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BIOMEDICAL SCIENCES STREAM

EVALUATION OF THE MOLLUSCICIDAL AND CERCARICIDAL PROPERTIES OF LEAVES OF *DATURA STRAMONIUM*

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 sciences)

By

Aster Sheshay

Addis Ababa

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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 accordingly acknowledged.

Name: Aster Sheshay

Signature: _____

Date:_____

Advisor: Prof. Yalemtsehay Mekonnen

Signature: _____

Date:_____

Advisor: Prof. Berhanu Erko

Signature: _____

Date:_____

Advisor: Dr. Mirutse Giday

Signature: _____

Date:_____

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List of Abbreviations

AAU	Addis Ababa University
ANOVA	Analysis of Variance
CDC	Centers for Disease Control
CI	Confidence Interval
PPM	Parts per million
SPSS	Statistical packages for social sciences
SD	Standard Deviation
WHO	World Health Organization

ABSTRACT

Schistosomiasis is one of the most devastating tropical diseases globally due its major cause of morbidity and mortality, and has been targeted for increased control strategies. Controlling snails through the use of molluscicides especially plant based ones is considered the best choice of controlling the disease. Studies on molluscicidal activity of plants have drawn increasing attention due to reasons such as ecological issues, high cost, and concern over emergence of resistance in snails to synthetic molluscicides. In line with this the present study evaluated the molluscicidal activity of aqueous and methanol extracts of *Datura stramonium* leaves against *Biomphalaria pfeifferi* snails as well as the cercaricidal activity against *Schistosoma mansoni*. Lethal concentration needed to kill 50% and 90% of the snail population of the aqueous and methanol extracts of the plant tested were computed. The L_{C50} value of aqueous extract for 24 and 48 h exposure against *B. pfeifferi* were 108.1 and 87.2 parts per million, respectively, while that of methanol extract were 111.3 and 104.1 ppm, respectively. On the other hand, the respective L_{C90} values of aqueous and methanol extracts for 48 h were 157.2 and 186.6 ppm. The *in vitro* mortality of cercariae exposed to aqueous extract of the leaves of the plant increased with increasing concentration. In conclusion, the aqueous extract of *D. stramonium* has moderate molluscicidal and cercaricidal activities.

Key words: *Datura stramonium*, *Biomphalaria pfeifferi*, aqueous extract, methanol extract, molluscicidal, cercaricidal, Schistosomiasis

1. INTRODUCTION

Schistosomiasis is a chronic disease caused by the platyhelminth worms of the genus *Schistosoma*. These trematodes are intravascular that live in the bloodstream of humans and animals. Schistosomiasis, also referred to as bilharzia, was discovered by Theodore Bilharz, a German physician in Cairo, who first identified the etiological agent *Schistosoma haematobium* in 1851 (Gryseels *et al*., 2006). Schistosomiasis affects millions of people, particularly children whose activities are around freshwater schistosome cercariae in the developing countries of Africa, Asia and Latin America. Although congenital transmission does not occur, exposure and infection can take place soon after birth, depending on an infant's environmental exposure to parasite-infested water. Until recently, the burden of disease from these early exposures was overlooked. However, infants and preschool children (aged 1–5 years) can have active disease and have poor access to deworming treatment because current measures are focused on preventive chemotherapy to school-age children aged 6–15 years (Bustinduy *et al*., 2014).

Schistosomiasis is multifactoral disease, including environmental, behavioral, parasitic, vector and host factors. It continues to be a significant cause of morbidity and mortality (Elbaz and Esmat, 2013). The disease is difficult to recognize at early ages, but later it can produce disability to people at their productive years. Schistosomiasis affects country's health and economy although people in areas of endemicity have light infections with no symptoms. Schistosomiasis is rarely fatal, but strongly linked to diarrhea, pain, fatigue, hemoglobin deficit, under nutrition, and reduced exercise tolerance. Schistosomiasis' effects are not negligible for those who are infected and live in endemic areas where recurring infections are possible (WHO, 2007). Schistosomiasis is a disease of poverty and still survives in poverty-stricken, remote areas where there is little or no safe water or sanitation, and health care is scarce or non-existent (WHO, 2008). Manmade lakes, dams, open irrigation systems and other agro engineering projects have sometimes increased breeding sites of the snail intermediate host and spread of the disease (The Carter Center, 2012).

Schistosomiasis transmission is highly associated with infested water contact activities. When the infected people continue excreting schistosome eggs into local water sources (lakes, rivers and canals) in which human are exposed to water body activities, the infection rate will be very high. Thus, re-infection rates remain high, and are likely to continue, unless the behavior associated with disease transmission does not change (Bruun and Aagaard-Hansen, 2008). As a mainly rural, often occupational disease, schistosomiasis principally affects people who are unable to avoid contact with water, either because of their profession (agriculture, fishing) or because of a lack of a reliable source of safe water for drinking, washing and bathing. Over the past few decades man-made reservoirs and irrigation systems have contributed to the spread of schistosomiasis. As a result of a low level of resistance and intensive water contact when playing and swimming, children are also at high risk of infection. Increased population movements help to spread the disease (Boelee and Madsen, 2006).

Currently twenty two different species of *Schistosoma* (S) are known, of which five are infective to human, namely, *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum, S. japonicum* and *S. mekongi* (Duval *et al*., 2015). The first three species are the most important ones in terms of geographical distribution and number of people infected. The distribution of the different species depends mainly on the ecology of the snail hosts. Schistosome species differ according to their snail intermediate hosts, egg morphology, and final location of the adult worms in the human body (Boelee and Madsen, 2006).

There are two major forms of schistosomiasis –intestinal and urogenital-caused by five species of the parasite. Intestinal schistosomiasis is caused by four species, namely S. *mansoni, S. japonicum, S. mekongi and S. intercalatum. S. mansoni* is the most prevalent species being endemic in 55 countries for instance, Arab peninsula, Egypt, Libya, Sudan, sub-Saharan Africa, Brazil, some Caribbean islands, Suriname and Venzuela. *S. japonicum* is endemic in China, Indonesia and the Philippines while *S. mekongi* prevails in several districts of Cambodia and the Lao Peoples' Democratic Republic and *S. intercalatum* prevails in rain forest areas of Central Africa (Gryseels *et al*., 2006). On the other hand, *S. haematobium* –which is the causative agent of urogenital schistosomiasis – is endemic in 53 countries in Africa and the Middle East (Barakat, 2013).

1.1 Biology of schistosomes and snail intermediate hosts

1.1.1 Human schistosomes and their life cycle

Biologically, the flukes belong to the Phylum Platyhelminthes, Class Trematoda, Subclass Digenea, Family Schistosomatidae, and Genus *Schistosoma*. Schistosomes are strange parasites; many aspects of their morphology, physiology and lifecycle tactics are unique when compared with the other members of the taxon (Cox, 1993). Adult schistosomes are white or grayish worms, threadlike in shape of 7–20 mm in length and 0.2–1.0 mm in width, cylindrical body features with two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs (Gryseels *et al*., 2006). Unlike other trematodes schistosomes are diecious which is, worms have separate sexes. Adults remain in copula during their life span, and live attached by their sucker disks to the endothelium of the veins. The male's body forms a groove or gynaecophoric channel, in which it holds the longer and thinner female. In most schistosomes, female sexual maturity only follows successful pairing with a male worm (Cox, 1993). Schistosomes are facultative anaerobes, deriving energy primarily through degradation of glucose and glycogen. The debris is regurgitated in the host's blood. As permanently embraced couples, the species causing intestinal schistosomiasis live in the mesenteric veins of the gut while *S. haematobium* occupies the vesical veins of the bladder wall (Gwadz and Knirsch, 2005).

The life cycles of all five human schistosome species are similar and involve a snail intermediate host (Fig. 1). Eggs are excreted in faeces and urine and can stay viable for up to 7 days. On contact with water, the egg releases ciliated miracidium, it searches for the intermediate host which is, freshwater snails, guiding by light and chemical stimuli. After penetrating the snail, miracidia starts to multiply asexually into multicellular sporocysts and then into cercariae. After infection and spin around in the water for up to 72 hours, cercariae seeking the skin of a suitable definitive host. When humans come in contact with infested freshwater cercariae get the chance to penetrate the human skin. Upon host location, cercariae attach to and penetrate the host skin via glandular secretions and lose their tails when they penetrate the skin, and they became young schistosomes called schistosomula (Gryslees *et al*., 2006). After spending days in the skin, the parasites burrow through the dermis, penetrate a blood vessel wall, and gain access into the circulatory system. The parasites migrate to the lungs and remain there for several days before

travelling to the liver afterwards; they emerge as male-female worm pairs, and inhabit either the portal or pelvic vessels. This habit of the parasite is exemplified by the four schistosome species except *S. hematobium* which prefers the urinary bladder venous plexus. The female begins to lay eggs within the mesenteric or pelvic vessels, and the lifecycle continues (Olveda *et al*., 2013).

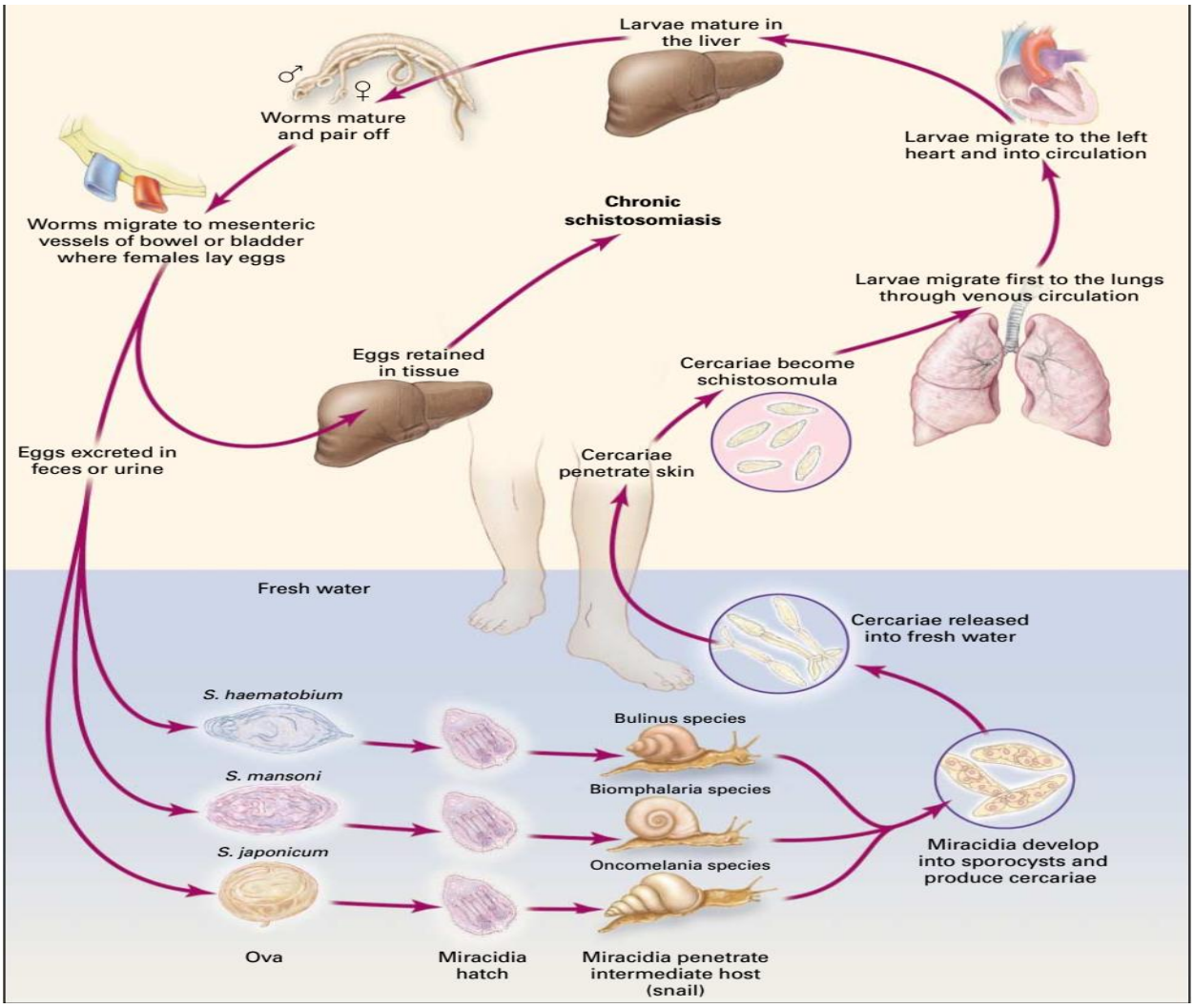

Figure 1.The life cycle of schistosomes (Ross *et al*., 2002)

1.1.2 Snail intermediate hosts

Families of the Pulmonata, the Gastropoda, the Planorbidae and Lymnaeidae are intermediate hosts of trematodes. Freshwater snails in the classes Gastropoda are either aquatic or terrestrial mollusks. Classification is based primarily on morphological characteristics, such as shell and radula, I.e. intermediate snail hosts for schistosomiasis are mainly weak-shelled aquatic animals (Paul, 2009).

Snails are hermaphrodite but cross-fertilization is usual. Eggs are laid in masses in water but *0ncomelania* is an exception, being dioecious and laying eggs out of water. Egg masses are up to 1cm in diameter and contain up to 30 eggs. Eggs hatch at tropical temperatures in 1-2 weeks, and growth to maturity takes 3-6 months. Snails have a big reproductive potential and egg-laying is usually most frequently recorded at the beginning of the rainy season, the stimulus being either, or a combination of change in temperature, the addition of nutrients, dilution of substances in solution and changes in size of habitat. The ecological requirements of snails are difficult to define in view of the combination of biological and a biotic variable to which they may be exposed. It is believed that snails require water at temperatures between 22 and 24^0C, which contains suspended solids, and is calcium-rich (for shell growth) and hence alkaline. Other factors which are measured in studies in snail ecology are water conductivity (to measure salinity), light intensity, pH which varies due to carbon dioxide fluctuation in water, geology of the area providing different ions in solution and rate of flow. Temperature reduction as well as extreme heat reduces breeding. Snail population benefit from the presence of a rich algal microflora and this can support snails in the absence of higher aquatic plants.

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 species involved can be identified by the shape of the outer shell. Simple regional keys are available for the determination of most species. 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*) (WHO, 1988).

In Africa and the South 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* (Gryseels *et al*., 2006).

In Ethiopia, two species of fresh water snails, namely, *Biomphalaria pfeifferi* and *Biomphalaria sudanica* are responsible for the transmission of *S. mansoni* . Unlike, *B. pfeifferi*, which is known

to have a wide geographical distribution, *B. sudanica* has very limited distribution in Ethiopia. Its presence has so far been reported from only three areas in Rift Valley, Ziway and Abaya Lakes and the interface between TikurWuha River and Awassa Lake (Birrie *et al*., 1995). It seems, therefore, that *B. pfeifferi* has ubiquitous distribution, while *B. sudanica* is limited in its distribution. *Bulinus abyssinicus* and *Bulinus africanus* are the only bulinid species found naturally transmitting *S. haematobium* in Ethiopia at lower altitudes ranging from 300 to 700 meters a.s.l., even though, about 10 Bulinus species are expected to occur (Lo *et al*., 1988). From the previous studies it is established that *B. abyssinicus* is the intermediate host for *S. heamtobium* in Awash valley, whereas *B. africanus* transmit the disease in Kurmuk (an area locating near to Sudan) (Kloos *et al*., 1988).

1.2 Global epidemiology of schistosomiasis

The World Health Organization recognizes schistosomiasis as one of the 17 neglected tropical diseases (NTDs), which are mostly persistent and prevalent in people and communities living in poverty and social exclusion (Makaula *et al*., 2014). According to WHO'S estimate schistosomiasis and soil-transmitted helminths represent more than 40% of the global disease burden caused by all tropical diseases, excluding malaria. Schistosomiasis is the third most devastating tropical disease globally (after malaria and other intestinal helminthiases) and is a major cause of morbidity and mortality for developing countries in Africa, South America, the Caribbean, the Middle East, and Asia (Olveda *et al*., 2013). Schistosomiasis is endemic in 77 countries in tropical and subtropical regions; estimates of infected individuals worldwide are 391–597 million; another 779 million people are at risk of being infected and more than 97% of all cases occur in Africa, mainly in sub-Saharan region. Almost 300,000 people die annually from schistosomiasis in Africa and the disability adjusted life years (DALYs) lost as a result of schistosomiasis was estimated to be 70 million. This global burden estimate exceeds that of malaria or tuberculosis, and is almost equivalent to the DALYs lost from HIV/AIDS (Hotez and Fenwick, 2009; El-Ridi and Tallima, 2013). Among the total infection, about 120 million people infected with schistosomiasis are estimated to be symptomatic; 20 million develop severe disease. the highest prevalence estimates and infection intensities are usually found in school-age children, adolescents and young adults as well as in infants and pre-school children (Makaula *et al*., 2014). In African countries south of the Sahara, poverty usually goes hand-in-hand with

poor hygiene and housing, limited access to clean water and improved sanitation, subsistence farming and low educational level all of them exacerbating the risk of acquiring schistosomiasis (Walz *et al*., 2015; WHO, 2008). In this part of the world, prevalence levels, particularly of *S. mansoni*, have increased due to water resources development projects, population increase or displacement, migration and competing priorities in the health sector. The distribution and epidemiology of schistosomiasis vary greatly with geographical, demographic and socioeconomic factors. Within a geographical or political boundary, the prevalence of schistosomiasis can vary among people of different age groups, sex or occupations (Yoon, 1995).

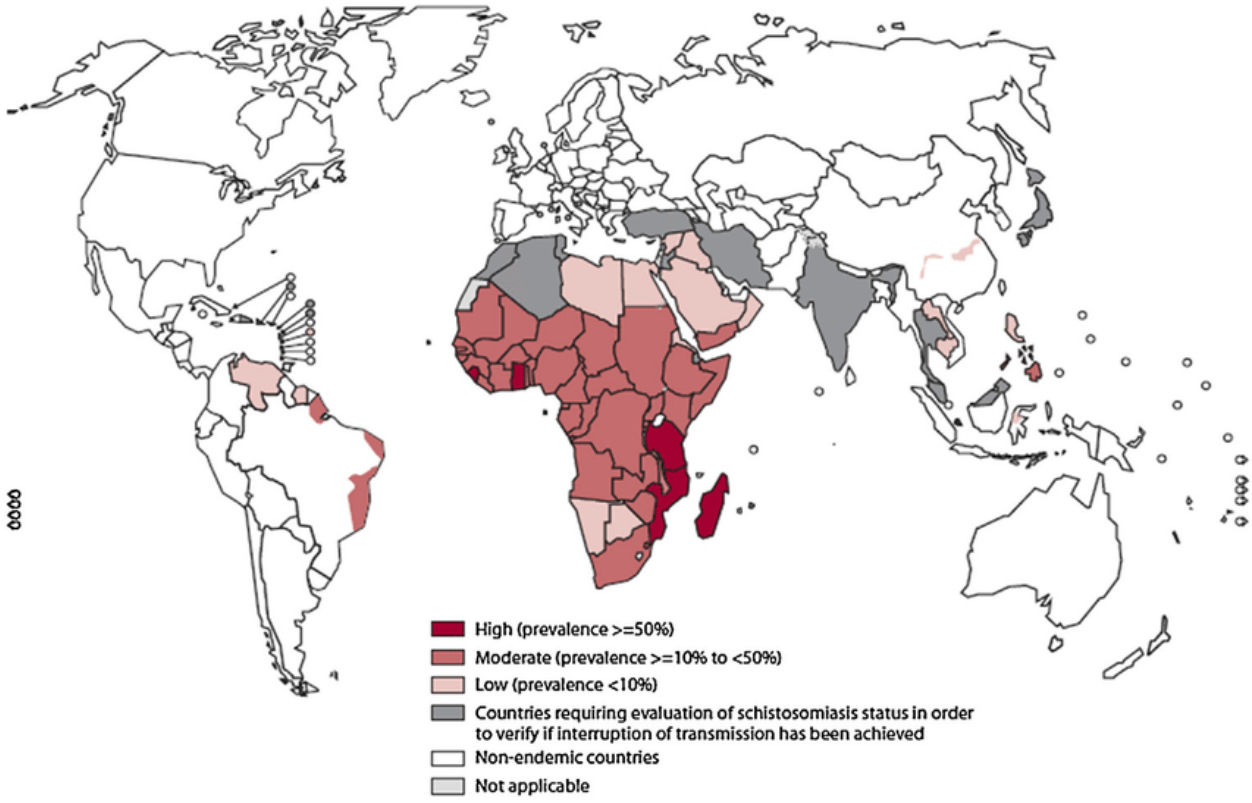

Figure 2 Global distribution of schistosomiasis showing the most at-risk populations within Africa (WHO, 2013).

Schistosomiasis infection is usually acquired in childhood when children tend to spend time swimming or bathing in water containing the larval form of the parasite. Prevalence and intensity of infection increase with age, peaking in the 5 to 14 year age group. Children also suffer the most side effects of the disease, especially poor growth and impaired cognitive development. The disease also contributes to malnutrition and disrupts school attendance. In older people,

there is a drastic decline in intensity of infection but not in the prevalence of the disease (WHO, 2008).

Schistosomiasis, which is generally restricted to the less affluent classes, is related to poor life conditions, to areas lacking basic services, and to lack of information and instruction. In these conditions people become infected through water linked activities, such as work, personal hygiene, laundry, fishing and recreation. The scarcity of latrines enhances transmission probabilities through indiscriminate defecation habits, generally carried out close to water bodies. The disease has been described as a "three factor disease" involving schistosomes, snails and humans, transmission takes place where the ecologies of these three converge in space and time in suitable water bodies (WHO, 2008). Transmission of the disease can take place in almost any type of habitat from large lakes and rivers to small seasonal ponds and streams. Although transmission may be intense in both natural and man-made water bodies, the latter seems particularly important as the human population density is often high near these. Within irrigation schemes transmission is focal and is primarily due to much localized contamination of habitats with human excreta or urine containing schistosome eggs and, also because of the high incidence of human water contact at a few points (Boelee and Madsen, 2006). Transmission of schistosomiasis is the result not only of interplay between humans, snails and parasites, but also of complex demographic, environmental, biological, technological, political, socioeconomic and cultural processes.

Both abiotic and biotic factors contribute to the schistosomiasis transmission cycle and these determine the spatial distribution of the disease. The abiotic factors affecting schistosomiasis transmission include climatic factors such as temperature, rainfall, water body type, water velocity and altitude can all have a significant effect on the schistosome lifecycle and survival of the snail intermediate host (Mas-Coma *et al*., 2009). These factors, however, can affect the prevalence of schistosomiasis indirectly as they affect the breeding and development of the intermediate snail hosts. For instance, the optimal temperature for snail development and survival is around 25^{o}c. Above 30^{o}c, snail mortality increases and thermal death occurs at 40^{o}c. However, snails are less sensitive to low temperatures than schistosome parasites. According to water flow velocity snails are likely to be found in stagnant than rapidly flowing water which is,

they do not tolerate water velocities higher than 0.3m/s (Boeele and madsen 2006). In addition, it has been reported that many biotic factors can affect the prevalence of schistosomiasis such as host sex and age as a result of behaviour, hormonal or genetic reasons (Cox, 1993).

A major man-made factor associated with the rise of schistosomiasis is water development projects, particularly manmade lakes (hydroelectric power) and irrigation schemes (agriculture), which can lead to shifts in snail vector populations (Steinmann *et al*., 2006). On the other hand, water stagnation and weed growing due to inadequate water management sustain the life of the snails to complete the life cycle of schistosomes (Boeele and Madsen, 2006). Schistosomiasis can also be regarded as one of the most sensitive and obvious indicator diseases of environmental change following the establishment of lakes, waterways, dams, irrigation schemes and reclamation projects in many areas of the world. If preventive measures and health services are insufficient or non-existent, infections appear a year or two after the change and the new symptoms are often recognized by the local population (WHO, 2008). As agricultural, hydroelectric and other water resource development projects expand in the endemic countries, transmission of schistosomiasis spreads and the degree of transmission is intensified (Yoon, 1995).

1.2.1 Schistosomiasis in Ethiopia

In Ethiopia, intestinal schistosomiasis caused by *S. mansoni* and urinary schistosomiasis caused by *S. haematobium* have been known to be endemic (Kloos *et al*., 1988). Even though the disease is recorded in all regions of the country and the prevalence is significantly high, but there is no specific policy launched to decrease the disease burden especially in endemic areas (Mesfin *et al*., 2015).The main determinants for the distribution, transmission and spreading of the schistosomiasis in Ethiopia include water temperature, local absence or presence of snail intermediate host, human population movement and water impoundment for irrigation and power (Mitiku *et al*., 2010; Hailu and Yimer 2014). With the construction of dams and expansion of irrigation based agriculture schemes and population movements, the incidence of schistosomiasis infection is on the rise in Ethiopia, affecting a substantial portion of the productive force, that in turn, affects the economy (Erko *et al*., 1996).

Schistosoma mansoni is widely distributed in the country and is found mainly at altitudes between 1200-1900 m above sea level. Two species of the genus Biomphalaria, *B. sudanica* and B. pfeifferi, are known to transmit *S. mansoni* in Ethiopia. Unlike, *B. pfeifferi*, which is known to have a wide geographical distribution, *B. sudanica* has very limited distribution in Ethiopia. Its presence has so far been reported from only three areas in the Rift Valley, Ziway and Abaya Lakes and the interface between TikurWuha River and Awassa Lake. It seems, therefore, that *B. pfeifferi* has ubiquitous distribution, while *B. sudanica* is limited in its distribution (Birrie *et al*., 1995).

Bulinus abyssinicus and *Bu. africanus* are the only bulinid species found naturally transmitting *S. haematobium* in Ethiopia, even though about 10 Bulinus species are expected to occur (Lo *et al*., 1988). From the previous studies it is established that *Bu. abyssinicus* is the intermediate host for *S. heamatobium* in Awash valley, whereas *Bu. africanus* transmit the disease in Kurmuk (an area located near to Sudan) (Kloos *et al*., 1988).

Generally new transmission foci are being discovered in different parts of the country over time. The reasons for the spreading of the disease to new areas seem to be the establishment of water resource development projects such as dams and irrigation and migration of people from endemic areas to previously non-endemic ones. Currently, the government of Ethiopia has given more attention to the development of dams for hydroelectric power such as Gilgel Gibe hydroelectric dam and irrigation based agriculture schemes. These developments inevitably lead to the spread of schistosomiasis unless careful planning is made and timely interventions are put in place in areas where transmission has already been established (Erko *et al*., 1996).

In Ethiopia, the endemicity of both intestinal and urinary schistosomiasis has long been established. Although there has been no recent national survey, estimates made in 1980s document that the number of people infected and at risk of infection with *S mansoni* were 2.5 and 18 million, respectively, whereas the number of people at risk of *S. haematobium* infection would be 4 million (Erko *et al.*, 2012).

1.3 Control of schistosomiasis

The control of the disease in known foci of transmission is possible by using one or a combination of the following measures: improved detection and treatment of sick people; improvement of sanitary facilities for safe and acceptable disposal of human excreta; provision of safe drinking-water; reduction of contact with contaminated water; and snail control (Gryseels, 2006). With the introduction of new and safer drugs for the treatment of schistosomiasis, and in many places, improvements in water supply and sanitation facilities, snail control is perhaps employed less often as a means of combating the disease (WHO, 1990). However, it remains an important and effective measure as part of the overall control strategy, to achieve sustained reduction in transmission by reducing exposure to cercariae infested water (WHO, 2017). Snail control measures can be classified into three categories; these are, environmental/ engineering, chemical and biological control.

Biological control of snail population is cheaper and offers environmentally acceptable alternatives over the other control measures. And it is based on the use of organisms to attack the snails that is, predators, competitor snails and sterilization of males to prevent reproduction, (Negron-Aponte and Jobin, 1979). The reduction of snail population by introducing sterile males, however, has not shown promise. An ideal method of biological control of disease vector should be based on competitive displacement by introduction of a non-vector species with similar ecological requirement as vector but with higher biological potential and adaptability. In the case of schistosomiasis, a snail species with higher growth rate, better utilization of food resources, longer life span, harmless to surrounding crops will be preferred (Frandsen and Madsen, 1979).

Despite more than half a century of international research on schistosomiasis control, this disease is still a public health menace in many developing countries, especially in Africa, Asia and South America. Chemotherapy and transmission reduction via intermediate host snail eradication are the two main tools in the control of schistosomiasis. Control of the intermediate snail host is still considered the most important means of schistosomiasis control where the water volume per head of human population at risk of infection is small (Boelee and madsen, 2006). However, safe

and effective drugs are now available for the treatment of schistosomiasis. Schistosomicides such as antimonials were introduced as the drugs of choice, and they continued to be used as such until the early 1960s. However, the severe side-effects of the antimonials made their application difficult and adversely affected their use in large-scale chemotherapy campaign. The antimonials were, therefore, replaced by hycanthone and lucanthone. These drugs also produced side-effects such as hepatotoxicity and gastrointestinal disturbances, and were consequently withdrawn. It was then decided that the alternative was to produce synthetic drugs that could be administered orally. Niridazole, oxamniquine and metrifonate were consequently introduced as schistosomicidal agents, while drugs like oltipraz and amoscanate were still at clinical trial phase. Therapeutic doses of drugs like hycanthone, niridazole and amoscanate were found to cause many major side-effects and were, therefore, considered unsafe (Dannso-appaiah *et al*., 2013).

The introduction of relatively safe, effective, broad spectrum oral antheminthic agent, praziquantel, constituted a significant landmark in the chemotherapeutic control of schistosomiasis. To date, praziquantel is the drug of choice for infections caused by all species of Schistosoma (Bergquist *et al*., 2017). Oxamniquine has also been used effectively in treating infections caused by S. mansoni in some cases where praziquantel is less effective. Studies have also shown that metrifonate is as effective as praziquantel in treating S. *haematobium* and *S. mansoni* infections. Because praziquantel is effective even in treating advanced hepatosplenic schistosomiasis, with fewer side-effects, the drug is currently the drug of choice for the treatment of any kind of schistosomiasis. Its only limitation is the cost which restricts its use in many developing countries. With the introduction of praziquantel, there has been a shift away from transmission control to the control of severe morbidity. However, despite the effectiveness of praziquantel, there is a high re-infectivity rate in endemic areas even after mass treatment. Together with chemotherapy, molluscicides are widely considered to be an important tool of schistosomiasis control that can be used at selected transmission sites to achieve quick results. Measures such as improved sanitation and health education are likely to take longer time to affect the disease spread and prevalence (Olveda *et al*., 2013).

Molluscicides are chemical substances or biocides developed specifically for destroying mollusks. The mode of action of many of these compounds is stress to the water balance system of mollusk species. Stress on the water balance system alone can cause death of mollusks additionally, the reduction of normal water flow in the mollusk body results in other disturbances in metabolism or physiological function, which will often lead to organism death. Other products cause toxic reactions to occur at gill membranes (Sprecher and Getsinger 2000).

There are a series of compounds with molluscicides action that are used in the control of schistosomiasis. Among these, pentachlorophenol (NAPCP) was identified as being promising however; this was subsequently discarded due to its toxicity for other organisms and is only used in china. Compounds containing lead and tin are highly active and but are also toxic for various organisms. In Japan, Yurimin (3, 5-dibromo-4 hydroxy -4-nitroazobenzene replaced NAPCP but its fabrication was stopped after only a few years of use. The same happened with Frescon (N-tritylmorpholin), one of the most active molluscicides for adult snails but which was not active against eggs. Copper was also used although in the presence of organic material, cleaved PH and certain solid in the water it lost activity. In Japan compound named B-2 (sodium 25, dichloro-4-bromophenol) was tested against the amphibian snails *oncomelania nosophora* (Coelho and Caldeira, 2016).

Niclosamide (BayluscideÒ, Bayer, Germany) is the only viable molluscicide in terms of efficacy, commercial availability, and complete evaluation which is recommended by WHO (MC Cullogh, 1992). It was initially developed as a sea lamprey larvicide, but had molluscicidal activity (sprecher and Getsinger *et al.*, 2002). Later on it has been used as a molluscicide since 1960 and is still a molluscicide of choice. It is highly active against all stages of snails' life cycle. Furthermore, niclosamide immobilize *S. mansoni* larvae within minutes of exposure at concentrations below 1 mg/L. Although highly effective, the relatively high cost of this compound, the expenses required for applying, and the long-term impact of synthetic chemicals on the environment limit its use (Boelee and Madsen, 2006). Moreover, the use of indigenous rather than imported materials is desirable, especially as strategies for schistosomiasis control programmes should be based on long-term operations (Ali, 2011).

1.3.1 Plants with molluscicidal and anti schistosomicidal activities

Medicinal plants have been one of the most ancient forms of medicinal practice of humankind, having been used in the treatment of many diseases. Based on information gathered along centuries, popular observations on the use and efficiency of medicinal plants have contributed significantly to the disclosure of their therapeutic properties (WHO, 2003). It is estimated that only 17% of all the plants in the world have been studied in one way or another regarding their medicinal use, in most cases, no deep analyses were carried out on their phytochemical and pharmacological properties. Many species are used empirically, with no scientific support on their efficiency and safety. These data show the enormous potential of plants for the discovery of new phytotherapeutic drugs. More than 1000 plant species have been screened for molluscicidal activity (El-Bolkiny *et al*., 1997)

The high cost, increasing concern over snail resistance and their toxicity to non-target organisms of synthetic molluscicides, has drawn much attention during recent years in renewed interest in the use of plant molluscicides (Mantawy and Mahmoud, 2002). These plant molluscicides can solve such problems and many plants have been screened for their intrinsic molluscicidal properties in an attempt to find an affordable alternative to niclosamide. However, despite the discovery of several promising plant molluscicides, none of them has yet been used in schistosomiasis control campaigns. Plants with molluscicidal activity may be exploited to contribute to schistosomiasis control, especially if they are already grown locally for other purposes. The use of plants with molluscicidal properties is a simple, inexpensive and appropriate technology for the snail control. Since the discovery of active saponins in the berries of *Phytolacca dodecandra* (L'Herit), naturally occurring molluscicides are receiving considerable attention. To date several types of plants have been tested for their molluscicidal and antischistomicidal activities.

Rug and Ruppel (2000) studied the toxic activity of methanolic extract of *Jatropha curcas* L. (Euphorbieacea) against snails transmitting *S. mansoni* and *S. haematobium*. It showed the highest toxicity with Lc_{100} values of 25 ppm for *B. glabrata* and 1ppm for *Bu. truncatus* and *Bu. natalesis*.

Dos santos *et al* (2006) evaluated the latex of *Euphorbia conspicua* (Euphorbiaceae) for its molluscicidal and cercariacidal activities. It exhibited high activities against adult snails with L_{C90} values of 4.87µg/mL and showed a lethal effect to the cercaria of *S.mansoni* at concentrations of 100µg/mL.

Hyptis suaveolens (Labiacae) was reported to have strong molluscicidal activity on *Bu. globosus* snails. The ethanolic extract of the plant which has been tested on different developmental stages which are one week old juveniles, three to four week old immature snails, adults and egg masses. And the LC50 values for the different developmental stages were 0.614, 0.196, 0.161 and 0.077 ppm, respectively, and that of L_{C90} values were 0.796, 0.353, 0.274 and 0.467 ppm, respectively. The use of plant extract of *H. suaveolens* gave impressive molluscicidal effects on the various stages of the snail intermediate host of *S. haematobium* (*Bu. globosus*) (Salawu and Odaibo, 2011).

Another study conducted by El-Sherbini *et al*., (2009) suggested that different solvent extracts of the plants *Solanum nigrum*, *Solanum sinaicum*, and *Solanum villosum* were found to be having molluscicial properties against snail *B. alexandrina*. Ethanol extract of *S. nigrum* was recorded as the highest mortality rate, followed by *S. sinaicum* and *S. villosum*.

Alstonia scholaris (Apocynaceae) the common medicinal plant in India has been studied for its toxic activity against harmful snail populations of *Lymnaea acuminata* and *Indoplanorbis exustus* and petrol extract of the stem bark was found highly toxic to both the snails, its sub lethal doses have ovicidal activity and also reduced the survival rate of hatchings of the snails (Chauhan and singh, 2014).

A study from Cameroon by Ndamukong *et al* (2006) investigated methanol extact of *Nicotiana tabacum* plant and found it best for its molluscicidal activity on *Bulinus* species. The results revealed that *N. tabacum* was the most toxic, with twenty four hours L_{C50}, L_{C90} and L_{C100} of 48.7ppm, 91.7ppm and 800ppm respectively for *Bu. truncatus*; 35.9ppm, 85.2ppm and 692ppm respectively for *B. camerunensis*.

From Chinese medicinal plants which have been studied for their molluscicidal properties Wang *et al.* (2006) has found that three plants *Nerium indicum* Mill, *Pterocarya stenoptera* DC, and *Rumex japonicum* Houtt for their strong molluscicidal potency on *Oncomelania hupensis* snails, and that of *N. indicum* Mill are stronger than those of the extracts of *P. stenoptera* DC and *R. japonicum* Houtt. Especially, the L_{D50} value of the water extract of *N. indicum* Mill is as low as 13.2 mg/L. although the molluscicidal activities of these plant extracts are lower than those of the synthetic molluscicides (the L_{D50} of niclosamide is around 0.1 mg/L).

Another study by Jean-Jacques *et al.* (2014) tested *Solanum szybrilifolium* (Solanaceae) for its cercariacidal activity against *S. mansoni* cercariae. The concentrations needed to kill all cercariae (L_{C100}) within 10 min of exposure were 0.01 mg/mL. The plant had high level of cercaricidal activity against free swimming cercariae and it seems to be ecologically safe, since it is known to have very low toxicity to fish.

The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants, of which about 12% are endemic. Plants have been used as a source of medicine in Ethiopia from time immemorial to treat different ailments. In Ethiopia, toxic plants, berries of *Phytolacca dodecandra* (Endod in Amharic) are being commonly used for washing clothes and to control fresh water snails. Since the discovery of Endod in 1965, there have been extensive studies on the chemistry, toxicity, and epidemiology of Lemma toxins and its cultivation. Endod-based *S. mansoni* control project was implemented in Ethiopia between 1994 and 1999. The aim was to develop an effective, cheap and sustainable method of controlling schistosomiasis. Endod is a proven botanical pesticide with L_{C50} of 1.85 ppm to control schistosomiasis transmitting snails (Karunamoorthi *et al*., 2008).

In Ethiopia, screening of local plants for molluscidal activity was limited to a few plants, despite the existence of variety of traditionally claimed medicinal plants. Of special interest in this regard is the locally grown plant *P. dodeccandra*. Berries of *P. dodeccandra* have been extensively studied and proved effective against molluscs in Ethiopia and elsewhere. Dried ground up berries of endod suspended in water at 15-30 ppm has a potent molluscicidal activity that kills *Biomphalaria* snails in 24 h of exposure. Water extracts of powdered endod berries

after deffating with benzene were more active in killing snails with 100% mortality of 2 ppm after 24 h exposure (Lemma, 1970). However, it's unnecessary effect on non- target organisms such as fish and amphibians and limited growth and cultivation area are cited to hinder its wider application (Woldemichael *et al*., 2006). Investigations into different medicinal species, is thus considered necessary in increasing the means of finding better alternative plants with molluscicidal activities.

A study by Molla *et al*. (2013) has also investigated the molluscicidal and cercariacidal effect of *Balanities aegyptica* fruit against *B. pfeifferi* and *S. mansoni* cercariae. The result showed that *B. egyptica* is active against *B. pfeifferi* with Lc_{50} value of 80.33 mg/L. He has also recorded 100% mortality of *S. mansoni* cercariae at 35 mg/L of 2-h exposure to the plant extract. Similarly Kiros *et al*. (2014) has also investigated that the molluscicidal and cercariacidal effect of *Glinus lotoides* fruit against *B. pfeifferi* and *S. mansoni* cercariae. The result showed that *G. lotoides* is active against *B. pfeifferi* with $L_{C\ 50}$ value for the aqueous and ethyl acetate was 47.1 and 66.1 mg/L, respectively. The *in vitro* cercariacidal activity was determined after 2 h of exposure to the aqueous plant extract. It was found out that the $L_{C50}$and L_{C90} values were 18.7 and 41.7 mg/L, respectively.

Datura stramonium L., a wild-growing plant of the Solanaceae family, is widely distributed and easily accessible. The species has originated in Tropical North America, is now a cosmopolitan weed. It occurs in most Ethiopian regions, and also in Eritrea, Sudan, Somalia, and throughout tropical Africa, Europe and parts of Asia (Dagne, 2011). Traditionally, *D. stramonium* has been used for mystic and religious purposes, and as an herbal medicine with narcotic effects or to treat asthma. The seed of *D. stramonium* is smoked to achieve hallucinogenic experiences as well. *D. stramonium* is toxic when consumed improperly. Accidental poisoning of humans and animals who consumed food sources contaminated with *D. stramonium* has been reported (Gair and Subedi, 2013).

Traditionally, when the leaves of *D. stramonium* are mixed with mustard oil and used in skin disorders. Juice of flower petals is used in ear pain and seeds are used as purgative, in cough, fever and asthma. Seeds are smoked due to its narcotic action. Leaf paste and extract are

externally used for injuries, wounds, bleedings and pains. Seeds in small quantity are used for asthma and tonsil problems. The extract of leaves is also used for baldness (Khan and Khatoon, 2008). Leaves are used externally for management of pains. The plant is frequently used as antiparasitics and repellents. Fruit oil is used for body pain. Leaf or whole plant is antiinflammatory and antispasmodic. Green leaves are applied for the softening of boils. Juice of the fruit is applied to scalp for falling hairs and as antidandruff. Juice of the flower of the plant is used in earache. Paste of leaves is topically applied for skin diseases. Dried leaves and seeds are used as anticholinergic and sedative. Seeds are used to make somebody unconscious (Rahmatullah *et al*., 2009).

In pharmacological studies conducted on *D. stramonium*, aqueous leaf extract of the plant showed efficient nematicidal activity on *Meloidogyne incognita* (Sharma and Trivedi, 2002). The methanol seed extract of the plant showed growth inhibitory effect on promastigotes and amastigotes of *Leishmania major*. Inhibitory concentrations, (I_{C50}) for promastigote assay was 155.15µg/mL. The extracts also reduced the number of amastigotes in macrophage cells (Nikmehr *et al*., 2014). In addition to this, the plant has potential for its antimicrobial activity for various bacteria and fungi infections. Its extracts showed significant antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusarium* species (Reddy, 2009; Girmay, 2015).

D. stramonium contains sixty-four tropane alkaloids. Two new tropane alkaloids, 3-phenylacetoxy-6, 7-epoxynortropane and 7- hydroxyapoatropine. The alkaloids scopoline, 3-(hydroxyacetoxy) tropane, 3-hydroxy-6-(2-methylbutyryloxy) tropane, 3a-tigloyloxy-6-hydroxytropane,3,7-dihydroxy-6-tigloyloxytropane,3-tigloyloxy-6 propionyloxytropane, 3-phenylacetoxy-6,7- epoxytropane, 3-phenylacetoxy-6-hydroxytropane,aponorscopolamine, 3a,6a-ditigloyloxytropane and 7- hydroxyhyoscyamine are reported for the first time for this species (Sayyed and Shah, 2014).

The reason for testing this plant for its molluscicidal activity is due to the presence of alkaloids, flavonoides and other chemical constituents (Pereira Filho *et al*., 2014). Molluscicidal activity of this herb has not previously been investigated. The present study aimed at evaluating the

molluscicidal and cercaricidal activity of aqueous and methanol extracts of *D. stramonium* leaf on *B. pfeifferi* and *S. mansoni*.

1.4 Significance of the Study

To date, several studies of molluscicidal and cercaricidal activities on different plant species have been conducted. And there is no study conducted on this plant so the current study dealt with evaluation of the potential molluscicidal activities of the plant *D. stramonium* against snail intermediate hosts, *B. pfiefferi*, and cercaricidal activity against human schistosome cercariae in Ethiopia. The results of this study would provide information on the use of this plant as molluscicidal and cercaricidal agent.

2. OBJECTIVES

2.1 General objective

To evaluate aqueous and methanol crude extracts of the plant *D. stramonium* leaves for its molluscicidal effect against schistosome snail intermediate hosts and cercaricidal activity.

2.2 Specific objectives

To determine the toxicity of crude extracts of the leaves of *D. stramonim* against *B. pfeifferi* and *S. mansoni* cercariae;

To determine the Lc_{50} and Lc_{90} values of *D. stramonim* leaf extracts against *B. pfeifferi* and *S. mansoni* cercariae;

3. HYPOTHESIS

The crude extracts of the plant *D. stramonium* possess molluscicidal activity against *B. pfeifferi* snail intermediate host, as well as cercaricidal property against *S. mansoni* cercariae because of its possession of bioactive substances that makes susceptible to both *B. pfeifferi* snail and *S. mansoni* cercariae.

4. MATERIALS AND METHODS

4.1 Plant material collection and authentication

Leaves of the plant *D. stramonium* were collected from their natural habitat found in Gondar town on roadside around Samuna-ber sub city. The plant materials were identified by a botanist at the University of Gondar. Voucher specimens were deposited at University of Gondar herbarium.

4.1.1 Description of the plant materials

D. stramonium is a wild weed belonging to family Solanaceae locally known as "Astefaris" in Amharic. It is a large and coarse annual herb, branching freely, of up to one meter high, or even two in rich soil. The root is long, thick and whitish, giving off many fibers. The stem is stout, erect, leafy and smooth, a pale yellowish green in color. Leaves are large and angular, uneven at the base, with a wavy and coarsely-toothed margin, and branching veins very plainly developed. Flowers are large and 8-10cm long, white or violet, growing singly on short stems springing from the axis of the leaves or at the forking of branches. The corolla, folded and only half opened, is funnel-shaped, of a pure white, with six prominent ribs. Flowers are succeeded by large, egg shaped seed capsules of a green color, and covered with numerous sharp spines (Fig. 3). When ripe, this seed-vessel opens at the top, throwing back four valve-like forms, leaving a long, central structure upon which numerous rough, dark-brown seeds can be found (Gair and Subedi, 2013; Dagne, 2011).

Figure 3. Photographs of ***Datura stramonium*** **plant**

4.2 Collection and maintenance of snails

The snails used in this study were B. *pfeifferi* and were collected from their natural habitats, from Shinta River in Gondar, which is located at729 Km in northwest of Addis Ababa. Uniform sized (8-12mm) *B. pfeifferi* snails were collected using dip-net scoop. Collected snails were brought to University of Gondar laboratory using open plastic buckets which contained some sand, weeds and water.

Snails were cleaned from debris attached to them and screened for any trematode infection. They were examined for natural infection according to Christensen and Frandsen (1985). Snail was placed in shedding vials, half filled with aged water. The vials (each with a snail) were exposed to the day light and left for one hour to allow cercariae to emerge. Snails that did not show trematode infections were then transferred into plastic bowls and left for 72 h to acclimatize the laboratory condition. The snails were maintained in aquaria with aged water, air circulation, and aquatic plants. Boiled and dried lettuce leaves (*Lactuca sativa*) were used as a source food for the snails (Otarigho and Morenikeji, 2012). Water was changed three times a week, and dead snails were removed from the aquaria as soon as possible to avoid contamination.

4.3 Laboratory studies

4.3.1 Preparation and extraction of the plant material

The leaves of *D. stramonium* were washed using tap water, and air dried at room temperature for a week. After completely dried, the leaves were ground into powder with the help of electric blender (M 20, KIKA^{2}WERKE, Germany). Dried and powdered plant material was sieved using 250 microns to find fine material. The powder was kept in a screw capped glass container until subsequent use in the extraction process. Successive extraction of this plant material was made using two solvents, water and methanol. Water extract was prepared by soaking 1g of the dried plant powder in 250ml of aged water with shaking for 24 h with (AMBALA CANTT, India made shaker) at room temperature. The solution was being shaken at 160 rpm. After shaking, the unfiltered suspension was directly used to prepare the stock solution (Abdalla *et al*., 2011). On the other hand, methanol extract was prepared by soaking 50 g of the dried plant powder in 250 ml of methanol. The solution was extracted by shaking for 72 h at 160 rpm under room temperature. The extracted material was then concentrated to dryness under reduced pressure

using UK made RE 200, BIBBYSTERIN Rota vapor at 45^{o}C. The crude extract obtained was then kept at -20ºC until the subsequent use in the bioassay (Salawu and Odaibo, 2011).

4.3.2. Preparation of the stock solution and serial dilution

For aqueous extract, the plant material soaked for 24 h was directly used to prepare the stock solution by adjusting to 1000 ml using aged water. For the methanol, stock solution was prepared by dissolving 1 g of a dried alcoholic extract and then made up to 1000 ppm concentration by adding a suitable volume of aged water. For each extract, further serial dilutions were made from the above stock solutions to which snails were exposed. Once the extent of the toxicity range was determined, several convenient concentrations of the stock solution were prepared to give mortalities between 0 to 100%. Serial dilutions for water extract were prepared in a final concentration of 0, 25, 50, 75, 100, 125, 150, 175, and 200 ppm. Similarly, the final concentrations for methanol extract were 0, 25, 50, 75, 100, 125, 150, 175, 200 and 225 ppm. In both cases the series of concentrations were prepared in a final volume of 1000 ml aged water. To achieve the desired concentration, appropriate volume was taken from the stock solution and then completed to 1000ml.

Dilution procedure: Serial dilutions for the aqueous and methanol extract were prepared from the stock solution using the simple dilution procedure that reads as follows.

$C_1V_1 = C_2V_2$; Where: C_1 = is the initial concentration (stock solution)

V_1= the initial volume

V_2= the final volume required

C_2= the final concentration required

4.3.3 Molluscicidal potency test of the aqueous and methanol extracts of *D. stramonium* leaves

The molluscicidal potency test of *D. stramonium* crude extract against *B. pfeifferi* was performed according to the established procedure (WHO, 1983). A group of ten snails were exposed per test to different concentrations of aqueous (25-200 ppm) and methanol (25-225 ppm) extracts. Snails were challenged by exposing to the extracts for 24 h under room temperature (25-27^{o}c) and were kept under normal diurnal lightening. After 24 h, the suspension was decanted; snails were rinsed

twice and transferred to a new recipient filled with aged water. The snails were then held under observation for another 24 h. A group of ten snails were run in aged water under the same experimental conditions as a control. The snails were not fed during the course of the experiment, it had been observed that snails preserved under a good laboratory condition can live up to 5days or more without food, provided other environmental conditions are constant (Adetunji and Salawu, 2010). Mortality rate was recorded after every 24 up to 48 h. Toxicity was expressed as Lc_{50} and Lc_{90} corresponding to concentrations that killed 50% and 90% of the tested snails, respectively. The Lc_{50} and Lc_{90} values were determined for both 24 and 48 hrs exposure (Changbunjong *et al*., 2010; Eissa *et al*., 2011). Snails were considered dead if they did not move and were either retracted well into or hanging out of the shell, with discolored body. Mortality was also assessed by probing the snails with a blunt wooden probe to elicit typical withdrawal movements. They were proved dead if they failed to show any response to mechanical stimulation with a blunt elongated wooden object (Changbunjong *et al*., 2010; Singaba *et al*., 2006). Dead snails were removed as soon as possible to avoid contamination.

4.3.4 Cercariacidal potency test of the aqueous extract of *D. stramonium* leaves

4.3.4.1Preparation of cercarial suspension

Naturally infected *B. pfeifferi* snails were obtained from field in Shinta River which is located at 729km northwest of Addis Ababa. Snails were individually placed in a Petri dish containing 5 ml of water followed by exposure to artificial light for 2 h. Emerging cercariae were checked using dissecting microscope. The cercariae shed from the snails were then pooled into a glass beaker and mixed well to have uniform distribution. After that, the number of cercariae in 100µl was counted using replicate samples and the average was used (Al-Zanbagi and Abuljadayel, 2005).

4.3.4.2 *In vitro* cercariacidal activity test of the aqueous and methanol leave extract of *D. stramonium* against *S. mansoni* cercariae

A series of concentrations containing 20, 25, 35, 40, 60 and 80 ppm were prepared in Petri dishes. An average of twenty freshly shed cercariae was transferred into each Petri dish using micro-pipette. The same number of cercariae was placed in Petri dishes containing aged water as a control. Each dilution, as well as control group, was tested in duplicate. The cercariae were

observed under a dissecting microscope for survival and mortality at a successive interval of 30min, 1h, 1 and half hour and 2h. Snails were considered to be dead, when they stopped movement, sinking down and detach their tail (Eissa *et al*., 2011). At the end of each experiment, iodine was added for clarity in counting of the total number of cercariae as a confirmation of accuracy of the counting procedure. The Lc_{50} and Lc_{90} values of aqueous extract of *D. stramonium* leaves on *S. mansoni* cercariae after 2h exposure were determined.

4 .4 Data Analysis

The experimental data was subjected to statistical analysis to derive mean and standard deviation. The significant difference in concentration of the plant extract was confirmed by one way analysis of variance (ANOVA). Further individual mean significant difference was calculated by Tukey-HSD post hoc test by using SPSS software version 20 and significance difference in exposure period was confirmed by paired sample t-test. The mean percentage snail mortality was subjected to probit analysis for L_{C50} and L_{C90} with 95% upper and lower confidence (LCL-UCL) limits (SPSS, 2010).

5. RESULTS

5.1 The molluscicidal and cercaricidal potency of aqueous and methanol leaf extract of *D. stramonium*

The molluscicidal activity of aqueous and methanol extracts of the leaves of *D. stramonium* against adult *B. pfeifferi* and as well as the cercariacidal activity against *S. mansoni* cercariae were investigated. Aqueous and methanol extracts of *D. stramonium* leaf showed moderate molluscicidal activity against *B. pfeifferi*. It was observed that snails in the untreated water (control), first retracted their heads and feet into the shells, but 30 minutes later they resumed normal behavior, i.e, moving with their foot extended outside of the shell. After exposure to the molluscicide, after some minutes the snails was shown to withdraw into their shells depending on the extract concentrations. After 24 h, the toxic effect of the active plant extracts became evident in the test snails. There was either a partial retraction in the partially dead snail or no retraction in the dead snails. Crawling of the snails out of the container containing the tested solution was also observed some hour later after exposure.

Snails in the untreated container (controls), withdrew into their shell but after a while they became active and continued moving around the container with its foot extended. When a mechanical stimulus was applied to the foot sole, the snail immediately retracted into their shells. The behavior shown by the snails agrees with those reported by El-Sherbini *et al*. (2009). Immediately after the application of the extract, or after some exposure time, depending on the extracts concentrations, the snails withdrew into their shells, but they started crawling out of the water and cluster together most staying at the water-air interface with their shell partially immersed in the water and this was similar with report by (Ahmed *et al*., 2014). After 24h, the toxic effects of the active plant extract became evident in the test snails. Partially dead snails retracted partially into their shells (withdrawal response) or no retraction in the dead snails to the mechanical stimulation of the foot-sole.

The extracts caused concentration dependent mortality of *B. pfeifferi* from 0 - 100% mortality in concentrations of (25-225ppm). The lethal concentration (Lc_{50} and Lc_{90}) values revealed that the aqueous extract has higher molluscicidal activity than methanol extract in both 24 and 48 h exposure time. Snail mortality was not recorded for the aqueous and methanol extract below 25

ppm. In contrast, 100% mortality was obtained at 200 ppm after 24 h contact, while after 48 h the concentration that produced 100% mortality was reduced to175 ppm for aqueous extract. On the other hand, 100% mortality of the same extract was obtained at 225 ppm after 24 h exposure and 200 ppm after recovery period.

For the *in vitro* cercaricidal activity, aqueous extract of the plant showed increasing activity with increasing concentration.

5.1.1 The molluscicidal potency of aqueous and methanol extracts of *D. stramonium* against *B. pfeifferi* snails

The molluscicidal activities of *D. stramonium* extract with aqueous and methanol at different concentrations (25-225 ppm) after 24 and 48 h exposure are presented in Tables 1 to 5. The mortality caused by these extracts was concentration dependent. Change in concentration was significantly associated with snail mortality rate, which is ($p<0.05$) as shown on table 1 (ANOVA). However, change in exposure time was not significantly associated with snail mortality rate, which is ($p>0.05$) as shown in table 2 (paired sample test). At 200 ppm concentration the aqueous extract caused 100 % mortality of adult *B. pfeifferi* for the 24 h exposure (Table 3). After recovery period (48 hour exposure) the peak molluscicidal activity of the aqueous extract was reduced to 175 ppm (Table 4). The methanol extract of *D. stramonium* leaf after 24 h post-exposure caused 100 % mortality of *B. pfeifferi* snails at 225 ppm (Table 5). However, the mortality of the same extract was reached 100% at 200 ppm, after recovery period of 48 h (Table 6).

The 24 and 48-hour- Lc_{50} and Lc_{90} values of aqueous extract of *D. stramonium* were higher than that of methanol (Tables 3-6). The 24 h Lc_{50} and Lc_{90} values for the aqueous extract were 108.1, 183.5 ppm, respectively. After recovery period, the Lc_{50} and Lc_{90} values of the same extract were reduced to 87.2, 157.2 ppm, respectively (Table 4). Table 5 on the other hand shows the 24 h Lc_{50} and Lc_{90} values of methanol extract to be 111.3 and 205.0 ppm, respectively. After 48 h of exposure, the Lc_{50} and Lc_{90} values were found to be 104.1 and 186.6 ppm, respectively (Table 6).

Table 1: Mean percentage mortality of adult *B. pfeifferi* snails exposed to various concentrations of aqueous and methanol extracts after 24 and 48h exposure.

Concentration (ppm)	Aqueous extract		Methanol extract	
	24 h	48 h	24 h	48 h
0	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}
25	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}
50	20 ± 0.0^{ab}	20 ± 0.0^{ab}	10 ± 14.1^{ab}	10 ± 14.1^{ab}
75	30 ± 14.1^{abc}	40 ± 0.0^{bc}	20 ± 0.0^{abc}	30 ± 14.1^{abc}
100	40 ± 0.0^{bc}	50 ± 14.1^{c}	40 ± 0.0^{bcd}	40 ± 0.0^{bcd}
125	50 ± 14.1^{bc}	60 ± 0.0^{c}	50 ± 14.1^{cd}	50 ± 14.1^{cd}
150	60 ± 0.0^{cd}	90 ± 14.1^{d}	$60 \pm 0.0d^{e}$	70 ± 14.1^{de}
175	90 ± 14.1^{de}	100 ± 0.0^{d}	90 ± 14.1^{ef}	90 ± 14.1^{e}
200	100 ± 0.0^{e}	100 ± 0.0^{d}	90 ± 14.1^{ef}	100 ± 0.0^{e}
225			100 ± 0.0^{f}	100 ± 0.0^{e}
F-value	38.3	71.4	36.8	31.3
P-value	<0.001*	<0.001*	<0.001*	<0.001*

*Significant
Different alphabets are statistically significant

Table 2. Effect of exposure time change on molluscicidal activity of aqueous and methanol leaf extracts of *D. stramonium* (Paired *t*-test).

Concentration (ppm)	Aqueous extract				Methanol extract			
	24 h	48 h	*t*-value	P-value	24 h	48 h	*t*-value	P-value
0	0 ± 0.0	0± 0.0	-	-	0±0.0	0±0.0	-	-
25	0 ± 0.0	0± 0.0	-	-	0±0.0	0±0.0	-	-
50	20 ± 0.0	20±0.0	-	-	10±14.1	10±14.1	-	-
75	30 ± 14.1	40±0.0	1.0	0.500	20±0.0	30±14.1	1.0	0.500
100	40 ± 0.0	50±14.1	1.0	0.500	40±0.0	40±0.0	-	-
125	50 ± 14.1	60±0.0	1.0	0.500	50±14.1	50±14.1	-	-
150	60 ± 0.0	90±14.1	3.0	0.205	60±0.0d	70±14.1	1.0	0.500
175	90 ± 14.1	100±0.0	1.0	0.500	90±14.1	90±14.1	-	-
200	100 ± 0.0	100±0.0	-	-	90±14.1	100±0.0	1.0	0.500
225					100±0.0	100±0.0	-	-

Table 3. The molluscicidal potency of aqueous extract of *D. stramonium* leaf against *B. pfeifferi* (n= 10 snails exposed) after 24 h exposure

Concentration (ppm)	Mortality	Mortality rate (%)	Lethal concentrations (ppm)	
			Lc_{50}	Lc_{90}
0	0	0		
25	0	0		
50	2	20		
75	3	30		
100	4	40	108.1	183.5
125	5	50	CI(86.00-131.43)	CI(148.61-267.47)
150	6	60		
175	9	90		
200	10	100		

Table 4. The molluscicidal potency of aqueous extract of *D. stramonium* leaf against *B. pfeifferi* (n= 10 snails exposed) after 48 h exposure.

Concentration (ppm)	Mortality	Mortality rate (%)	Lethal concentrations (ppm)	
			Lc_{50}	Lc_{90}
0	0	0		
25	0	0		
50	2	20		
75	4	40	87.2	157.2
100	5	50	CI(77.46-102.56)	CI(130.77-215.84)
125	6	60		
150	9	90		
175	10	100		

Table 5. The molluscicidal potency of methanol extract of *D. stramonium* leaf against *B. pfeifferi* (n= 10 snails exposed) after 24 h exposure period

Concentration (ppm)	Mortality	Mortality rate (%)	Lethal concentrations (ppm)	
			Lc_{50}	Lc_{90}
0	0	0		
25	0	0		
50	1	10		
75	2	20		
100	4	40	111.3	205.0
125	5	50	CI(92.23-130.27)	CI(168.46-295.83)
150	6	60		
175	9	90		
200	9	90		
225	10	100		

Table 6. The molluscicidal potency of methanol extract of *D. stramonium* leaf against *B. pfeifferi* (n= 10 snails exposed) after 48 h exposure period

Concentration (ppm)	Mortality	Mortality rate (%)	Lethal concentrations (ppm)	
			Lc_{50}	Lc_{90}
0	0	0		
25	0	0		
50	1	10		
75	3	30		
100	4	40	104.1	186.6
125	5	50	CI(86.13-121.37)	CI(155.01-260.60)
150	7	70		
175	9	90		
200	10	100		

5.1.2. *In vitro* cercaricidal potency of the aqueous extract of *D. stramonium* leaf against *S. mansoni* cercariae.

In vitro cercaricidal activity test for the aqueous extract of *D. stramonium* leaf showed that the plant possessed cercariacidal activity against *S. mansoni* cercariae. It was found that at the first 30 minutes of exposure to 20 ppm, no mortality of *S. mansoni* cercariae was observed. However, after 2 hour exposure, mortality at the same concentration was elevated to 10%. The Lc_{50} and Lc_{90} values were determined after two hour exposure to the plant extract. The Lc_{50} value was 35.3 ppm with 95% CI = 28.20-43.65 (Table 7). While, the respective Lc_{90} value was 64.6 ppm with 95 % CI =50.36-118.20. In general, the cercaricidal activity of the plant was increased with increase in concentration of the extract.

Table 7. Mean percentage mortality of *S. mansoni* cercaria exposed to various concentrations of aqueous extract of *D.stramonium* after 2h exposure.

Concentration (ppm)	**Cercaria mortality (mean±SD)**
0	0 ± 0.0^{a}
20	10 ± 7.1^{ab}
25	22.5 ± 10.6^{ab}
35	47.5 ± 17.7^{abc}
40	72.5 ± 10.6^{cd}
60	75 ± 7.1^{cd}
80	100 ± 0.0^{d}
F-value	30.8
P-value	<0.001*
LC_{50}	35.3
(LCL-UCL)	28.20-43.65
LC_{90}	64.6
(LCL-UCL)	50.36-118.2

Sufficient concentration needed to kill 50 and 90% of the cercaria (probit analysis)

Concentration change was significantly associated with cercaria mortality ($p<0.01$) (ANOVA)

6. Discussion

In the present study, *B. pfeifferi* was susceptible to *D. Stramonium* extracts at different concentrations similar to the report by (Mkoji *et al*., 1989) who screened the molluscicidal activity of plants under the family *Solanaceae* and showed varying degrees of potency. The molluscicidal potency of *D. stramonium* leaf extract against *B. pfeifferi* was higher than the previously studied related plants such as *Withania somnifera* Linn., which showed 90% mortality of *B. pfeifferi* only at 200-400 mg/L (Ojewole, 2004). This difference in potency or activity strength is due to seasonal influence and other confounding factors, which may include age of the plant, environmental stresses on the plant, individual snail susceptibility, parts of the plant locality of the plant species, time of collection, storage conditions , method of extraction , and solvent type used. concerning the target snail, toxicity of the plants depends on strength of toxin, quantity consumed, time of exposure, individual body chemistry and genetic differences within the species (Tariwari *et al*., 2014).

Molluscicidal activities of plants reported in different phytochemical studies showed, the presence of substances of plant secondary metabolites. These bioactive phytochemical constituents produce definite physiological action in the targeted organism (Kindiki, 2014). Some of the most important phytochemical constituents are tannins, saponins, flavonoids, terpenoids, coumarins, steroids and phenolic compounds (Pereira-Filho *et al*., 2014). Methanol extract from *Datura innoxia* exhibited a remarkable toxic effect against the snails *B. alexandrina*, *B. trancatus* and *L. caillaudi*, and this could be due to the presence of a compound from the coronaridine glycoside derivatives (Hamad, 1999). Singh and Singh (2013) confirmed that the plant *D.stramonium* contains alkaloids, tannin, glycosides and flavonoides. Due to the presence of these substances the plant *D.stramonium* could be responsible for its molluscicidal activity. Moreover, antibacterial, antifungal and nematicidal properties of *D.stramonium* have been recognized to its possession of alkaloids, glycosides, flavonoides and the like secondary metabolites (Singh and Singh 2013).

The molluscicidal potency with its respective Lc_{50} and Lc_{90} for 24 and 48h exposure evidenced that the aqueous extract showed higher potency than that of methanol extract. This variation in activity might be due to solubility of the plant's secondary metabolites such as alkaloids,

glycosides and flavonoides which is responsible for their activity. These secondary metabolites are extracted in greater measures with more polar solvent that is water than less polar solvent methanol. This finding give support to other studies that reported differences in the activities of extracts using different solvents. For instance, the aqueous extract of *G. lottoides* is more potent than the ethanol extract of the same plant (Kiros *et al*., 2014).

The current findings showed that the 24 h- Lc_{50} and Lc_{90} values of aqueous extract of *D. stramonium* were 108.1 and 183.5 ppm, respectively. This was in agreement with the study conducted by Ojwole *et al*., (2004). A closely related result was reported where he found Lc_{50} and Lc_{90} values of *Solanum nigrum* to be 100 and 200 ppm for *B. pfeifferi*, respectively. The present study also proved the molluscicidal potency of methanol extracts of *D. stramonium* leaves. The 24 and 48 h-Lc50 value of *D. stramonium* was similar to that of Kabbashi *et al*. (2016) who evaluated the molluscicidal activity of methanol bark extract of *Acacia seyal* to be 80.79 and 34.33 ppm after 24 and 48 hour exposure respectively.

According to Leitchfield and Wilcoxon (1949) a plant to be considered as moderate molluscicide, a crude aqueous extract of the plant material should be active at 100-200 ppm or less and kill 90% of snails exposed for 48 h. Lc50 > 100 < 500 mg/L indicates that the substance is moderately toxic. In the present study, aqueous extract of *D. stramonium* leaf was active at a concentration 157.2 ppm and killed 90% of the snails within 48 h contact to the plant extract. Thus, *D. stramonium* leaf aqueous extract can be considered as a moderate molluscicide. On the other hand, in the present study, methanol extract of *D. stramonium* fruit showed Lc_{90} value of 205 ppm after 24 h exposure to the methanol extract which showed weak molluscidal activity.

Regarding to toxicity of *D. stramonium* to non-target organisms, seeds of the plant have been tested in mice. At concentrations of 0.5% or more in the diet produced adverse physiological changes in rats (Singh and Singh, 2013). This indicates that the plant might be toxic to non-target organisms. The drawback in which toxicity to non-target organism may limit its wide spread application in schistosomiasis control as it was reported by (Woldemichael *et al*., 2006) on the use of *P. dodeccandra* (Endod) as a potential molluscicide. Besides this, D. *stramonium* has many important characteristics to be used as an alternative molluscicide. Among them, the plant

can easily be extracted with water, inexpensive and uses simple techniques for application in endemic areas and eco-friendly to the environment.

As far as cercaricidal potency of the plant *D. stramonium* is concerned, the plant showed reasonable cercariacidal potency against *S. mansoni* with respect to concentration-time dependence. This result is in agreement with that of Tekwu *et al*. (2017) stem bark extract of *Rauwolfia vomitoria* showed cercariacidal potency, and the result was both concentration and time dependent. There are other findings also in accordance with this investigation, such as, *G. lottoides* fruit extract by Kiros *et al*. (2014), *B. aegypiaca* by Molla *et al*. (2013), *Iris germanica* leaf and rhizome extract by Singaba *et al*. (2006) showed that the effect of time and concentration with relation to its cercaricidal potency.

The Lc_{50} and Lc_{90} values for the *in vitro* cercaricidal activity after 2h of exposure was 35.3 and 64.6 ppm, respectively. This toxicity result is higher than others reported for other cercaricidal plants such as, Tekwu *et al*. (2017) reported that the Lc_{50} of *Rauwolfia vomitoria* stem bark extract was potent at 61.18 ppm against *S. mansoni* cercariae after 2hr exposure. Similarly, Rug and Ruppel (2000) L_{C50} of plant *Jatropha curcas* was 100 ppm. But neither as active as that of Kiros *et al* (2014) L_{C50} and L_{C90} values 18.7 and 41.7 mg/l; Kindiki (2014) L_{C50} value of *Bridelia micrantha* aqueous extract was 4.465 mg/L. The toxic effect suggested that the flavonoids content of plant extract may also interfere with the electron transport pathway of cercariae, as reported by Lyddiard and Whitfield (2001).

Customarily, the plant *D. stamonium* has been used for the cure of various ailments in different countries. Concerning to Ethiopia's experience, the plant used for wound treatment against wound causing bacteria (Singh and Singh, 2013). Sharma and Trivedi (2002) reported that aqueous extract prepared from the leaf of *D. stramonium* showed efficient nematicidal activity. The current study proved that *D. stramonium* possessed both molluscicidal and cecaricidal activity. Due to its antiheminthic and other activities, the present study would give attention to the use of this plant as alternative molluscicide and cercaricide in the control of schistosomiasis.

The limitation of this study is, *in vivo* test and toxicity test to non-target organisms using mice was not conducted due to an unavailability of mice for conducting the study.

7. CONCLUSION

In this study, water and methanol were used for extraction of crude plant extracts; water was proved to be better for the extraction of the plant. The tested plant extract sample has proven to have molluscicidal effects against *B. piefferi* snails. In addition, the plant extract also had ability to destroy *S. mansoni* cercariae. Aqueous extract of *D. stramonium* was more efficacious than the methanol extract, and this gives more emphasis to the water extract in terms of its cost for extraction and easiness in its application in endemic areas.

Generally, this study has been able to demonstrate significant molluscicidal and cercaricidal activity in *D. stramonium*, the results suggest that *D. stramonium* extract has a promise to be used in schistosomiasis control programs.

8. RECOMMENDATIONS

Based on the present findings, the following recommendations are forwarded; the extracts of *D. stramonium* should be further analyzed, that is, bioactivity guided fractionation to isolate the specific compounds responsible for its molluscicidal as well as cercaricidal activity is needed. Regarding to plant extraction, *D. stramonium* extracts were made from only one part of the plant, it is suggested that many plant parts be used since there are variations in the accumulation of the chemical compounds in various plant parts. In line with this Mode of action of those secondary metabolites/active compounds on the death of snail and cercariea should be investigated. Eventually, in order to check in which life stage does the plant extract is potent, activity of the plant extract against different life stages of the *S.mansoni* including the eggs, miracidia and adult worm should be determined.

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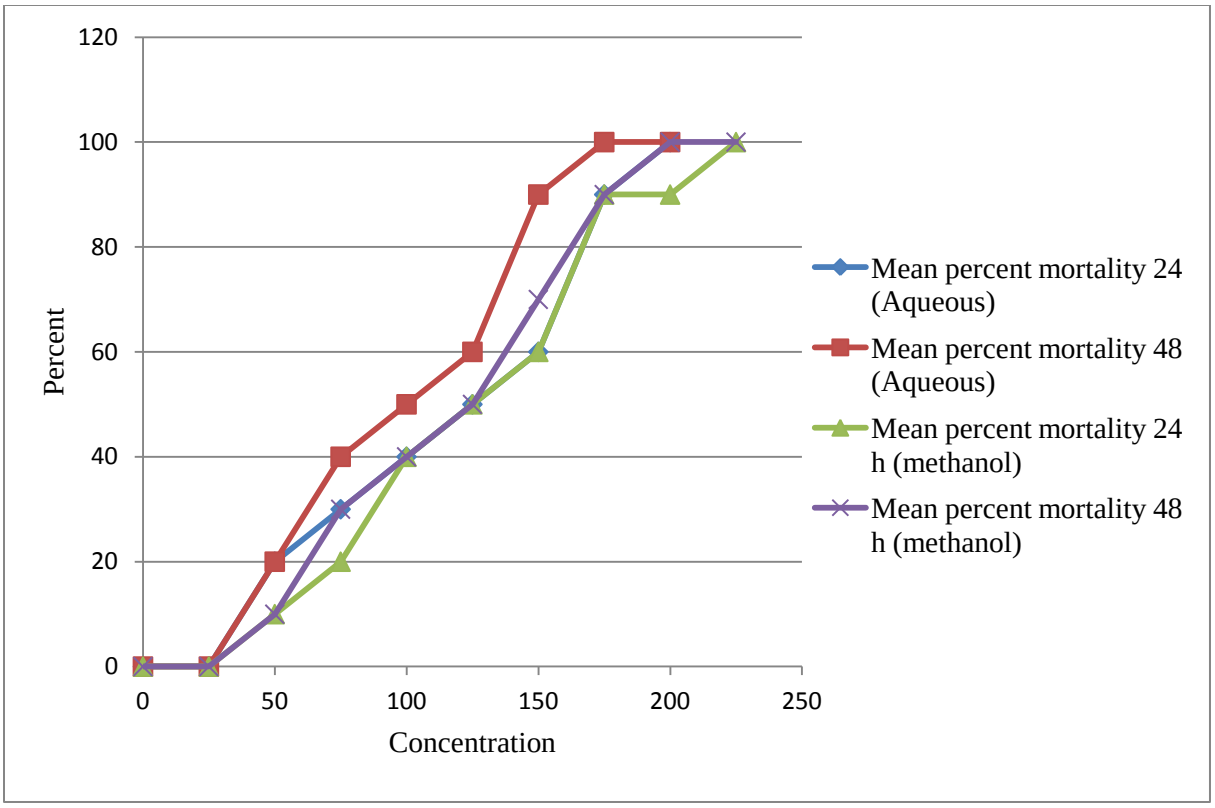
10. ANNEXES

Annex 1: collection of leaves of *D. stramonium*

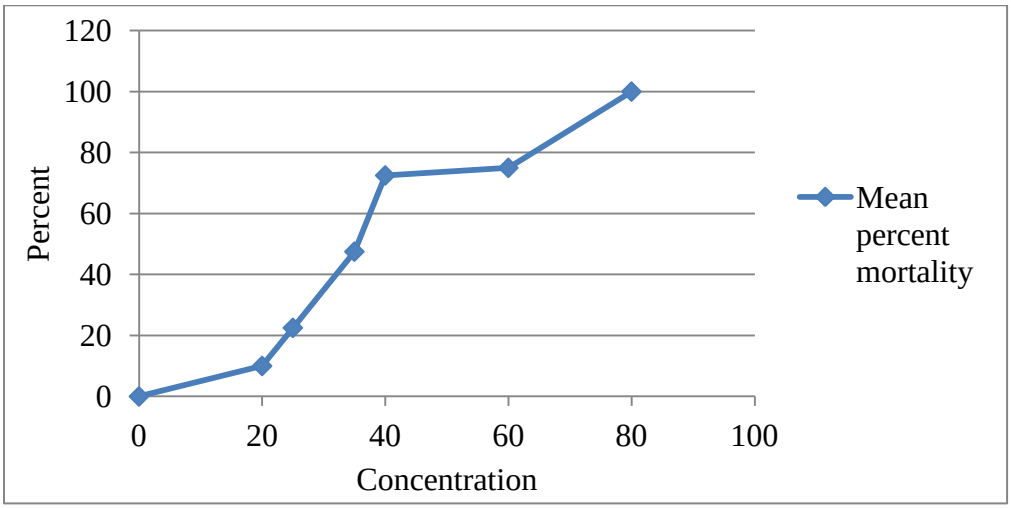
Annex 2: extraction of the plant material (*D. stramonium)*

Annex 3: *B. pfiefferi* snail collection in shinta river, Gondar

Annex 4: exposing *B. pfiefferi* snails to the extracted plant material



Annex 5: Graph showing as concentration increases snail mortality increases i.e. change in concentrations is significantly associated with snail mortality.



Annex 6: Graph showing as concentration increases cercaria mortality increases i.e. change in concentration is significantly associated with cercaria mortality.