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MOLLUSCICIDAL AND CERCARIACIDAL ACTIVITIES OF *BALANITES AEGYPTIACA*

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

(Biomedical Science)

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List of Abbreviations and Acronyms

AAU	Addis Ababa University
ALIPB	Aklilu Lemma Institute of Pathobiology
ANOVA	Analysis of variance
CDC	Center for Disease Control
CI	Confidence interval
Epg	Eggs per gram
HF	Human fascioliasis
LC_{50}	Concentration of testing material that produces 50% death of tested snails
LC_{90}	Concentration of testing material that produces 90% death of tested snails
ppm	Parts per million
SPSS	Statistical package for social sciences
TCZ	Triclabendazole
WHO	World Health Organization

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ABSTRACT

Studies on molluscicidal activity of plants have drawn increasing attention due to such issues as environmental preservation, high cost, and concern over emerging of resistance in snails to synthetic molluscicides. In line with this the present study evaluated the molluscicidal activity of aqueous extracts of *Balanites aegyptiaca* seeds and fruits against adult *Biomphalaria pfeifferi* and *Lymnaea natalensis* snails as well as the cercariacidal activity against *Schistosoma mansoni*. Snail mortalities were compared between each plant part and snail species, and LC_{50} and LC_{90} values for the plant parts tested were computed. For the molluscicidal activities of seed, endocarp, mesocarp and whole fruit part, the LC_{50} values against *B. pfeifferi* were 56.32, 77.53, 65.51 and 66.63 ppm, respectively, while the respective LC_{90} values were 77.70, 120.04, 89.50 and 97.55 ppm. Similarly, the LC_{50} values for the seed, endocarp, mesocarp and whole fruit against *L. natalensis* were 80.33, 92.61, 83.52 and 87.84 ppm, respectively, while the respective LC_{90} values were 102.30, 138.21, 115.42 and 127.69 ppm. *B. pfeifferi* were found to be more susceptible to *B. aegyptiaca* than *L. natalensis*. As regards the cercariacidal activities of the plant, the *in vitro* mortality of cercariae exposed to aqueous extract of seeds increased with increasing concentration and exposure time. Infectivity evaluation test showed that *S. mansoni* cercariae exposed to 15 ppm of aqueous extract of seeds were incapable of infecting mice. In particular, the mean egg load of tissue was reduced in mice infected with the cercariae exposed to 5 and 10 ppm (ANOVA, $p<0.05$). In conclusion, the aqueous extracts of *B. aegyptiaca* have reasonable molluscicidal and cercariacidal activities. Yet, comprehensive laboratory evaluation of this plant is required before its field use.

Key words: *Balanites aegyptiaca, Biomphalaria pfeifferi, Lymnaea natalensis, aqueous extract, molluscicidal, cercariacidal, schistosomiasis, fascioliasis*

1. INTRODUCTION

Schistosomiasis, a parasitic disease transmitted by snail intermediate hosts, is one of the most widespread human health problems in the tropics (Yadav and Singh, 2007). It is a chronic and debilitating disease that affects people who have contact with water harboring infected snails. The disease affects millions of people in several countries and is one of the world's main public health problems (Augusto A dos Santos *et al*., 2003).

Fascioliasis, also variously referred to as fasciolosis, distomatosis or liver rot, is a worldwide problem caused by the liver fluke (Hussein *et al*., 2010). It is a serious infectious parasitic disease infecting domestic ruminants (Soliman, 2008). Fascioliasis may occasionally affect man. The public health importance of human fascioliasis (HF) has, however, increased in recent years (Haseeb *et al*., 2002).

The five most common species of schistosome infecting humans are *Schistosoma mansoni*, *S. japonicum*, *S. haematobium*, S. *mekongi*, and *S. intercalatum* (McManus and Loukas, 2008). Other species of *Schistosoma* which have common veterinary importance include *Schistosoma mattheei*, *S. bovis*, *S. sprinadalis* and *S. rodhaini* (Okpala *et al*., 2004). While fascioliasis, is caused by a liver fluke belonging to genus *Fasciola*. The common causative agents of fascioliasis (liver fluke disease) are *Fasciola hepatica* and *F. gigantica* (Soliman, 2008).

The snails of the genera *Biomphalaria, Bulinus and Oncomelania* serve as intermediate hosts of schistosomes and play a crucial role in the transmission of the disease (Abadi, 2010). Where there are snail hosts, a single patient discharging viable eggs would suffice to establish a new infection site (Vinaud *et al.,* 2008). Species of the freshwater snail Lymnaidae family are well known for their role as intermediate hosts in the life cycle of fascioliasis. The common species of *Lymnaea* as intermediate host for fascioliasis are *Lymnaea natalensis*, *L. columella*, *L. acuminata*, *L. tomentosa*, *L. truncatula*, *L. auricularia* and *L. bulimoides* (Soliman, 2008).

Treatment of schistosomiasis relies heavily on praziquantel (Chitsulo *et al*., 2000). The problem in the use of chemotherapeutic agents is the common occurrence of re-infection after treatment due to the resistance of the strains of schistosomes to schistosomicide drugs. In addition, praziquantel was reported to induce hemorrhage in the lung, abdominal pain and diarrhea (Kabatereine *et al*., 2003).

The drug of choice to treat human cases of fascioliasis is now triclabendazole, a benzimidazole compound, which has been used in veterinary practice since 1983 to treat the disease (Savioli *et al*., 2002). These drugs are costly and may lead not only to anthelmintic resistance, but also to undesirable residues in food or the environment (Mahmoud *et al*., 2010).

Many plant species have been used throughout the world in traditional medicine for the treatment of both veterinary and human helminth infections and evaluated for their molluscicidal activity against snail intermediate hosts (Al-Sharkawi *et al*., 2007). The high cost of imported synthetic compounds, the negative impacts of synthetic molluscicides on the environment and increasing snail resistance to these compounds have given a new impetus to the study of molluscicidal plants (Sharma *et al*., 2009; EL-Kamali *et al*., 2010; Augusto A dos Santos *et al*., 2003; Changbunjong *et al*., 2010).

In this regard the plant *Balanites aegyptiaca* (L.) Del. (family Balanitaceae), has been reported to possess molluscicidal and cercariacidal activities. Its fruit, bark and other parts have properties lethal to snail intermediate hosts, schistosome miracidia and cercariae, and the cercariae of other trematodes (Koko *et al*., 2000; Archibald, 2004; Yadav and Panghal, 2010; Chothani and Vaghasiya, 2011). Nevertheless, the molluscicidal and cercariacidal properties of the plant have not been studied in Ethiopia. The purpose of the present study, therefore, is to evaluate the molluscicidal and cercariacidal activity of aqueous extracts of *B. aegyptiaca* seed and fruit in Ethiopia.

1.1. The Biology of Trematodes and Snail Intermediate Hosts

1.1.1. The Schistosomes

Adult schistosomes share all the fundamental features of the digenea. They have a basic bilateral symmetry, oral and ventral suckers, a body covering of a syncytial tegument, a blind-ending digestive system consisting of mouth, oesophagus and bifurcated caeca. Schistosomes exhibit sexual dimorphism and have distinct separate sexes. Adult worms are about 1.2 cm in length, live in the blood vessels around the intestine or bladder. The mature male worm claps the female in a special gynaecophoric canal. The female worm after copulation is set free to lay its eggs. Each mature female produces about two hundred ova per day (Rollinson and Johnson, 1996).

Schistosomes have a typical trematode vertebrate-invertebrate lifecycle, with humans being the definitive host. The life cycle of *Schistosoma mansoni* provides an example for all species of schistosomes. After the eggs of the human-dwelling parasites are emitted in the feces (or urine in the case of *S. haematobium*) into the water the ripe miracidium hatches out of the egg. The miracidium searches for a suitable fresh water snail to act as an intermediate host and penetrates it. Following this, the parasite develops via a so-called mother-sporocyst and daughter-sporocyst generation to the cercaria. From a single miracidium results of a few thousand cercaria, every one of which is capable of infecting man. The cercariae propel themselves in water with the aid of their bifurcated tail and actively seek out their final host. When they recognize human skin, they penetrate it within a very short time (Hamed, 2010). Following migration through the body within the bloodstream, if they meet a partner of the opposite sex, they develop into sexually mature adults, laying eggs and complete its life cycle (Figure 1) (Ross *et al*., 2002; Hamed, 2010).

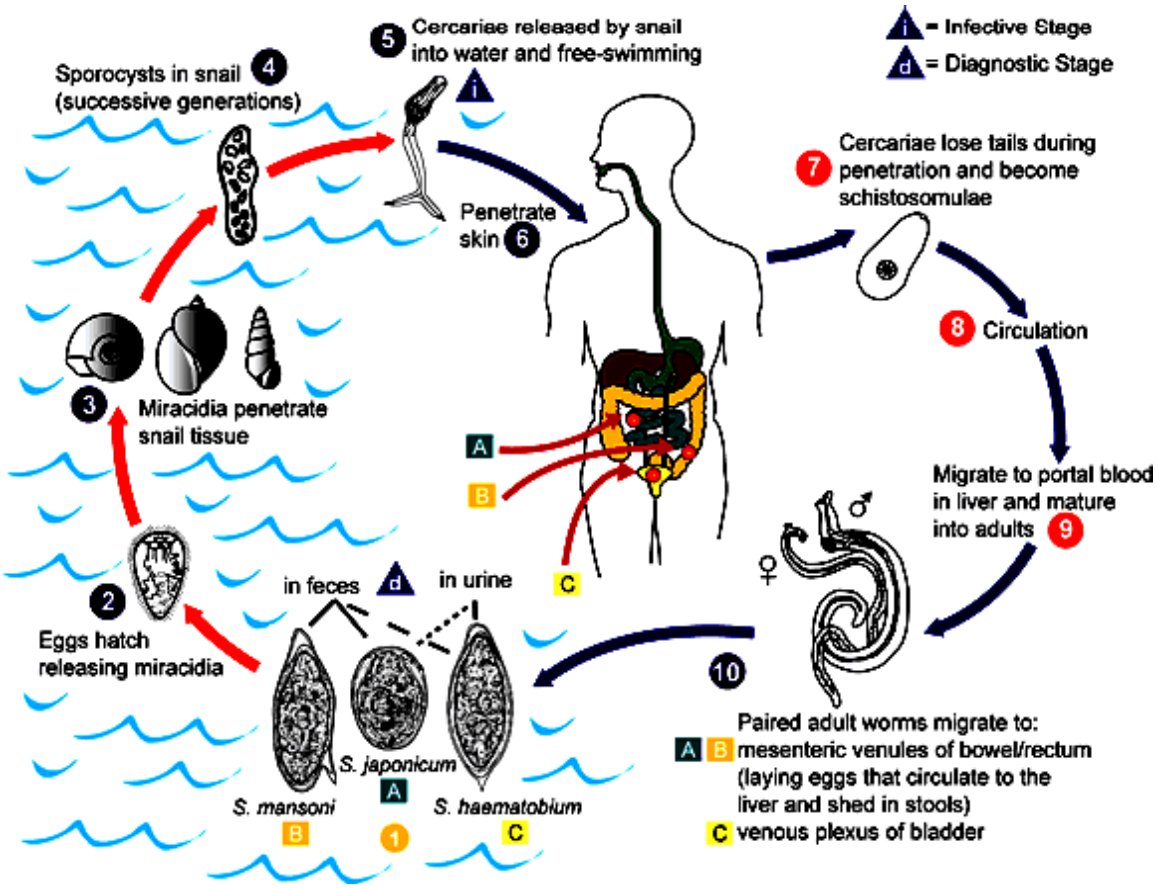


Figure 1: The life cycle of human schistosomes

(Source: CDC, 2003)

1.1.2. The *Fasciola*

The adult *Fasciola* worms have a flat leaf-like body, typical of flukes, and measures 20 to 75 mm long by 8 to 15 mm wide. They have an anterior elongation (a cephalic cone) on which the oral and ventral suckers, which are approximately of equal size, are located (Soulsby, 1982). In order to complete its life cycle, *Fasciola* require a freshwater snail as an intermediate host. The life cycle of *Fasciola* spp. is a typical of digenetic trematodes. Infection with *Fasciola* spp. occurs when metacercariae are accidentally ingested on raw vegetation (Figure 2) (Soliman, 2008).

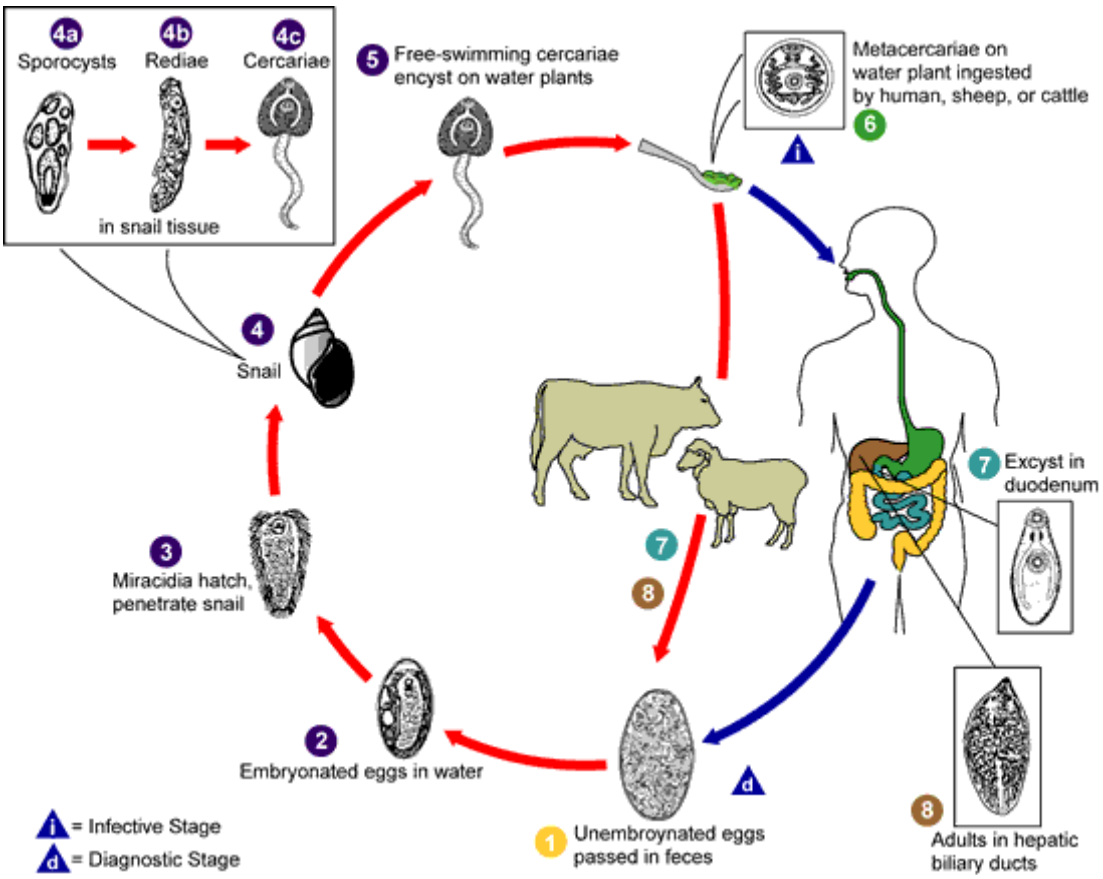


Figure 2: The life cycle of fascioliasis

(Source: CDC, 2004)

1.1.3. The Snail Intermediate Hosts

Some 350 snail species are estimated to be of possible medical or veterinary importance. Most intermediate hosts of human *Schistosoma* parasites belong to three genera, *Biomphalaria, Bulinus* and *Oncomelania*. The snails can be divided into two main groups: aquatic snails that live under water and cannot usually survive elsewhere (*Biomphalaria, Bulinus*), and amphibious snails adapted for living in and out of water (*Oncomelania*). A large population of snails inhabits freshwaters, where the larvae of parasitic trematodes also pass part of their life (Singh *et al.,* 2004). In Africa and the Americas, snails of the genus *Biomphalaria* serve as intermediate hosts of *S. mansoni*. Snails of the genus *Bulinus* serve as

the intermediate hosts of *S. haematobium* in Africa and the Eastern Mediterranean, as well as of *S. intercalatum* in Africa. In south-east Asia, *Oncomelania* serves as the intermediate host of *S. japonicum*, and *Tricula* as the intermediate host of *S. mekongi* (WHO, 1984).

Among the snail intermediate hosts of trematodes, the species belonging to the genus *Lymnaea* are of importance in the transmission of liver flukes. Common *Lymnaea* species, most important in the transmission of *F. hepatica,* include *L. truncatula,* widespread in Europe, Asia, Africa and North America; *L. bulimoides* in North America and *L. columella* in USA and Australia. The most important intermediate hosts of *F. gigantica* are *L. natalensis* and *L. auricularia. Lymnaea* species may be either aquatic or amphibious. *L. natalensis* is a strictly aquatic snail often found in Africa (Soulsby, 1982).

1.2. Epidemiology of Schistosomiasis and Fascioliasis

1.2.1. Schistosomiasis

Schistosomiasis remains one of the most prevalent parasitic infections and has significant economic and public health consequences (Chitsulo *et al*., 2000). The disease is widespread in various parts of the world, especially in the most parts of Africa, South America and Asia (Al-Sharkawi *et al*., 2007). The infection is second only to malaria as a cause of human morbidity and mortality (Singh *et al*., 2010). Data indicate that schistosomiasis affects between 200 and 300 million people in around 74 countries. Over 600 million people are reported to be at risk of this trematode disease (Ibrahim, 2009). The great majority (80-85%) of schistosomiasis is found in sub-Saharan Africa. The disease is more prevalent in Africa, perhaps due to low socio-economy or favorable climate for transmission of the disease (Liang *et al*., 2007).

In Ethiopia, an estimated number of 29.89 million people are at risk of acquiring schistosomiasis and of these 4 million are infected (Chitsulo *et al*., 2000). Intestinal schistosomiasis, caused by *Schistosoma mansoni*, and the urinary form caused by *S. haematobium* are endemic in Ethiopia with a wider distribution of the former than the latter. The construction of dams and expansion of

irrigation based agriculture schemes and population movements, have increased the incidence of schistosomiasis in Ethiopia (Woldemichael *et al*., 2006).

Schistosomiasis transmission takes place where the ecologies of the schistosome parasites, the aquatic snail intermediate hosts, and the human definitive hosts meet in space and time in surface waters. Both abiotic factors (temperature, rainfall, and water current velocity) and biotic factors (host sex and age) contribute to the schistosomiasis transmission cycle and these determine the spatial distribution of the disease (Zhou *et al*., 2008).

1.2.2. Fascioliasis

Fascioliasis has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. It may occasionally affect man. Human infection with fascioliasis was very sporadic until the last three decades where clinical cases and outbreaks were reported. However, the increasing importance of human fascioliasis worldwide has re-launched interest in fascioliasis. Studies have shown that human fascioliasis presents marked transmission patterns in different endemic areas and several geographical areas have been described as endemic for the disease in humans (Mas-Coma *et al*., 2009).

Fascioliasis is increasingly recognized as causing significant human disease, with 2.4 million people infected. Prevalence is highest in areas where extensive sheep and cattle raising occurs and where dietary practices include the consumption of raw aquatic vegetables (Soliman, 2008). Fascioliasis is endemic in five continents and 61 countries and has become trematode infection of public health importance in parts of the world such as the Andean Highlands of Bolivia, Ecuador and Peru, the Nile Delta of Egypt, Turkey and Northern Iran. Over 180 million people are reported to be at risk of these trematode infections throughout the world (Haseeb *et al*., 2002).

The largest numbers of infected people have been reported from Bolivia, China, Ecuador, Egypt, France, Islamic Republic of Iran, Peru and Portugal. Disease prevalence is particularly high in

specific regions of Bolivia (65-92%), Ecuador (24-53%), Egypt (2-17%), and Peru (10%) (Soliman, 2008).

The presence of fascioliasis due to *Fasciola hepatica* and *F. gigantica* in Ethiopia has long been known (Yilma and Mesfin, 2000). It is mainly an animal disease, causing a great economic burden particularly in the highland areas of the country. There are only a few reported cases of the disease in humans (Bayu *et al*., 2005). It was widespread, particularly in the north and the west of the Great Rift Valley. The prevalence and distribution of the disease varies from 11% in the Rift Valley to 100% in the central highlands of Ethiopia (Ahmed *et al*., 2007).

1.3. Control of Schistosomiasis and Fascioliasis

Elimination of schistosomiasis has been possible in certain areas or even countries and has already been achieved in some, but there are no agreed criteria for determining elimination of the disease. Elimination will require a combination of methods, including chemotherapy, molluscicide, health education, environmental management, provision of safe water and adequate sanitation (WHO, 2009).

Schistosomiasis control through molluscicide is the most important approach in complementing chemotherapy. At present niclosamide is the only molluscicide applied on a large scale. Since this synthetic molluscicide is also toxic to fish and frequently not affordable in poor, schistosomiasis-endemic areas, alternative possibilities for snail control need to be evaluated. Plants with molluscicidal activity may be exploited to contribute to schistosomiasis control. The advances in the battle against the snails using natural molluscicides must be encouraged in order to minimize the negative side effects to the environment. A number of tropical plants have been investigated for their molluscicidal activity (Musman, 2006).

The huge negative impact of fascioliasis on human communities demands rapid action (Soliman *et al*., 2008). And with the development of triclabendazole for human use, fascioliasis can now

move up the list of priorities to be addressed urgently in endemic areas. WHO has spearheaded and recommended community-based chemotherapy for the control of helminthic infections in endemic communities (Savioli *et al*., 2002).

According to WHO (2007), Egypt is the only country that is currently implementing control activities against HF. Activities started in 1996 with the identification of the six endemic districts in Beheira Governorate. Since then, school surveys have been conducted in all the Delta governorates and in some Upper Egypt governorates.

Fascioliasis is controlled by a combination of anthelmintic therapy and management measures. These methods are costly and may lead not only to anthelmintic resistance, but also to undesirable residues in food or the environment (Mahmoud *et al*., 2010). In addition to treatment, the application of molluscicides to decrease the population of snail intermediate hosts is another important approach to control the disease (Soliman *et al*., 2008).

1.3.1 Chemotherapy

Chemotherapy is the most effective method for reducing morbidity and infection rates of trematodiasis. Access to very effective drug in the last three decades has resulted in reducing the prevalence and morbidity of the disease in many areas of the world and reducing the public health importance of the disease in some countries. Treatment of infected cases reduces morbidity and rate of the transmission (Castro *et al*., 2002). Treatment of cases as a control method require proper planning such as defining objectives of treatment, using the most reliable case finding method, selection of appropriate drug, correct dosage schedule and having adequate information on the drug and its side-effects. Because case detection is the first approach for planning chemotherapy and selection of most effective chemotherapy, diagnostic techniques available will be described first (Ali, 2010).

Praziquantel (PZQ), a pyrazinoisoquinoline derivative, is the mainstay of treatment and a critical part of community-based schistosomiasis control programs. This drug is effective, generally in a

single dose, against all species of schistosomes. Recent reports on praziquantel elucidate its failure to stop reinfection as a result of development of drug resistant *Schistosoma* strain, beside it induces hemorrhages in the lung tissue of the host as well as abdominal pain and diarrhea by long term application of the drug (Kabatereine *et al*., 2003; Coura and Conceição, 2010).

Praziquantel, the drug of choice for treating all human foodborne trematode infections, is ineffective against fascioliasis (Savioli *et al*., 2002). Triclabendazole (TCZ) is the treatment of choice for fascioliasis and is effective at a single dose of 10 mg/kg body weight against the adult parasites in the bile ducts and immature flukes migrating through the liver. TCZ has been used during outbreaks in several countries and for selective treatment of infected individuals in a control program, especially in the Nile Delta of Egypt. It has not been used in large community-based control programs because of its limited availability (WHO, 2007).

Oxamniquine, a tetrahydroquinoline derivative is used for the treatment of schistosomiasis due to *S. mansoni* both in the acute stage and in patients with hepatosplenic involvement. Some strains of *S. mansoni* are resistant to this drug. Side effects of oxamniquine are dizziness, drowsiness and headache (Ali, 2010).

1.3.2. Snail Control

Snail control is one of the methods of choice for the control of transmission of trematodiasis. It may entail the use of synthetic molluscicides, plant molluscicides, environmental management and biological agents (WHO, 1993). Snail control could be regarded as a rapid and efficient method of eliminating transmission and remains among the methods of choice for the control of this disease. Existing control methods are aimed principally at the management of snail populations that inhabit endemic areas. Where feasible, environmental management including elimination of natural water bodies such as marshes and ponds, and regulation of human settlement in areas with significant risk, has proven to be efficient (Lardans and Dissous, 1998). Generally, snail host control could be achieved through molluscicidal, environmental and biological control of snails.

1.3.2.1. Molluscicides

Molluscicides are crucial for controlling schistosomiasis and fascioliasis in complementing chemotherapy. The molluscicides currently in use are either synthetic or from natural origin (Toche *et al*., 2009). The use of molluscicides has always been considered to be a major supportive procedure in integrated schistosomiasis and fascioliasis control.

Perrett and Whitfield (1996) provided an overview of currently available synthetic and natural molluscicides. Among synthetic compounds, niclosamide is still the molluscicide of choice, being highly active at all stages of the snail life cycle and also effective on the schistosome larvae. While niclosamide is not toxic to humans, domestic animals and crops, the molluscicide is costly and toxic to fish. Moreover, the application of niclosamide did not prevent recolonization of sites by remaining snails, which could lead to the selection of molluscicide-resistant populations. This is due to the amphibious nature and reproductive nature of snail hosts. For example, all species of *Biomphalaria* and *Bulinus* are hermaphrodite, possessing both male and female organs and being capable of self- or cross-fertilization. A single specimen can repopulate a new habitat in less than a month. So it is difficult to control them (Lardans and Dissous, 1998). Plant molluscicides that are environmentally sound, stable in storage for long periods of time and less expensive have high contribution in controlling snail hosts (Singh *et al.,* 2004).

1.3.2.1.1. Synthetic Molluscicides

Application of synthetic molluscicides in snail habitats is a common method for elimination of intermediate host snails, in addition to environmental modification such as cementing canals, re-adjusting irrigation systems, etc. The use of molluscicides as one of the strategies to control schistosomiasis began by using niclosamide. Niclosamide, a chemical molluscicide, is recommended by the WHO as a sole chemical molluscicide since the 1960s and is still the molluscicide of choice (Yang *et al*., 2010). The high cost, the possibility of recolonization of

breeding grounds by snails and the ecological toxicity of this product were limitations on its use as an official molluscicide program of schistosomiasis control (Mello-Silva *et al*., 2006). On the other hand, Takourgang *et al*. (2006) studied the effect of lower bayluscide doses on snail host and fish. Niclosamide concentration of 0.50 g m^{-3} applied to ponds under investigation resulted in high snail mortality and show low lethality to fish.

Fenitrothion and anilofos (aniloguard) were tested as molluscicides against *Biomphalaria alexandrina*. The results obtained showed that sublethal concentrations of fenitrothion caused reduction in growth rate of *B. alexandrina* and reduction in the hatchability of snail's eggs. The mortality rates of miracidia and cercariae were elevated by increasing both the concentrations of fenitrothion and the time of exposure (Tantawy, 2006).

Other synthetic organic molluscicides such as carbomate, organophosphorus and pyrethoroids are used for the control of infected snails. However, these synthetic molluscicides are causing serious environmental hazards (Sharma *et al*., 2009). Some other synthetic molluscicides like Thymol, Linalool and Eugenol (monoterpenes) showed considerable molluscicidal effect against *B. alexandrina*, *Bulinus truncatus* and *Lymnaea natalensis* (El-Din, 2006).

1.3.2.1.2. Plant Molluscicides

The high cost of synthetic molluscicides used in the control of the intermediate snail hosts of schistosomiasis and fascioliasis, serious environmental hazards, and increasing concern over the possible built up of snail resistance of these molluscicides and their toxicity to non-target organisms have drawn much attention during recent years in renewed interest in the use of plant molluscicides (Sharma *et al*., 2009; Shekhawat and Vijayvergia, 2010). Molluscicides of plant origin have gained greater importance since it is believed that natural products are ecologically and culturally sound than synthetic ones. In addition, the use of local plants as molluscicides is beneficial in reducing burden of purchasing expensive synthetic molluscicides used in snail control (Shekhawat and Vijayvergia, 2010). A large number of plant families which possess natural molluscicidal activity have been identified (Sharma *et al*., 2009).

The search for more effective molluscicides, at lower costs and with less impact on the environment, stimulated the search for potential active substances from native plants. For instance, active molluscicides can be derived from specific parts of *Phytolacca dodecandra*, *Croton macrostachyus, Jatropha curcas, Ambrosia maritima, Anarcardium occidentale*. These plant molluscicides may provide cheap, locally produced, biodegradable and effective control agents in rural areas of developing countries where schistosomiasis and fascioliasis are endemic. Plant molluscicides could be used in low doses at transmission foci to reduce trematode infected snails (Augusto A dos Santos *et al*., 2003).

Mantawy and Mahmoud (2002) added that, *Allium cepa* (onion) and *Allium sativum* (garlic) have molluscicidal effect through disturbance in the protein profile, glucose and glycogen content of *B. alexandrina* snails. Dry powder of *Capparis spinosa* and *Acacia arabica* plant leaves seem to have a molluscicidal potency against *B. alexandrina* snails (Mantawy *et al*., 2004). El-Sayed (2006) stated that the dry powder of the aerial part of plant *Cupressus macrocarpa* (Cupressacea) have a significant molluscicidal activity against *B. alexandrina*.

Mello-Silva *et al*. (2006) considered that the latex of *Euphorbia splendens* var. *hislopii* is the most promising plant molluscicides. They have observed that the latex of *Euphorbia splendens* var. *hislopii* causes a sharp reduction in the reserves of glycogen in the digestive gland of *B. glabrata*.

Dos Santos *et al*. (2007) evaluated the latex of *Euphorbia conspicua* (Euphorbiaceae) for its molluscicidal and cercariacidal activities. The plant exhibited high activities against adult snails with LC_{90} values of 4.87 μg mL^{-1} and showed a lethal effect against the cercariae of *Schistosoma mansoni* at concentrations of 100 μg mL^{-1}.

Rug and Ruppel (2000) studied the toxic activity of methanolic extract of *Jatropha curcas* L. (Euphorbiaceae) against snails transmitting *Schistosoma mansoni* and *S. haematobium*. The

study showed the highest toxicity with LC_{100} values of 25 ppm for *Biomphalaria glabrata* and 1 ppm for *Bulinus truncatus* and *Lymnaea natalensis*.

Other investigation in Egypt showed that three species of *Solanum* (*S. nigrum, S. sinaicum, and S. villosum*) possess molluscicidal properties against the snail *B. alexandrina*. Ethanol extracts of *S. nigrum* had a stronger molluscicidal activity than the other extracts, and, therefore, it is the most suitable for biological application which offers a potentially simple, readily available, inexpensive and environmentally safe molluscicidal agent of plant origin for controlling schistosomiasis and fascioliasis (El-Sherbini *et al*., 2009).

The toxicity of active ingredients of certain plants to freshwater snails usually leads to death or low density of freshwater snails in such an environment (Olofintoye and Akinbile, 2007). Therefore, the use of plant molluscicides should be justified in that the effect of the dose needed as a molluscicide is nontoxic to other non target aquatic animals especially fish, which share the same habitat with snails (Ibrahim, 2009). The need for control measure of the freshwater snails is crucial and method with low cost and free of environmental pollution could be adopted (Olofintoye and Akinbile, 2007).

Endod as a Molluscicide

The discovery of molluscicidal properties of endod by Aklilu Lemma in 1964 has been a major breakthrough in combating intermediate snail hosts in schistosomiasis control (Erko *et al*., 2002). Endod *(Phytolacca dodecandra*), also called the soap-berry plant in Ethiopia, has proven to be a potent molluscicide, killing snail intermediate hosts of trematodes. The species occurs throughout Africa and is familiar to rural populations. In Ethiopia, the plant has been used by local people as laundry soap for centuries (Kloos and McCullough, 1987). The fact that endod grows locally and is biodegradable, makes it ecologically and economically more acceptable for use in schistosomiasis control compared to synthetic molluscicides (Erko *et al*., 2002).

It is the most thoroughly studied and promising of the currently available plant molluscicide (WHO, 1983). Its berries are the most thoroughly studied as source of the molluscicidal saponins with no major toxic properties for other animals and plants (Ali, 2010; Kloos and McCullough, 1987). Endod is an effective schistosomiasis and fascioliasis control agent, particularly when combined with chemotherapy (Abebe *et al*., 2005). Its active principle, a monodesmosidic saponin with an oleanolic acid glucoside base, is most concentrated in the pericarp of immature fruits (WHO, 1983).

The water extract of the dried and powdered fruit has a potency of killing snails (*Biomphalaria*, *Bulinus* and *Lymnaea* spp.) at a concentration of 10 ppm within 24 hours at room temperature. Comparisons of the molluscicidal potency of the green leaves, lower and buds showed that the green dried fruit extract was the most potent. Higher potencies were reported from the green and semi-ripe than from the fully ripe berries (Tilahun *et al*., 1998).

The potency of endod is temperature dependent. At higher water temperatures (30°C-35°C) less than 20ppm are needed to kill snails within 6 hours exposure period, whereas 60ppm is needed to produce the same effect at room temperatures. Endod's molluscicidal potency remains stable over a wide range of water acidity/alkalinity (pH values 4-9), in the presence of various concentrations of organic and inorganic matter in the water (Lemma *et al*., 1983).

Although the preparation and application of the endod berries is a safe procedure and that the molluscicidal saponins are decomposed to water and carbon dioxide in the environment, some outstanding questions are still to be solved. One of the future projects should deal with ecotoxicology in respect of the biological impact of the endod berries on the environment. Toxicity testing of endod is a prerequisite for its safe use as a molluscicide. The berries may be less detrimental to the ecosphere than are the synthetic molluscicides; however, they are harmful to the snails that are not host snails for trematodes and to fish (Lemma *et al*., 1983). In addition, it lacks an ovicidal activity (Kloos and McCullough, 1987).

Desirable Characteristics of Molluscicides

Vast arrays of compounds are molluscicidal that are used in the control of trematodiasis. Amongst these, pentachlorophenol was identified as being promising however; this was subsequently discarded due to its toxicity for other organisms. Compounds containing lead and tin are highly active but are also toxic for various organisms (McCullough, 1992). Niclosamide is now the molluscicide of choice and is virtually the sole commercially available compound at the present time. Its greatest disadvantage is its rising cost (Toche *et al*., 2009).

The perfect molluscicide does not yet exist and indeed may never do so. Instead, a list of characteristics has been laid down in a WHO publication. Generally, the minimum requirements for a good candidate molluscicide are a) toxicity for snails at low concentrations; b) absence of toxicity for mammals, neither presenting acute nor chronic problems of toxicity; c) lack of adverse effects if it enters the food chain; d) stable in storage for at least 18 months. In addition to these characteristics, low cost, proven efficacy, specificity for snails, low toxicity for other organisms, simple means of application and easy availability are desirable (WHO, 1985; McCullough, 1992).

1.3.2.2. Environmental Control

Schistosomiasis can only be transmitted by water contacts mostly for domestic and recreational purposes and also occupational. Therefore, providing safe water supply including safe drinking-water, washing facilities, cattle watering facilities and bathing reduce the risk of infection with schistosomiasis (Ali, 2010). Since human infection with fascioliasis is occurred after eating aquatic plants on which encysted organisms are present or by drinking contaminated water, providing clean water and cooking and washing aquatic vegetables is an important approach to control the disease (Soliman, 2008). It is apparent that since the snails all require water, at least for breeding, reduction in the size and number of potential breeding areas will reduce the total population and so tend to reduce human infections of both schistosomiasis and fascioliasis. Generally weed clearance, repeated cleaning of irrigation canals, good ditch and stream

management and good farming practices led to a substantial reduction in density of snail intermediate hosts of schistosomiasis and fascioliasis as well as their egg masses (Boelee and Laamrani, 2004).

1.3.2.3. Biological Control

Biological control is the control of snails through micro-parasites, trematodes, predators and competitor snail species (Arijo *et al*., 2007). Some proposed agents such as birds, turtles, fish and crayfish, have been observed eating snails in the field. Many more, such as leaches, nematodes, rotifers and ostracods, caused extensive mortality when they accidentally infested snail colonies. While most undoubtedly can exterminate snails in the laboratory, such predators are rarely effective in the field. Snails form only part of their diet and they turn to other food sources when snail numbers drop. So they eat snails only when they are abundant and their main effect is to limit the size of established snail populations (Sturrock, 1995).

In the case of biological control of snails using competitor snails, species with higher fecundity rates, voraciously feeding snails, snails resistant to parasites, species with longer life spans and snails that are harmless to other animals and surrounding crops are preferable as competitors (Giovanelli *et al*., 2003; Sturrock, 1995).

1.4. Significance of the Study

A number of studies have been conducted on different plant species of molluscicidal and cercariacidal activities. This study was an attempt to evaluate the potential molluscicidal activities of the plant *B. aegyptiaca* against snail intermediate hosts of trematodes, together with the cercariacidal activity against human schistosome cercariae in Ethiopia setting. The results of this study would provide further information on the use of this plant as molluscicidal and cercariacidal agent, particularly in Ethiopia.

2. OBJECTIVES

2.1. General Objective

- To find out molluscicidal and cercariacidal activities of the plant *Balanites aegyptiaca*.

2.2. Specific Objectives

- To investigate molluscicidal properties of aqueous extracts of fruits and seeds of *Balanites aegyptiaca* against *Biomphalaria pfeifferi*
- To investigate molluscicidal properties of aqueous extracts of fruits and seeds of *Balanites aegyptiaca* against *Lymnaea natalensis*
- To assess the lethal effect of *B. aegyptiaca* seeds to *Schistosoma mansoni* cercariae
- To provide further insight into molluscicidal and cercariacidal activities of *B. aegyptiaca* in Ethiopia

3. HYPOTHESIS

The seeds and fruits of *Balanites aegyptiaca*, which are used in traditional medicine, exhibit molluscicidal activities against Ethiopian biomphalarid and lymnaeid snails as well as cercariacidal property.

4. MATERIALS AND METHODS

4.1. Description of the Plant *Balanites aegyptiaca*

Balanites aegyptiaca (L.) Del. (‘*bedena’* in Amharic) is a species of tree, classified as a member of the family Balanitaceae. It is commonly known as thorn tree/desert date/soapberry tree. The plant is well adapted to different agro-climatic regions characterized by arid and semi-arid climatic features. It grows extensively even when neglected. It is a woody tree up to 10m high, and very variable. It have much larger, thick and acuminate leaves and fruits with round, almost spherical and slightly tomentose flowering buds and often bears forked spines (Martin, 1989). The fruits are generally ovoid, with a thin epicarp, a thick and often fleshy mesocarp, and a hard endocarp (Gour *et al*., 2007).

Balanites aegyptiaca is widely grown in the Sudano-Sahielian region of Africa, the Middle East and South Asia (Yadav and Panghal, 2010). Generally, this tree is distributed from Senegal to Somalia and from Egypt south into Zimbabwe, also to Japan Valley and Arabian Peninsula. This plant is also common in Ethiopia particularly in the Rift Valley areas in Gamo Gofa, Sidamo and Shoa regions, and upland Harerge (Martin, 1989). In addition, *B. aegyptiaca* is one of the dominant tree species deliberately retained on farm fields in the Tigray region of Ethiopia (Hailemariam *et al*., 2010).

Various parts of *B. aegyptiaca* tree have been used for folk medicines in many regions of Africa and Asia (Chapagain and Wiesman, 2005). It is medicinally used by many countries like Sudan, Nigeria, Egypt, Israel, etc. The fruit, kernel, bark and other parts of the tree have activities lethal to molluscs, miracidia and cercariae of schistosomes and *Fasciola gigantica*, and the cercariae of other animal trematodes (Archibald, 2004; Yadav and Panghal, 2010; Koko *et al*., 2000). The plant, in the collected areas (Ziway and Arsi Negele), traditionally is used for treating snake bites and stomach illnesses.

A number of saponins, that have medicinal role, have already been isolated from the seed and fruit of *B. aegyptiaca* and others from the bark and root (Brimer *et al*., 2007). The study conducted in Belgium indicated that the bark of this tree is used as purgative and analgesic in the treatment of the colics while the barks of roots are employed for the treatment of psychoses and epilepsy (Gnoula *et al*., 2007). The other study in India showed that the aqueous extract of root bark of *B. aegyptiaca* showed marked and potent anthelmintic activity than the standard drug albendazole (Dwivedi *et al*., 2009).

Balanites aegyptiaca was also investigated as a mosquito larvicidal agent (Yadav and Panghal, 2010) in Sudan. Chapagain and Wiesman (2005) in Israel demonstrated the potency of *B. aegyptiaca* extract in the control of *Culex pipiens* mosquito. All parts of *B. aegyptiaca* contain larvicidal properties and used as natural insecticides for the control of mosquitoes. In Ethiopia, *B. aegyptiaca* tree is preferred by farmers in Tigray region of Ethiopia for fuel wood, provision of shade, fodder and as a medicinal plant to treat intestinal illnesses (Hailemariam *et al*., 2010). The plant is taken as a purgative for colic and stomachache in Ethiopia as well as in Senegal, Nigeria and Morocco (Chothani and Vaghasiya, 2011).

4.2. Collection of the Plant and Snails

4.2.1. Collection of the Plant Parts

The plant parts (fruits and seeds of *Balanites aegyptiaca*) that are used in this study were collected from Ziway and Arsi Negele areas, 163 to 225 kms south of Addis Ababa in the Oromiya Regional State of Ethiopia during a month of January, 2011. Fruits and seeds of *B. aegyptiaca* were collected 3 kms from Ziway and 10 kms from Arsi Negele nearby forests. The collected plant parts were covered with wet sacks to avoid the direct sunlight, and then transported to the laboratory at Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. The plant was authenticated by a taxonomist/botanist, and voucher specimens were deposited at the National Herbarium of Addis Ababa University, (specimen no., Eshetu 001).

4.2.2. Collection of the Snails

The snails *Biomphalaria pfeifferi* (intermediate hosts of schistosomiasis) and *Lymnaea natalensis* (intermediate hosts of fascioliasis) were collected from different areas. *B. pfeifferi* snails were collected from Chacha, Senbete (North Shoa, Amhara Regional State) and Mekele (Tigray Regional State) areas, about 110, 270 and 783 kms north of Addis Ababa respectively. *L. natalensis* snails were collected from Wondo Genet 284 kms south of Addis Ababa and from Jiga (Gojjam) about 375 kms northwest of Addis Ababa. The snails were collected from the irrigation canals and drainage networks and were collected using a flat dip-net scoop. The collected snails were placed in a plastic bucket containing some dam water with few leaves. The snails were then transported to the laboratory in ALIPB, Addis Ababa University for identification and maintenance. The snails were identified using standard identification keys and were left to acclimatize to laboratory conditions before being used in the experiments.

4.3. Laboratory Studies

4.3.1. Preparation of Plant Materials

Following collection, mature unripe *B. aegyptiaca* fruits were washed with tap water. The epicarp (outer cover) of the fruits was removed by using sterile sharp surgical blade, and the fruits pulp/mesocarp were scraped manually and collected. The endocarp of the fruits was broken manually and the seed was then collected. The prepared fruit parts (endocarp and mesocarp), the whole fruit part and seeds were air dried under shade in the laboratory for 5-6 days, separately (Chapagain and Wiesman, 2005). Thereafter, the dried specimens were manually grounded to powder using a mortar and pestle. The powder, then, was sieved through 250 microns mesh to obtain a fine material. The powdered fine plant material was then transferred into closed containers until use.

4.3.2. Maintenance of the Snails

The snails used in this study were *Biomphalaria pfeifferi* and *Lymnaea natalensis*. All collected snails were cleaned from the vegetation cover and the attached debris. Both *B. pfeifferi* and *L. natalensis* snails that shed cercariae were identified using the shedding method described by Frandsen and Christensen (1984), by placing all the snails in a beaker with aged water. The beakers were then exposed to the day light and left for 40 minutes to allow the cercariae to emerge. The snails that shed cercariae were separated individually into small beakers half filled with aged water to identify the infected snails. Snails which shed cercariae were excluded from molluscicidal testing. *Biomphalaria* and *Lymnaea* snails, which did not shed cercariae, were maintained in separate plastic aquaria containing aged water and maintained at room temperature, and the aquaria were kept under fluorescent light for 12-hours daily. The snails were fed every second day with boiled dried lettuce, and water was changed thrice a week, whenever necessary or otherwise added daily to replace the loss of evaporation. Dead snails were removed as soon as possible from the troughs to prevent water fouling.

4.3.3. Preparation of the Stock Solution and Serial Dilution

The concentrations used in the bioassays were prepared from fruits (endocarp and mesocarp), seeds and the whole fruit part in successive dilutions with aged water. One gram of each powdered dry plant part used in the screening tests was weighed using an electric balance. The weighed powdered dry part was soaked in 1000 ml of aged water for 24 hours with occasional vigorous shaking using a shaker. Then, the suspension was filtered using filter paper (Whatman No.1). The marc/unfiltered part was washed three times with aged water. All filtrates were mixed together and using volumetric flasks, final volume was adjusted to 1000 ml by adding aged water to get stock solution of 1000 ppm. This stock solution was then used to prepare different working concentrations. The plant extracts were used immediately after the extraction to insure their freshness.

After determining one hundred percent (100%) mortalities of both snail species, different working concentrations were prepared for both species of snails. After the preparation of the mother (stock) solution of 1000 mg/l, successive concentrations of the aqueous extract were prepared to obtain the final concentrations of 65, 70, 75, 80, 85, 90, 95, 100 ppm for testing *B. pfeifferi* snails. And for *L. natalensis* snails, 85, 90, 95, 100, 105, 110, 115 and 120 ppm, in a final volume of 500 ml in each solution were prepared. For each test, there was a control with aged water, without plant material, with the same volume of the solution (Changbunjong *et al*., 2010).

Dilution Procedure

The different concentrations of the aqueous extracts tested in the experiments were prepared from the stock solutions using the simple dilution procedure (Ibrahim, 2009), which reads as follows:

$C_1 V_1 = C_2 V_2$

Where:

C_1= Concentration of stock solution.

V_1= Volume of the stock solution used

C_2= Test concentration

V_2= Final volume

4.3.4. Molluscicidal Potency Tests of Aqueous Extracts of *Balanites aegyptiaca* against Adult *Biomphalaria pfeifferi* and *Lymnaea natalensis* Snails

Water extracts of the plant parts, the seeds, endocarp, mesocarp and the whole fruit were tested against adult *B. pfeifferi* and *L. natalensis* snails according to the method recommended by WHO (1983).

To each concentration of plant parts used, 20 adult snails of both *B. pfeifferi* and *L. natalensis* were immersed. Tests were carried out at room temperature. The exposure period to the plant extracts was 24 hours. After 24 hours of exposure, the suspension was decanted; the snails were rinsed thrice with aged water and transferred to another aged water and maintained there for another 24 hours, recovery period. 20 snails will be immersed in separate aged water that will be served as control with the same volumes of the solvent used in the treated cases. All groups were watched carefully after 24 hours, the number and the percentages of death in each group were calculated. The numbers of dead snails were converted to probit and the LC_{50} and LC_{90} values were calculated (Mohamed *et al*., 1999). No food was supplied during the experiments.

Confirmation of the snails' death: The deaths of the snails during the tests were confirmed by the change in the shell color and failure of the flesh portion to withdraw into the shell upon mechanical stimuli (probe). Snails were considered dead if they could not move or retracted well into or hanging out of the shell, with the body and shell discolored (Vijay, 2010).

4.3.5. Cercariacidal Potency Tests of Aqueous Extracts of *Balanites aegyptiaca* against *Schistosoma mansoni* Cercariae

S. mansoni cercariae were obtained from field-collected snails (collected from Senbete, North Shoa). Snails were individually placed in shedding vials containing 5 ml of aged water and exposed to artificial light for 30 minutes. Then the cercariae obtained from as many positive snails as possible were pooled into a glass beaker. This pool was gently mixed to make the distribution of the cercariae in the beaker uniform. From this cercarial suspension, 0.50 ml was taken using fine pipettes and transferred into a counting chamber. The number of cercariae per 0.50 ml was obtained and the volume of cercarial suspension needed to get about 200 cercariae was then estimated.

In Vitro Observation on the Lethal Effect of *Balanites aegyptiaca* Seeds on Cercariae

Five petri dishes containing 5, 10, 15, 25 and 35 ppm water extracts of *B. aegyptiaca* seeds were set up in duplicates. To each petri dish, 20 freshly shed cercariae were added. The motility and mortality status of the cercariae in each concentration were then observed and recorded under a dissecting microscope at 30 min, 1 hr, $1^1/_2$ hr and 2 hr intervals. Results were recorded as very actively motile, actively motile and weakly motile/sluggish. As controls, the same numbers of cercariae were placed in petri dishes containing aged water and set up in duplicates and were observed at similar time intervals for their motility and /or mortality status (Birrie *et al*., 1998).

The *In Vivo* Observation on Mice Exposed to Cercariae Pre-treated with Aqueous Extracts of *B. aegyptiaca* Seeds

Three glass beakers that contain 500 ml of 5, 10 and 15 ppm of water extract of *B. aegyptiaca* seeds prepared in aged water were set up in duplicates. Slightly over 300 cercariae were caught with pipettes and transferred to each beaker and allowed to stand for two and four hours. Similarly, six glass beakers (3 for two and 3 for four hours of exposure of cercariae to the extract) containing 500 ml aged water were set up in duplicates. After exposing the cercariae for two and four hours to these concentrations, 200 cercariae (pre-determined infective dose) were again transferred from beaker to beaker containing aged water. In each of the beakers one mouse was placed and allowed to stay for 40 minutes for exposure by the paddling method (through skin penetration of the legs). After 40 minutes of exposure to cercariae the mice were returned to their cages and maintained in the animal house under standard conditions. As a control mice were exposed to the same numbers of cercariae that were not exposed to the seed extract for the same exposure periods, and set up in duplicates (Birrie *et al*., 1998).

After 45 days post exposure to the cercariae, the faecal samples of mice were collected and checked for the presence of schistosome eggs. Each infected mice were sacrificed to

determine the mean egg load in each tissue (liver, small and large intestines) (Ogboli *et al*., 2000). Tissue egg load was determined by digesting known weights of liver, small intestine and large intestine by mechanical grinder. After grinding each tissue, about 0.10 gm of each tissue was observed in microscope and the eggs were counted. The number of eggs obtained were then extrapolated for the total weight of each tissue and finally converted to number of eggs per gram of tissue (Birrie *et al*., 1998). The mean of numbers of egg loads for the total mice perfused for each concentration was calculated by geometric mean (Montresor *et al*., 1998), using the formula:

$$\text{Geometric mean} = \text{Exp}^{\frac{\sum \log (epg + 1)}{n}} - 1$$

Where,

epg = the number of eggs per gram of tissue

Exp = exponential or antilogarithm

log (epg + 1) = the sum of the logarithm of each mouse epg

n = the number of mice perfused in each concentration.

4.4. Data Analysis

Probit regression analysis of SPSS program version 13.0 was carried out for all tested parts of the plant to determine the LC_{50} and LC_{90} values against both snail species with 95% confidence intervals (Changbunjong *et al*., 2010). The 95% confidence limits (lower and upper), from probit regression analysis, were calculated and significant differences between each snail species and all tested parts of the plant were determined. Analysis for variance (One-way ANOVA) was used to determine the significant reduction in tissue egg load in the mice (Birrie *et al*., 1998). A p value < 0.05 was considered as statistically significant.

5. RESULTS

5.1. Molluscicidal and Cercariacidal Potency Tests of Aqueous Extracts of Different Plant Parts of *Balanites aegyptiaca*

The molluscicidal activity of aqueous extracts of seed, endocarp, mesocarp and the whole fruit of *B. aegyptiaca* against adult *Biomphalaria pfeifferi* and *Lymnaea natalensis* as well as the cercariacidal activity of its seed on *S. mansoni* cercariae were investigated. The molluscicidal potency of all tested parts of *B. aegyptiaca* against both *B. pfeifferi* and *L. natalensis* were concentration dependent. Generally, mortality increased with increase in extracts' concentrations (Tables 1-8). With regards to the snail species, *B. pfeifferi* were more susceptible to the plant extract than *L. natalensis* for both seeds and fruits (Figure 3). No difference of molluscicidal activity was observed with respect to plant and snail collection sites.

No mortality was observed for *B. pfeifferi* exposed to extracts' concentrations of 2, 5 and 8 ppm of all tested plant parts after 24 hours exposure. One hundred percent mortality rates were observed on *B. pfeifferi* exposed to a concentration of 100 ppm for the seeds and mesocarp, whereas on *L. natalensis* 100% mortality was observed at 120 ppm. No mortality was observed at 24 hours exposure period below the concentrations of 15 ppm.

From the cercariacidal investigation, the *in vitro* cercariacidal activity of the plant on *S. mansoni* cercariae showed that the mortality rates of cercariae were elevated by increasing both the concentrations of seeds and the time of exposure. The *in vivo* observation of the infectivity of *S. mansoni* cercariae was evaluated by pre-exposing the cercariae with seed extracts and then exposing to mice, it was found that infectivity of cercariae was completely inhibited at 15 ppm. And a significant reduction in tissue egg deposition occurred even at lower concentrations than 15 ppm ($p<0.05$).

5.1.1. Molluscicidal Potency Tests of Aqueous Extracts of *Balanites aegyptiaca* Seeds and Fruits on Adult *Biomphalaria pfeifferi*

The lethal concentrations for aqueous extracts of *Balanites aegyptiaca* seed, endocarp, mesocarp and the whole fruit part and that killed 50% (LC_{50}) of adult *Biomphalaria pfeifferi* were 56.32, 77.53, 65.51 and 66.63 ppm, respectively, while the respective LC_{90} values were 77.70, 120.04, 89.50 and 97.55 ppm (Tables 1-4 and Figure 3).

Comparing the LC_{50} and LC_{90} of the plant parts, seeds showed the highest molluscicidal activity, followed by mesocarp, whole fruit part and then the endocarp. The LC_{50} of the seeds was significantly different from the LC_{50} of endocarp. The LC_{90} of the seed was also significantly different with whole fruit part and endocarp. And the LC_{90} of the mesocarp was significantly different from the LC_{90} of the endocarp ($p<0.05$).

Table 1: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* seed on mortality rates of *Biomphalaria pfeifferi*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (mg/l)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	65	20	15	75		
	70	20	15	75		
	75	20	17	85	56.32	77.70
	80	20	18	90	(36.99-	(71.97-
	85	20	19	95	63.61)	86.63)
	90	20	19	95		
	95	20	20	100		
	100	20	20	100		

Table 2: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* endocarp on mortality rates of *Biomphalaria pfeifferi*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (mg/l)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	65	20	7	35		
	70	20	8	40		
	75	20	8	40	77.53 (70.15-83.11)	120.04 (104.18-178.95)
	80	20	10	50		
	85	20	12	60		
	90	20	13	65		
	95	20	15	75		
	100	20	16	80		

Table 3: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* mesocarp on mortality rates of *Biomphalaria pfeifferi*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (mg/l)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	65	20	11	55		
	70	20	12	60		
	75	20	14	70	65.51	89.50
	80	20	15	75	(56.31-	(83.81-
	85	20	16	80	70.35)	102.16)
	90	20	18	90		
	95	20	19	95		
	100	20	20	100		

Table 4: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* fruit on mortality rates of *Biomphalaria pfeifferi*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (mg/l)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	65	20	10	50		
	70	20	11	55		
	75	20	13	65	66.63	97.55
	80	20	14	70	(55.64-	(89.42-
	85	20	16	80	72.09)	120.35)
	90	20	17	85		
	95	20	17	85		
	100	20	19	95		

5.1.2. Molluscicidal Potency Tests of Aqueous Extracts of *Balanites aegyptiaca* Seeds and Fruits on Adult *Lymnaea natalensis*

The lethal concentrations for aqueous extracts of *Balanites aegyptiaca* seed, endocarp, mesocarp and the whole fruit part and that killed 50% (LC_{50}) of adult *Lymnaea natalensis* were, 80.33, 92.61, 83.52 and 87.84 ppm, respectively, while the respective LC_{90} values were 102.30, 138.21, 115.42 and 127.69 ppm (Tables 5-8 and Figure 3).

Comparing the LC_{50} and LC_{90} of the plant parts, seeds showed the highest molluscicidal activity, followed by mesocarp, whole fruit part and then the endocarp. There was no significant difference occurred between each plant part in terms of LC_{50} ($p>0.05$). But the LC_{90} of the seed was significantly different from the LC_{90} of the whole fruit part and the endocarp ($p<0.05$).

Table 5: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* seed on mortality rates of *Lymnaea natalensis*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (ppm)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	85	20	13	65		
	90	20	15	75		
	95	20	16	80	80.33	102.30
	100	20	17	85	(66.38-	(97.35-
	105	20	17	85	86.38)	111.92)
	110	20	19	95		
	115	20	20	100		
	120	20	20	100		

Table 6: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* endocarp on mortality rates of *Lymnaea natalensis*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (ppm)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	85	20	8	40		
	90	20	10	50		
	95	20	11	55	92.61 (79.88-98.76)	138.21 (121.97-212.11)
	100	20	11	55		
	105	20	12	60		
	110	20	14	70		
	115	20	15	75		
	120	20	17	85		

Table 7: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* mesocarp on mortality rates of *Lymnaea natalensis*

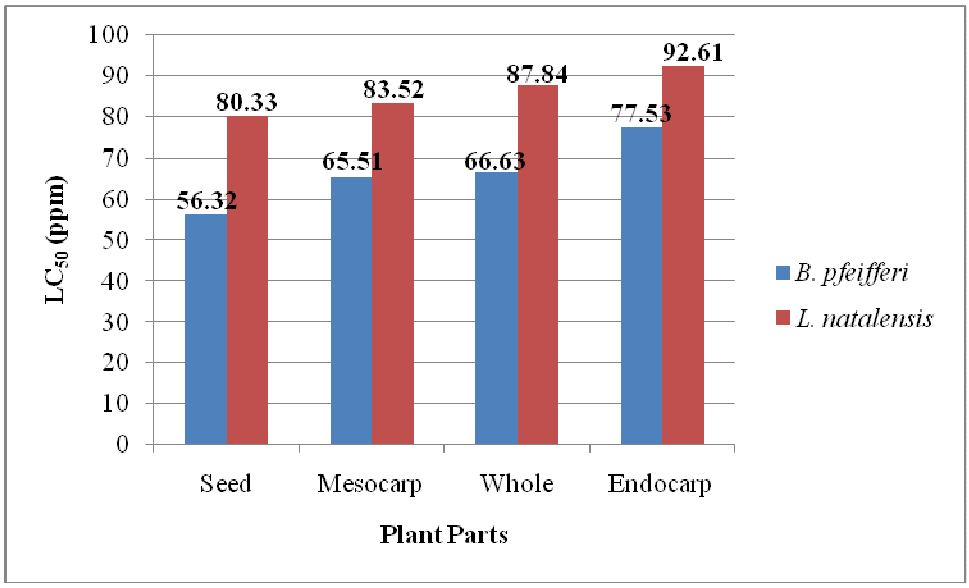
Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (ppm)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	85	20	11	55		
	90	20	13	65		
	95	20	14	70	83.52 (68.15-90.03)	115.42 (107.53-139.06)
	100	20	15	75		
	105	20	15	75		
	110	20	16	80		
	115	20	18	90		
	120	20	20	100		

Table 8: Molluscicidal potency of aqueous extract of *Balanites aegyptiaca* fruit on mortality rates of *Lymnaea natalensis*

Exposure/ recovery time	Conc. (mg/l) (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC (ppm)	
					LC_{50} (95% CI)	LC_{90} (95% CI)
24 hour/ 24 hour	Control	20	0	0	-	-
	85	20	10	50		
	90	20	11	55		
	95	20	12	60	87.84 (72.93-94.19)	127.69 (115.59-174.56)
	100	20	12	60		
	105	20	14	70		
	110	20	15	75		
	115	20	17	85		
	120	20	18	90		

Comparison of the LC_{50} and LC_{90} values of the seed and fruit extracts of *B. aegyptiaca* against the two snail species showed that *B. pfeifferi* were more susceptible to all tested parts of the plant than *L. natalensis* (Figure 3). Both LC_{50} and LC_{90} values obtained for the seeds against *B. pfeifferi* were significantly different from the LC_{50} and LC_{90} values against *L. natalensis*. The LC_{90} value of the mesocarp against *B. pfeifferi* was significantly different from the LC_{90} value against *L. natalensis*. The LC_{50} value obtained for the whole fruit part against *B. pfeifferi* was also significantly different from the LC_{50} value against *L. natalensis*. But no significant difference of LC_{50} and LC_{90} values occurred between the two snail species for endocarp ($p>0.05$).

a)



b)

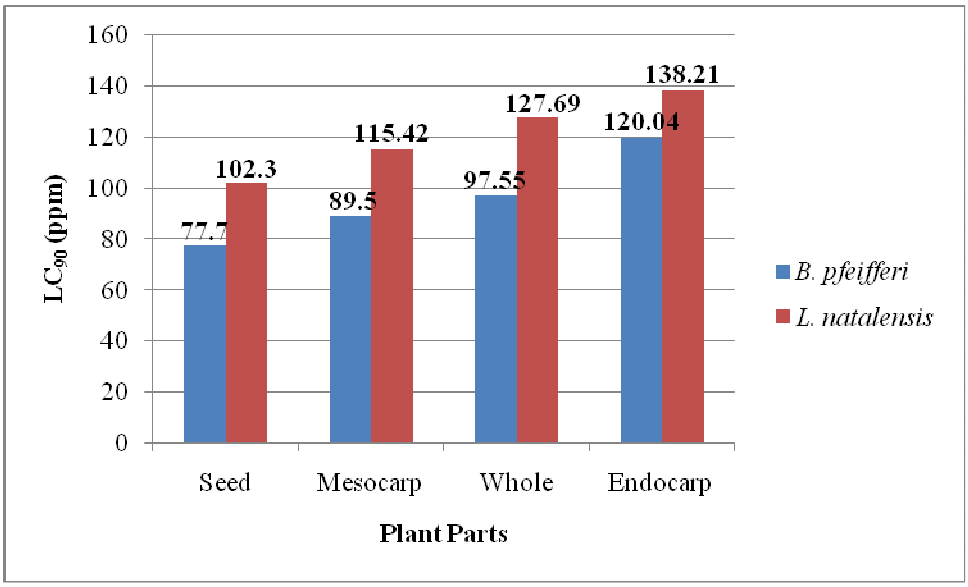


Figure 3: LC_{50} (a) and LC_{90} (b) values for aqueous extracts of *Balanites aegyptiaca* against adult *Biomphalaria pfeifferi* and *Lymnaea natalensis* snails

5.1.3. Cercariacidal Potency Tests of Aqueous Extracts of *Balanites aegyptiaca* Seeds on *Schistosoma mansoni* Cercariae

In Vitro Observation on *S. mansoni* Cercariacidal Activity

The mortality rate of cercariae exposed to aqueous extracts of *B. aegyptiaca* seeds increased with increasing time of exposure and concentrations. At a concentration of 10 ppm and exposure periods of 2 hours, less than 25% of the cercariae died. At a concentration of 15 ppm and exposure period of 2 hours, about 25-50% of the cercariae died. A concentration of 25 ppm increased the mortality rate to about 51-75%. In a concentration of 35 ppm cercariae mortality increased to about 76-100% with in one hour. The motility rates of the remaining cercariae were also reduced with increase in the exposure time and concentrations (Table 9).

Table 9: The *in vitro* observation on cercariacidal activity of aqueous extract of *Balanites aegyptiaca* on *S. mansoni* cercariae

Seed concentration (ppm)	Time interval in minutes	200 Cercariae							
		Mortality					Motility		
		0	<25%	25-50%	51-75%	76-100%	+++	++	+
Control	30						**X**		
	60						**X**		
	90						**X**		
	120						**X**		
5	30						**X**		
	60		**X**				**X**		
	90		**X**				**X**		
	120		**X**					**X**	
10	30						**X**		
	60		**X**				**X**		
	90		**X**					**X**	
	120		**X**					**X**	
15	30		**X**					**X**	
	60		**X**						**X**
	90		**X**						**X**
	120			**X**					**X**
25	30		**X**						**X**
	60		**X**						**X**
	90			**X**					**X**
	120				**X**				**X**
35	60					**X**			

+++= very active ++= active += sluggish

***In Vivo* Observation on *Schistosoma mansoni* Cercarial Infectivity of Aqueous Extract of *Balanites aegyptiaca* Seeds**

The infectivity of the cercariae to mice was evaluated by exposing the mice to the cercariae pre-exposed to aqueous extract of *B. aegyptiaca* seeds. The infection was completely inhibited at 15 ppm, at two and four hours of exposure of cercariae to the seed extract. Concentrations lower than 15 ppm significantly reduced tissue egg load ($p<0.05$) (Table 10).

Table 10: The *in vivo* observation on *S. mansoni* cercarial infectivity of aqueous extract of *Balanites aegyptiaca* seeds

Exposure time	Concentration (ppm)	No. of mice	GM		
			SI	Liver	LI
2 hours	Control	2	80.44	52.94	48.00
	5	2	50.40	35.55	39.09
	10	2	37.60	36.70	25.93
	15	2	0	0	0
4 hours	Control	2	62.18	59.74	47.46
	5	2	27.46	32.50	29.29
	10	2	9.32	25.82	10.94
	15	2	0	0	0

LI= Large intestine; SI= Small intestine; GM= Geometric mean of egg counts per gram of tissue

6. DISCUSSION

The present study showed that aqueous extracts of *Balanites aegyptiaca* seeds and fruits possess molluscicidal and cercariacidal properties. Their activities are time and concentration-dependent. Between the test snail hosts of trematodes, *Biomphalaria pfeifferi* were more susceptible to the plant extract than *Lymnaea natalensis*. Aqueous extracts of *B. aegyptiaca* seeds showed the highest molluscicidal activity followed by mesocarp, whole fruit and endocarp on both test snail species after 24 hours of exposure period. The seeds and fruits showed high molluscicidal activity against both snails. Previous investigators also reported similar observations (Vijay, 2010; El-Sheihk, 1994; Adewunmi and Sofowora, 1980).

The LC_{50} values for water extract of *Balanites aegyptiaca* against *B. pfeifferi* and *L. natalensis* were 56.32 and 80.33 mg/l for seeds; 77.53 and 92.61 mg/l for endocarps; 65.51 and 83.52 mg/l for mesocarps; 66.63 and 87.84 mg/l for whole fruit part, respectively. The respective LC_{90} values of the plant against *B. pfeifferi* and *L. natalensis* snails were 77.70 and 102.30 mg/l for seeds; 120.04 and 138.21 mg/l for endocarps; 89.50 and 115.42 mg/l for mesocarps; and 97.55 and 127.69 mg/l for whole fruit part, respectively. The varying potencies of each plant part may be due to the differences in the concentration and/or the type of the active ingredient(s) present in each part (Brimer *et al.*, 2007). In this observation, water extract of seeds and mesocarps were more potent than the water extract of endocarp, due to the more concentrations of saponins in the mesocarps and seeds, and also presence of high amount of deltonin in the seeds (Bah *et al.*, 2006; Brimer *et al.*, 2007). Therefore, the presence of these two ingredients in seeds could be the most probable reason for the high molluscicidal property of seeds over the mesocarps and endocarps.

The LC_{50} values of aqueous extracts of the seed and fruit observed against *L. natalensis* in this study were higher than the values reported by Vijay (2010). He obtained LC_{50} values of 60 ppm after the exposure of *Lymnaea acuminata* to aqueous extracts of *B. aegyptiaca* fruits for 24 hours. In the present study, the LC_{50} values against *L. natalensis* (with 80.33 ppm for

seed, 92.61 ppm for endocarp, 83.52 ppm for mesocarp and 87.84 ppm for fruit) were higher than 60 ppm. On the other hand, the LC_{50} value of the seed against *B. pfeifferi* was below 60 ppm with 56.32 ppm but for other plant parts the LC_{50} values were above 60 ppm (with 77.53 ppm for endocarp, 65.51 ppm for mesocarp and 66.63 ppm for whole fruit part). In addition, Anto *et al*. (2005) reported that extracts of the freeze-dried, ripe fruit of *B. aegyptiaca* have molluscicidal activity, giving LC_{95} at 16.9 and 19.7 ppm for the juveniles and adults of *Bulinus globosus*, and at 14.2 and 12.0 ppm for the juveniles and adults of *B. truncatus*, respectively.

In the Sudan, Ragab *et al*. (2005) studied the molluscicidal activity of this plant and found out that *B. aegyptiaca* saponins (seeds) produced significant effect on *Bulinus truncatus* at different concentrations. They also observed that the mortality of snails increased with increasing concentration and exposure period. In addition, Ndabaneze *et al*. (1994) observed that *B. aegyptiaca* fruits exhibited greatest toxicity at 10 mg of powder/liter and 100% mortality at 100 mg/l. The present observation on *B. aegyptiaca* against *Biomphalaria pfeifferi* (100% mortality at 100 mg/l in both seed and mesocarp) was similar with the findings of Ndabaneze and his colleagues. But the results obtained for *L. natalensis* do not agree with their result with 100% mortality at a concentration of 120 mg/l in both the seed and mesocarp.

On the contrary, the LC_{50} values for endod against snail hosts of schistosomes are less than 10 mg/l (Ndamba, 1993). In addition, Monkiedje *et al*. (1990) showed that the LC_{50} and LC_{90} values for endod berries against *Biomphalaria glabrata* were 2.57 and 2.92 mg/l, respectively. Lemma *et al*. (1972) also observed that the LC_{90} values of powdered endod berries and butanol extract against *B. glabrata* were 22.3 and 3.0 ppm after 24 hours of exposure time. But the LC_{90} values of *B. aegyptiaca* seeds and fruits obtained in the current study were higher than those produced by endod.

The LC_{90} values of *B. aegyptiaca* seed, endocarp, mesocarp and whole fruit part against *B. pfeifferi* and *L. natalensis* were higher than the LC_{90} values reported by Dos Santos *et al*.

(2007). Dos Santos and his colleagues reported that the latex of *Euphorbia conspicua* exhibited high activities against adult *Biomphalaria glabrata*, that is, they observed that LC_{90} values of 4.87 μg mL^{-1} for *E. conspicua*.

On the other hand, the molluscicidal activity observed for *B. aegyptiaca* in the present study was higher than that reported by Abdalla *et al*. (2011). Abdalla and his colleagues observed that the LC_{50} and LC_{95} values for *Calotropis procera*, *Nicotiana tabacum* and *Trigonella foenum* against *Bulinus truncatus* were over 619 ppm.

Results obtained in the current study for aqueous extracts of *B. aegyptiaca* seed, endocarp, mesocarp and whole fruit part, which demonstrated LC_{50} of 56.32, 77.53, 65.51 and 66.63 ppm, respectively for *B. pfeifferi* and LC_{50} of 80.33, 92.61, 83.52 and 87.84 ppm respectively for *L. natalensis*, could compare with the activity of nutmeg and mace in the study of Jaiswal and Jingh (2009). They observed that the LC_{50} values of nutmeg and mace powder against *Lymnaea acuminata* after 24 hour exposure time were 74.15 mg/l and 38.61 mg/l, respectively. In the present observation *B. aegyptiaca* seed, mesocap and whole fruit part showed higher potency than nutmeg powder against *B pfeifferi*. But *B. aegyptiaca* seeds and fruits showed lower molluscicidal potency against *L. natalensis* than nutmeg and mace.

In the present study, the molluscicidal properties of *Balanites aegyptiaca* were found to vary with snail species. Similarly, Changbunjong *et al*. (2010) observed that the ethanolic crude extract of *Solanum xanthocarpum* produced varying mortalities of snail hosts. In this study, *S. xanthocarpum* produced LC_{50} and LC_{90} values of 163.85 and 219.33 ppm against *B. glabrata*, respectively. In addition, they also observed the LC_{50} and LC_{90} values of 198.00 and 236.80 ppm for *S. xanthocarpum* against *Inoplanorbis exustus*, respectively. Ahmed (1997), in determining the effect of the water crude extract of *S. nigrum* against the snails of *B. alexandrina*, *B. truncatus*, and *L. natalensis*, showed that LC_{50} values were 18.6, 14.5 and 17.7 mg/l, respectively. Chimbari and Shiff (2008) also observed that *Biomphalaria glabrata* was most susceptible to *J. curcas* seed extract (with LC_{50} value of 282 ppm) than *Bulinus*

globosus (with LC_{50} value of 605 ppm). The reasons for differences in susceptibility may need to be investigated.

In order for a plant to be considered as candidate molluscicide, its crude extract should be active at 100 ppm or lower when 90% of the snails are killed after 24 hours exposure (Mott, 1987). The results of the current observation indicated that aqueous extracts of the seed, mesocarp and whole fruit part caused 90% *B. pfeifferi* mortality after 24 hours exposure time at 77.70, 89.50 and 97.55 ppm, respectively. Hence, this plant could be a candidate plant molluscicide for *B. pfeifferi*. On the other hand, the LC_{90} values for *B. aegyptiaca* against *L. natalensis* were above 100 ppm, that is, 102.30, 115.42 and 127.69 ppm for seeds, mesocarps and fruits, respectively indicating that aqueous extract of this plant parts are less potent against *L. natalensis*.

The present preliminary *in vitro* observation on cercariacidal properties of *Balanites aegyptiaca* showed that aqueous extracts of seeds have cercariacidal property. This is in agreement with the study of Guyatt and Evens (1992) who reported that the seed and fruit extracts of this plant have lethal effect on miracidia and cercariae of schistosomes. The cercariacidal activity of this plant is also consistent with Birrie *et al*. (1998) using endod, who showed that the mortality of *S. mansoni* cercariae exposed to aqueous extracts of endod berries increased with increasing concentration and exposure time.

In addition, Dos Santos *et al*. (2007) showed lethal effect of *Euphorbia conspicua* to the cercariae of *S. mansoni* at a concentration of 100 μg mL^{-1}. On the other hand, in the present study only a concentration of 35 ppm of aqueous extracts of *B. aegyptiaca* seeds produced up to 100% cercarial mortality. The cercariacidal activity of aqueous extract of *B. aegyptiaca* seed is also in accordance with Rug and Ruppel (2000) using *Jatropha curcas* and Ahmed and El-Hamshary (2005) using *Iris pseudacorus*.

Similarly, it was observed that water extract of *B. aegyptiaca* seeds made *S. mansoni* cercariae less infective to mice at lower concentrations. *S. mansoni* cercariae exposed to 15

ppm of aqueous extract of seeds were incapable of infecting the mice. In addition, at 5 and 10 ppm, the mean tissue egg load was significantly reduced. This observation is in agreement with those of others who used different plant species as schistosomicide. Birrie *et al*. (1998) showed that pre-treatment of the cercariae with 12 ppm of the endod berries completely inhibited infection of mice by cercariae and a significant reduction in tissue egg deposition occurred even below a concentration of 12 ppm. Ojewole *et al*. (2004) also demonstrated that aqueous extract of *Barringtonia racemosa* fruit have a remarkable cercariacidal activity.

Koko *et al*. (2000) reported that *B. aegyptiaca* possesses anthelminthic activity against *Fasciola gigantica* compared with albendazole. A single dose of 200 mg/kg body weight of *B. aegyptiaca* fruit showed activity against *S. mansoni* in infected mice when compared with praziquantel. A significant reduction was observed in epg and egg burden in tissues for both the extract- and the drug-treated mice (Koko et al., 2005). In the present study, *B. aegyptiaca* showed potential cercariacidal properties. Thus, further studies should be made to determine its anthelminthic activities, particularly for its schistosomicidal property.

7. CONCLUSION AND RECOMMENDATIONS

From the present study, it can be concluded that aqueous extracts of seeds and fruits of *Balanites aegyptiaca* have reasonable molluscicidal activity. Different parts of the plant showed different level of molluscicidal potencies. The aqueous extract of seeds exhibited stronger molluscicidal activity compared to the mesocarps and endocarps. In regards to the snail species tested, *B. pfeifferi* were more susceptible to this plant than *L. natalensis*. The seeds of this plant also showed reasonable cercariacidal property against *Schistosoma mansoni* cercariae. The aqueous extracts of *B. aegyptiaca* seeds were more toxic to the cercariae of *S. mansoni* than to their snail hosts.

Based on the present results the following recommendations are made:

- Test the effects of varying temperatures and pH on the molluscicidal and cercariacidal potency of *Balanites aegyptiaca*
- Active principles of the seeds and fruits responsible for molluscicidal and cercariacidal activity should be identified
- Investigate the mechanism of action of *B. aegyptiaca* extracts on snails and cercariae
- It is strongly recommended that the toxic effects of this plant to fish, cercariae, snails, snail eggs and mammals be further investigated so as to determine the right concentration, particularly for use in fish ponds.

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9. ANNEX

Plate 1: *Balanites aegyptiaca* fruits in laboratory

Plate 2: Fruits of *Balanites aegyptiaca*

Plate 3. Adult *Biomphalaria pfeifferi* snails

Plate 4. Adult *Lymnaea natalensis* snails

Declaration

I, the undersigned, declare that this thesis is my own work. It has not been presented in other university, colleges or institutions, seeking for similar degree or other purposes. All source of materials used for the thesis has been duly acknowledged.

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