IN VIVO ANTI SCHISTOSOMAL ACTIVITIES OF ECHINOPS KEBERICHO MESFIN ROOT AND HAGENIA ABYSSINICA (BRUCE) J.F GMEL FLOWER PART HYDROALCOHOLIC EXTRACTS ON SCHISTOSOMA MANSONI INFECTED SWISS ALBINO MICE

BY

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A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF LABORATORY SCIENCES AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN MEDICAL PARASITOLOGY.

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ABSTRACT

Introduction: - Schistosomiasis is a chronic helminthic infection caused by trematodes of the genus *schistosoma* resulting a great public health and socio-economic impact in many developing countries of the tropics. However, its control solely depends up on praziquantel treatment which now a day's fear of drug resistance emergence occurred and has drawn attention for alternative drugs. Therefore, evaluation of safety and effectiveness of traditional medicinal products is important to supplement the process of development of new candidate drug.

Objective: - To evaluate the *in vivo* anti schistosomal activities of *Echinops kebericho* Mesfin root and *Hagenia abyssinica* (Bruce) J.F Gmel flower part hydroalcoholic extracts on *schistosoma mansoni* infected swiss albino mice.

Methods and materials: - Soxhlet extraction technique was employed for the extraction of *Echinops kebericho* root part using 70% ethanol whereas maceration technique was performed for the extraction of *Hagenia abyssinica* flower part using 80% methanol. Swiss albino mice were then orally administered the plant extracts by oral gavage needle in a single dose of 5000mg/kg, 2500mg/kg, 1250mg/kg and 3% tween 80 as vehicle during oral acute toxicity assessment of *Echinops kebericho* and five days administration of both *Echinops kebericho* and *Hagenia abyssinica* at doses of 1200mg/kg, 600mg/kg and 300mg/kg in anti schistosomal activity evaluation with control groups praziquantel and 3% tween 80 run concurrently.

Besides, mice were infected by the paddling technique each with 150±10 cercaria and kato katz technique of stool examination was performed for egg count on the eighth week and two weeks after the end of treatment. Liver granuloma score and worm count were performed at the end of experiment on the eleventh week post cercarial exposure. Statistical analysis was performed using spss version 16 employing the non parametric statistical options considering significance at $p - value < 0.05$.

Results: - No death was observed up to a dose of 5000mg/kg *Echinops kebericho* extract single dose oral administration indicating LD_{50} to be above 5000mg/kg. However, weakness, hair errection and loss of appetite were observed in a dose dependent manner

with no significant difference ($p>0.05$) in complete blood count and body weight measurement across the group. Liver and kidney histological findings were also normal except obliterated urinary space of kidney at dose 5000mg/kg. Moreover, *Echinops kebericho* and *Hagenia abyssinica* showed statistically significant reduction ($p<0.05$) in egg count, worm burden and liver granuloma score in anti schistosomal activity evaluation of all the treatment doses.

Conclusions and recommendations: - *Echinops kebericho* root extract was safe up to a dose of 5000mg/kg and both *Echinops kebericho* root and *Hagenia abyssinica* flower extracts possessed anti schistosomal activity in all the treatment doses with *Hagenia abyssinica* showing better activity. Therefore, *Hagenia abyssinica* (Bruce) J.F Gmel involving further studies should be conducted for its future use.

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ACRONYMS AND ABBREVIATIONS

ANOVA:	Analysis of Variance
Ca^{++}:	Calcium Ion
DALYs:	Disability Adjusted Life Years
EDTA:	Ethylene Diamine Tetra Acetic acid
EHNRI:	Ethiopian Health and Nutrition Research Institute
EPG:	Egg per Gram
ERR:	Egg Reduction Rate
Hct:	Hematocrite
Hgb:	Hemoglobin
LD_{50}:	Lethal Dose 50
Lymph:	lymphocyte
Plt:	Platelet
PZQ:	Praziquantel
RBC:	Red Blood Cell
TM:	Traditional Medicine
WBC:	White Blood Cell
WBR:	Worm Burden Reduction
WR:	Worm Recovery
WHO:	World Health Organization

INTRODUCTION

BACKGROUND

Schistosomiasis (also known as bilharziasis) is a chronic parasitic disease caused by blood flukes or trematodes of the genus *schistosoma*. *Schistosoma mansoni, Schistosoma haematobium* and *Schistosoma japonicum* are among the five species of schistosomes that can cause disease in human and result significant public health problem. Intestinal schistosomiasis is caused by *S. mansoni*, *S.japonicum, S.mekongi* and *S. intercalatum* whereas *S. haematobium* is responsible for urinary schistosomiasis. Other mammalian species such as *S. bovis, S. matheei, S. curasoni* and *S. capense* can also produce infection in man (Cheesbrough, M., 2009; Payet, M., 1966; Richard, L.G. *et al.*, 2004). The life cycle of schistosomiasis is complex involving man and other warm blooded animals being the definitive hosts with the freshwater snails (*Biomphalaria* spps., *Bulinus* spps. and *Oncomelania spps.*) as intermediate hosts, while water bodies provide the link between them (Domo, A.G., *et al.*, 2009).

As shown in Figure-1 of schistosome parasites life cycle, humans are infected upon water contact containing schistosoma cercaria which can penetrate the skin and transform into a larval stage called the schistosomulum. As a result, early stage clinical features occur with the skin local irritation 'swimmers itch,' which may appear immediately or within few days. Then schistosomulum enter blood vessels and pass round the venous circulation, through the heart, lungs, back to heart then reach the liver and mature over about a month to adult worms in the portal vein. As the viable schistosomulum begin their migration to the liver, the rash disappears and the patient experiences fever, headache and abdominal pain for 1-2 weeks. The adult paired worms (female residing in gynecophoral canal of males) migrate distally to the veins around the bladder and the mesenteric veins of the intestine where they can usually live for 3–5 years and even up to 30 years (Gryseels, B., 2012; Mahmoud, A.F., 2008; Peter. M.L., *et al*., 2010; Ryan, K. J. and Ray, C.G., 2004).

As a matter of fact, adult *S. mansoni, S. haematobium* and *S. japonicum* females lay between 100-300 eggs/day, 20-200 eggs/day and 500–3500 eggs/day, respectively. Accordingly, the intermediate stage begins with oviposition and is associated with a

complex of clinical manifestations. In addition to the fever and chills, patients experience cough, urticaria, arthralgia, lymphadenopathy, splenomegaly, abdominal pain and diarrhea. Nonetheless, about half of these eggs succeed in passing through the wall of the bladder or intestine to emerge in urine or feces. Those retained eggs induce inflammation and scarring, initiating the final and most morbid phase of the chronic stage of schistosomiasis. Upon contact with fresh water in lakes, rivers and streams, the eggs release a ciliated larval form called the miracidium. Miracidia go on to infect specific aquatic snails and within the snails they multiply asexually into sporocysts from which arises the cercaria (Cheesbrough, M., 2009; Mahmoud, A.F., 2008; Peter. M.L., *et al.*, 2010; Ryan, K. J. and Ray, C.G., 2004).

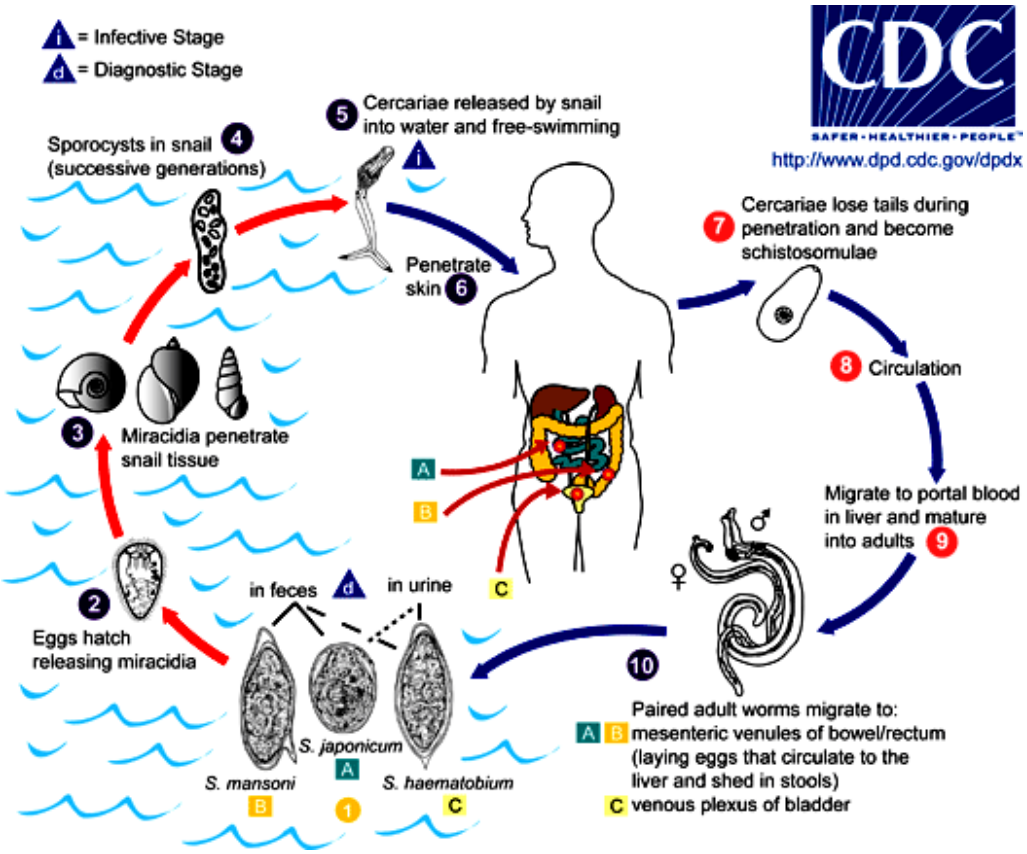

Figure 1. The life cycle of schistosome parasites (source: http://www.dpd.cdc.gov/dpdx)

Moreover, the diagnosis of schistosomiasis can be made by microscopic examination through finding eggs in multiple urine or stool specimens, as egg shedding is variable. The shape of the egg and the position of the spine are characteristic for each species of schistosoma. *S. haematobium* has a terminal spine while *S. mansoni* and *S. japonicum*

have lateral spines. As a result, the most widely used kato-katz technique, concentration techniques, biopsy, serologic tests, endoscopic examination, ultrasonography and enzyme immunoassay based dipstick tests are among the techniques used for the diagnosis of schistosomiasis (Gillespie, S. H. & Pearson, R. D., 2001; Peter. M.L., *et al.*, 2010).

Besides, prevention depends on health education, control of snail intermediate host, reducing contaminated water contact and mass chemotherapy to treat infected individuals and reduce egg shedding. Although several drugs were used in the past, now only a few drugs are used for the treatment of schistosomiasis. Praziquantel (PZQ), which acts against all species of the parasite, is effective against the adult worms and cannot be used very soon after infection before adults have matured. Metrifonate is active against *S.hematobium* while oxamniquine is effective against *S.mansoni* and artemisinin derivatives with anti schistosomulum activity (Ali, S. A., 2011;Caffrey, C. R., 2007).

Nevertheless, traditional systems of medicine also continue to be widely practiced as a source of medicines for a wide variety of human ailments due to population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects and development of resistance to several allopathic drugs (WHO, 2011). Of course, traditional medicine includes all health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises applied to treat, diagnose and prevent illnesses or maintain well being which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (WHO, 2002a; 2003a,b). The clinical effects of herbal medicines are therefore related to traditional knowledge, the skill of individual practitioners and the theories of traditional medicine (WHO, 1998).

As a result, Ethiopian traditional medicine is commonly used to treat a variety of diseases recommended by traditional healers. Accordingly, among the many are gastrointestinal diseases, respiratory disorders, sexually transmitted infections, tuberculosis, impotency, hemorrhoids, rabies, intestinal parasites, skin problems, liver diseases, mental disorders, hypertension, diabetes, gynecological conditions, rheumatism, malaria and others (Deribe, K. *et al*. 2006).

STATEMENT OF THE PROBLEM

Schistosomiasis is one of the most widespread parasitic infections that affect human and it is second only to malaria in terms of socioeconomic and public health importance in tropical and subtropical areas. It is endemic in 76 tropical developing countries with 779 million population at risk of infection globally (85% in Africa), 207 million people are infected (> 97 % in Africa) and 120 million people morbidity (20 million is severe). The annual mortality is 15,000–280,000 and 1.7– 4.5 million disability adjusted life years (DALYs) lost (Engels, D., *et al.* 2002; Steinmann, P., *et al.*, 2006; WHO, 2004).

Likewise, *S. mansoni* is endemic in 52 countries and is wide-spread in many African countries, Madagascar, parts of the Middle East, South America and the West Indies (Cheesbrough, M., 2009; Richard, L.G. *et al.* 2004). It is estimated that 393 million individuals are at risk of *S. mansoni* infection in Sub-Saharan Africa with 54 million being infected and 4.5 million DALYs lost due to infections. There are 31 countries in Africa including Ethiopia where 1-10 million inhabitants are infected with schistosome. But five African countries where the estimated number of infections is in excess of 10 million in descending order of number of infections are Nigeria (28.8 million), Tanzania (19. 0 million), Ghana (15.2 million), Congo (14.9 million) and Mozambique (13.2 million) (Steinmann, P., *et al*., 2006; WHO, 2002b).

In Ethiopia, the endemicity of both intestinal and urinary schistosomiasis has long been established and estimates made in the 1980's documented that the number of people at risk of infection with *S. mansoni* were 18 million and 2.5million infected whereas the number of people at risk of *S. haematobium* infection would be about 4 million (Lo C.T., *et al.*, 1988). However, Chitsulo, L., *et al*., 2000 reported an estimated number of 29.89 million people are at risk of acquiring schistosomiasis and of these 4 million are infected. Besides, the prevalence of *S.mansoni* ranges from less than 1% up to more than 90% in Ethiopia (Ali, A., *et al.*, 2006). Similarly, prevalence studies in Jimma zone showed 16.4% (Mengistu, A., *et al.*, 2007), 26.3% (Mengistu, M., *et al.*, 2011) and 10% (Mekonnen, Z., *et al.*, 2012) in Jimma town. Whereas, 12.38% in Agaro health center

(Mekonnen, Z., *et al.*, 2007) and 10.61% at a health center near Gilgel Gibe dam (Abebe, G., *et al.*, 2009).

Furthermore, water development projects for conservation, irrigation and hydroelectric power have contributed to the spread of schistosomiasis worldwide. By 2000, some 45,000 large dams had been built globally with a distinct shift towards construction in developing countries. There are over 1,100 large dams in Africa including the tens of thousands of small dams built for agricultural irrigation, to supply drinking water and water for livestock; the cumulative effect of these on schistosomiasis transmission may be greater. It is also expected that dam building will increase in Africa south of the Sahara, the area with the greatest risks for the spread and intensification of schistosomiasis (Cheesbrough, M., 2009; Richard, L.G., *et al*., 2004; WHO, 2002b).

Accordingly, the introduction of *S. mansoni* into the upper and middle Awash valley, Ethiopia, following the establishment of large scale irrigation schemes has been documented for the Wonji sugar estate, where irrigation commenced in 1954 and the first case of *S. mansoni* was diagnosed in 1964. Prevalence steadily increased up to 20% in 1980 and 81.9% in 1988 among children of a village in the irrigation scheme. The prevalence of *S. mansoni* also increased from 29.7% to 48.4% as a result of small dam constraction in Tigray region (Ghebreyesus, T.A, *et al*., 2002; Kloos, H., *et al.*, 1988; Simonsen, P.E, *et al.*, 1990). Therefore, continous surveying is required around the constracted dams as the water bodies are suitable for the survival of intermediate host and there is posiblity of case occurrence (Alemeshet, Y., *et al.*, 2010).

As to the control of schistosomiasis, chemotherapy and the reduction in rate of transmission by either use of snail molluscicdes or reduction of human water contact are considered the two main actions. As a result, the treatment of schistosomiasis has improved significantly in view of the introduction of drug of choice praziquantel. Its oral administration in the dose of 40 to 60 mg/kg is efficient in reducing the morbidity, but the results of treatments with this drug have been less promising than expected. The reason is that cases of tolerance and resistance to the treatment for schistosomiasis have been reported for PZQ (Parise-Filho & Silveira, 2001). The relative lack of efficacy of PZQ

against juvenile schistosome worms invivo and invitro is also a potentially significant deficiency in the pharmacological profile of this drug (Pica-Mattoccia, L. and Cioli, D., 2004). Oxamniquine which is effective only against *S. mansoni*, in alternative to PZQ, is unlikely to be used much in Africa because the price has remained high (Pica-Mattoccia, L. *et al*., 2006).

Furthermore, in an efficacy study in northern Senegal, PZQ treatment gave cure rates of only 18–39 % (Stelma, F. F., *et al*., 1995). These were alarmingly low compared with the normally expected 60 – 90 % and increasing the dose gave no significant improvement (Tchuente, T. *et al*., 2001). In Egypt, 1607 *S. mansoni* infected patients in the Nile delta region were treated with PZQ at 40 mg/kg and after an additional two treatments, the last at 60 mg/kg, 1 .6 % of the patients were still passing viable eggs (Ismail, M., *et al.*, 1996). Further evidence for isolates showing PZQ insusceptibility has also been found in Kenyan car washers as shown in both *in vitro* assay on miracidia hatched from eggs excreted by *S. mansoni* infected individuals and *in vivo* study (Melman, S.D., 2009).

Additionally, efficacy and side effects study of PZQ in the treatment of schistosomiasis in Ethiopia, Wendo Genet reported lower cure rate and egg reduction rate of 73.6% and 68.2%, respectively (Erko, B., *et al.*, 2012). However, the result of efficacy study in fincha river showed cure rate of 80.9% and egg reduction rate of 99.5% (Haile, S., *et al.*, 2012) while cure rate of 93.44% and 88.99% was observed in North Ethiopia, Tumuga and Waja respectively, (Dejenie, T., *et al.*, 2010) which indicate no evidence for resistance of *S. mansoni* against PZQ. Nevertheless, the possibility of drug resistance should be considered when the egg reduction rate (ERR) is less than 70% in the case of schistosome infections (WHO, 1998).

Therefore, there is a great need to develop new candidate drug for the treatment of schistosomiasis. The possibility of obtaining new chemotherapeutic agents from natural sources was also shown in some studies conducted on medicinal plants in evaluating their anti schistosomal activities. However, evaluation of the safety and effectiveness of traditional medicinal products and practices is limited (Mostafa, O.M. *et al.*, 2011; WHO, 2002a). So it is necessary to focus on all aspects of medicinal plant research: from

cultivation, Ethnopharmacology, utilization, isolation and identification of active constituents to efficacy evaluation, pharmacology, safety, standardization, formulation and clinical evaluation. Animal toxicity studies are also required to establish the potential adverse effects of traditional medicinal plants (WHO, 2003c).

LITERATURE REVIEW

Between 70% - 95% of citizens in a majority of developing countries, especially those in Asia, Africa, Latin America and the Middle East, use traditional medicine for the management of health and as primary health care to address their health care needs and concerns. Accordingly, African countries such as Ethiopia (80-95%), Mali (75%), Rwanda (70%), Myanmar (70%), Tanzania (60%) and Uganda (60%) of the population use traditional medicine for primary health care while populations in those developed countries such as Germany(80%), Canada (70%), France (49%), Australia (48%) and USA (42%) use as complementary and alternative medicine at least once (WHO, 2011).

It is estimated that at least 25% of all modern medicines are derived either directly or indirectly from medicinal plants, primarily through the application of modern technology to traditional knowledge (WHO, 2011). So, the importance of plants as sources of natural product bioactive molecules to medicine lies not only in their pharmacological or chemotherapeutic effect but also in their role as template molecules for the production of new drug substances (Phillipson, J. D., 1994). Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use early in China, Syrians, Babylonians, Hebrews and Egyptians for their important contributions in the healthcare system of local communities as the main source of medicine for the majority of the rural population (Abbink, J., 1995; Dery, B., *et al*., 1999).

Likewise, more than 80% of Ethiopian communities are dependent on traditional medical preparations for a long time and of which more than 95% are of plant origin (Abebe, D. 1986; Assefa, B., *et al*., 2010). Of the 250,000 higher plant species on earth, more than 50,000 are used for medicinal purposes. The flora of Ethiopia consists of some 6,000 to 7,000 species and 10% of which are believed to be endemic to the country. About 800 species of plants are used in the traditional health care system to treat nearly 300 mental and physical disorders. These medicinal plants occur throughout the country's diverse highland areas as well as in south west and central lowlands (Fassil, H., 2005; Tilahun, T. and Mirutse, G., 2007).

ECHINOPS KEBERICHO MESFIN

Echinops kebericho Mesfin, locally called 'kebericho', is an erect massive root stock bearing perennial herb that grows up to a height of 1.2 m. It belongs to the genus *Echinops* that comprises 125-130 species in the family Asteraceae/Compositae and distributed in semi-humid zones of tropical and North Africa, mediterranean basin and temperate regions up to central Asia. However, 12 species occur confined to the highlands of Ethiopia (Hedberg, *et al.*, 2004; Tadesse and Abegaz, 1990 and Teresa, *et al*., 2005). Reports and ethno botanical surveys evidence long traditional use of the plant for preparation of medicines against migraine, mental illness, heart pain, tuberculosis, leprosy, kidney disease, malaria, bilharzias, syphilis and amoebic dysentery (Abebe and Ahadu, 1993; Ashebir and Ashenafi, 1999; Hymete, *et al.*, 2005).

The roots of *E. kebericho* are traditionally used as a fumigant, mainly after childbirth. It is claimed that the smoke is effective against typhus, fever and to repel snakes from their vicinity. It is also indicated that the roots are chewed to reduce stomach ache in humans. A decoction of the roots is used to cure intestinal diseases in cattle (Hymete, A., *et al.*, 2007). Powdered root of *E. kebericho* is also sprinkled on burning charcoal and smoke is inhaled for evil eye (Tilahun, T. and Mirutse, G., 2007) for headache and infusion taken orally for coughing (Teklehaymanot, T., *et al.*, 2007). On the other hand activity studies of ethanol extract of the roots of *E. kebericho* has been shown to have antimalarial activity *in vivo* study (Biruksew, A., 2012. unpublished) and a very strong lethal activity against earthworm. Worms kept in a cabinet together with the powdered roots of *E. kebericho* were also found dead after a few hours suggesting that the volatile constituents could be responsible for the lethal activity. Some other species of this genus are as well claimed to exhibit vermicidal activities (Hymete and Kidane, 1991). Extracts and essential oils of the roots of *E. kebericho* were also assessed for their antimicrobial, antihelminthic and molluscicidal activities (Hymete, *et al.*, 2005). Volatile oils of *E. kebericho* shows

activity on *Haemonchus cortortus* ova (Hussien, J., *et al*., 2011) and strong antileishmanial activity (Tariku, Y., *et al.*, 2011).

Moreover, Echinops species were reported to contain a wide range of chemical structures comprising alkaloids, saponins, phytosterols, polyphenols, carotenoids, sesquiterpene lactones/alcohols, lignans, fatty acids, essential oil, acetylenic and thiophene compounds (Hymete, *et al.*, 2005; Tadesse and Abegaz, 1990). Generally, *in vitro* study on the toxicity of *Echinops kebericho* Mesfin essential oil on the human monocytic leukemia (THP-1) cell line and red blood cells showed weak hemolytic effect (Tariku, Y., *et al.*, 2011).

HAGENIA ABYSSINICA (BRUCE) J.F. GMEL

Hagenia abyssinica (Bruce) J.F. Gmel is a mono-specific genus belonging to the family Rosaceae with local names kosso. The species is abundant in the semi-humid mountain woodlands of Ethiopia above 2000m altitude and sparsely distributed in mountainous central, central west and south eastern parts of Ethiopia. The species also occurs in Kenya, Tanzania, Uganda, Sudan, Congo, Malawi, Burundi and Rwanda. (Friis, I. 1992; Hedberg, O., 1989; Jansen, P.C.M. and Getachew, A., 2002; Negash, L. 1995). It is a slender tree growing up to 20 m tall, with a short trunk and thick branches; branchlets covered in silky brown hairs and ringed with leaf scars. Trees have either male or female flowers which are handsome polygamo-dioecious, female heads pinkish-red, clearly veined, bulkier than the more feathery orange-buff to white male heads. Flowering and seeding can be observed throughout the year with a break in the months with the coldest temperatures, during the dry season between October and February (Jansen, P.C.M. & Getachew, A., 2002; Orwa, C., *et al*., 2009).

Throughout history, *H. abyssinica* has been used as an antihelminthic especially to expel tapeworm in Ethiopia and other parts of Africa. Honey obtained from beehives located near *H. abyssinica* trees and collected immediately after their flowering, is also said to be

effective in expelling tapeworms. It is also used, often in a mixture with parts of other plants, as a medicine to treat syphilis, scrofula, malaria, fever and cough (Desta, B., 1995; Giday, M., *et al.*, 2003; Jansen, P.C.M. & Getachew, A., 2002). Even so, bark may be pounded, added to cold water and the liquid drunk as a remedy for diarrhea and stomach-ache. It is a strong medicine that must not be taken in large quantities even sometimes taken as an abortifacient (Assefa, B., *et al*., 2010; Orwa, C., *et al*., 2009). It was also used by mixing the powder with honey and a little bit of water, then boiled and drunk before breakfast for five days or the powder is mixed with local 'tella' and drunk before breakfast after leaving for overnight for three days for Ascariasis (Mesfin, F., *et al.*, 2009). The dried and powdered female kosso flowers are used also as decoction or macerated in water (Woldemariam, T.Z, *et al.*, 1992).

The active principles in *H. abyssinica* flowers are phloroglucinol derivatives, called kosins: kosotoxin, protokosin, kosidin, α-kosins and β-kosins. All are mixtures of isobutyryl, isovaleryl and 2-methylbutyryl side chain homologues of methylene-bis-pseudo-aspidinol (Jansen, P.C.M. & Getachew, A., 2002). Although the non polar kosotoxin is believed to be the active principle, some contributing components may well exist in the crude water extract of *H. abyssinica* and influence its activity (Woldemariam, T.Z, *et al.*, 1992).

Also, volatile oil, a bitter acrid resin and tannic acid are other constituents of kosso. The bitter principle called α-kosins and β-kosins, which are found in kosso, are thought to be decomposition products. Kosotoxin, a yellow amorphous powder possibly related to filicia acid and rottlerin is the principal constituent of kosso whereas protokosin and kosidin are inactive colorless bodies found in the extract of kosso (Yohannes, P. and Dagne E., 1983). Typical Rosaceae polar constituents such as quercetin 3-*O*-β-glucuronide, quercetin 3-*O*-β-glucoside, rutin flavonoids and ellagic acid were also detected in the *H. abyssinica* extract (Thomsen, H., *et al*., 2012). Furthermore, trypanocidal activity of essential oil (Nibret, E. and Wink, M., 2010), antihelminthic for cestodes control in goats (Abebe, G., *et al*., 2000), anti malaria activity (Kassa, M., *et al.*, 1998) and *in vitro* anti schistosomal activity on *S. mansoni* adult worm (Thomsen, H., *et al.*, 2012) were reported.

Moreover, study done on the toxicity in mice of aqueous and hydroalcoholic extracts of *H. abyssinica* given intraperitoneally showed LD_{50} at 2014 ± 301 and 1980 ± 179, respectively (Desta, B., 1995). The aqueous flower extract was also tested for its single and repeated dose toxic effect on rats of both sex. Studies at 5000 mg/kg and at three dose levels (500, 1000, and 2000 mg/kg) were conducted orally on 5 rats per sex per dose with concurrent control receiving distilled water in single dose study. No toxic signs were noted at all dose levels and there were no significant differences in mean body weights of the treated group compared to control. Therefore, LD_{50} of the extract was reported greater than 5000 mg/kg. Cage side observation, body weight, biochemical and morphologic pathology observation did not show significant differences across the group and also none of the organs have shown to be the target (Demma, J., 2005).

Extract of *Commiphora molmol* (myrrh) has been licensed and marketed for clinical use against fasciola and schistosome infections in Egypt. The extract has some anti schistosomal properties that cause worm pairs to separate. The female worm then shifts to the liver, where they are destroyed (Sher, Z.A., 2001). Moreover, vernonodalin which is a highly toxic sesquiterpene lactose compound, extracted from *Vernonia amyglandina* showed significant activity against Schistosomes, Plasmodium and Leishmania *spps*. Anti schistosomal activities were also shown in a studies conducted on *Nigella sativa* crushed seeds (Mohamed, A.M., *et al.*, 2005), *Ginger* rhizome (Mostafa, O.M., *et al.*, 2011), *Clerodendrum umbellatum poir* leaves (Jatsa, H.B., *et al.*, 2009), *Maytenus senegalensis, Terminalia glaucescens, Colocassia antiquorum* (Domo, A.G., 2009), *Solanum incanum, Carica papaya in vivo* and *in vitro* studies (John M.M., *et al.*, 2012; Muchika, S., *et al.*, 2011), *Punica granatum* (Zeinab H.F., *et al.*, 2009), *Curcuma longa (*Afaf, K.E., *et al.*, 2007*)* and *Garlic* (Nahed, H.A, *et al.*, 2009*)* which indicate the promise of traditional medicinal plants for future clinical studies.

On the other hand, the currently in use drug praziquantel has activity against a wide spectrum of trematodes and cestodes. In schistosomes and other trematodes, PZQ directly kills the parasite, possibly by increasing calcium ion (Ca^{++}) flux into the worm. There is experimental evidence that PZQ increases the permeability of the membranes of parasite cells for Ca^{++}. The drug thereby induces contraction of the parasites resulting in paralysis

in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation or may be destroyed by host immune reaction or phagocytosis (Badreldin, H.A., 2006). PZQ although generally well tolerated, it may induce abdominal discomfort, bloody diarrhea, nausea, headache, dizziness, urticaria and rectal bleeding in patients with heavy worm loads (Ali, S. A., 2011).

Besides, the efficacy of PZQ is most often measured as a reduction in schistosome egg excretion rates and the result expressed in terms of cure rate. Cure rates of 60% or greater and sometimes 85–90% are generally achieved (Doenhoff, M. J., *et al.*, 2009) with egg reductions of 90-95% in those not cured. As well, parameters such as reduced miracidial hatch, reduction in the number of living worms; altered distribution of living worms, reduction in macroscopic liver damage, reduction in the number of egg load in the host tissues and oogram pattern can be used in therapeutic efficacy study in mice (Duvall, R. H. and Dewitt, W. B., 1967; Katz, N. and Pellegrino, J., 1974). However, the reduction in the worm recovery and egg density in treated mice was considered by several authors as a strong evidence of the efficiency of anti schistosomal drugs (Mati, V.L., *et al.*, 2010; Suleiman, M.I., *et al.*, 2004; Utzinger, J., *et al.*, 2002).

SIGNIFICANCE OF THE STUDY

Our study plants, Echinops kebericho Mesfin and *Hagenia abyssinica* (Bruce) J.F Gmel are traditionally used for the treatment of wide variety of human ailments including intestinal parasites especially helminthes. In addition, there was no report of safety of *Echinops kebericho* and *in vivo* anti schistosomal activities of *Echinops kebericho* and *Hagenia abyssinica* so as to evaluate for possibilities of these natural products potential for anti schistosomal activity in Ethiopia.

Therefore, the current study will be helpful in providing information of the oral acute toxicity of *Echinops kebericho* in mice and anti schistosomal activities of *Echinops kebericho* and *Hagenia abyssinica* on *Schistosoma mansoni* infected mice. This study will thus use as a baseline data for further investigation of natural products and pave the way to come up with new, safe, effective and economically sustainable substances from locally sourced raw materials so as to supplement or replace the existing drug(s).

OBJECTIVES

GENERAL OBJECTIVE

The general objective of this study was to evaluate the *in vivo* anti schistosomal activities of *Echinops kebericho* Mesfin root and *Hagenia abyssinica* (Bruce) J.F. Gmel flower part hydroalcoholic extracts on *Schistosoma mansoni* infected swiss albino mice.

SPECIFIC OBJECTIVES

- ✓ To determine the extraction yields of 70% ethanol *Echinops kebericho* Mesfin root using soxhlet extraction and 80% methanol *Hagenia abyssinica* flower part maceration extraction techniques.
- ✓ To assess the oral acute toxicity profiles of 70% ethanol root extract of *Echinops kebericho* Mesfin on swiss albino mice.
- ✓ To evaluate the *in vivo* anti schistosomal activity of 70% ethanol root extract of *Echinops kebericho* Mesfin on *S.mansoni* infected swiss albino mice.
- ✓ To evaluate the *in vivo* anti schistosomal activity of 80% methanol flower extract of *Hagenia abyssinica* (Bruce) J.F. Gmel on *S.mansoni* infected swiss albino mice.

MATERIALS AND METHODS

STUDY AREA AND PERIOD

The study was conducted in Jimma University laboratory of drug quality in collaboration with research laboratory of veterinary parasitology from March to April 2012 for toxicity and November to February 2012/13 for activity investigation.

STUDY DESIGN

An experimental study design was employed to evaluate the oral acute toxicity profile of *Echinops kebericho* Mesfin and *in vivo* anti schistosomal activities of *Echinops kebericho* Mesfin and *Hagenia abyssinica* (Bruce) J.F. Gmel on *Schistosoma mansoni* infected swiss albino mice.

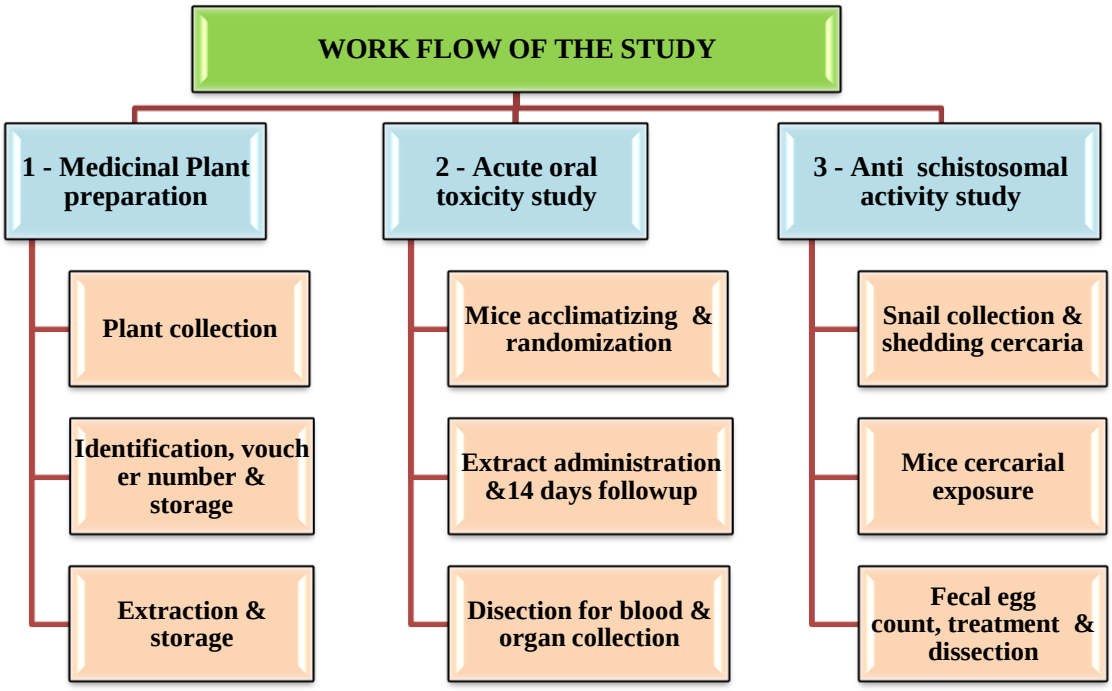

Figure 2. Chart showing the overall experimental work flow and activities performed during the study period.

PLANT MATERIAL COLLECTION AND EXTRACTION

The roots of *Echinops kebericho* Mesfin and flower of *Hagenia abyssinica* (Bruce) J.F. Gmel were collected from Jimma zone, Sigimo woreda and Setema woreda with the period of January to February, 2012 based on information gathered from traditional healers and internet browser. The collected plant parts were then confirmed by Dr

Remesa Moochikkal, plant taxonomist and voucher specimens were stored in Jimma University herbarium under the voucher specimen numbers Yonas-2359-Echinops and Yonas-2349-Hagenia.

The plant materials were air-dried at room temperature with no direct sunlight. The air dried and powdered 200g *Echinops kebericho* root and 200g *Hagenia abyssinica* flower were extracted with 400ml 70% ethanol using soxhlet and 400ml 80% methanol using maceration technique, respectively. Extracts were evaporated using rota vapor and finally the concentrated layers were further dried in an oven at 40°c. The percent yields were calculated as dry weight of the extracts divided by the original weight of the powder used multiplied by 100 and the dried extracts were stored at 4ºc until use (Debella, A., 2002 and Sukhdev, S. H., *et al.*, 2008).

EXPERIMENTAL ANIMALS

Adult male swiss albino mice were procured from breeding colony of school of veterinary medicine of Jimma University and maintained in clean cages with free access to water and commercial pellet.

STUDY VARIABLES

INDEPENDENT VARIABLES

- Doses of plant extracts and drugs

DEPENDENT VARIABLES

- Body weight, food consumption, gross observation (weakness, hair errection and diarrhea), complete blood count, histopathology of liver and kidney for toxicity study and
- Egg count, liver granuloma score and worm count for activity study.

ACUTE ORAL TOXICITY ASSESMENT

Acute oral toxicity study of *Echinops kebericho* was done as per the Organization of Economic Co-operation Development guideline (OECD, 2001). Twenty four male mice of age 6 to 8 weeks weighing 24 to 38gm were systematically randomized using lottery method in to four treatment groups of 6 mice each in to labeled cage of dose levels based on tail mark for identification and acclimatized to the laboratory environment for one

week prior to study. Treatment doses of 0.5ml 3% Tween 80 as control, 5000mg/kg, 2500mg/kg and 1250mg/kg of *E. kebericho* extract were administered based on body weight after overnight starvation.

Individual mice observation was done at least once during the first 30 minutes after dosing and periodically within 6 hours during the first 24 hours (with special attention given during the first 4 hours at 30 minutes interval) and daily thereafter, for a total of 14 days. Parameters for observations were hair errection, walking status, diarrhea, death, body weight measurement (1^{st}, 7^{th} and 14^{th} day) and food appetite (food consumption measured on day 7 and 14). The mice were anesthetized using diethyl ether upon termination of the experiment and blood collection was done into EDTA test tubes by the cardiac puncture technique for the analysis of complete blood count (CBC). Liver and kidney were then collected and preserved in 10% formalin for histological examination.

ANTISCHISTOSOMAL ACTIVITY EVALUATION

Rivers found in Jimma town: Awetu, Boye, Kito and Cehore were visited for snail collection. However, the collected snails failed to shed cercaria despite three trials of collection. Finally, successful trial was achieved on snails collected from Aniso river of Manna woreda, Jimma zone in November 2012. The identification of snail *spps* was done based on shell size and morphology (Brown, D.S., 1994; Mandahl, B.G., 1962) and Shedding of *S. mansoni* bifurcated tail cercaria was done as per the procedure described in (Lewis, F., and Barnes. F., 2012).

Fortyfive mice of age 4 - 6 weeks weighing 22 - 34gm were then infected by the paddling technique (Frandsen, F., 1981) each with 150±10 *Schistosoma mansoni* cercaria. Direct wet mount was used for checking egg shedding of mice starting from the fourth week post cercarial exposure. Systematic randomization using lottery method based on tail mark for identification of infected mice in to eight treatment groups of five mice each was done on the 8^{th} week post infection for extracts of *E. kebericho* and *H. abyssinica,* Praziquantel at 200mg/kg (Adamu, S. U., *et al.*, 2006) and 3% Tween 80 as negative control (figure 3). Treatment was given orally in 0.5ml amount by oral gavage needle every day for five days.

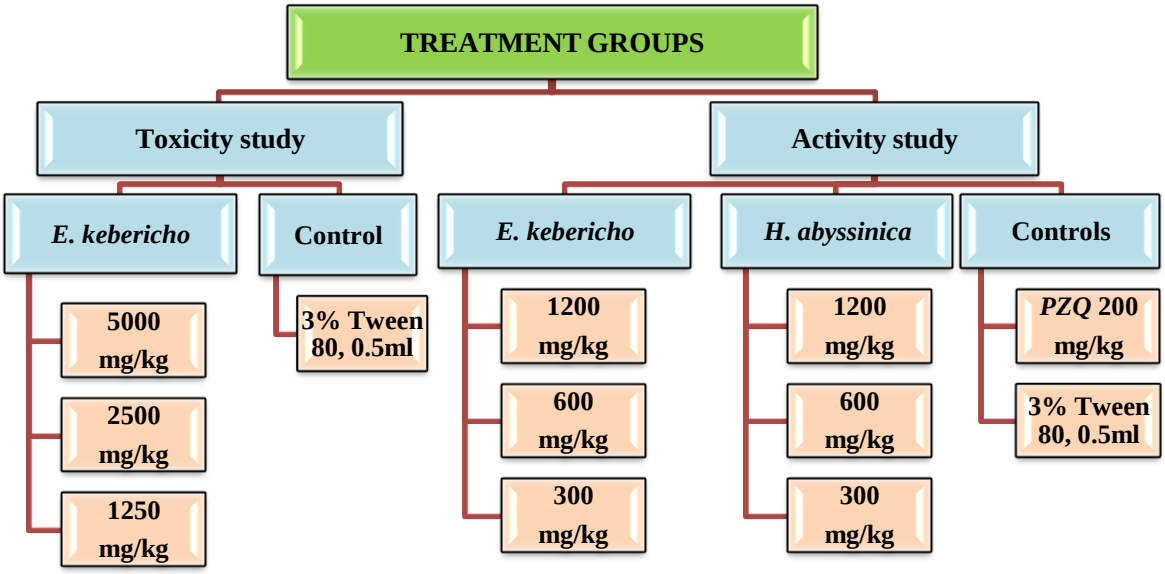

Figure 3. Mice group arrangement during *Echinops kebericho* extract toxicity study and *Echinops kebericho* and *Hagenia abyssinica* extract during activity study, Praziquantel and 3% Tween 80 administered as control.

S. mansoni ova count was done using kato katz technique (Katz, N., *et al.*, 1970) before and two weeks after treatment and the mean number of eggs per gram (EPG) of feces for each group was calculated to compare the egg reduction. The percentage egg reduction rate was calculated as:

$$\% \text{ ERR} = \left(\frac{\text{egg count before treatment} - \text{egg count after treatment}}{\text{egg count before treatment}}\right) \times 100$$

Two weeks post treatment; mice were anesthetized using 60mg/kg sodium pentobarbital (Nembutal®) via intra peritoneal injection. Upon mice dissection; gross pathological changes of liver and spleen were observed for granuloma formation and size enlargement. As a result, a score of;

- ✓ 0; assigned to a liver that had no surface lesion,
- ✓ +1; assigned to a very light mottling of lesion on the hepatic surface,
- ✓ +2; assigned to moderate mottling of lesion on the liver and
- ✓ +4; assigned to livers with densely mottled surfaces (Campbell, W.C., *et al.*, 1978).

Then the recovery of adult worms of *S. mansoni* from the hepatic portal vein and mesenteric veins were done as per the procedure described in (Duvall, R. H. and Dewitt, W. B., 1967). The adult worms were counted based on sex and the percentage of worm recovery, worm burden reduction and worm maturation calculated as:

$$\%\text{Worm maturation} = \frac{\text{number of worm recovered from infected untreated}}{\text{initial number of infecting parasites}} \times 100$$

$$\%\text{Worm Recovery} = \frac{\text{number of worm recovered from treatments}}{\text{number of worm recovered from infected untreated}} \times 100$$

$$\%\ \text{WBR} = \frac{\text{worm recovered from untreated} - \text{worm recovered from treated}}{\text{worm recovered from untreated}} \times 100$$

OPERATIONAL DEFINITIONS

Acute oral toxicity: - refers to those toxic effects occurring within 14 days following oral administration of a single dose of a substance.

Anti schistosomal activity: - refers to either reduction or complete death of adult worms or decrease or complete cessation of egg shedding or reduced pathological changes of liver and spleen.

QUALITY CONTROL

The collected plant materials were identified by the plant taxonomist and standard laboratory bred normal animals were used for the experiment. Snails were identified by taxonomist based on keys for snail identification and training required for the experiment was taken at Addis Ababa University Institute of Pathobiology. The overall experimental study was carried out by senior laboratory technologists under the follow up of the principal investigator in a sterile condition and suitable laboratory environment as per the protocol adopted for the study. The overall study was held under the supervision of senior advisors. The positive controls were subjected to praziquantel treatment (Distocide®).

DATA ANALYSIS

All data were analyzed with the software package SPSS version 16.0 and results were expressed as the mean value ± standard deviation. The results of parameters of toxicity effects and activity studies were analyzed for statistical significant differences using the

Wilcoxon, Friedman, Mann Whitney and Kruskal Wallis non parametric statistical options and all results were considered significant at $p < 0.05$ of 95% confidence interval.

ETHICAL CONSIDERATION

The ethical clearance was obtained from Jimma University ethical review board. Animal involving study was done after securing permission from school of veterinary medicine and the Ethiopian Health and Nutrition Research Institute (EHNRI) Guideline for laboratory animal use and care was strictly followed. All animals were handled with care in standard living conditions and anesthesia used during all invasive experiments and upon the termination of an experiment.

DISSEMINATION PLAN

The result of this study was submitted to Jimma University College of post graduate studies and department of laboratory sciences and pathology. A presentation was held to the Jimma University scientific community as Msc thesis defense, will progress on different research symposiums and attempt will be done to publish the paper on reputable scientific journals.

RESULTS

1. Plant parts extraction

The extraction yields of flower of *Hagenia abyssinica* using maceration technique and root of *Echinops kebericho* using soxhlet technique were 13.3% and 14.7%, respectively (Table: 1).

Table 1. Extraction techniques employed and percent yields of *Echinops kebericho* Mesfin root and *Hagenia abyssinica* flower parts.

Part of plant	Extraction technique	Solvent used	% yield
***Echinops kebericho* Mesfin root**	Soxhlet	70% Ethanol, 400ml	14.7% (28.14g/200g)
***Hagenia abyssinica* (Bruce) J.F Gmel flower**	Maceration	80% Methanol, 400ml	13.3% (26.6g/200g)

2. Acute oral toxicity profile of *Echinops kebericho* Mesfin

The 70% ethanol *E. kebericho* root extract was administered orally at a single dose of 1250mg/kg, 2500mg/kg and 5000mg/kg. Weakness, hair errection, diarrhea and presence of death were followed with a special attention during the first 4 hours and daily thereafter for 14 days post extract administration. No death was observed (6/6, 100% survival) after treatment up to a dose of 5000mg/kg during the first 24 hour (table: 2).

Hair errection and weakness were observed during the first two days in a dose dependent manner whereas no diarrhea observed throughout the study groups. As a result, treatment groups were likely to show hair errection and weakness during the first two days ($p<0.05$, Cochran's Q test). In addition, significant difference in the body weight was observed during the study period in all the study groups after extract administration ($p^1 < 0.05$, table: 2). Similarly, no statistically significant body weight difference was observed across the treatment groups with respect to days of measurement during the study period ($p^2 > 0.05$, table: 2).

Table 2. The effect of *Echinops kebericho* Mesfin 70% ethanol root extract on body weight measurement and survival of swiss albino mice.

Treatment groups	Body weight measurement			p-value[1]	Animals survived
	On D_O	On D_7	On D_{14}		
Control	30.17 ± 2.23	34.33 ± 2.34	38.00 ± 1.26	0.002	6/6 (100%)
5000mg/kg	33.00 ± 4.05	32.67 ± 3.50	35.00 ± 3.09	0.013	6/6 (100%)
2500mg/kg	31.33 ± 2.34	36.00 ± 2.00	38.33 ± 1.51	0.002	6/6 (100%)
1250mg/kg	28.50 ± 2.43	32.83 ± 2.40	37.00 ± 2.00	0.027	6/6 (100%)
P – value[2]	0.055	0.154	0.176		

Key: Statistical significance at P < 0.05; **D_O, D_7** and **D_{14}** – first, seventh and fourteenth day body weight measurement, **p – value[1]** –Friedman asymp significance, **p – value[2]** – Kruskal Wallis asymp significance

Moreover, the effect of extract on food appetite was also assessed after food provision of 1000gm on day 0 and consumption measured on day 7 and day 14. As a result, dose dependant increasing food consumption of 147gm, 231gm and 259gm was observed in decreasing treatment doses of 5000mg/kg, 2500mg/kg and 1250mg/kg, respectively while 356gm in control groups during the first week. However, no differences in food consumption measurement or recovery of appetite in treatment groups were observed during the second week. Generally, the 14 days food consumption shows loss of appetite in increasing a dose of extract treatment (Fig: 4).

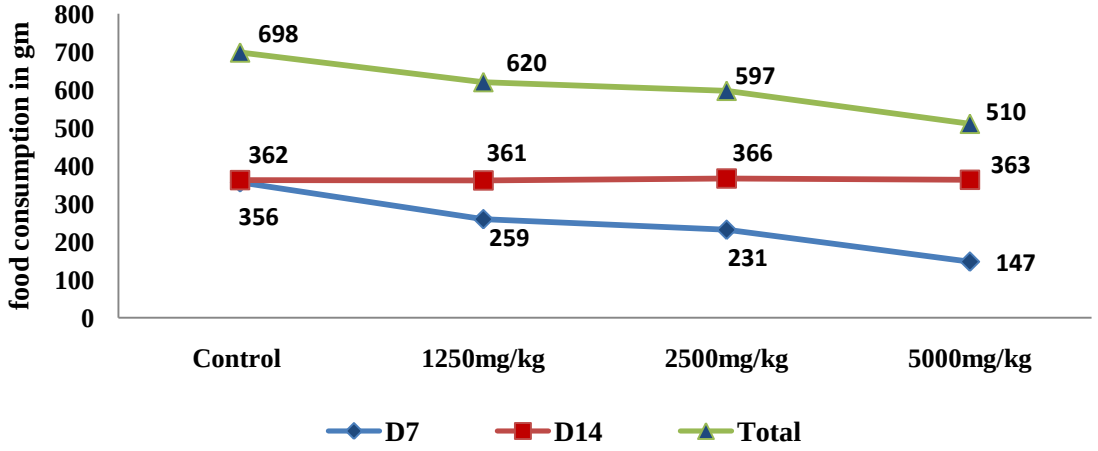

Figure 4. The effect of 70% ethanol root extract of *Echinops kebericho* Mesfin on food appetite observed by food consumption measurement on the 7th and 14th day.

About 1.5-2ml volume of blood was drawn into EDTA test tube by cardiac puncture technique for the analysis of hematological parameters such as the white blood cell (WBC), red blood cell (RBC), hemoglobin (Hgb), hematocrite (Hct), lymphocyte (Lymph) and platelet (Plt) count. As a result, no statistically significant differences in all the hematological parameters were observed within the treatment group ($p > 0.05$, table: 3).

Table 3. The effect of 70% ethanol root extract of *Echinops kebericho* Mesfin on hematological parameters of swiss albino mice.

CBC values	Control	5000mg/kg	2500mg/kg	1250mg/kg	p-value
WBC (10^3/µl)	3.17±0.37	4.06±0.98	3.07±1.27	4.14±1.46	0.130
RBC (10^6/µl)	8.78±0.87	9.23±1.28	9.78±1.27	10.26±1.54	0.249
Hgb (g/dl)	12.88±1.11	12.62±1.56	13.32±1.55	13.97±1.75	0.399
Hct (%)	49.62±2.41	46.8±6.28	50.22±6.67	54.13±8.27	0.275
Lymph (%)	77.28±24.68	71.17±10.87	66.50±21.06	79.43±7.34	0.329
Plt (10^3/µl)	974.83±486.6	1029.8±443.2	1184.7±643.9	1329.0±506.1	0.788

Key: Statistical significance difference at $P < 0.05$, **p – value** – Kruskal Wallis asymp sig

Additionally, the histological examination of liver and kidney of each mouse were performed after staining with hematoxylin and eosin and slides compared to the control group. As a result, histological examination of liver at doses of 5000mg/kg, 2500mg/kg and 1250mg/kg showed normal central vein (CV), and normal surrounding hepatocytes (HC), sinusoid (S) and kupffer cells (KC) similar to the control groups. Similarly normal appearance of the kidney of mice was observed for glomerulus (G), vascular pole (VP), urinary pole (UP), proximal convoluted tubule (PCT) and red blood cell (RBC) as compared to the control group. However, mice group treated at a dose of 5000mg/kg showed obliterated urinary space different from the control mice (fig 9 – 12, annex 5).

3. *In vivo* anti schistosomal activity study

The life cycle of *Schistosoma mansoni* was maintained in the research laboratory of veterinary parasitology as per the procedure adopted for the study. About 80 snails were brought to the laboratory during each trial and 15 of them were found infected during the fourth experiment. Forty five mice were then infected by paddling technique and four of

which died during the third and fourth week post cercarial exposure. Establishment of infection was successful in all mice observed upon egg excretion on the eighth week post cercarial exposure and resulted in 17.95% of adult worms' maturation.

Moreover, egg count was done on the 8th week post cercarial exposure and two weeks after treatment to observe the treatment effect on the fecundity of adult worm. As a result, egg reduction of 99.58% was observed in mice treated with *praziquantel* 200mg/kg. The extract of *Hagenia abyssinica* at the doses of 1200mg/kg, 600mg/kg and 300mg/kg also induced in 91.57%, 78.85% and 65.07% egg reduction, respectively (table: 4). Whereas the egg reduction rate in *Echinops kebericho* 1200mg/kg, 600mg/kg and 300mg/kg treated mice was observed to be 64.09%, 42.63% and 25.78%, respectively (table: 4). The egg burden reduction was statistically significant in all the treatment groups ($p^1<0.05$, table:4) after treatment and across the group as well ($p^2 < 0.05$, table: 4). Statistically significant difference ($p<0.05$, statistics not displayed) in egg count after treatment were observed in all *Hagenia abyssinica* and *Echinops kebericho* treated groups as compared to infected and untreated control groups.

Table 4. The mean *Schistosoma mansoni* egg per gram of feces before and after treatment of extracts of *Echinops kebericho* root, *Hagenia abyssinica* flower part, Praziquantel and 3%Tween 80.

Treatment groups	**Egg count before treatment**	**Egg count after treatment**	**ERR%**	**p-value[1]**
E_1	241.07±52.03	86.57±13.15	64.09	0.042
E_2	242.42±46.16	139.07±20.08	42.63	0.041
E_3	244.80±57.79	181.70±27.36	25.78	0.042
C_1	240.19±57.29	1.00±0.00	99.58	0.043
C_2	249.99±55.25	314.22±52.03		
p-value[2]	0.994	0.000		

Key: Statistical significance at $P < 0.05$, **p – value[1]**, Wilcoxon signed rank test asymp sig**, p – value[2]**, Kruskal Wallis asymp sig

Treatment groups	EPG before treatment	EPG after treatment	ERR%	p-value[1]
H_1	252.51±43.59	21.29±27.36	91.57	0.041
H_2	251.39±49.77	53.17±27.36	78.85	0.042
H_3	249.99±55.25	87.33±31.29	65.07	0.039
C_1	240.19±57.29	1.00±0.00	99.58	0.043
C_2	249.99±55.25	314.22±52.03		
p-value[2]	0.998	0.000		

Key: Statistical significance at P < 0.05, **p – value[1]**, Wilcoxon signed rank test asymp sig**, p – value[2]**, Kruskal Wallis asymp sig

E_1 – *E. kebericho* 1200mg/kg, **E_2** – *E. kebericho* 600mg/kg, **E_3** – *E. kebericho* 300mg/kg, **H_1** – *H. abyssinica* 1200mg/kg, **H_2** – *H. abyssinica* 600mg/kg, **H_3** – *H. abyssinica* 300mg/kg, **C_1** – *PZQ* 2/00mg/kg, **C_2** – 3% Tween 80

Additionally, the highly mottled surface and enlargement in size seen upon mice dissection and gross observation of liver and spleen in infected non treated groups indicates the establishment of pathology clearly. On the contrary, significantly lower the liver damage was observed in all the *Hagenia abyssinica* treated groups and *Echinops kebericho* at 1200mg/kg and 600mg/kg treatment doses as compared to the infected non treated groups ($p^2 < 0.05$, table: 5). Significant difference (p – value[1] < 0.05) was observed in liver granuloma score within treatment groups. As a result no significant difference were observed in *Echinops kebericho* at a dose 300mg/kg compared to infected untreated group ($p^2 > 0.05$, table: 5). *Hagenia abyssinica* at a dose levels of 1200mg/kg and 600mg/kg showed no significant difference compared to praziquantel treated groups ($p^3 > 0.05$, table: 5).

Table 5. The mean *liver granuloma score of mice* treated with extracts of *Echinops kebericho* root, *Hagenia abyssinica* flower part, Praziquantel and 3%Tween 80.

Treatment groups	E_1	E_2	E_3	C_1	C_2	p-value[1]
Mean liver score	1.6	1.8	2.2	0	3.6	0.002
p-value[2]	0.014	0.041	0.065*	0.004		
p-value[3]	0.005	0.005	0.005			

Key: Statistical significance at P < 0.05, **p – value[1]**: Kruskal Wallis, **p-value[2/3]**: Mann Whitney: p – value[2] no difference compared to C_2 (*), p – value[3] no difference compared to C_1 (**)

Treatment groups	**H_1**	**H_2**	**H_3**	**C_1**	**C_2**	**p-value[1]**
Mean liver score	0.4	0.8	1.0	0	3.6	0.002
p-value[2]	0.006	0.009	0.009	0.004		
p-value[3]	0.134**	0.053**	0.017			

Key: Statistical significance at P < 0.05, **p – value[1]**: Kruskal Wallis, **p-value[2/3]**: Mann Whitney: p – value[2] no difference compared to C_2 (*), p – value[3] no difference compared to C_1 (**)

E_1 – *E. kebericho* 1200mg/kg, **E_2** – *E. kebericho* 600mg/kg, **E_3** – *E. kebericho* 300mg/kg, **H_1** – *H. abyssinica* 1200mg/kg, **H_2** – *H. abyssinica* 600mg/kg, **H_3** – *H. abyssinica* 300mg/kg, **C_1** – *PZQ* 2/00mg/kg, **C_2** – 3% Tween 80

Finally, the experiment ends with perfusion of mice for the recovery of adult worms to observe the treatment effect on the survival of adult worm. As a result, worm recovery of 4.27% was observed in mice treated with *praziquantel* 200mg/kg indicating the 95.73% worm burden reduction. *Hagenia abyssinica* extracts of 1200mg/kg, 600mg/kg and 300mg/kg showed 4.91%, 14.78% and 26.59% worm recovery and 95.09%, 85.22% and 73.41% worm burden reduction, respectively. Treatment with *Echinops kebericho* 1200mg/kg, 600mg/kg and 300mg/kg also showed 31.01%, 50.09% and 67.43% worm recovery and 68.99%, 49.91% and 32.57% worm burden reduction (table: 6). Additionally, higher proportion of male adult worms were recovered along the treatment groups. Besides, statistically significant differences in male, female and total worm counts were observed across the treatment group's (p < 0.05).

Table 6. The mean number of *Schistosoma mansoni* adult worms perfused from mice after the treatment of *Echinops kebericho* root, *Hagenia abyssinica* flower parts extract, 3%Tween 80 and Praziquantel.

Treatment groups	Male worm	Female worm	Total worm	WBR%	%WR
E_1	5.47±1.34	3.89±1.00	8.35±2.30	68.99	31.01
E_2	7.92±1.22	6.55±0.89	13.49±1.95	49.91	50.09
E_3	10.17±0.84	8.98±0.71	18.16±1.30	32.57	67.43
C_1	1.15±0.45	1.00±0.00	1.15±0.45	95.73	4.27
C_2	14.97±1.00	12.94±1.41	26.93±2.24		
P - value	0.000	0.000	0.000		

Key: Statistical significance at p-value < 0.05, **p - value –** Kruskal Wallis asymp sig, **WBR-** Worm Burden Reduction, **WR-** Worm Recovery

Treatment groups	Male worm	Female worm	Total worm	WBR%	%WR
H_1	1.32±0.55	1.00±0.00	1.32±0.55	95.09	4.91
H_2	3.10±0.84	1.89±0.71	3.98±1.30	85.22	14.78
H_3	3.98±1.30	2.55±1.09	7.16±0.84	73.41	26.59
C_1	1.15±0.45	1.00±0.00	1.15±0.45	95.73	4.27
C_2	14.97±1.00	12.94±1.41	26.93±2.24		
P - value	0.000	0.000	0.000		

Key: Statistical significance at p-value < 0.05, **p - value –** Kruskal Wallis asymp sig, **WBR-** Worm Burden Reduction, **WR-** Worm Recovery

E_1 _ *E. kebericho* 1200mg/kg, **E_2** _ *E. kebericho* 600mg/kg, **E_3** _ *E. kebericho* 300mg/kg, **H_1** _ *H. abyssinica* 1200mg/kg, **H_2** _ *H. abyssinica* 600mg/kg, **H_3** _ *H. abyssinica* 300mg/kg, **C_1** – *PZQ* 2/00mg/kg, **C_2** – 3% Tween 80

DISCUSSION

The oral route of administration is the most convenient and commonly used when studying the acute toxicity. As a result, the extract of *Echinops kebericho* was found safe up to 5000mg/kg due to observations of absence of death indicating LD_{50} to be greater than 5000mg/kg. Moreover, substances with LD_{50} higher than 5000mg/kg by oral rout are regarded as being safe or practically non toxic (Kenedy, G.L., *et al.*, 1986). Besides, mice in the treatment group of 5000mg/kg dose showed weakness and hair errection during the first two days after treatment which could be due to the extract dose and taste discomfort.

However, no diarrhea but dose dependant appetite loss was observed during the study period. The body weight change was also significant across the group. Thus, the observed dose dependant weakness could be attributed to the suppression of the animals' appetite by the extract leading to reduced food intake. The phytochemicals found in the extract might also contribute for the changes observed. Generally, the reduction in body weight gain is simple and sensitive indices of toxicity after exposure to toxic substances. As a matter of fact, body weight loss of more than 10% from the initial body weight will be significant in toxicity studies (Raza, M., *et al.*, 2002; Teo, S., 2002).

The present study therefore revealed the oral acute toxicity of E. *kebericho Mesfin* extract at doses of 1250mg/kg and 2500 mg/kg didn't exhibit any signs of toxic effects. Mice treated with dose of 5000 mg/kg exhibited a little toxic effect but no mortality, suggesting that toxic effects will be increased progressively with increasing the dose. In addition, hematological indices in animals are important to determine the toxicity risk since the changes in the blood system have a higher predictive value for human toxicity (Oslon, H., 2000). However, no statistically significant difference were observed in the WBC, RBC, Hgb, Lymph, Plt and Hct measurements across the treatment group ($p > 0.05$, table: 3). Therefore, hematological parameters studied revealed that the extract couldn't bring any alteration on measurements.

Whereas, the liver is known to be a key organ in the metabolism and detoxification of xenobiotics and kidneys, also due to their high rate of perfusion and ability to concentrate a range of substances in the tubular lumen, are vulnerable to damage induced by a huge

variety of chemicals (Hatschek, W., *et al.*, 2002; Kumar, V., 2004). Thus, histological examinations of liver of mice treated with doses of 1250mg/kg and 2500mg/kg showed normal central vein, and normal surrounding hepatocytes, sinusoid and kupffer cells. The observed histological appearance of the kidney was also normal and the same as the control group at these treatment doses. This showed that the plant extract had no effect on the organs of the animals at these doses. In addition, histological examination of liver of mice treated with 5000mg/kg showed normal cells as the control groups. However, histological examination of kidney of mice treated with 5000mg/kg showed obliterated urinary space different from the control mice. This might be due to the toxic effects of the plant extract at this dose.

The oral acute toxicity of both of our study plants is therefore safe due to the LD_{50} greater than 5000mg/kg of a single administration. As a result, a maximum dose of 1200mg/kg for five days was used for anti-schistosomal activity study to achieve a maximum efficacy with no toxic effects. Therefore, mice were infected with the pooled cercaria shed from infected snails for the extract activity study and also infection was successful in all exposed mice. However, four of mice were found dead on the third and fourth week post cercarial exposure. This could be due to the overdoses of cercaria, weakness because of clinical manifestations and were young in age during exposure.

Schistosoma mansoni infection in mice then resulted in excretion of half of the eggs in faeces of the total shaded eggs by the adult worms living in the portal and mesenteric veins as the rest eggs will be lodged in the tissue of intestine and liver. The major pathology of schistosomiasis is therefore granulomatous inflammation observed due to those retained eggs, a cellular immune response to antigens secreted by schistosomes' ova trapped in organs as lung, liver and spleen. Therefore, the reduction of hepatomegaly, splenomegaly and granuloma after treatment might suggest a possible anti-inflammatory role of plant extracts. As a result, significant reduction in liver score was observed in all *Hagenia abyssinica* and *Echinops kebericho* at 1200mg/kg and 600mg/kg treatment doses ($p < 0.05$, table: 5). Liver score of mice in *Hagenia abyssinica* 1200mg/kg and 600mg/kg were found similar to *praziquantel* treated group ($p > 0.05$, table: 5). However,

no significant difference was observed in *Echinops kebericho* 300mg/kg as compared to infected untreated groups ($p > 0.05$, table: 5).

Nevertheless, the reduction in the egg density and worm burden in treated mice was considered by several authors as a strong evidence of the efficiency of antischistosomal drugs (Mati, V.L., *et al*., 2010; Mostafa, O.M.S., 2001). Thus, the egg reduction rate observed in mice treated with praziquantel was the highest indicating the potency of the drug. The egg count in *Hagenia abyssinica* treated mice also shows remarkable reduction as compared to the infected untreated control group ($p < 0.05$, table: 4). Also statistically significant difference in egg count was observed in mice after the treatment with *Echinops kebericho* extracts ($p < 0.05$, table: 4). Besides, the egg count in *Hagenia abyssinica* 1200mg/kg and 600mg/kg treated mice groups were comparable to Praziquantel treated group ($p > 0.05$, table: 4). Therefore, both of the plant extracts reduced the egg burden in a dose dependant manner even if their potency differs.

The reduction of the egg load in the faeces in treated mice may be attributed to the reduction in the worm burden as a result of treatment, the low productivity of the female already present and the active destruction of the few eggs produced by the host's tissue reaction (Mostafa, O.M.S., 2001). Moreover, the total worm burden recovered from the untreated control group gave an idea of the rate of experimental infection. The mean total of 26.93 worms recovered represents an infection rate of 17.95 %. This corroborates with other work (Standen, O. D, 1963) where the rate of development of cercaria to adult schistosomes worms was found to be between 18-22%.

The present study showed higher proportion of male worms from the total adult worms recovered along the group (table: 6). This could be due to the fact that male female interactions could take an important place in the establishment of higher male proportion due to the development of a lot of genotypes of the parasites, inducing a strong competition between females and thus an increase in the male proportion (Boissier, J. and Moné, H., 2000, 2001). Therefore, the proportions of adult female worms recovered were lower in number across the treatment groups. The higher the egg reduction observed in this study could indicate the egg reduction emanates either from the lower the worm

recovery due to death of the adult worms or reduced fecundity of female worms already present. Thus, the lowest worm recovery and higher worm burden reduction in praziquantel treated group was expected and can be attributed to the fact that praziquantel has good efficacy against the adult *S. mansoni* worm. The invitro antischistosomal property of *Hagenia abyssinica* was supported in the present study indicating the potential of the plant for future study (Thomsen, H., *et al.*, 2012). Our finding indicates the extract of *Hagenia abyssinica* possess better potency in reducing the total adult worms including female worms which could contribute for reduced fecundity. *Echinops kebericho* also revealed significant reduction of adult worm in all the three treatment doses.

Thus, polar constituents of our plant extracts possess anti schistosomal activity especially with a better potency of *Hagenia abyssinica* extract treatment. The chemical compounds present in this extract could be responsible for their bioactivity. It has been also shown that antimicrobial and antiparasitic properties of plant extracts are assigned to some chemicals compounds as tannins, terpenes, flavonoids, phenols, and alkaloids present in plants extract (Perrett, S., *et al.*, 1995; Cowan, M.M., 1999; Lydiard, J.R., *et al.*, 2002). Activity against *Plasmodium falciparum* and Gram-positive bacteria of Indoloquinoline alkaloids extracted from *Sida acuta* (Karou, D., *et al.*, 2003, 2005), analgesic and anti-inflammatory activities of alkaloids and flavones (Sutradhar, R.K., 2006) and activity against *S. japonicum* of sesquiterpene lactones isolated from *Vernonia amygdalina* (Jisaka, M., 1992) were reported as responsible chemicals.

CONCLUSION

The oral acute toxicity evaluation of *Echinops kebericho Mesfin* 70% ethanol root extract showed no death in mice up to 5000mg/kg indicating the LD_{50} to be above 5000mg/kg. Additionally, no significant toxic effects were observed after oral administration of a single dose extract except the loss of appetite and obliterated urinary space of the kidney indicating a dose dependant toxicity effect of the extract. Therefore, the plant was found safe or non toxic in mice up to 5000mg/kg.

Moreover, evaluation of the anti schistosomal activity showed a significant egg reduction and worm reduction in both plant extracts treated mice of all treatment groups even if their potency of activity varies. As a result, *Hagenia abyssinica (Bruce) J.F Gmel* oral administration induced 91.57% egg reduction and 95.09% worm reduction while 64.09% and 68.99%, respectively in *Echinops kebericho Mesfin at the higher dose level.* Similarly, significant liver granuloma reduction was seen in all *Hagenia abyssinica* treatment doses and *Echinops kebericho* treatment at the higher dose level.

Therefore, this study revealed out both plant extracts possessed anti schistosomal activity with significant effect on worm count, ova count and also liver granuloma.

RECOMMENDATION

- ✓ Further toxicity studies including pathological examination of other organs to observe the potential toxic effects upon long term administration of the *Echinops kebericho Mesfin* extract should be conducted.
- ✓ Evaluation of anti schistosomal activity of *Echinops kebericho Mesfin* using non polar solvents and oil extracts including invitro activity study should be conducted.
- ✓ Further studies involving isolation, identification of active constituents, standardization, formulation and clinical trial including activity on snails, other developmental stages and mechanism of action of *Hagenia abyssinica (Bruce) J.F Gmel* should be well illustrated for its future use.

REFERENCES

Abbink, J., (1995). Medicinal and ritual plants of the Ethiopian South-west: An account of recent research. Indig. Knowl. and devel. monitor. 3(2): 6-8.

Abebe, D., (1986). Traditional medicine in Ethiopia, The attempt being made to promote it for effective and better utilization. Ethiop. J. Science 9: 61-69.

Abebe, G., Dawson, L.J., Detweiler, G., Gipsonand, T.A. and Sahlu, T., (2000). *Hagenia abyssinica* (kosso) for internal parasite control in goats. In: Proceedings of a conference held at Debub University, Awassa, Ethiopia from November 10 - 12, 2000. E (Kika) de la Garza Institute for Goat Research, Langston University, Langston, OK.

Abebe, G., Kiros, M., Golasa, L. and Zeynudin, A., (2009). *Schistosomiasis mansoni* infection among patients visiting a health center near Gilgel Gibe dam, Jimma, South Western Ethiopia. East Afr. J. Publ. Health: 6(3), 300-2.

Adamu, S.U., Kela, S.L. and Suleiman M.M., (2006). Antischistosomal properties of extracts of *jatropha curcas (l)* on *Schistosoma mansoni* infection in mice. A*fr. J. Trad. Cam.* 3(2): 37–41.

Afaf, K.E., Samia, A.A. and Sanaa, A.A., (2007). Anti schistosomal and liver protective effects of *Curcuma longa* extract in *Schistosoma mansoni* infected mice. Indian j. of exp. biology: 45, 791-801.

Alemeshet Y., Sileshi K. and Yoseph M., (2010). Impact assessment of Gilgel Gibe hydroelectric dam on schitosomiasis: a cross sectional study in South West Ethiopia. Ethiop. J. Heal. Science: 20(2).

Ali, A., Erko, B., Woldemichael, T., Kloos, H., (2006). Schistosomiasis. In: Berhane, Y., Hailemariam, D., Kloos, H. eds. Epidemiology and ecology of health and diseases in Ethiopia. 1st edition, Addis Ababa: Shama books, 660-673.

Ali, S. A., (2011). Natural Products as therapeutic agents for schistosomiasis. Res. J. of Med. Plant. 5(1): 1-20.

Assefa, B., Glatzel, G., Buchmann, C., (2010). Ethnomedicinal uses of *Hagenia abyssinica* (Bruce) J. F Gmel among rural communities of Ethiopia. J. of Ethnobiol. & Ethnomed. 6(1): 20.

Badreldin, H.A., (2006). A short review of some pharmacological, therapeutic and toxicological properties of praziquantel in man and animals. Pak. J. Publ. Science: 19(2), 170-75.

Biruksew, A., (2012). Anti malarial activity of *Echinops kebericho Mesfin* and *Zingiber officinale* against rodent *plasmodium burghie* invivo. Unpublished.

Boissier, J. and Moné, H., (2000). Experimental observations on the sex ratio of adult *S. mansoni*, with comments on the natural male bias. Parasitology: 121, 379–83

Boissier, J. and Moné, H., (2001). Relationship between worm burden and male proportion in *Schistosoma mansoni* experimentally infected rodents and primates. A meta-analytical approach. Int. J. Paras. 31:1597–1599.

Broome, R.L, Feng, L., Zhou, Q., Smith, A., Hahn, N., Matsui, S.M., Omary, M.B., (1999). Non-invasive transgenic mouse genotyping using stool analysis. Federation of European Biochemical Societies: Letters: 462, 159-160.

Brown, D.S., (1994). Freshwater snails of Africa and their medical importance. London: Taylor and Francis. 2nd editions.

Caffrey, C. R., (2007). Chemotherapy of schistosomiasis: present and future. Curr. Opin. in chem. Biol. 11(4): 433-439.

Campbell, W. C., Eva, B. and Cuckler, A. C., (1978). A method for detecting therapeutic activity against *schistosoma mansoni* in mice. J. of Paras: 64(1), 69-77.

CDC: Schistosomiasis life cycle. http://www.dpd.cdc.gov/dpdx. Accessed date 12/11/2012.

Cheesbrough, M., (2009). District laboratory practice in tropical countries. Cambridge University Press: part 1, 2nd edition.

Chitsulo, L., Engels, D., Montresor, A. and Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Trop.* 77: 41-51.

Cowan, M.M., (1999). Plant products as antimicrobial agents. Clin. Micr. Rev., 12: 564- 82.

Debella, A., (2002). Manual for phytochemical screening of medicinal plants. Department of drug research, Ethiopian Health and Nutrition Research Institute: Addis Ababa, Ethiopia.

Dejenie, T., Asmelash T. and Abdelkadir, M., (2010). Efficacy of PZQ in treating *S. mansoni* infected school children in Tumuga and Waja, Ethiopia. MEJS: 2(2); 3-11.

Demma, J., (2005). Toxicological study of *Glinus lotoides* and *Hagenia abyssinica*: Traditionally used taenicidal herbs in Ethiopia. Unpublished: 1-98.

Deribe, K., Amberbir, A., Getachew, B. and Mussema, Y., (2006). A historical overview of traditional medicine practices and policy in Ethiopia. Eth. J. of Heal. Devel. 20(2): 127-134.

Dery, B., Otsyina, R., et al. (1999). Indigenous knowledge of medicinal trees and setting priorities for their domestication in Shinyanga Region, Tanzania, World Agroforestry Centre.

Desta, B., (1995). Ethiopian traditional herbal drugs. Part I: Studies on the toxicity and therapeutic activity of local taenicidal medications. J. of Ethnophar. 45(1): 27-33.

Doenhoff, M. J., Hagan, P., Cioli, D., Southgate, V., Pica-Mattoccia, L., Botros, S., Coles, G., Tchuem Tchuente, L. A., Mbaye, G, A. and Engels, D., (2009). Praziquantel: its use in control of schistosomiasis in SSA and current research needs. Parasitology: 136, 1825-35.

Domo, A.G., Kela, S.L., Istifanus, W.A. and Umar, I., (2009). Comparative worm load recovered from laboratory animals treated with local plant extracts and praziquantel. Int. J. Biomed. & Heal. Science: Vol. 5, No. 4.

Duvall, R. H. and Dewitt, W. B., (1967). An improved perfusion technique for recovering adult schistosomes from laboratory animals. The Am. J. of trop. Med. and hygiene: 16(4).

Engels, D., Chitsulo, L., Montresor, A. and Savioli L., (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta Tropica. 82(2): 139-146.

Erko, B., Degarege, A., Tadesse, K., Mathiwos, A. and Legesse M., (2012). Efficacy and side effects of praziquantel in the treatment of *Schistosomiasis mansoni* in school children in Shesha kebele elementary school, Wondo Genet, Southern Ethiopia. As. Pac. J. of T. Bio.: 235-239.

Fabricant, D.S. and Farnsworth, N.R., (2001). The value of plants used in traditional medicine for drug discovery. Envir. Heal. Perspectives. 109, 69–75.

Fassil, H., (2005). Beyond plants, professionals & parchments: The role of home-based medicinal plant use and traditional health knowledge in primary health care in Ethiopia. Ethnobot. Res. & Appl. 3:037-049.

Frandsen, F., (1981). Cultivation of schistosomes for chemotherapeutic studies. Acta Pharmacologica et Toxicologica: 49, 118-122.

Friis, I. (1992). Forest and forest trees of North East tropical Africa. Their natural habitats and distribution patterns in Ethiopia, Djibouti and Somalia. Kew Bulletin, additional series XV. Her majesty's stationary office, London.

Ghebreyesus, T.A, Witten, K.H, Getachew, A., Haile, M., Yohannes, M., Lindsay, S.W., Byass, P., (2002). Schistosome transmission, water resource development and altitude in Northern Ethiopia. Ann. Trop. Med. Parasitol. 96:489–495.

Giday, M., Asfaw, Z., et al. (2003). An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. J. of ethnopharm. 85(1): 43-52.

Gillespie, S. H. & Pearson, R. D., (2001). Principles and Practice of Clinical Parasitology. John Wiley & Sons Ltd: 369-405.

Gryseels, B., (2012). Schistosomiasis. Infect Dis Clin N Am. 26: 383–397.

Haile, S., Golassa, L. and Mekonnen, Z., (2012). Prevalence of *Schistosoma mansoni* and effectiveness of praziquantel in school children in Finchaa valley, Ethiopia. J. of Paras. and Vect. Biology. Vol. 4(3), pp. 25 -30.

Hatschek, W., Rousseaux, C., Wallig, M., (2002). Handbook of toxicologic pathology. Vol1, 2nd ed. Academic press, New York. p. 187-362.

Hedberg, I., Friis, I. and Edwards, S. (2004). Flora of Ethiopia and Eritrea. Vol. 4, part 2, Addis Ababa University, Addis Ababa. pp. 221–223.

Hedberg, O., (1989). Rosaceae. In: Hedberg, I. & Edwards, S. (Eds). Flora of Ethiopia, Pittosporaceae to Araliaceae, Vol. 3. National Herbarium, AAU, Addis Ababa, Ethiopia.

Hussien, J., Urgessa, K., Regassa, F., Jemal, A., Abajebel, S. and Hussien, N., (2011). Antihelminthic activities of the essential oil extracts of selected medicinal plants against *Haemonchus cortortus*. Inter. J. of Agric. Research: 6(3), 290-298.

Hymete, A. and Kidane, A., (1991). Screening for antihelminthic activity in two *Echinops spp*. Ethiop. Pharm. J: 9 (1), 67–71.

Hymete, A., Iversen, T.H., Rohloff, J. and Erko, B., (2005). Screening of *E. ellenbeckii* and *E. longisetus* for biological activities and chemical constituents. Phytomed. 12: 675-679.

Hymete, A., Rohloff, J., Iversen, T.H. and Kjøsen, H., (2007). Volatile constituents of the roots of *Echinops kebericho Mesfin*. Flavour and Fragr. J. 22: 35 -38.

Ismail, M., Metwally, A., Farghaly, A., Bruce, J., Tao, L.F, Bennett, J.L., (1996). Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. The Am. J. of Trop. Med. and Hyg. 55(2): 214.

Jaijoy, K., Vannasiri, S., Piyabhan, P., Lerdvuthisopon, N., Boonraeng, S., Khonsung, P., Lertprasertsuke, N. and Sireeratawong S., (2011). Acute and subchronic toxicity study of the water extract from the fruits of *Piper chaba Hunter* in rats. Inter. J. of Appl. Res. in Nat. Products: 3 (4), 29-35.

Jansen, P.C.M. & Getachew, A., (2002). *Hagenia abyssinica (Bruce) J.F.Gmel*. Record from Protabase. Oyen, L.P.A. & Lemmens, R.H.M.J. (Editors). PROTA (Plant Resources of Tropical Africa/ Ressources végétales de l'Afrique tropicale), Wageningen, the Netherlands.

Jatsa, H.B., Ngo Sock, E.T., Tchuem Tchuente, L.A., Kamtchouing, P., (2009). Evaluation of the *in vivo* activity of different concentrations of *Clerodendrum umbellatum poir* against *Schistosoma mansoni* infection in mice. Afr. J. Trad., CAM: 6 (3): 216 – 221.

Jisaka, M., Kawanaka, M., Sugiyama, H., Takegawa, K., Huffman, M.A., Ohigashi, H. and Koshimizu, K., (1992). Antischistosomal activities of sesquiterpenes lactones and steroid glucosides from *Vernonia amygdalina*, possibly used by wild chimpanzees against parasite-related diseases. Biosc Biotech Bioch; 56: 845-846.

John M.M., Helen L. K., Susy M., Rebecca W., Dorcas S. Y., (2012). The evaluation of the effects of aqueous and methanol extract of the *Solanum Incanum* on *Schistosoma mansoni* infected mice. As. J. of pharm. and heal. Science: 2(1).

Karou, D., Dicko, M.H., Sanon, S., Simpore, J. and Traore, A.S., (2003). Antimalarial activity of *Sida acuta Burm. f.* (Malvaceae) and *Pterocarpus erinaceus* Poir. (Fabaceae). J Ethnopharm; 89: 291-294.

Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpore, J., Colizzi, V. and Traore, A. S., (2005). Antibacterial activity of alkaloids from *Sida acuta*. Afr J Biot; 4: 1452-57.

Kassa, M., Mshana, R., Regassa, A. and Assefa, G., (1998). Invitro test of five Ethiopian medicinal plants for anti-malarial activity against *P. falciparum*. SINET: EJSc; 21:81-89.

Katz, N., Coelho, P.M. and Pellegrino, J., (1970). Evaluation of kato's quantitative method through the recovery of *S. mansoni* eggs added to human feces. J. Paras. 56: 1032– 33.

Kenedy, G.L., Ferenz, R.L.J. and Burgess, B.A., (1986). Estimation of acute toxicity in rats by determination of the approximate lethal dose rather than the LD50. J. A. Toxicology: 6, 145-48.

Kloos, H., Lo, C.T., Birrie, H., Ayele, T., Tedla, S., Tsegay, F., (1988). Schistosomiasis in Ethiopia. Soc Sci Med. 26(8):803–827.

Kumar, V., Abbas, A. K., Fausto, N., (2004). Robbins & Cotran pathologic basis of disease, 7th ed. Philadelphia: Elservier Saunders, New York. p. 831-981.

Lewis, F. and Liang, Y.S., (2012). Control of rotifers and other commensals on/within *Biomphalaria glabrata* snails. NIAID Schisto Resource Center.

Lewis, F., (2012). Perfusion of adult worms from mice. NIAID Schisto Resource Center.

Lewis, F., and Barnes. F., (2012). Shedding snails. NIAID Schisto Resource Center.

Lewis, F.A., Stirewalt, M.A., Souza, C.P., Gazzinelli, G., (1986). Large scale laboratory maintenance of *Schistosoma mansoni*, with observations on three schistosome/snail host combinations. The J. of paras. 72(6): 813-829.

Lo C.T., Kloos, H. and Birrie H., (1988). The ecology of health and disease in Ethiopia. In: Ahmed, Z.Z, Kloos, H., editors. Schistosomiasis. Addis Ababa, Ministry of Health: 196-213.

Lyddiard, J.R., Whitfield, P.J. and Bartlett, A., (2002). Antischistosomal bioactivity of isoflavonoids from *Millettia thonningii* (Leguminosae). J. Paras., 88: 163 – 170.

Mahmoud, A.F., (2008). In Harrison's principles of internal medicine. Schistosomiasis and other trematode infections. McGraw-Hill: 17th Ed, pp 1330-1334.

Mandahl, B.G., (1962). Key to the identification of East and Central African freshwater snails of medical and veterinary importance. Bull. WHO: 27; 135-150.

Mati, V.L., Freitas, R. M., Melo, A.L., (2010). Effects of pentoxifylline during *Schistosoma mansoni* infection in swiss mice: an analysis of worm burden, fecundity and liver histopathology. J. Helminthol, 29:1–7.

Mekonnen, Z., Hussen, Y., Suleman, S., Van Mark, E. and Chatterjee, S., (2007). Prevalence of *schistosoma mansoni* infection among patients visting Agaro health centre, Jimma zone, south west Ethiopa. Abstract.

Mekonnen, Z., Meka, S., Ayana, M., Bogers, J. P., Vercruysse, J. and Levecke, B., (2012). Pooling stool samples: a cost effective strategy to assess infection intensity of soil-

transmitted helminthes and to monitor drug efficacy? International workshop proceeding on Schisto/STH.

Melman, S. D., Steinauer, M. L., Cunningham, C., Kubatko, L.S., Mwangi, I. N., Wynn, N. B., Mutuku, M. W., Karanja, D. M., Colley, D. G., Black, C. L., Secor, W. E., Mkoji, G. M. and Loker, E. S., (2009). Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PLoS Negl. Trop. Dis. 3(8), e504. doi: 10.1371/journal.pntd.0000504.

Mengistu, A., Gebre-Selassie, S. and Kassa, T., (2007). Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. *Ethiop.J.Health Dev.* 21:1, 1-6.

Mengistu, M., Shimelis, T., Torben, W., Terefe, A., Kassa, T. And Hailu, A., (2011). Human intestinal schistosomiasis in communities living near three rivers of Jimma town, South Western Ethiopia. Ethiop. J. Health Sci. 21(2), p 1-8.

Mesfin, F., Demissew, S. and Teklehaymanot, T., (2009). An ethnobotanical study of medicinal plants in Wonago woreda, SNNPR, Ethiopia. J. of Ethnobiol. and Ethnomed. 5:28.

Milligan, J.N. and Jolly, E.R., (2011). Cercarial transformation and invitro cultivation of *Schistosoma mansoni* schistosomules. J. of Visual. Experiments: 54.

Mohamed, A.M., Metwally, N.M., Mahamoud, S.S., (2005). Sativa seeds against *Schistosoma mansoni* different stages. Mem. Inst. Osw. Cruze: 100, 205-211).

Mostafa, O.M., Eid, R.A. and Adly, M.A., (2011). Antischistosomal activity of ginger (*Zingiber officinale*) against *Schistosoma mansoni* harbored in C57 mice. Paras. research. 109: 395-403.

Mostafa, O.M.S., (2001). Experimental use of black seed oil against *Schistosoma mansoni* in albino mice: I. some parasitological and biochemical parameters. Egypt J Med Lab Sci. 10(2):99–113.

Muchika, S., Kutima, H.L., Maranga, R.O., Yole, D.S., (2011). Antischistosomal effects of *Solanum incanum* and *Carica papaya* crude extracts on the parasite *Schistosoma mansoni* invivo and invitro. The Internet J of Trop Medicine: 7(2).

Nahed, H.A, Hoda, A.T. and Yomna, I.M., (2009). Effects of *Garlic* on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study. Trop Biomed: 26(1), 40–50.

Negash, L. (1995). Indigenous trees of Ethiopia: biology, uses and propagation techniques. pp. 125–147. Printed by the SLU Reprocentralen, Umea˚, Sweden. ISBN 91-7191-105-7.

Nibret, E. and Wink, M., (2010). Trypanocidal and antileukaemic effects of the essential oils of *Hagenia abyssinica*, *Leonotis ocymifolia*, *Moringa stenopetala* and their main individual constituents. The Free Library. http://www.thefreelibrary.com/Trypanocidal and antileukaemic effects of the essential oils of...-a0242964338 (accessed May 15, 2013).

OECD, (2001). The Organization of Economic Co-operation Development (OECD) guideline for testing of chemical: 425 acute oral toxicity up and down procedure.

Olivier, L. and Stirewalt, M., (1952). An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. The J. of paras.: 38(1),19-23.

Orwa, C., Mutua, A., et al. (2009). Agroforestree database: a tree reference and selection guide version 4.0. world agroforestry centre: Nairobi, Kenya. Pp 1-5.

Oslon, H., Betton, G., Robinson, D., Thomas, K., Monro, A. and Kolaja, G., (2000). Concordance of toxicity of pharmaceuticals in humans and in animals. Regul. Toxic. and pharmacy. 32: 56–67.

Parise-Filho, R. & Silveira, M.A., (2001). Panorama atual da esquistossomíase no mundo. Braz. J. of Pharmac. Sciences: 37(2), 123-135.

Payet, M., Perie, P. and Sankale, M. (1966). Cliniques Africaines. Gauthier-Villars Paris: pp. 67-80.

Perrett, S., Whitfield, P.J., Sanderson, L. and Bartlett, A., (1995). The plant molluscicide *Millettiath onningii* (Leguminosae) as a topical anti schistosomal agent. J. Ethnopharm., 47: 49 – 54.

Peter, M.L., Michael, F.C., John, H., William, L.I., Nino P., Pradhib, V. and Katherine, N.W., (2010). Case studies in infectious disease. Garland Science, Taylor & Francis Group: 393-402.

Phillipson, J. D., (1994). Natural products as drugs. Trans R Soc.Trop.Med.Hyg. 88:17-19.

Pica- Mattoccia, L. and Cioli, D., (2004). Sex and stage related sensitivity of *Schistosoma mansoni* to invivo and invitro praziquantel treatment. Int. J. for paras. 34(4): 527-533.

Pica-Mattoccia, L., Carlini, D., Guidi, A., Cimica, V., Vigorosi, F., Cioli, D., (2006). The schistosome enzyme that activates oxamniquine has the characteristics of a sulfotransferase. MemÃ³rias do Instituto Oswaldo Cruz. 101: 307-312.

Raza, M., Al- Shabanah, O.A., El-Hadiyah and Al-Majed, A. A., (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of swiss albino mice. Sci. pharmaceut., 70: 135-145.

Richard, L.G., David, H.W. and Peter, F.W., (2004). Tropical infectious diseases principles, pathogens and practice. vol 1: 2nd edition.

Ryan, K. J. and Ray, C.G., (2004). Sherris medical microbiology, an introduction to infectious diseases. Mcgraw-hill: 4th edition.

Sher, Z.A., (2001). Safe, effective herbal antischistosomal therapy derived from *myrrh*. Am. J. Trop. Med. Hyg: 65, 700-704.

Simonsen, P.E., Nega, A., Furu, P., (1990). Intestinal schistosomiasis among children in a labour village of Wonji sugar estate, Ethiopia. East Afr Med J. 67:532–538.

Sourabh, S.B., Sonal, D., Prashant, S., Priya, S. and Yogesh, S., (2011). Acute toxicity study of aqueous extract of *Coccinia indica* (roots). Asian J. Res. Pharm. Sci. 1(1), 23-25.

Standen, O. D., (1963). Chemotherapy of helminthes infections; In experimental chemotherapy vol I (Schnitzer, R. J and hawking, F. Eds). Academic press inc. London. Pp 701-892.

Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J., (2006). Schistosomiasis and water resources development: systematic review, meta-analysis and estimates of people at risk. The Lanc. Inf. Dis. 6(7): 411-425.

Stelma, F.F., Talla, I., Sow, S., Kongs, A., Niang, M., Polman, K., Deelder, A.M. and Gryseels, B., (1995). Efficacy and side effects of praziquantel in an epidemic fFocus of *Schistosoma mansoni*. The Am. J. of Trop. Med. and Hyg. 53(2): 167-170.

Sukhdev, S. H., Suman, P.S., Gennaro, L. and Dev, D.R., (2008). Extraction technologies for medicinal and aromatic plants. Trieste: international centre for science and high technology.

Suleiman, M.I., Akarim, E.I., Ibrahim, K.E., Saad, A.M., Mohammed, A.E., Ahmed, B.M., Suleiman, S.M., (2004). Antischistosomal effects of praziquantel, its alkaline hydrolysis and sun decomposed products on experimentally *S. mansoni* infected albino mice. (A)

Efficacy assessment based on clinicopathological findings. J. Egy. Soc Paras. 34(1):131–142.

Sutradhar, R.K., Rahman, A.M., Ahmad, M., Bachar, S.C., Saha, A. and Guha, S.K., (2006). Bioactive alkaloid from *Sida cordifolia* Linn with analgesic and anti-inflammatory activities. Iran. J. Pharm. Therap; 5: 175-178.

Tadesse, M. and Abegaz, B. (1990). A review of the genus Echinops (Composite, craude) in Ethiopia with notes on phytogeography and chemistry. Proceedings of the 12th plenary meeting of AETFAT: pp 605-629.

Tariku, Y., Hymete, A. Hailu, A. and Rohloff, J., (2011). Invitro evaluation of antileishmanial activity and toxicity of essential oils of *Artemisia absinthium* and *Echinops kebericho*. Chemistry & Biodiversity: Vol. 8.

Taylor, J.L., Rabe, T., McGaw, L.J., Jäger, A.K. and van Staden, J., (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation: 34, 23–37.

Tchuem Tchuenté, L.A., Southgate, V.R., Mbaye, A., Engels, D. and Gryseels, B., (2001). The efficacy of praziquantel against *Schistosoma mansoni* infection in Ndombo, Northern Senegal. Trans. of the roy. Soc. of Trop. Med. and Hyg. 95(1): 65-66.

Teklehaymanot, T., Giday, M., Medhin, G. and Mekonnen, Y., (2007). Knowledge and use of medicinal plants by people around Debre Libanos monastery in Ethiopia. J. of Ethnopharm. 111: 271–283.

Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Vikram, K., (2002). A 90 day oral gavage toxicity study of D-methlphenidate and D, L methylphenidate in sprague-dawley rats. Toxicology, 179: 183-196.

Thomsen, H., Reider, K., Franke, K., Wessjohann, L.A., Keiser, J., Dagne, E. and Arnold N., (2012). Characterization of constituents and antihelminthic properties of *Hagenia abyssinica*. Sci. Pharm. 80: 433–446.

Tilahun, T. and Mirutse, G., (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. J. of Ethnobiol. and Ethnomed: 3(12).

Utzinger, J., Chollet, J., Tu, Z., Xiao, S., and Tanner, M., (2002). Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. Trans. R. Soc. Trop. Med. Hyg. 96(3):318–323.

WHO, (1998). Report of the WHO informal consultation on monitoring of drug efficacy in the control of schistosomiasis and intestinal nematodes. Geneva. pp. 1-45.

WHO, (2002a). Traditional medicine strategy 2002–2005. WHO/EDM/TRM/2002.1. World Health Organization Geneva.

WHO, (2002b). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee, World Health Organization.

WHO, (2003a). Traditional medicine. Fact sheet 134.

WHO, (2003b). African traditional medicine: our culture, our future. African Health Monitor 4(1).

WHO, (2003c). WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. Geneva, Switzerland.

WHO, (2004). The world health report 2004: changing history, annex table 3: burden of disease in DALYs by cause, sex and mortality stratum in WHO regions, estimates for 2002. Geneva.

WHO, (2011). The world medicines situation; traditional medicines: global situation, issues and challenges. World Health Organisation, Geneva. 3rd ed: 2(3).

Woldemariam, T.Z, Fell, A.F, Linley, P.A, Bibby, M.C. and Phillips, R.M., (1992). Evaluation of the anti-tumor action and acute toxicity of kosins from *Hagenia abyssinica*. J. Pharm. Biomed. Anal; 10: 555–560.

Yohannes, P. and Dagne E., (1983). Comparative study of the male and female flowers of *Hagenia abyssinica*. SINET: Ethiop. J. Scie. 6 (1): 31-34.

Zeinab H.F., Amal M.E., Wafaa E., Eman A. and Sayed S. A., (2009). Potential antiparasitic activity of pomegranate extracts against shistosomules and mature worms of *Schistosoma mansoni*: invitro and invivo study. Aus. J. of Bas. and Ap. Sciences. 3(4): 4634-43.

ANNEXES

ANNEX - 1 MEDICINAL PLANT PARTS COLLECTION, IDENTIFICATION AND EXTRACTION PROCEDURES

MEDICINAL PLANT PARTS COLLECTION, IDENTIFICATION AND EXTRACTION

Specimen collection is an essential part of any further traditional medicine study for identification purpose, storage as a voucher (reference materials), bioassay, toxicity tests, & phytochemical screening tests. Follow the steps described below:

- Identify plant parts and the local use, gather information from traditional healers
- Register the date of collection, site of collection (location and habitat), season of collection, name of collector, local use if available and collect for identification
- Identification by botanist and storage at herbarium with voucher number
- Dry at room temperature under shaded area and grind into powder

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The soxhlet ethanol for *E. kebericho* and methanol maceration for *H. abyssinica* extraction is selected for this study.

SOXHLET EXTRACTION

- The finely ground plant part (100gm) is placed in a porous bag or "thimble" and placed in chamber of the Soxhlet apparatus.
- The extracting solvent (400ml) added in flask is heated and its vapors condense in condenser. The condensed extractant drops into the thimble and extracts it by contact.
- When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon drops into flask.
- This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.
- Evaporate with rota vapor and dry under oven at 40-50 ^{o}c and store at 4^{o}c after calculating the percentage yield of the dried and powdered product.

MACERATION EXTRACTION

- The finely ground plant part is measured (200mg) and placed in a closed vessel and extracting solvent added until covering over the powder.

- Daily shaking, after 3 days filter with filter paper, evaporate using rota vapor, dry under oven and finally store at 4^{o}c for the later use.

(Debella, A., 2002; Sukhdev, S. H. *et al.*, 2008).

*** Use distilled water, washed and steralized equipments, follow the storage refrigerator for the proper functioning and electric supply and keep the extract uncontaminated. In case any contamination or growth on the storage material is seen discard the extract.

ANNEX - 2 ACUTE ORAL TOXICITY PROFILE STUDY PROCEDURES

ANIMAL SPECIES SELECTION, HOUSING AND FEEDING CONDITIONS

- Adult swiss white mice that are healthy, female/male that are nulliparous and non-pregnant kept in a clean cages. Age between 8 and 12 weeks old six per test group.
- Room temperature with 22°C ± 3°C and Relative humidity between 30 % and 70 %
- Artificial lighting with 12 hours light and 12 hours dark. Unlimited supply of food commercial pellet and drinking water. Keep clean the cage daily and working environment.
- Kept in their cages for at least a weak prior to dosing to allow for acclimatization to the laboratory conditions.

PREPARATION OF ANIMALS, TREATMENT AND ADMINISTRATION OF DOSES

- The animals are randomly selected based on lottery of tail marked number for individual identification
- Use the volume of 1-2ml/100g of body weight ~ 0.2-0.5ml for oral dosage.
- The test substance is administered in a single dose by oral gavage needle.
- Animals should be overnight fasted prior to dosing 12hr (food but not water).
- Following the period of fasting, weigh the animals and calculate the extract dose for administration. Use sensitive analytical balance for weighing.
- Administer maximum dose of 5000mg/kg for six mice and the next dose with half of the first at least for three doses and follow for 24hrs strictly for follow up parameters.
- Provide food after the substance has been administered.

Example: Let average weight of mice is 30gm then 5000mg/kg dose will be:

5000mg _ 1000g, $X = \underline{150mg}$,

Xmg _ 30g

Dose calculation will be [150mg X (6+1 mice/group) = 1050mg/3.5ml 3% Twn 80]
+ 1 is for contingency

OBSERVATIONS

- Observe animals individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days.
- Parameters include hair errection, walking status, diarrhea, body weight (1^{st}, 7^{th} and 14^{th} day), food appetite or food consumption (1^{st}, 7^{th} and 14^{th}), histopathology of liver and kidney and CBC hematology. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible. Collect the blood (1.5-2ml) by cardiac puncture and store the organs in 10% formalin solution for histopathology under proper anesthesia (Diethyl ether).
  (OECD, 2001).

ANNEX -3 *IN VIVO* ANTI SCHISTOSOMAL EXTRACT ACTIVITY TESTING PROCEDURES

SNAIL COLLECTION

Snails normally inhabit shallow rocky or stable wooden structures along the shore line where they feed on complex mixture of algae and other microorganisms growing on the structures. Snails are located visually, collected by gloved hand or scoop and identified to be *Biomphalaria pfeiferi* based on the shell morphology and size measurement (Brown, D.S., 1994; Mandahl, B. G. 1962).

- Upon arrival at a collection site, conduct a visual search for snails.
- As snails are observed, collect them by gloved hand or scoop.
- After verifying that a snail is alive, place the snail in a pre-labeled sample jar or clean tray filled with water from the site.
- Measure the size of snail (*Biomphalaria spps* are planorbids with size up to 14mm)

CERCARIA SHEDDING FROM SNAILS

After cercaria mature within the snail, they are then liberated into the surrounding water depending on the lighting conditions of investigator's choosing (Lewis, F., and Barnes, F., 2012).

Equipment, Materials and reagents

Incubator that can hold a steady temperature around 26ºC or you can use artificial light, featherweight forceps, 200 ml beakers, aged tap water

Procedure

- Using featherweight forceps, remove snails from the maintenance pan, and place them in a 200 ml glass beaker with 50 ml aged tap water.
- Place the beaker under a strong artificial light source, in a 26ºC incubator, water bath, expose to sun light for 1-2 hours, taking care not to overheat the snails.
- Remove the snails, and place them back into the maintenance pan.
- Pour the cercaria suspension into a clean beaker.

COUNTING CERCARIA

Known numbers of cercaria may be delivered by two general methods depending on the conditions of the experiment (Olivier, L. and Stirewalt, M., 1952).

- In the first method cercaria are transferred drop by drop and the number of cercaria in each drop is counted at the time of transfer with the aid of a dissecting microscope. It is somewhat tedious, time consuming, employed when great accuracy is necessary and when the number of cercaria used is small.
- When large numbers of cercaria per mouse are required they may be delivered into the tubes by aliquot from a stirred suspension of known concentration. This concentration can be determined by delivering a series of aliquots into ruled syracuse watch dishes and counting the cercaria.
- Few drops of iodine can be used for making ease the counting procedure. Then simple for estimating number of cercaria in required volume of cercarial suspension.

Equipment, Materials and reagents

- Dissecting microscope, suspension of *S. mansoni* cercaria, adjustable eppendorf pipette (200 µl), Aged tap water, 60mm diameter scored petridish

Procedure

- Withdraw 200 µl of a cercaria suspension. It is important to mix the suspension gently to evenly distribute cercaria before withdrawing aliquots.
- Place the aliquot in a scored, 60 mm diameter petridish.
- Add a sufficient amount of aged tap water to cover the bottom of the petri dish.
- Under a dissecting microscope count the cercaria.

Ξ *For our experiment count 150 ± 10 cercaria*

MICE CERCARIAL EXPOSURE

Animals are infected either by skin penetration, usually through the tail or shaved abdominal skin or via sub-cutaneous or intra peritoneal injection of cercaria.

Paddling technique of mice exposure

Equipment, Materials and reagents

- Beaker, pipette, dissecting microscope, aged tap water

Procedure

- Pipette a pre-calculated number of cercaria into the beaker of 600ml filled with 60ml aged tap water.
- Pick up a mouse by its tail and put in the beaker.
- After 45-60 minutes remove the mouse, release it to its cage.

EGG COUNTING AFTER MICE CERCARIA EXPOSURE

Use direct wet mount for fecal examination of mice starting from 4^{th} week post cercarial exposure. Employ kato technique for counting egg in mice found infected before and two weeks after treatment twice for observing the egg reduction.

Fecal sample collection

- Collect mice stool after holding the mouse by grasping the skin around the neck and positioning the mouse upright until a stool droplet is excreted (requires < 30sec) directly into a stool cup (Broome, R., *et al.*, 1999) or by placing the mouse in a clean cage/ beaker then collecting the stool droplet.

Kato Katz technique

Materials and reagents

Plastic template, fresh stool, nylon screen or mesh, spatula, cellophane, microscopic slides, glycerol, gloves, malachite green, news paper

Procedure

- Place a small amount of saline moistened fresh mice stool on news paper.
- Press the screen over the feces to remove large particles and scrap the filtered feces with spatula.
- Transfer to the hole of a template placed on a slide and remove the template gently.

- Cover with a piece of cellophane soaked in 50% glycerol (glycerin) added 3% malachite green (50ml glycerol, 50ml distilled water, 0.5ml 3% malachite green).
- Press the cellophane to distribute preparation evenly or invert the slide over the cellophane and press over smooth surface.
- The glycerol clears the fecal material from around the eggs. Wait 24 hrs for reading the slides systematically and the counted number of eggs is then multiplied by 24 to calculate egg per gram of faces.

  (Cheesbrough, M., 2009).

SAFETY MEASURES

Schistosoma cercaria can directly penetrate the skin without the needs of a cut or pores, and cause the chronic disease schistosomiasis. Therefore proper personal protective equipment and proper safety measures must be followed. Latex gloves should be worn, as should a lab coat, a waterproof pair of shoes. In case of an accidental spill, immediately spray contaminated area with 70% ethanol or 1% bleach. This will be sufficient to disable the parasite, but all possible infections should be reported and taken seriously. Disinfect all surfaces that come into contact with any potentially infectious substance after the experiment is performed and discard waste in an approved biohazard container (Milligan, J.N. and Jolly, E.R., 2011).

GENERAL CONSIDERATIONS OF COMMON PROBLEM

- Continuous water aeration for 4-5 days or up to 1 week to reduce chlorine levels may be sufficient treatment.
- Optimal snail growth, sporocysts development and cercaria shedding occur at 26 ^{O}c.
- Flashing tap water on snail shell, snail feces continuous removal and restarting the snail colony with uncontaminated snails or egg masses will keep maintenance ease.
- Of natural foodstuffs, lettuce (fresh, boiled, or baked and dried) serves as food.
- No more than 10-15 prepatent snails should be kept per liter and the water should be changed at least twice per wk**.**

(Lewis, F. A., *et al.*, 1986; Lewis, F. and Liang, Y.S. 2012)

TREATMENT OF MICE

The plant extract and the control drug *praziquantel* will be administered for those mice found infected.

Rout of administration

The most common routs of administration of drugs are oral, subcutaneous, intramuscular, intravenous, intrathecal, rectal, nasal and topical application.

Oral gavage

- Feeding needles with a ball tip helps prevent introduction of the needle into the trachea and prevents trauma to the oral cavity.
- With the mouse restrained in one hand the feeding needle is introduced.
- As the needle approaches the pharynx the mouse will usually swallow allowing introduction into the esophagus.
- Using the feeding needle to gently extend the neck facilitates introduction into the stomach.
- With the stomach tube fitted to a syringe or aspirator, materials may be administered or withdrawn as required.
- Care must be taken that the tube or needle does not enter the trachea or puncture the esophagus or stomach.
- A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea.

Dose of administration

- Maximum dose with 1200mg/kg, Intermediate dose with 600mg/kg and Minimum dose with 300mg/kg daily for five days period of both plant extracts for in vivo activity test via 0.5ml/mice 3% Tween 80 as a vehicle solution.
- Praziquantel as positive control with 200mg/kg daily for five days

Example: let the average weight of mice for the group is 30g.

Then 1200mg _ 1000g X = 36mg

Xmg _ 30g

So [36mg X (5+1 mice/group) X 5 days / 0.5ml X (5+1 mice/group) X 5 days] = 1080mg/ 15ml 3% Tween 80

For Praziquantel: weight 20 tablets and take the average weight, then powder the tablet

Let average weight be 0.9045g or 904.5mg then

200mg = 1000g X = 6mg

Xmg = 30g

Then 1 tablet = 904.5mg = 600mg active ingredient *X = 9.045mg*

X mg = 6mg

So [9.045mg X (5+1 mice/group) X 5 days] = 271.35mg

LIVER GRANULOMA SCORE

Upon dissection, torn open the abdominal cavity and observe the liver and spleen of each mice for pathological changes in the enlargement of size and granuloma formation. Liver granuloma score will be done as described in (Campbell, W.C., *et al.*, 1978). A score of 0; assigned to a liver that had no surface lesion, +1; assigned to a very light mottling of lesion on the hepatic surface or 1-3 granuloma per lobe, +2; assigned to moderate mottling of lesion on the liver or 4-10 granuloma per lobe and +4; assigned to livers with densely mottled surfaces or >10 granuloma per lobe.

PERFUSION OF ADULT WORMS FROM MICE

For chemotherapeutic studies it is common to compare the number of schistosoma worms in treated and untreated control animals, which is usually done after schistosoma have matured (6 - 8 weeks after infection). In a bisexual worm infection in the mouse, adult *S. mansoni* typically reside in the mesenteric veins, from which they can be harvested by perfusion of the portal venous system (Lewis, F. 2012; Duvall, R. H. and Dewitt, W. B., 1967).

Materials and Reagents

- 22 or 24# injection needle, petridish, anesthetic anti- coagulant solution, perfusion fluid
- Anesthetic anti-coagulant solution: 3000 units of heparin in 100ml of Na-pentobarbital 60mg/ml).
- Perfusion fluid: 8.5gm of NaCl, 7.5gm of Na-citrate and 1000ml of water

Procedure

- Kill the mouse by intraperitonal injection of 0.3ml of anesthetic anti-coagulant solution.
- Tear off the skin of mouse to avoid the worms sticking to the hair and Cut open peritoneal and thoracic cavity as well as diaphragm to expose the aorta.
- Pin down the animal on the perfusion board and tear open the portal vein by lifting the liver.

- Insert #22 or #24 injection needle into the aorta and start pumping the perfusion fluid. This step requires keen eye sight and you must succeed the first time.
- Rinse the body cavity with the perfusion fluid thoroughly.
- Pour the perfusion fluid into a petridish and examine for worms after 3 minutes.

ANNEX – 4 MATERIALS, EQUIPMENTS, REAGENTS AND OTHER NECESSARY REQUIREMENTS FOR THE STUDY

-22 or 24# injection needle
-70% alcohol
-Adhesive tape
-Aged tap water
-Analytical balance
-Aquarium
-Artificial light source
-Beaker
-Binocular microscope
-Bleach
-Cage
-Caliper
-Cellophane
-Centrifuge
-Cercaria
-Cotton
-Dissecting microscope
-Distilled water
-*Echinops kebericho Mesfin* root
-Ethanol
-Filter paper
-Flask
-Forceps
-Formalin
-Gauze pads
-Glove
-Glycerol
-*Hagenia abyssinica (Bruce) J.F Gmel* flower
-Heating mantle
-Hematology analyzer
-Heparin
-Incubator
-Iodine
-Kato template
-Laboratory coat
-Lettuce
-Malachite green
-Marker
-Methanol
-Microscope slides
-Mortal and pistil
-NaCl
-Normal saline
-Nylon mesh
-Oral gavage needle
-Oven
-Pencil
-Petridish
-Pipette
-Praziquantel
-Refrigerator
-Rota vapor
-Scissor
-Scoop
-Snails
-Sodium citrate
-Sodium pentobarbital
-Soxhlet apparatus
-Spatula
-Swiss white mice
-Test tube
-Test tube rack
-Water bath

ANNEX – 5 PICTURE PLATES SHOWING STUDY WORK FLOW

Figure 5. *Hagenia abyssinica* (Bruce) J.F Gmel tree and *Echinops kebericho* Mesfin root.

Figure 6. The soxhlet (right) and maceration (left) techniques of extraction used for the study.

Figure 7. Standard mice cage with commercial pellet and drinking water.

Figure 8. Cardiac puncture technique of blood collection (right) and liver and kidney of mice (left) collected during toxicity study.

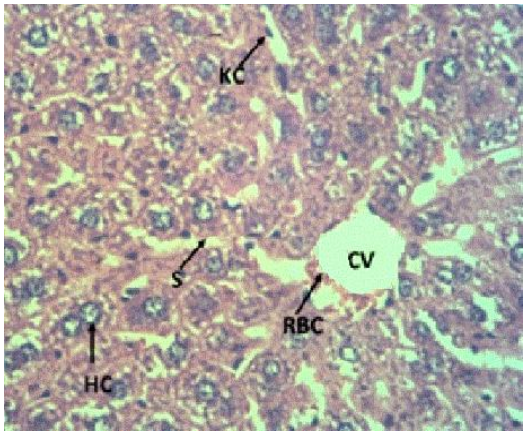

Figure 9. Photomicrograph of the hematoxylin and eosin stained liver sections of control group showing the central vein (CV), and surrounding normal hepatocytes (HC), sinusoid (S) and kupffer cells (KC) (H and E, X400)

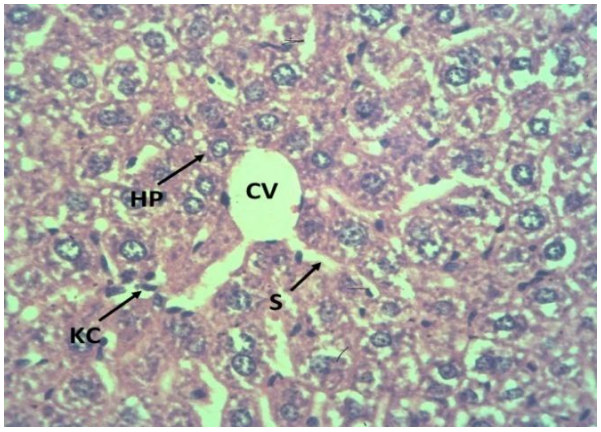

Figure 10. Photomicrograph of the hematoxylin and eosin stained liver sections of mice treated with a dose of 5000mg/kg of 70 % ethanol extract of *Echinops kebericho* showing the central vein (CV), and surrounding normal hepatocytes (HC), sinusoid (S) and kupffer cells (KC) (H and E, X400)

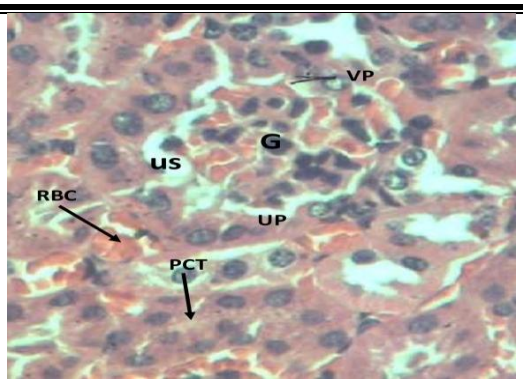

Figure 11. Photomicrograph of hematoxylin and eosin stained kidney section of the control group showing normal histological appearance of glomerulus (G), vascular pole (VP), urinary pole (UP), proximal convoluted tubule (PCT), red blood cell (RBC), (H and E, X400)

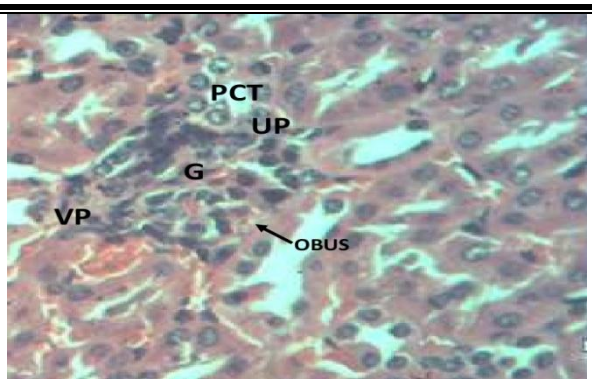

Figure 12. Photomicrograph of hematoxylin and eosin stained kidney section of mice treated with a dose of 5000mg/kg of 70 % ethanol extract of *Echinops kebericho* showing normal histological appearance of glomerulus (G), vascular pole (VP), urinary pole (UP), proximal convoluted tubule (PCT), red blood cell (RBC) but obliterated urinary space (OUBS), (H and E, X 400)

Figure 13. Snail collection using scoop and the collected snails from the Aniso river.

Figure 14. Identified snails of *Biomphalaria pfeiferi spps* 10 -12mm.

Figure 15. An artificial snail maintaining aquarium and wilted lettuce in it as snail food.

Figure 16. Snails kept in water bath (right) and under artificial light (left) to shed cercaria.

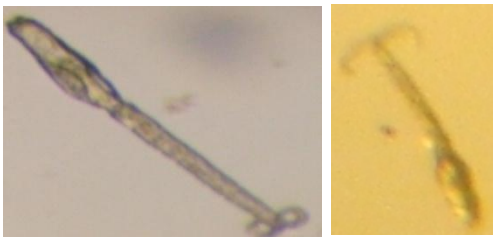

Figure 17. *Schistosoma mansoni* bifurcated tail cercaria shaded from snails.

Figure 18. Exposure of experimental animals to cercaria using paddling technique.

Figure 19. Stool prepation of kato katz smear for *schistosoma mansoni* ova counting.

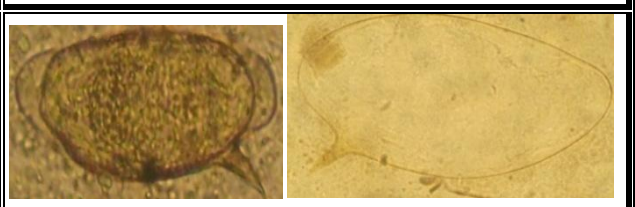

Figure 20. *Schistosoma mansoni* ova examined in wet mount (right) and kato katz (left), X 40.

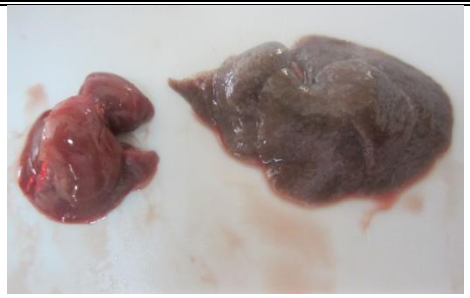

Figure 21. Liver of mice treated *praziquantel* (right) and infected untreated (left).

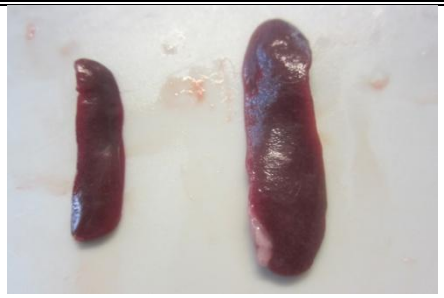

Figure 22. Spleen of mice treated Praziquantel and infected but left untreated mice

Figure 23. Perfusion of the adult worm from the hepatic portal and mesenteric veins of mice**.**

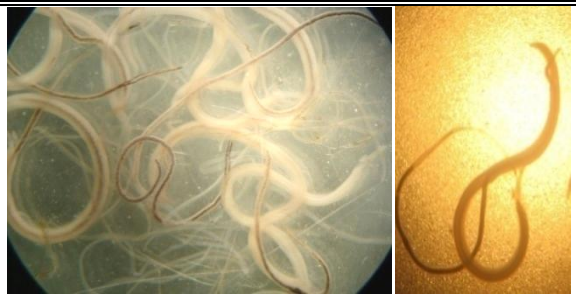

Figure 24. *Schistosoma mansoni* adult worms recovered from mice.