_{6, 21} = 31.636; p < 0.05$). Dunnett 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 respectively. The other comparison of the other ethanolic 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: Miracidal activity

The various concentrations of the aqueous and ethanol extracts of *B. micrantha* had significantly different miraculous activity within 60 minutes (*F*<sub>4, 15</sub> = 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 miraculous 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*<sub>4, 15</sub> = 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<sub>50</sub> Determination

LD<sub>50</sub> 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 logarithms. Plotting the logarithms 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.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. luxurians</em></td>
<td>100.94 ± 20.63</td>
<td>1422.69±329.90</td>
</tr>
<tr>
<td><em>B. micrantha</em></td>
<td>24.98 ± 3.35</td>
<td>19.01 ± 3.61</td>
</tr>
<tr>
<td><em>O. americanum</em></td>
<td>100.94 ± 20.63</td>
<td>100.93 ± 20.67</td>
</tr>
<tr>
<td><em>A. secundiflora</em></td>
<td>100.94 ± 20.63</td>
<td>106.83 ± 17.82</td>
</tr>
<tr>
<td><em>C. megalocarpus</em></td>
<td>106.83 ± 17.83</td>
<td>100.93 ± 20.67</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>6.37 ± 2.72</td>
<td>6.37 ± 2.72</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1422.69±329.90</td>
<td>1422.69±329.90</td>
</tr>
</tbody>
</table>
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**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. luxurians</em></td>
<td>1422.69±329.90</td>
<td>1422.69±329.90</td>
</tr>
<tr>
<td><em>B. micrantha</em></td>
<td>22.86±4.69</td>
<td>26.30±4.58</td>
</tr>
<tr>
<td><em>O. americanum</em></td>
<td>100.93±20.67</td>
<td>1422.69±329.90</td>
</tr>
<tr>
<td><em>A. secundiflora</em></td>
<td>1422.69±329.90</td>
<td>100.93±20.67</td>
</tr>
<tr>
<td><em>C. megalocarpus</em></td>
<td>1422.69±329.90</td>
<td>1422.69±329.90</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>6.37±2.72</td>
<td>6.37±2.72</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1422.69±329.90</td>
<td>1422.69±329.90</td>
</tr>
</tbody>
</table>
3.6.3: LD<sub>50</sub> Determination for Miracidical and Cercaricidal activity

*Br sidelines micrantha* aqueous extract had a LD<sub>50</sub> value of 11.108 mg/l on the *S. mansoni* miracidia and a LD<sub>50</sub> value of 4.465 mg/l on the cercariae. The ethanol extract was found to have a LD<sub>50</sub> value of above 40 mg/l for the miracidia and the cercariae. The results are shown on Table 5.

**Table 5**: LD<sub>50</sub> values of aqueous and ethanol extracts of *B. micrantha* on *Schistosoma mansoni* miracidia and cercariae

<table>
<thead>
<tr>
<th>Extract</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; value (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Miracidia</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>11.108</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>67.011</td>
</tr>
</tbody>
</table>
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, \textit{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, \textit{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 \textit{Sonchus luxurians}, \textit{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 soluble 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 respectively. 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-potassium and calcium-magnesium ATPase activity or insertion of the hydrophobic saponin nucleus into the lipid bilayer. Others have the ability to lower serum cholesterol and 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 research 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: Miracidal 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 miracidal 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 20 mg/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 miracidal 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<sub>50</sub> Determination**

LD<sub>50</sub> is the dose required to kill half the members of a tested population after specified test duration. LD<sub>50</sub> of the extracts was determined to measure the dose that killed or immobilised 50% of the target organisms within the treatment period. LD<sub>50</sub> figures are frequently used as a general indicator of a substance's acute toxicity (Fleming & Hunt, 2000). A large LD<sub>50</sub> means it takes a large quantity of the material to cause a toxic response (Walker & Lupien, 2000). LD<sub>50</sub> < 100 mg/l indicates that the the substance is highly toxic, LD<sub>50</sub> > 100 < 500 mg/l indicates that the the substance is moderately toxic, LD<sub>50</sub> > 500 < 1000 mg/l indicates that the the substance is weakly toxic while LD<sub>50</sub> > 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<sub>50</sub><100mg/l while *S. luxurians*,
*O. americanum*, *A. secundiflora* and *C. megalocarpus* aqueous extracts were moderately toxic; LD$_{50}$>100<500 mg/l. *B. micrantha* ethanol extract was highly toxic; LD$_{50}$<100 mg/l, *O. americanum*, *A. secundiflora* and *C. megalocarpus* were moderately toxic; LD$_{50}$>100<500 mg/l while *S. luxurians* was non toxic on the adult snails; LD$_{50}$>1000 mg/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$_{50}$<100 mg/l, *O. americanum* was moderately toxic; LD$_{50}$>100<500 mg/l while *S. luxurians*, *A. secundiflora*, and *C. megalocarpus* were non toxic on juvenile snails; LD$_{50}$>1000 mg/l. For the ethanol extracts, *B. micrantha* was highly toxic; LD$_{50}$<100 mg/l, *A. secundiflora* was moderately toxic; LD$_{50}$>100<500 mg/l, *S. luxurians*, *O. americanum*, and *C. megalocarpus*, were non toxic on juvenile snails; LD$_{50}$>1000 mg/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 extractor. 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, miracical 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, miracical 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


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 molluscidal activity against the schistosome vector snails Oncomelania, Biomphalaria and Bulinus. Tropical Medicine and International Health 2, 179-188.


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Appendix I: Multiple comparisons-Molluscicidal activity

Ethanol extract vs Juvenile snails

Dunnett t (2-sided)

<table>
<thead>
<tr>
<th>(I) Treatments</th>
<th>(J) Treatments</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
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<td><strong>-10.0000</strong></td>
<td>.000</td>
<td><strong>-12.6448</strong></td>
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<tr>
<td>Distilled water</td>
<td>Niclosamide</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S.luxurians</td>
<td>Niclosamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.micrantha</td>
<td>Niclosamide</td>
<td><strong>-6.5000</strong></td>
<td>.073</td>
<td><strong>-9.1448</strong></td>
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<tr>
<td>O.amERICANUM</td>
<td>Niclosamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.secundiflora</td>
<td>Niclosamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.megalocarpus</td>
<td>Niclosamide</td>
<td></td>
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</tr>
</tbody>
</table>

Ethanol extract vs adult snails

Dunnett t (2-sided)

<table>
<thead>
<tr>
<th>(I) Treatments</th>
<th>(J) Treatments</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
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<td><strong>-13.0283</strong></td>
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</tr>
<tr>
<td>A.secundiflora</td>
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<td>.000</td>
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<td>.000</td>
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</table>
Appendix II: Multiple Comparisons- Molluscicidal activity

Aqueous extracts vs Juvenile snails

Dunnett t (2-sided)

<table>
<thead>
<tr>
<th>(I) Treatments</th>
<th>(J) Treatment</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Niclosamide</td>
<td>10.00000*</td>
<td>1.17006</td>
<td>.000</td>
<td>-13.2642 - 6.7358</td>
</tr>
<tr>
<td><em>S. luxurians</em></td>
<td>Niclosamide</td>
<td>10.00000*</td>
<td>1.17006</td>
<td>.000</td>
<td>-13.2642 - 6.7358</td>
</tr>
<tr>
<td><em>B. micrantha</em></td>
<td>Niclosamide</td>
<td>-6.25000*</td>
<td>1.17006</td>
<td>.062</td>
<td>-9.5142 - 2.9858</td>
</tr>
<tr>
<td><em>O. americanum</em></td>
<td>Niclosamide</td>
<td>-9.75000*</td>
<td>1.17006</td>
<td>.000</td>
<td>-13.0142 - 6.4858</td>
</tr>
<tr>
<td><em>A. secundiflora</em></td>
<td>Niclosamide</td>
<td>-5.75000*</td>
<td>1.17006</td>
<td>.000</td>
<td>-9.5142 - 2.9858</td>
</tr>
<tr>
<td><em>C. megalocarpus</em></td>
<td>Niclosamide</td>
<td>-9.75000*</td>
<td>1.17006</td>
<td>.000</td>
<td>-13.0142 - 6.4858</td>
</tr>
</tbody>
</table>

Aqueous extract vs adult snails

Dunnett t (2-sided)

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<tr>
<th>(I) Treatments</th>
<th>(J) Treatment</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>Distilled water</td>
<td>Niclosamide</td>
<td>10.00000*</td>
<td>1.37148</td>
<td>.000</td>
<td>-13.8260 - 6.1740</td>
</tr>
<tr>
<td><em>S. luxurians</em></td>
<td>Niclosamide</td>
<td>10.00000*</td>
<td>1.37148</td>
<td>.000</td>
<td>-13.8260 - 6.1740</td>
</tr>
<tr>
<td><em>B. micrantha</em></td>
<td>Niclosamide</td>
<td>-5.75000*</td>
<td>1.37148</td>
<td>.053</td>
<td>-9.5760 - 1.9240</td>
</tr>
<tr>
<td><em>O. americanum</em></td>
<td>Niclosamide</td>
<td>-9.75000*</td>
<td>1.37148</td>
<td>.000</td>
<td>-13.5760 - 5.9240</td>
</tr>
<tr>
<td></td>
<td>Niclosamide</td>
<td></td>
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<td></td>
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<td>-------------</td>
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<tr>
<td><em>A. secundiflora</em></td>
<td>-9.75000</td>
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<td>.000</td>
<td>-13.5760</td>
<td>-5.9240</td>
</tr>
<tr>
<td><em>C. megalocarpus</em></td>
<td>-9.75000</td>
<td>1.37148</td>
<td>.000</td>
<td>-13.5760</td>
<td>-5.9240</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
Appendix III: Multiple Comparisons -Miracidal activity

Multiple Comparisons using Dunnet test-Miracidal activity

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

Dunnett t (2-sided)

<table>
<thead>
<tr>
<th>(I) Concentration of <em>B. micrantha</em> extracts</th>
<th>(J) Concentration of <em>B. micrantha</em> extracts</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound     Upper Bound</td>
</tr>
<tr>
<td>5mg/l Niclosamide</td>
<td></td>
<td>-1.75000</td>
<td>1.82802</td>
<td>.744</td>
<td>-6.7356     3.2356</td>
</tr>
<tr>
<td>10 mg/l Niclosamide</td>
<td></td>
<td>-1.00000</td>
<td>1.82802</td>
<td>.949</td>
<td>-5.9856     3.9856</td>
</tr>
<tr>
<td>20 mg/l Niclosamide</td>
<td></td>
<td>-0.75000</td>
<td>1.82802</td>
<td>.981</td>
<td>-5.7356     4.2356</td>
</tr>
<tr>
<td>40 mg/l Niclosamide</td>
<td></td>
<td>0.00000</td>
<td>1.82802</td>
<td>1.000</td>
<td>-4.9856     4.9856</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

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

Dunnett t (2-sided)

<table>
<thead>
<tr>
<th>(I) Concentration of <em>B. micrantha</em> extracts</th>
<th>(J) Concentration of <em>B. micrantha</em> extracts</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound     Upper Bound</td>
</tr>
<tr>
<td>5mg/l Niclosamide</td>
<td></td>
<td>-3.75000</td>
<td>1.19373</td>
<td>.022</td>
<td>-7.0057     -.4943</td>
</tr>
<tr>
<td>10 mg/l Niclosamide</td>
<td></td>
<td>-3.75000</td>
<td>1.19373</td>
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</tr>
<tr>
<td>20 mg/l Niclosamide</td>
<td></td>
<td>-0.75000</td>
<td>1.19373</td>
<td>.921</td>
<td>-4.0057     2.5057</td>
</tr>
<tr>
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<td></td>
<td>-0.75000</td>
<td>1.19373</td>
<td>.921</td>
<td>-4.0057     2.5057</td>
</tr>
</tbody>
</table>

*. 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)

<table>
<thead>
<tr>
<th>(I) Concentration of <em>B. micrantha</em> extracts</th>
<th>(J) Concentration of <em>B. micrantha</em> extracts</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mg/l Niclosamide</td>
<td></td>
<td>-3.50000</td>
<td>1.31972</td>
<td>.058</td>
<td>7.0993 - .0993</td>
</tr>
<tr>
<td>10 mg/l Niclosamide</td>
<td></td>
<td>-2.25000</td>
<td>1.31972</td>
<td>.298</td>
<td>5.8493 - 1.3493</td>
</tr>
<tr>
<td>20 mg/l Niclosamide</td>
<td></td>
<td>-4.00000*</td>
<td>1.31972</td>
<td>.058</td>
<td>7.5993 - .4007</td>
</tr>
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<td>40 mg/l Niclosamide</td>
<td></td>
<td>-2.25000</td>
<td>1.31972</td>
<td>.298</td>
<td>5.8493 - 1.3493</td>
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</table>

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

### Dunnett t (2-sided)

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<th>(J) Concentration of <em>B. micrantha</em> extracts</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mg/l Niclosamide</td>
<td></td>
<td>-3.75000*</td>
<td>1.17615</td>
<td>.020</td>
<td>6.9577 - .5423</td>
</tr>
<tr>
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<td></td>
<td>-3.50000*</td>
<td>1.17615</td>
<td>.031</td>
<td>6.7077 - .2923</td>
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<tr>
<td>20 mg/l Niclosamide</td>
<td></td>
<td>-3.75000*</td>
<td>1.17615</td>
<td>.020</td>
<td>6.9577 - .5423</td>
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<tr>
<td>40 mg/l Niclosamide</td>
<td></td>
<td>-1.75000</td>
<td>1.17615</td>
<td>.407</td>
<td>4.9577 - 1.4577</td>
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Cercaricidal activity of the Aqueous extracts of *B. micrantha*

Dunnett t (2-sided)

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<tr>
<th>Concentration of <em>B. micrantha</em> extracts</th>
<th>Concentration of <em>B. micrantha</em> extracts</th>
<th>Mean Difference (I-J)</th>
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<tr>
<td>5 mg/l Niclosamide</td>
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Cercaricidal activity of the Ethanol extracts of *B. micrantha*

Dunnett t (2-sided)

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<th>Concentration of <em>B. micrantha</em> extracts</th>
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<th>Std. Error</th>
<th>Sig.</th>
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<tr>
<td>5 mg/l Niclosamide</td>
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<td>-3.75000*</td>
<td>1.17615</td>
<td>.020</td>
<td>-6.9577 - -1.5423</td>
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<td>10 mg/l Niclosamide</td>
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<td>4.9577 - 1.4577</td>
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</tbody>
</table>

* 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 miracidial activity of aqueous extract of *Bridelia Micrantha*
Appendix XI: Graph showing miracidial activity of ethanol extract of *Bridelia micrantha*

![Graph showing miracidial activity of *Bridelia micrantha*](image1)

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

![Graph showing cercaricidal activity of *Bridelia micrantha*](image2)
Appendix XIII: Graph showing cercaricidal activity of ethanol extract of *Bridelia micrantha*