KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

ANTI-PARASITIC ACTIVITIES OF SOME SELECTED MEDICINAL PLANTS AND MARINE ALGAE IN GHANA

By

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmaceutics, KNUST and Center for Discovery and Innovation in Parasitic Diseases, University of California-San Diego, United States of America. All the necessary acknowledgements have been made. I hereby declare that this work has not been submitted to any university or institution for the award of a degree.

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ABSTRACT

Neglected tropical diseases (NTDs) are a group of major chronic conditions that affects people in the poor regions of sub-Saharan Africa, Asia, Latin America and the Caribbean. Current medications have untoward side effects and the microorganisms responsible for these NTDs have grown resistant to some of these medicines. There is the need to get new medicines for the treatment of NTDs. Ghana as a country is biodiversitically rich in medicinal plants and marine flora. In this study, extracts, fractions of extracts and isolated compounds of some plants and marine algae (*U. fasciata, S. vulgare* and *H. dentata*) were screened against *Giardia lamblia, Entamoeba histolytica, Trypanosoma brucei brucei, Schistosoma mansoni* and *Naegleria fowleri*: *N. fowleri, G. lamblia* and *E. histolytica* were screened using the CellTitre Glo luminescent cell viability assay while *T. b. brucei* and *Schistosoma mansoni* (juvenile and adult stages) were, respectively screened using the SYBR-Green assay and phenotypic observation of physical changes of the worms. The ethanol extracts of the barks of *A. glaberrima* and *M. angolensis*, pet ether fraction of *M. nobilis* bark and ethyl acetate fraction of *E. ivorense* bark exhibited IC_{50}s ranging between 13.76 and 44.25 µg/mL against *G. lamblia*. For *N. fowleri*, ethanol bark extract of *A. glaberrima* had IC_{50} of 38.70 µg/mL. The acetone and ethyl acetate fractions of the bark of *E. ivorense*, the aqueous leaf extract of *P. muellerianus* and the pet ether fraction of *M.* nobilis exhibited activity against *T. b. brucei* (IC_{50}: 10.53 - 25.60 µg/mL). The ethyl acetate and acetone fractions of *E. ivorense* caused rounding of the schistosomulae at concentrations of 0.31 and 0.63 µg/mL respectively, whiles the adult worms were killed at 1.25 µg/mL (both fractions) within 48 h. Among the algae studied, only *U. fasciata* showed inhibitory activity against *G. lamblia* (IC_{50} of 35.86 µg/mL). None of the plant extracts and fractions exhibited activity against *E. histolytica*. However, xylopic acid and geraniin inhibited *E. histolytica* with IC_{50}s of 4.80 and 34.71 µg/mL respectively. Xylopic acid also exhibited anti-parasitic activity against *G. lamblia* (IC_{50} of 11.45),

T. b. brucei (IC_{50} of 13.42) and *N. fowleri* (IC_{50} of 16.06 µg/mL). Geraniin as well showed inhibitory activity against *T. b. brucei* (IC_{50} of 6. 41 µg/mL). Phytochemical screening of the algae revealed the presence of saponins, tannins and alkaloids in all the three species. *H. dentata* and *S. vulgare* also possessed glycosides and flavonoids while sterols were present in *H. dentata* and *U. fasciata*. The plant extracts and compounds exhibited activity against the parasites tested and hence the use of these medicinal plants in the treatment of the studied parasitic infections may be justified.

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DEDICATION

I dedicate this work to Dr. Christian Agyare and Mr Benjamin Kyere-Davies. The two gentlemen who have kept me going.

Ms. Martha Lilian Obeng. The woman who made it all possible.

LIST OF PUBLICATIONS

CONFERENCE PAPERS/ABSTRACTS

1. Kyere-Davies, G., Agyare, C., Suzuki, B., Caffrey, C. (2016). Antischistosomal activity of some selected Ghanaian plant medicines. Ghana Biomedical Convention 2016, Ho, Ghana, 1st to 5th August, 2016. Abstract number 810.
2. Kyere-Davies, G., Agyare, C., Debnath, A., Caffrey, C., McKerrow, J. (2016). Antigiardial activity of some plant extracts and fractions from Ghana. 9th Joint Natural Products Conference, Copenhagen, Denmark, 24th to 27th July. 2016. Abstract number P199.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

APIs	Active Pharmaceutical ingredients
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
Ca^{2+}	Calcium ion
CATT	Card Agglutination Test for Trypanosomiasis
Cl^-	Chloride ion
CSF	Cerebrospinal Fluid
CO_2	Carbon dioxide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIAs	Enzyme Immunoassays
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
$H+$	Hydrogen ion
HAT	Human Africa Trypanosomiasis
HCL	Hydrochloric acid
H_2O	Water
H_2SO_4	Sulphuric acid
IACUC	Institutional Animal Care and Use Committee
$K+$	Potassium ion
MNZ	Metronidazole
MRI	Magnetic resonance imaging
NTDs	Neglected tropical diseases
NaOH	Sodium hydroxide
PCR	Polymerase chain reaction
TNF-α	Tumor necrosis factor alpha
WHO	World Health Organisation
UCSD	University of California, San Diego

CHAPTER ONE

1.0 INTRODUCTION

Neglected tropical diseases (NTDs) are a group of 13 major chronic conditions. They are a subset of infectious diseases caused by pathogens (Hotez *et al.*, 2007) and represent some of the most common infections of people in the poor regions of sub-Saharan Africa, Asia, Latin America and the Caribbean (Lindoso and Lindoso, 2009; Hotez *et al.*, 2008).

Mathers *et al.* (2007) reported that NTDs affected about 177,000 people globally in 2002 with 20 million disability adjusted life and years and they represent 1.3% of the world's global burden of diseases and injuries. Hotez *et al.* (2006a) also reported that the NTDs affected about 543,000 people annually and about 57 million disability adjusted life. They all together represent the fourth most important communicable disease worldwide. This shows the degree of the seriousness of NTDs. Ghana as a country in the sub Saharan Africa has not been spared from NTDs. Nkegbe (2013) reported that about 50% of the people in the Volta and Greater Accra regions of Ghana suffered from schistosomiasis. *Giardia lamblia* was responsible for about 89.5% of intestinal protozoan infections in children and adolescents under the age of 18 years in Ghana (Nkrumah and Nguah, 2011). About 47.8% of school going children are infected with schsistosomiasis in the rural north of Ghana (Anto *et al*., 2014)

The NTDs can be parasitic, bacterial, protozoan, viral and fungal (Hotez *et al.*, 2008). The NTDs include three vector-borne protozoan infections, leishmaniasis, human African trypanosomiasis, and Chagas disease; three bacterial infections, trachoma, leprosy, and Buruli ulcer; and seven helminth infections, hookworm, ascariasis, trichuriasis, lymphatic filariasis, onchocerciasis, guinea worm (drancunculiasis) and schistosomiasis. Cysticercosis, food-borne trematodiases and

some other infections such as giardiasis and amoebiasis could be included in this list depending on the region (Hotez *et al.,* 2006a; Keiser and Utzinger, 2005; Lun *et al.,* 2005). The causative organisms of these NTDs are; *Ascaris lumbricoides, Trichuris trichiura* and the hookworm (*Necator americanus* and *Ancylostoma duodenale*) and the two schistosomes (*Schistosoma haematobium* and *Schistosoma mansoni*) which are highly endemic to sub-Saharan Africa. Other parasites include *Trypanasoma brucei, Trypanasoma cruzi*, *Leishmania donovani, Mycobacterium lepri, Brugia malayi, Wuchereria bancrofti* and *Onchocerca volvulus*. Other NTDs agents are *Giardia lamblia* and *Entamoeba histolytic*.

These NTDs cause childhood growth impairment, lack of fitness and anaemia through mechanisms like intestinal blood loss, autoimmune haemolysis, haematuria, hypersplenism, red blood cell sequestration and chronic inflammation (Fleming and de Silva, 2004; Friedman *et al*., 2005). They are also able to cause long term disability, disfigurement, reduced economic productivity, social stigma, poverty and death (Hotez *et al.,* 2006b; Hotez and Kamath, 2009). There are few medicines used in the management and treatment of these NTDs which have been in use and remained unchanged since the middle of the 20th century (Hotez *et al.,* 2006b). According to the WHO 2003, failure to use existing tools effectively, inadequate or non-existent tools and insufficient knowledge of the disease are the three factors contributing to the burden of illness associated with these NTDs. Trouiller *et al.* (2002) reported that there has been insufficient allocation of resources to fight diseases that affect the poor such as the NTDs by society despite advances in medicine and healthcare. These could be some of the reasons why the NTDs still thrive and affect lots of people today.

The best way of controlling NTDs at this point is through the expanded use of mass drug administration (Hotez and Kamath, 2009). This is to facilitate the eradication of NTDs. This calls

for the attention of scientists and researchers in the field of medicine to discover more drugs that are cost effective, cheap and readily available.

For over 25 years, studies have revealed that medicinal plants can be a good source of active pharmaceutical ingredients (APIs) (Cordell *et al.,* 2001). Farnsworth and Morris (1976) reported that about 50% of community pharmacy-based prescription products were obtained from plant products. O'Neill and Lewis (1993) also reported that the leading pharmaceutical products in 1991 were from natural products or had a natural product as its core. Grifo *et al.* (1997) also reported that out of the 150 top products that were prescribed, according to the US National Prescription Audit, 57% of them had either a natural product as the active compound or was once derived from or patterned after compounds from natural products. Of the 25 drugs that were sold mostly in Dutch in 1996, 47% of them were derived from or produced to mimic natural products (De Smet, 1997). According to WHO, 2003, some African countres including Ghana use traditional as first line treatment for children ith fever and the global market for herbal medicine currently stands at at over US $ 60 billion annually and is steadily growing. This shows that natural products have been a part of the pharmaceutical industry. These natural products could be important in the discovery of new active drug compounds and pharmaceuticals for the treatment and management of NTDs.

When it comes to plants that have prospects as active natural compounds, the tropical forest is considered the most promising habitat since it has high biodiversity and endemism (Gentry, 1993; Macilwain, 1998). All the above evidence proves that falling on plants for the search for new active pharmaceutical ingredients is in the right direction and will be good for the pharmaceutical industry.

Ghana has a rich biodiversity (Brown, 1992). This biodiversity contains lots of active medicinal compounds. Several studies have been conducted to determine the antioxidant, anti-inflammatory, antimicrobial (Agyare *et al.,* 2013), anti-analgesic activity (Duwiejua *et al.,* 2002), anti-malarial activity (Asaase *et al.,* 2005) of several plant species from Ghana. This shows that the flora in Ghana could be a good source of active compounds for the treatment of many ailments including NTDs.

1.1 **Main objective**

The main aim of the study was to determine the anti-parasitic activity of some selected medicinal plants and some marine algae commonly used in Ghana.

1.1.1 **Specific objectives**

The specific objectives were to

- Collect, identify and prepare plant and algae extracts
- Perform preliminary phytochemical screening of the plant and algae extracts
- Determine the anti-giardial activity of the extracts
- Determine the activity of the extracts against *E. histolytica* and *N. fowleri*
- Determine the anti-trypanosomial activity of the extracts
- Determine the anti-schistosomal activity of the extracts

CHAPTER TWO

2.0 LITERATURE REVIEW

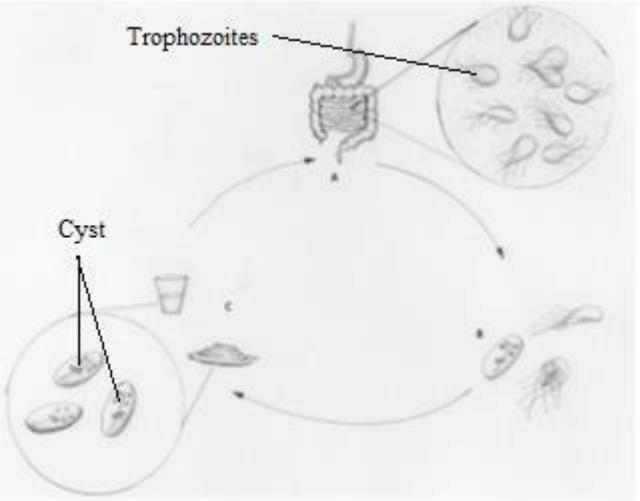
2.1 Selected Negleced Tropical Diseases

2.1.1 Giardiasis

Giardisasis, an intestinal parasitic infection is caused by the flagellate unicellular eukaryotic protozoan *Giardia lamblia* (syn: *Giardia intestinalis, Giardia duodenalis*) (Adam, 1991). It has been estimated that about 200 million people are infected each year in Africa, Asia and Latin America. It is a disease that has a high rate of prevalence and distribution worldwide and poses public health issues in developing countries as a result of poor sanitation, over population, unsafe water supply and inadequate control of the vector. It was known to affect about 280 million people in 2013 (Esch and Petersen, 2013) It affects mainly infants and school going children but can affect adults too (Calzada *et al.,* 2006; Noor *et al.,* 2007). This parasite is found in mammals including humans, cats, dogs and cattle (Faubert, 1988; Baruch *et al.,* 1996). The parasite is transmitted through the ingestion of contaminated water, food and by direct contact with feacal matter.

Giardia lamblia in its life cycle has two forms, the trophozoites and cyst (Fig. 2.1). The cyst which is the infectious form is what is transmitted through water, food and feacal matter (Rendtorff, 1954). It is inert and environmentally resistant. The cyst is ingested and a process known as excystation follows. In excystation, there is contact of the cyst with gastric acid content and pancreatitic enzymes including chymotrypsin and trypsin (Hill, 1993; Feely *et al.,* 1991). This leads to the production of two trophozoites (vegetative form) which replicate in the crypt of the duodenum and upper jejunum (Fig 2.1 and 2.2). This reproduction takes place asexually by binary

fission. Some of the trophozoites encyst as a result of bile acid exposure or cholesterol starvation (Gillin *et al.*, 1988; Lujan *et al.*, 1996).



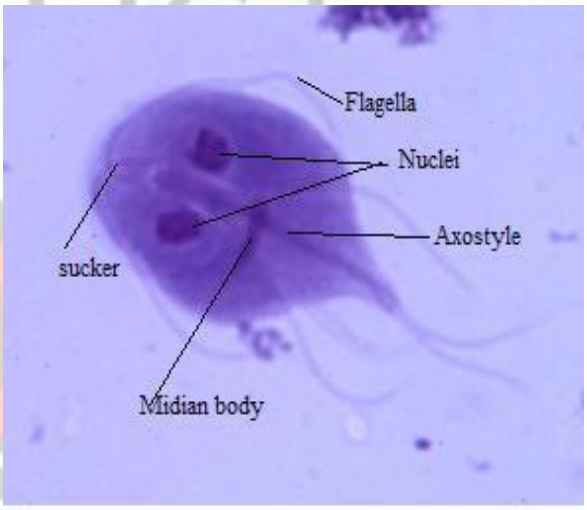


Fig 2.1 Life cycle of *Giardia lamblia*. (Source: www.microbewiki.kenyon.edu, accessed on 20th July, 2016)

Fig 2.2 Stained diagram of *G. lamblia*. (Source: www.microbewiki.kenyon.edu, accessed on 20th July, 2016)

The symptoms of giardiasis are diarrhea with foul smelling stools which may contain increased amount of fat and mucus, flatulence, bloating, abdominal cramps, nausea and vomiting, anorexia, malaise and weight loss. Giardiasis is most likely to resolve spontaneously but it may also last for weeks and sometimes months if left untreated (Ortega and Adam, 1997). Malabsorption occurs which leads to the weight loss. In cases of asymptomatic infection, malabsorption of fats, carbohydrates, sugars and vitamins could occur leading to malnutrition (Hill, 1993).

Giardiasis is mostly diagnosed microscopically by the identification of cyst or trophozoites in stool samples stained with trichrome or iron hematoxylin (Adam, 1991). Because motile trophozoites are associated with symptomatic giardiasis, directly examining fresh stool samples can be useful in diagnosing the disease (Marshal *et al.*, 1997). Diagnosis can also be done by the detection of

Giardia antigen using techniques like enzyme immunoassays (EIAs), direct and indirect immunofluorescent assays and direct fluorescent assays. These procedures are all highly specific and sensitive for environmental and stool samples analysis (Marshal *et al.*, 1997).

The main treatment for giardiasis is the use of metronidazole or quinacrine. Most patients respond to the single course of treatment of only metronidazole. In cases of recurrence, combination treatment of metronidazole and quinacrine HCl may be used. In children, furazolidine is approved for the treatment of the condition (Hill 1993). Metronidazole works by damaging the DNA of the parasite and prevents the formation of new DNA thereby inhibiting the survival of the parasite. Common side effects include nausea and vomiting, metallic taste in the mouth, dry mouth and headache. The uncommon side effects include urticarial, pruritis and dark coloured urine (Lau *et al.*, 1992; Khan *et al*., 2000).

Giardiasis is prevented by ensuring water sources are free from contamination. Filtration of water is another effective means of prevention. Boiling is also quite effective for destroying the cyst. Washing of hands frequently and rigorously also prevents the feacal-oral route of transmission. Food must be freshly cooked and eaten hot to prevent transmission through food (Ortega and Adam, 1997).

2.1.2 Amoebiasis

This disease is caused by the organism *Entamoeba histolytica*, an enteric protozoan parasite which is exclusive for humans. Stanley (2003) reported that it is the second leading cause of death in parasitic infections. Debnath *et al.* (2012) also reported that it is the fourth and third leading cause of death and morbidity, respectively. Amoebiasis is known to affect about 50 million people annually (Petri and Singh, 1999) and also cause about 40,000 to 100,000 deaths annually (Walsh,

1988; Loranzo *et al.*, 2013). *E. histolytica* is found all over the world making the disease a worldwide distributed disease. It is a substantial health risk in countries where there is inadequate sanitation facility. This makes it a major public health issue (Stanley, 2003).

The mode of transmission of amoebiasis is through the ingestion of food and water contaminated with the cyst form of the *E. histolytica* (Watanabe and Petri, 2015). Humans and other non-human primates are the only natural host of the organism (Stanley, 2003). Amoebiasis causes amoebic liver abscess and amoebic colitis (Petri *et al.*, 2002; Stanley, 2003).

The condition ranges from asymptomatic to symptomatic. Patients with amoebic colitis present with several weeks of gradual onset of abdominal tenderness and pain, diarrhea which presents with bloody stool. There is ulceration of the epithelium of the intestines and the trophozoites invade the lamina propia. This contributes to the pathological lesions observed in amoebic colitis. There is inflammation as well (Petri and Singh, 1999). Patients with amoebic liver abscess may present with acute fever, right upper abdominal tenderness and pain, prominent weight loss and abdominal pain (Petri *et al.*, 1987; Petri *et al.*, 2002). Patients with liver abscess may not have concurrent colitis although there may be some assocaited dysentery (Petri *et al.*, 2002).

The life cycle consists of two parts and these are the infectious cyst form and the trophozoites stage (Reeves, 1984; Stanly, 2003). The cyst is ingested in contaminated food or water to commence infection. The cyst travels through the small intestine into the terminal ileum or colon surviving the stomach acid to excyst forming the trophozoites (Reeves, 1984). The trophozoites feed on bacteria and food particles in the gut. They are highly motile and reproduce by binary fission. The trophozoite encyst within the colon and the cycle is completed when cysts are excreted through

the stool into the environment (Fig 2.3 and 2.4). When excreted in the environment the trophozoites are not able to survive (Eichinger, 2001).

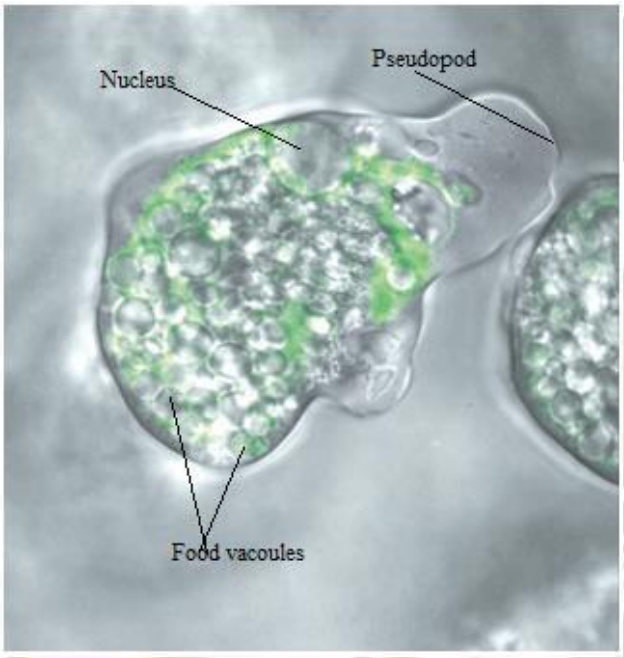


Fig. 2.3 Microscopic view of *E. histolytica* Magnification: X5. (Source: www.cdc.hindawi.com, accessed on 20th July, 2016)

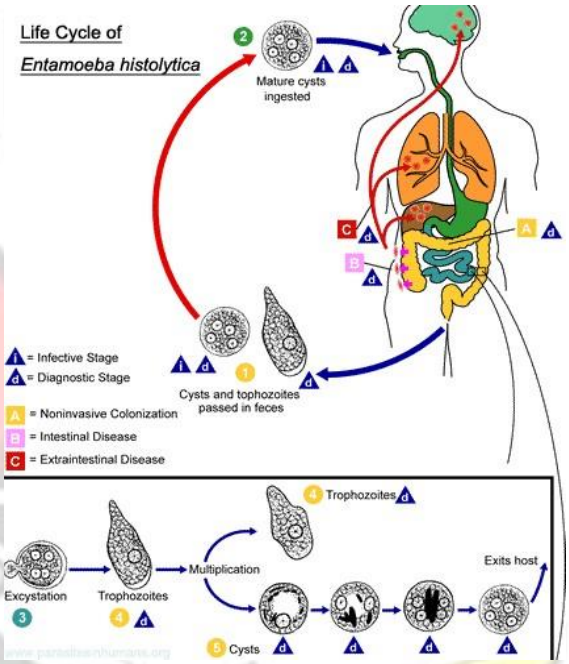


Fig. 2.4 Life cycle of *E. histolytica*. (Source: www.cdc.hindawi.com, accessed on 20th July, 2016)

Amoebiasis is diagnosed by the use of enzyme-linked immunosorbent assay (ELISA) which identifies the *E. histolytica* antigen (eg. EIA 34964X) in the stool. Other techniques that are used are polymerase chain reaction (PCR) (Clark and Diamond, 1993) and microscopy. The latter is outmoded since it is not able to differentiate between the various species of *Entamoeba* (Healy, 1971; Krogstad *et al.*, 1978). In cases where *E. histolytica* is not examined in the stool, colonoscopy or flexible sigmoidoscopy with biopsy sample that are examined for amoebic trophozoites may be useful (Blumencranz *et al.*, 1983). The treatment of amoebiasis depends on the use of the nitroimidazoles including metronidazole, tinidazole and ornidazole. Metronidazole

is the most widely used (Stanley, 2003). Metronidazole works by damaging the DNA of the parasite and prevents the formation of new DNA thereby inhibiting the survival of the parasite. Major side effects include nausea and vomiting, metallic taste in the mouth, dry mouth and headache. The minor side effects include urticarial, pruritis and dark coloured urine (Lau *et al.,* 1992; Khan *et al*., 2000). Amoebiasis can be prevented by filtering or boiling water before use. Washing of hands to prevent feacal oral transmission and provision of adequate sanitation worldwide are other ways of peventing the disease (Stanley, 2003).

2.1.3 Schistosomiasis

Schistosomiasis also known as Bilharzia is a disease caused by the blood fluke of the genus *Schistosoma* (Savioli *et al.,* 2004; Abdullah *et al.,* 2009). It is a disease with high chronicity and morbidity (King, 2010). It affects the tropical and subtropical regions of sub-Saharan Africa, South America, China, middle and south-Eastern Asia (Utzinger *et al.,* 2010; Caffrey and Secor, 2011). It is estimated that about 200 million people are infected annually with about 400 million people being at risk of getting infected (Steinmann *et al.,* 2006). This makes it a major public health issue in the endemic countries.

There are five major species of the schistosomes that infect humans; *S. mansoni*, *S. haematobium, S. japonicum, S. intercalatum* and *S. mekongi*. There are two forms of the disease; urinary and intestinal schistosomiasis (Gryseels *et al.,* 2006; Davis, 2009). The *S. haematobium* causes urinary schistosomiasis in which there are lesions in the walls of the bladder and haematuria (Hatz, 2001; van der Werf *et al.,* 2003; Gryseels *et al.,* 2006; Davis, 2009) while *S. mansoni*, *S. intercalatum, S. mekongi* and *S. japonicum* cause intestinal schistosomiasis (Hatz, 2001; Gryseels *et al.,* 2006; Lambertucci *et al.,* 2008; Davis, 2009). In areas with high endemicity, the disease is associated

with the construction and operation of large hydroelectric dams for power production and irrigation fed agriculture, multipurpose small dams and irrigation systems (Hunter *et al.*, 1993; Jobin, 1999; Amerasinghe, 2003; Steinmann *et al.*, 2006). Schistosomiasis affects all age groups of people (Caffrey and Secor, 2011) and it is known to be a disease of poverty affecting areas where there is lack in access to clean water and proper sanitation, weak health systems and substandard hygiene (Utzinger *et al.*, 2009; King, 2010).

Schistomiasis is a zoonotic disease affecting humans, other primates and birds. The vector for transmission is a fresh water snail; *S. mansoni* is transmitted by *Biomphalaria* snail; *S. haematobium* by the *Bulinus* snail and *S. japonicum* by the amphibian snail known as *Oncomelania*. The type of species of schistosome of found in different places depends largely on the presence of the immediate host snail available (Gryseels *et al.*, 2006).

For the life cycle of the parasite, the egg is excreted from a carrier's urine or feaces (Fig 2.5) that is contact with fresh water body. The egg releases the miracidium when it gets into contact with the water. The egg can stay viable for 7 days. The released miracidium swims in search of an intermediate host snail guided by light and chemical stimulus. The miracidium penetrates the snail and multiplies asexually to form the mother and daughter sporocysts and later develops into the cercariae. The cercariae have an embryonic sucker and bifurcated tail. The matured cercariae stay in the snail for 4 to 6 weeks and are released on exposure to light. Upon release, the infective cercariae swim to a definitive human host and penetrate the skin after shedding off the bifurcated tail. They migrate through the blood stream to the lungs and liver and transforms into the juvenile stage. One miracidium that infects a snail can produce thousands of cercariae. The schistosomulum matures in 4 to 6 weeks to the adult worm and migrate to their perivesicular or mesenteric destination. *S. haematobium* moves to the bladder and *S. mansoni* to the large intestines. The adult

worms lay eggs which passes out through the urine or feaces. The cycle then continues (Gryseels *et al.,* 2006).

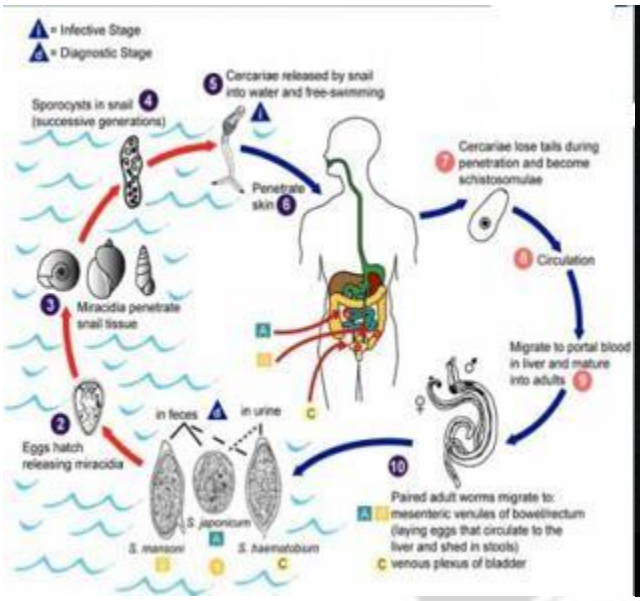


Fig 2.5 Life cycle of *Schistosoma mansoni*. (Source: www.cdc.gov.parasite.schistosoma, accessed on 22nd July, 2016).

Fig 2.6 An adult Schistosoma showing male and female adult worm. (Source: www.yourarticlelibrary.com, accessed on 22nd July, 2016).

There are urinary and intestinal schistosomiasis as a result of where the adult worm resides in the body (Utzinger *et al.*, 2011). Urinary schistosomiasis occurs when the *S. haematobium* migrate to the lumen of the bladder (Gryseels *et al.*, 2006). The eggs laid provoke granulomatous inflammation, ulceration and pseudopolyposis of the vesical and ureteral walls (Cheever *et al.,* 1978). This leads to early signs like pollakisuria, dysuria, proteinuria and haematuria (Chen and Mott, 1989; Gryseels, 1989). In highly endemic regions, these signs are the red flag in children from ages 5 to 10 (Jordan, 2000). From adolescence, these signs are less obvious but there are lesions formed and these evolve to fibrosis or calcification of the bladder and lower ureters. This can lead to hydroureters and hydronephrosis which can further lead to parenchymal damage or kidney failure (Gryseels *et al.*, 2006; Davis, 2009). Clinical observation and autopsy show that

patients, particularly elderly people die from schistosomiasis-induced renal damage (Smith *et al.*, 1975).

In intestinal schistosomiasis, the eggs laid from *S. mansoni*, *S. intercalatum*, *S. japonicum* and *S. mekongi* migrate to the intestinal wall (Hatz, 2001) and provoke mucosal granulomatous inflammation, pseudopolyposis, micro-ulcerations, and superficial bleeding (Cheever, 1968; Cheever *et al.*, 1978). The lesions that occur are found in the large intestines and rectum (Gryseels *et al.*, 2006). The commonest signs and symptoms of this condition are abdominal pain and discomfort which can be chronic or intermittent, loss of appetite and diarrhoea which can be bloody (Chen and Mott, 1988). The severity of the symptoms depends on the intensity of the infection (Gryseels *et al.*, 2006).

Intestinal schistosomiasis may result in hepatomegaly which consists of two combined characteristic syndromes; early inflammatory hepatic disease and late fibrotic hepatic disease (Gryseels and Polderman, 1991). The inflammatory hepatic disease is the early symptom and it is a reaction to the ova of the parasite trapped in the presinusoidal periportal spaces of the liver. In children and adolescents, it the major cause of schistosomiasis-induced hepatomegaly (Gryseels and Polderman, 1991; Gryseels, 1992). Hepatic fibrosis occurs years later as the infection progresses (Homeida *et al.*, 1988; Dessein *et al.*, 1999). This comes as a results of a massive deposition of diffuse collagen deposits in the periportal spaces leading to pathognomonic periportal or Symmer's pipestem fibrosis (Cheever, 1968; Colley *et al*., 2014). The fibrosis then leads to progressive occlusion of the portal vein, portal hypertension, splenomegaly, portocaval shunting, gastrointestinal varices and collateral venous circulation. In this case, the liver is generally hard and nodular (Gryseels and Polderman, 1987). This is different from cirrhosis in that hepatocellular function and indices are unchanged (Homeida *et al.*, 1988).

Schistosomiasis is diagnosed by microscopic examination of the excreta (Feldmeier and Poggensee, 1993). The eggs are sought for in the excreta using their size, shape, lateral spine and the living status of the miracidium, the egg. Urine and feaces are concentrated by methods such as sedimentation, centrifugation or filtration before examination. The urine is passed through nitrocellular filter and the eggs counted on slides under the microscope while the eggs in the feaces are counted via the Kato-Katz or feacal thick smear methods (Gryseels *et al.,* 2006). Antigen-based assays are also available for rapid diagnosis of patients who are travelers, migrants and those who are occasionally exposed (Bottieau *et al.,* 2006). These assays though expensive cannot differentiate between history of exposure and active infection (Gryseels *et al.,* 2006). Cystoscopy and endoscopy can also be used to visualize bladder lesions and oesophageal varices (Richter *et al.,* 1998). Laparoscopy and wedge biopsy can enhance visualization of the macroscopic and histological appearance of granulomatous inflammation or periportal fibrosis (Hayashi *et al.,* 2000).

Treatment of schistosomiasis relies on the use of praziquantel as single therapy. Praziquntel works by causing severe spasms and paralysis of the worms' muscles. This paralysis is accompanied and probably caused by a rapid Ca^{2+} influx inside the schistosome. Morphological alterations are another early effect of praziquantel. Common side effects include headache, dizziness, stomach pain, tiredness, weakness, joint and muscle pain, skin rash and sweating (Caffrey and Secor, 2011). It is a medication recommended by the WHO for either mass or individual treatment (Doenhoff *et al.,* 2009). Schistosomiasis can be prevented by the practice of good sanitation and supply of clean safe water to the population in endemic communities (Minai *et al.,* 2003). The intermediate snail host can be controlled by the use of molluscicides, physical measures and biological control (Gryseels *et al.,* 2006).

2.1.4 Trypanosomiasis

Human African trypanosomiasis (HAT) also known as 'Sleeping sickness' is a parasitic infection caused by the parasite *Trypanosoma brucei* (Hoet *et al.,* 2004). There is *T. brucei brucei* that causes nagana disease in cattle and other livestock. There are two main subspecies that cause distinct infection in humans. *T. brucei rhodesiense* which is found in east to south Africa and *T. brucei gambiense* which is found in west and central Africa (Brun *et al.,* 2010). The disease affects both children and adult especially those in the rural settings (Robays *et al.,* 2004). WHO reported in 1998 that HAT is known to affect about half a million Africans annually with an estimated 60 million people being at risk of getting the disease (Barret, 1999). Simarro *et al*, (2012) also reported that approximately 70 million people are at risk worldwide. As a result of control measures such as biological control of the tsetsefly, introduction of net traps for the fly, there was a significant reduction in the incidence of *T. gambiense* (69% reduction) and *T. rhodesiense* (4% reduction) in 2006 (Lutumba, 2005; Barret, 2006). The rate of infection differs with regards to the species of the *Trypanosoma brucei* (Brun *et al.,* 2010).

The vector for transmission is the haemtophageus tsetse fly of the genus *Glossina* (Freiburghaus *et al.,* 1996). There are about 30 different species and sub-species of the tsetse fly and these are grouped based on the preferred habitat (Jordan, 1993) and the sub-species of the parasite they transmit (Brun *et al.,* 2010).

The parasite has a 2-host life cycle; the host and the vector (Macleod *et al*., 2007). The life cycle begins with a bite from the tsetse fly during feeding on the mammalian host. The fly injects into the mammalian host metacyclic trypomastigotes which transform into blood stream

trypomastigotes and are carried to other sites in the body like the lymph system, spinal fluid and the blood. At this stage the trypomastigotes undergoes binary fission to multiply in various body fluids. This is the mammalian cycle (Fig 2.7). The vector cycle begins when the fly feeds on the infected mammalian blood and the bloodstream trypomastigotes enter the digestive tract. In the midgut, they transform to procyclic trypomastigotes and multiply by binary fission (Fig 2.8). They move from the midgut and transform to the epimastigotes. The epimastigotes reach the salivary gland and continue multiplication by binary fission. They mature to become the infective metacyclic trypomastigotes. The whole cycle in the fly takes about 3 to 5 weeks. Several factors affect this process (Macleod *et al.*, 2007).

At the initial stages of the infection, the parasites are seen only in the blood and lymph. As the infection progresses, they migrate to the brain parenchyma and cerebrospinal fluid (Stevens *et al*., 2004).

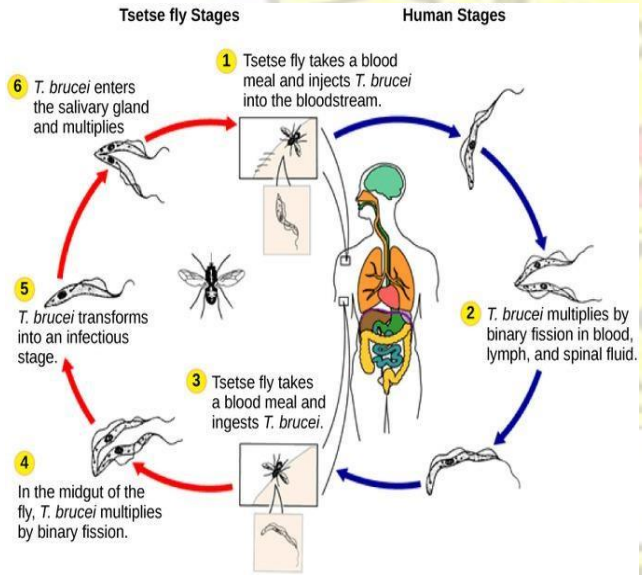
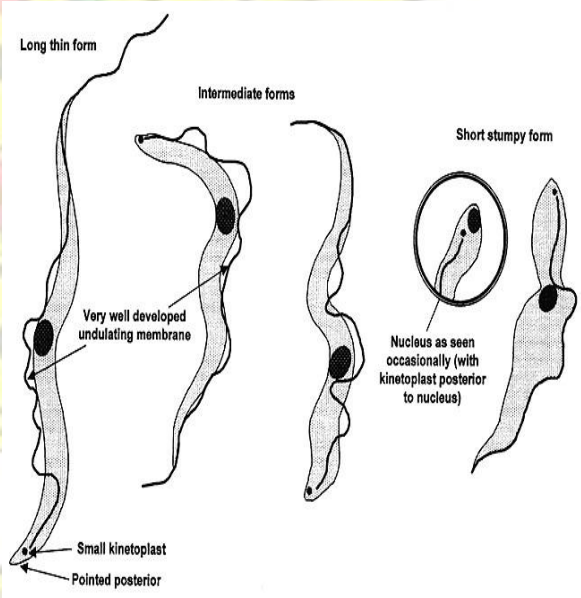


Fig. 2.7. Life cycle of *T. brucei*.

(accessed on 22Source: www.microbewiki.kenyon.ed$_{nd}$ July, 2016).



u **Fig. 2.8.** Diagram of *T. brucei* species. (Source: www.fao.org, accessed on 23rd July, 2016).

Laboratory examinations are required to be able to diagnose the infection caused by the trypanosomes. This is because clinical features are not specific. Laboratory examinations include the Card Agglutination Test for Trypanosomiasis (CATT). This is done with serum or blood from a finger prick to detect antibodies generated against the parasite (Noireau *et al.,* 1991; Chappuis *et al.,* 2002). Although CATT is sensitive (87 to 98%) and specific (93 to 95%) (WHO, 1998; Truc *et al.,* 2002), it cannot be used to confirm the presence of an already existing infection (Dukes *et al.,* 1992). Its uses are therefore limited to highly endemic areas where clinical features are indicative of an infection (Lutumba *et al.,* 2005). Another laboratory examination employed is microscopy. Microscopically examining the blood, spinal fluid and lymph node aspirates for the parasite is employed to confirm the presence of an infection (van Meirvenne, 1999). Other serological tests that are sensitive and can be employed are the immunofluorescence or enzymelinked immunosorbent assays (ELISA) and PCR for examining the parasite's nucleic. These assay are highly sensitive for less endemic areas (Brun *et al.,* 2010).

For *T. brucei rhodesiense,* no serological test is available for diagnosing. A thick blood smear is sufficient to confirm diagnosis because the density of blood with circulating parasite is always high in *T. b. rhodesiense* (Brun *et al.,* 2010).

Drugs for the treatment of trypanosomiasis include pentamidine, eflornithne, suramin and melarsoprol. Pentamidine and suramin are used in the initial or first stage of the infection when the parasites are found in the blood and lymph. Pentamidine is used against *T. b. gambiense* whereas suramin is used against *T. b. rhodesiense*. Pentamidine works by binding to DNA and RNA thereby inhibiting protein synthesis, cell production and synthesis in the parasite (Gustafsson *et al.,* 1987). Side effects include coughing, difficulty in breathing, difficulty in swallowing, burning pain in the throat and skin rash (Walter, 2005). Suramin inhibits growth factors within the

parasites. It reduces the worms' ability to produce insulin, platelets and dermal cells and this leads to reduction in ability of the parasite to replace old cells and produce energy. Side effects include itching, nausea, vomiting, numbness to the arms and feet, swellings on the skin, joint pain and irritability (Walter, 2005).

Melarsoprol is used for treating the second stage of the infection caused by *T. b. gambiense* in countries that lack adequate resources for eflornithine or its availability is lacking. Its mechanism of action is by inactivating pyruvate kinase of the parasite which is responsible for the production of adenosine triphosphate (ATP) which is the energy required for the parasite's survival. This leads to the death of the parasite as a result of lack of energy. Side effects include confusion, convulsion, headaches, numbness of the limbs, fever and sweating (Van Voorhis, 1990). It is however the only drug for the second stage of *T. b. rhodesiense* infection (Schmid *et al.,* 2005). Eflornithine is the recommended drug for the second line treatment for *T. b. gambiense* infection. It is safer than melarsoprol and not recommended for *T. b. rhodesiense* infection (Balasegaram *et al.,* 2006; Checchi *et al.,* 2007).

Trypanosomiasis can be prevented by controlling the intermediate host, the tsetsefly, from getting into contact with the mammalian host (Bruns *et al.,* 2010). This could be done by the use of repellents to avoid bites from the fly. Long clothes that reach the knees and wrist, socks and hand gloves can be worn to avoid bites as well. Thick clothing should be worn since the fly can bite through thin clothes. The use of insecticides, fly traps or screens and repellants can also be used as prevention mechanism. Cattle and other animals that serve as a reservoir for the parasite can be sprayed with insecticides (Maudlin, 2006; Torr *et al.,* 2007).

2.1.5 *Naegleria fowleri* infection

Naegleria fowleri is a free living protist amoeba that survives in water and soil. It dwells in the host central nervous system (De Jonckheere, 2011). It affects both children and adults and its associated with amoebic meningoencephalitis (Visvesvara *et al.,* 2007). The *N. fowleri* is a thermophilic organism as well. It infects the host by passing through the nostrils during water activities such as swimming, diving or water skiing (Jarolim *et al.,* 2000). Recreational water activities like nasal irrigation, ayuverda and nasal ablution can lead to contracting the organism (Yoder *et al.,* 2012).

The parasite attaches itself to the intact or disrupted nasal mucosa and this is followed by locomotion along the olfactory nerve and through the cribriform plate to reach the olfactory bulbs and to the cerebellum. There is deep penetration of the cortex to the periventricular system leading to meningoencephalitis. This happens with cerebral oedema that results in cerebellar herniation (Rojas-Hernandez *et al.,* 2004; Guarner *et al.,* 2007).

The life cycle of *N. fowleri* is in three stages; the cyst, trophozoites and flagellates (Fig 2.9). The infective stage is the trophozoites. They are 10 to 35 um long, granular and possess a single nucleus. They replicate by binary fission during which the nucleus remains intact. This process is called promitosis (Marciano-Cabral, 1988). They travel via pseudopodia. The trophozoites are found in the cerebrospinal fluid (CSF) and in other body tissues. The trophozoites occasionally turn into the flagellated form. This form is non-feeding and occurs when there are unfavourable and adverse conditions such as absence of food for the trophozoites. These flagellated forms can also be found in the cerebrospinal fluid and the water where the trophozoites are found. This form

can be inhaled into the nasal cavity during water activities such as swimming or diving. The cysts are smooth and spherical with a single layered wall and nucleus. They are resistant to harsh environment conditions such temperature and can survive these conditions (Marciano-Cabral, 1988). The trophozoites encyst during conditions such as unavailability of food, overcrowding, desiccation, cold temperatures and presence of accumulated waste products (Chang, 1978). In favourable, conditions the trophozoites escape from the cyst through the ostiole in its middle (Marciano-Cabral, 1988).

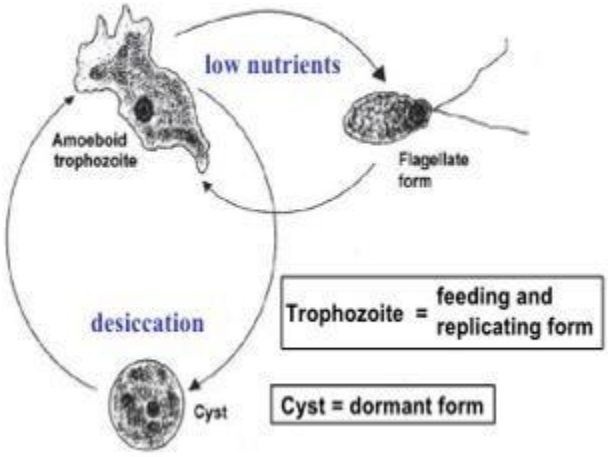


Fig. 2.9. Life cycle of *N. fowleri*. (Source: www.slideshare.net, accessed on 23 rd July, 2016).

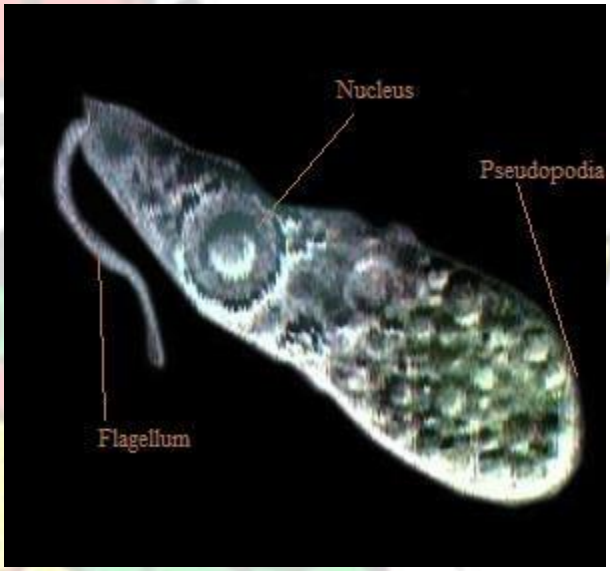


Fig. 2.10. Microscopic view of *N. fowleri*. Magnification X5

(Source: www.microscopy.uk.org.uk, accessed on 23rd July, 2016).

Amoebic meningoencephalitis is diagnosed with symptoms such as severe headaches, chills, fever, Brudzinski and Kernig signs, seizures, confusion, photophobia and a possibility of coma (Martinez, 1985). The pressure of the cerebrospinal fluid is measured and a pressure of more than 600 mm Hg can be indicative of *N. fowleri* infection (Martinez, 1985). The analysis of CSF shows certain

abnormalities and that can also be indicative of *N. fowleri* infection. These abnormalities include colour changes which ranges from grey at the initial stages of the infection through red at the later stage, increase in polymorphonuclear cells concentration and presence of trophozoites (Martinez, 1985; Visvesvara et al, 2007). Magnetic resonance imaging (MRI) can be used to stage the brain and abnormalities in the midbrain and subarachnoid space due to *N. fowleri* infection (Martinez, 1985; Visvesvara *et al*, 2007).

The most widely used and accepted drug for the treatment of *N. fowleri* infections is amphotericin B. Amphotericin B destroys the parasite by creating channels in the cell membrane as a result of binding to ergosterol. This causes leakage of monovalent ions such as K^+, H^+ and Cl^- and subsequent death of the parasite. Side effects include fever, chills, hypotension, dyspnea, tachypnea, vomiting and headaches (Mesa-Arango *et al*., 2012). Other anti-infectives include fluconazole, miconazole, azithromycin, clarithromycin and erythromycin (Kim *et al.,* 2008).

The *N. fowleri* can be prevented by avoiding freshwater sports such as swimming and water diving. If such activities cannot be avoided, nose masks or clips should be used to avoid the water coming into contact with the nostrils (Grace *et al.,* 2015).

2.2 Plants as source of medicines

Plants have been exploited for their medicinal use since around 1500 BC (Chopra, 2002). They serve as a source of medicine and are used to treat and prevent several infections, diseases and other ailments. The use of plants medicines is widely accepted in the culture and traditions of the indigenous Africans and other nationalities such as India, China and Sri Lanka (Calixto, 2005; Ayyanar and Ignacimuthu, 2011).

Currently almost 25% of the active medicinal compounds used in USA and UK were derived from higher plants and it is recorded that about 80% of the world population depends on traditional medicine for their primary healthcare (Anthony *et al.*, 2005; Nath *et al.*, 2011). About 30% of medicines employed for therapeutic purposes have their source from natural products such as plants (Ramos *et al.*, 2008). Examples are artemether for the treatment of malaria, digoxin for management of heart failure, morphine and acetyl salicylic acid for pain management and vincristine and vinblastine for cancer management (Akharaiyi, 2011). Some societies have their primary health care system incorporated with their unique traditional medicines (Akinyemi *et al.*, 2005; Toledo *et al.*, 2009).

This has led to increase in research into plant medicines for their active therapeutic compounds. The search for new drugs have taken a different route in that the science of ethnobotany and ethnopharmacognosy are being used as a guide for identification and isolation of different sources and classes of compounds (Heinrich, 2000; Anthony *et al.*, 2005).

Compounds derived from plants are a great source of antimicrobial agents and the use of these compounds as potential drugs for treatment of infectious diseases was well accepted even before microorganisms were discovered (Rios and Recio, 2005). These compounds have numerous therapeutic potentials and are effective in treating infectious diseases. Their safety profiles are usually minimal since they do not come with the side effects associated with synthetic drugs. These compounds isolated from plants have been developed into various medications which have aided in the management of parasitic, bacterial, fungal and other microbial infections (Agyare *et al.*, 2006).

Several researches have been carried out over the years and it has been proven that plants and their isolates can be a source of anti-parasitic agents. Asuzu and Chineme (1990) reported that methanol leaf extracts of *Morinda lucida* had activity against *T. brucei brucei*. Barbosa *et al.* (2007) also reported that epicatechin, a flavonoid isolated from the *Geranium mexicanum* exhibited potent activity against *G. lamblia* more than metronidazole which is widely used as the main chemotherapy. Hoet *et al.* (2004) reported on the antitrypanosomal activity of some selected Beninese plants. de Moraes *et al.* (2011) also reported on the antischistosoicidal activity of piplartine, an isolate from *Piper tuberculatum* (Piperaceae) against *S. mansoni*. All these show how plants are contributing to the search for newer anti parasitic agents.

There have been several reports on the use of medicinal plants in Ghana for wound infections and other diseases. Adu-Amoah *et al*. (2014) reported of the use of *Erythropheum ivorense* (A. Chev.) in treating wounds. Agyare *et al*. (2014) also reported of the use *Myrianthus arboreus* and *Alchornea cordifolia* for treating wounds and other infections in Ghana. Woode *et al*. (2011) reported on the nociceptive property of *Hilleria latifolia*. The analgesic and anti-inflammatory activities of *Xylopia aethiopica* have been reported (Woode *et al*., 2012). Boakye *et al*. (2016a) reported on the wound healing property of *Phyllanthus muellerianus*.

2.3 Plants under study

2.3.1 *Erythrophleum ivorense* (A Chev.)

Description

It is a huge tree that grows in the tropical parts of Africa example Ghana, Congo, Cameroon, Gabon, Nigeria Liberia (Adu-Amoh *et al.*, 2014). It is a legume and belongs to the family

Euphorbiaceae. In Ghana, it is popularly known in Asante-Twi as “Potrodom”. It has a cylindrical bole and fluted at the base sometimes. It usually grows to a height of a 40 m and has a diameter of 60 to 90 cm (Fig 2.11). The leaves are compound, alternate and bipinnate with 2 to 4 pairs of pinnae. The leaflets which are elliptical to ovate also have about 6 to 14 pairs of pinnae. It has a scaly outer bark which is fissured and grey with a reddish and granular inner bark. The plant has asymmetrical base and acuminate apex (Irvine, 1961; Burkill, 1995).

Fig 2.11 Leaves and stem of *E. ivorense.* (Source: Photographed by Chi Lim, https://vi.wikipedia.org, accessed on 24th July, 2016).

Medicinal uses

Its bark is used as a laxative, an emetic and an analgesic in Sierra Leone (Loder *et al.,* 1974). In Cote d’Ivoire, it is used to treat small pox and other skin infection. The bark is also applied on the skin to relieve pain (Dongmo *et al.,* 2001). The leaves are used to prepare decoctions to treat menstrual disorders and the twigs used for pulmonary infections (Burkhill, 1995).

Chemical constituents

The *E. ivorense* contains alkaloids including erythrophleine. The alkaloids of the plant are esters of tricyclic diterpene acids. Two main type exists and they are dimethylaminoethylesters; examples of these are cassaine, cassaidine and erythrophleguine and monomethylaminoethylesters, also known as nor-alkaloids. The dimethylaminoethylesters are found mostly in the bark of the plant (Bosch, 2006). Prelimienary phytochemical screening of of methanol extracts of both leaves and bark revealed the presence of saponins and flavonoids. The methanol leaf extract contained condensed tannins and sterols whereas the methanol bark extract contained hydrolysable tannins and terpnenoids (Adu-Amoah *et al*., 2014).

Biological activity

The alkaloids from the plant are known to exhibit stimulant effect on the heart similar to the alkaloids digitoxine and ouabain from *Digitalis* and *Strophanthus gratus*, respectively. The effect is short acting due to metabolism. They are also known to exhibit strong uterine and intestinal contractions. Cassaine and cassaidine cause anaesthetic and diuretic effect. While cassaine cause excitation, cassaidine cause depression (Cronlund, 1976). Adu-Amoah *et al*. (2014) also reported the antimicrobial activity of the methanol leaf and bark extracts of *E. ivorense* and the toxicity profile of these parts of the plant on Wistar rats.

2.3.2 *Margaritaria nobilis* L.f (Müll Arg).

Description

This is a deciduous tree with an open, globose crown. It usually grows to a height of 8 to 16 m tall. Its cylindrical bole can measure up to about 40 to 70 m in diameter (Fig 2.12). It is also known as

Phyllanthus nobilis and belongs to the family Phyllanthaceae. It is a fruit bearing plant and can be found in Mexico, South America, Central America and the West Indies. It is commonly known as bastard hogberry in English and 'bunasub' by the Konkonbas in Ghana (Cazetta *et al.*, 2008). It is found in the northern part of Ghana (Jato, 2015)

Medicinal and non-medicinal uses

In Brazil, the plant is used to treat infectious diarrhea, manage arthritis and wounds (Marcio *et al.*, 2010). For the non-medicinal uses, the plant is used for ornamental purposes such as landscaping. The wood is used in making boxes, crates, linings, toys and also burnt for fuel (Govaerts and Radcliffe-Smith, 1996).

Chemical constituents and biological activity

The *M. nobilis* contains flavonoids, alkaloids and polyphenolic tannins. The fruit is reported to contain gallic acid, methyl gallate and campherol. The plant is known to possess anti-inflammatory activity (Marcio *et al.*, 2010).

Fig 2.12. Leaves and fruits of *M. nobilis*. (Source: www.refloresta-bahia.org, accessed on 24th July, 2014).

2.3.3 *Hilleria latifolia* (Lam.) H. Walt.

Description

The *H. latifolia is a* perennial herb of the family Phytolaccaceae. It grows to a height of about 30 to 120 cm. It is known in Ghana as '*Anafranaku'* by the Asantes and '*Avegboma* or *Kukluigbe* by the Ewes. Its habitat is the rainforest and shady places and can be found in the tropical region of Africa and South America (Dokosi, 1998). The leaves of *H. latifolia* are simple, alternate and entire with petiole of 1 to 7 cm in length and the blade is ovate or elliptic to broadly lanceolate (Fig 2.13). They are 15 cm long and 6 cm wide. The leaves are also obtuse or sub-acute with weak bristly hairs on the surface and on the young branches. The fruits are red and smooth at maturity. They can also be reticulate and glabrous (Dokosi, 1998; Schmelzer and Gurib-Fakim, 2008)

Fig 2.13. Aerial part of *H. latifolia*. (Source: Dapaah S. O. Department of Pharmaceutics, KNUST, Kumasi. August, 2015).

Medicinal uses

Agyare *et al. (*2009) reported that the leaves of *H. latifolia* is used for the management of wounds, boils and rheumatism in Ghana. In Congo it is used to cure skin diseases (Mshana, 2000). It also reported that the leaves have purgative action and an enema of them can be given to treat gynaecological disorders, ascites and food poisoning (Schmelzer and Gurib-Fakim, 2008). The plant can be boiled and eaten to cure gonorrhea and vapour inhaled to cure jaundice. The leaves are grounded and the paste is given with orange juice to treat asthma (Mshana, 2000). The sap is also used to treat ear infections (Mshana, 2000).

Chemical constituents

The whole plant has been found to contain triterpenoids, phytosterols, reducing sugars and tannins (Amponsah *et al.,* 2014). The ethanolic aerial extract has been reported to contain to saponins, tannins, steroids, terpenoids, glycosides and small portions of flavonoids and alkaloids (Woode *et al.,* 2011). Another report indicated that the methanol extract contains glycosides, coumarins, reducing sugars and small proportions of triterpines and sterols (Assob *et al.,* 2011).

Biological activities

The leaves of *H. latifolia* have been found to possess anti-inflammatory, antioxidant, antimicrobial, anxiolytic and antidepressant-like properties (Woode *et al*., 2011; Abotsi *et al.*, 2012; Dapaah *et al*., 2016b). It is also known to contain anti-nociceptive and neuro-behavourial properties (Woode and Abotsi, 2011). The methanol leaf extract has also been reported to modulate the *in vitro* activity of some antibiotics (Dapaah *et al.,* 2016a).

2.3.4 *Laportea ovalifolia* (Shumach. and Thonn) Chew.

Description

The *L. ovaliofolia* is a perennial herbaceous plant of the family Urticaceae. It mainly spreads across the ground in stolons and roots at the nodes and can grow to about 1 m long with short stinging hair (Fig 2.14) (Chew, 1969; Ruffo *et al*., 2002). In Ghana, the Asante's call it 'akyekyenwonsa', 'aberewa nom taa' and 'Kumasi otuo'. The *L. ovalifolia* has perennial stem which is cylindrical in shape and a colour that ranges from greenish to reddish to brownish (Chew, 1969). It is primarily a rainforest plant and grows in the tropical African region from Sierra Leone through to the moist

regions of Sudan, Kenya, Uganda, Angola, Tanzania and Zimbabwe (Letouzey, 1968). It comes in the form of a male and female and it is only the male that has the stem growing erect to about 50 cm with a woody base. The female grows to a height of about 5 cm and it is almost underground. The male also has bigger leaves whereas the leaves of the female are smaller (Essiett *et al.,* 2011).

Fig 2.14. Leaves and flowers of *L. ovalifolia*. (Source: Dapaah S. O. Department of Pharmaceutics, KNUST, Kumasi. August, 2015).

Medicinal uses

The leaves are used externally to treat wounds and cuts and to relieve headache (Agyare *et al.,* 2009). The root is boiled and the decoction drank to prevent excessive menstrual bleeding (Sofowora, 1996; Ruffo *et al.,* 2002). The leaves are soaked in water and the infusion drank to help deliver the placenta after child birth in Kenya (Ruffo *et al.*, 2002). The fruit serves as a poison antidote in southern Sudan (Bouch, 2004).

Non-medicinal uses

The leaves and the tender shoots of the plants are use as pot herbs and vegetables in soups and stews by the people of Ibibio tribe in Nigeria. The leaves are also used as a mild flavourant and can be added to beans or peas and served with a staple such as rice (Ruffo *et al.,* 2002).

Chemical constituents

Methanol leaf extract has been analysed and found to contain saponins, tannins, flavonoids, phlobatanins and cardiac glycosides (Essiett *et al.,* 2011; Dapaah, 2015).

Biological activity

The *L. ovalifolia* possess antimicrobial, antioxidant (Dapaah *et al.,* 2016b), anti-hyperglycaemic (Iffen and Usoro, 2010) and wound healing activities (Dapaah, 2015). It is also known to reduce oxidative stress in diabetics (Okwulehie and Akanwa, 2013;). It has been reported to also possess hypolipidaemic effect in alloxan induced diabetic rats (Momo *et al.*, 2006). The methanol leaf extract has also been reported to influence *in vitro* activity of some antibiotics (Dapaah *et al.,* 2016a).

2.3.5 *Albizia glaberrima* (Schum. and Thonn) Benth

Description

Albizia glaberrima is a deciduous plant that has an umbrella shaped crown. It belongs to the family Leguminosae (Babu *et al.,* 2009). It can grow up to about 30 m tall and 100 cm in diameter (Fig 2.15). It grows in tropical Africa from Guinea Bissau through to Kenya and Angola. In Ghana, the Akans call it 'okuro-fi'. It is a less studied species and it is confused with *Albizia zygia* (Jato, 2015).

Fig 2.15. Aerial parts of *A. glaberrima*. (Source: www.mozabiqueflora.com, accessed on 2 nd August, 2016).

Medicinal uses

Albizia glaberrima is used as cough suppressant and also as a detoxifier by the people of Northern Ghana. The root is mixed with the leaves and bark and a concoction prepared to treat anaemia. Its leaves can be used to treat skin infections and sore throat. The bark is used as a wash on the skin to treat fever. Cold water extract of the root is used bilharzia (Babu *et al.*, 2009).

Chemical constituents

Phytochemical screening of the ethanol leaf extract of *A. glaberrima* revealed the presence of alkaloids, reducing sugars, saponins, cyanogenic glycosides, tannins, triterpenes and steroids (Jato, 2015).

Biological activities

The *A. glaberrima* has been found to possess anti-inflammatory, anti-microbial and antioxidant properties. It is also known to possess antifungal activity (Pezzuto *et al.,* 1992; Jato, 2015).

2.3.6 *Phyllanthus muellerianus* (Kuntze) Exell.

Description

Phyllanthus muellerianus (Kuntze) (syn. *Phyllanthus floribundus* (Baill.) Mull. Arg.) belongs to the family Euphorbiaceae. It is a monoecious, glabrous or climbing shrub which grows up to about 12 m in height. Its leaves are alternate, ovate-elliptic with rounded base and are about 8 x 4 cm. The flowers are greenish white or yellow in colour and they are unisexual (Fig 2.16). The fruits when matured measure about 3 to 4 mm in diameter, are fleshy, numerous, globose and red in colour and later turns black. The seeds are angular, long, possess faint ridges and are bright reddish brown or yellowish brown in appearance (Irvine *et al.,* 1961; Burkill, 2000).

The plant is found in tropical forest of Africa in countries such as Ghana, Nigeria, Cameroun, Sierra Leone, Cote d'Ivoire, Sudan and DR Congo. It is also found in sub-tropical regions of the world such as Cuba, Guam and Philippines. It is known as 'Awobe'by the Asantes in Ghana (Burkill, 2000; Saleem *et al.*, 2009).

Fig 2.16. Leaves and fruits of *P. muellerianus.* (Source: www.tropicaltheferns.info, accessed on 25th July, 2016).

Medicinal uses

The plant is widely used to treat intestinal troubles. In Sierra Leone and the southern part of Nigeria, the fruits are eaten and the leaves cooked with food or soup to treat severe dysentery. In Ghana, the leaves are added to palm fruits to make soup for new mothers for post-natal strength and vitality. A decoction is made from the leaves to treat anaemia and constipation. In Cote d'Ivoire, the root is made into a decoction and used to treat gonorrhea and in Ghana, dysentery. A decoction from the bark is prepared and given as a drink or enema for treating sore throat, mumps and fever. Infusion of the root and leaves is given to children to treat eruptive fevers in Togo. A paste is made from the fresh leaves and applied to boils and wounds as dressing and also for treating fevers and skin eruptions in Sierra Leone, Ghana, Nigeria and Cameroun (Burkill, 2000;

Agyare *et al.*, 2009). The twigs are roasted and powdered and eaten to treat dysmenorrhea and other menstrual problems. The fresh leaves are used as purgative and also to treat bronchitis and urethral discharge. The leaves are given to manage heart, liver and kidney disorders (Osmeobo, 2009).

Chemical constituents

Doughari and Sunday (2008) reported that the leaves contain secondary metabolites such as alkaloids, tannins, flavonoids and anthraquinones. Aside these, various other phytochemicals have been isolated from other parts of the plant such as the aerial parts, the leaves, the stem and the bark. Geraniin, furosin, corilagin, chlorogenic acid, gallic acid, methylgallate, caffeic acid, 3,5-odicaffeoylquinic acid, astragalin, rutin, phaselic acid and isoquercitrin have been isolated from the leaves and the aerial part of the plant (Agyare *et al.*, 2011). Other compounds isolated from the leaves are bis (2-ethyloctyl) phthalate, bis (2-ethylicosyl) phthalate, 3-friedelanone and β-sitosterol (Saleem *et al.*, 2009). Other compounds isolated from the bark are 22-β-hydroxyfriedel-1-ene and 1β,22β-hydroxyfriedel-1-ene (Adesida *et al.*, 1972).

Biological activities

The aqueous leaf stem bark extracts of *P. muellerianus* are reported to possess antimicrobial activity (Doughari and Sunady, 2008; Boake *et al.*, 2016a). The ethanol leaf extract is also known to have antiplasmodial activity against chloroquine-resistant *Plasmodium falciparum* (Doughari and Sunady, 2008). Additionally, the ethanol leaf extract of the plant is known to cause stimulation of the HaCaT keratinocytes and dermal fibroblasts (Agyare *et al.*, 2009). Boakye *et al.* (2016b) also reported that aqueous leaf extract of *P. meullerianus* possess anti-inflammatory property. It is found to possess wound healing and antioxidant activities (Boakye, 2015).

2.3.7 *Maerua angolensis* DC

Maerua angolensis belongs to the family Capparaceae (Leroy, 1982). It is a semi deciduous plant that is widely found in tropical Africa in countries like Ghana, Nigeria, Cameroun, Burkina Faso, Niger Kenya, Mauritania and Togo and other arid regions. The tree can grow up to about 10 m and has a rounded crown with smooth grey bark which sometimes flake to show yellow-orange patches (Burkill, 2000). The twigs and branches possess lenticels. The plant has soft drooping leaves with petioles which are same size as the leaves but thicker than the leaves. The leaves are also alternate, broadly elliptic to ovate and the tips are rounded (von Maydell, 1990).

Fig 2.17. Whole plant (**A**) and aerial parts (**B**) of *M. angolensis*. (Source: www.ispotnature.com, accessed on 25th July, 2016).

Medicinal uses

The leaves of *M. angolensis* can be given as a purgative, to treat anorexia and also used to manage asthenia (Nacoulma, 1996). Decoctions of the leaves are given to treat various stomach problems

and rheumatism. The leaves can be applied to the body to relieve pain (Mothana *et al.*, 2009). The root of the plant can be given to treat toothache and influenza. The root is also given to treat skin rashes, sores, sexually transmitted diseases and can be used for womb cleansing (Okatch *et al.*, 2012).

Phytochemical constituents

Phytochemical investigation conducted on *M. angolensis* indicated that the methanol leaf extracts contained flavonoids, phenol, tannins and glucosinolates (Mothana *et al.*, 2009; Meda *et al.*, 2013).

Biological activity

The *M. angolensis* has been found to possess antioxidant, anticancer and anti-inflammatory properties. It is also known to stimulate the humoral and anti-inflammatory immune response (Muchuweti *et al.*, 2007).

2.4 Marine Algae Samples

2.4.1 *Ulva fasciata* Delile

Description

It is a green algae known as sea lettuce and of the family Ulvaceae. It is also known as pimu palahalaha in Hawaii. The *U. fasciata* is thalli thin and consists of wide blades of about 10 to 15 cm in width at the base and 2.5 cm at the tip. It measures about 1 m long. The base is broad but the upper portions are divided into many ribbon-like segments. The *U. fasciata* has smooth margins and often undulate. Its colour is bright green to brown green with gold margins (Fig 2.18). However, when distressed, it may be colourless (Huisman *et al.*, 2007).

Ulva fasciata is found in the Eastern Atlantic, Africa, Carribean, Indian and Pacific Oceans. It is commonly found on intertidal rocks, tidepools and on reef flats and in areas of abundant fresh water with high nutrients contents (Beach *et al.*, 1995).

Fig 2.18. Freshly uprooted *U. faciata*. (Source: www.marinelife.photography.com, accessed on 25th July, 2016).

2.4.2 *Sargassum vulgare* C. Agardh

Description

Sargassum vulgare is a brown macro-algae of the class Phaeophyceae and family Sargassaceae. It is found in both the temperate and tropical oceans across the world. It is brown or dark green in appearance. It is a free floating species macro-algae (Abbot and Hollenberg, 1976). The *sargassum*

species were first found in the Sargaso river by some Portuguese sailors hence its name (Gomez de Silva, 1988). Much study has not been done on this species but it is known that the Chinese dissolve the powdered alga in hot water and drink it to resolve heat phlegm (Xu and Wang, 2002).

2.4.3 *Hydropuntia dentata* (J. Agardh) M. J. Wynne

Description

Hydropuntia dentata (syn. *Hydropuntia rangiferina* or *Gracilaria dentata*) is a red algae of the class Florideophyceae and family Gracilariaceae (Gurgel, 2004). Agar can be obtained from *H. dentata* since the agar is obtained mainly from the Gracilariaceae family (Armisen, 1995).

2.5 Isolated compounds

2.5.1 Geraniin

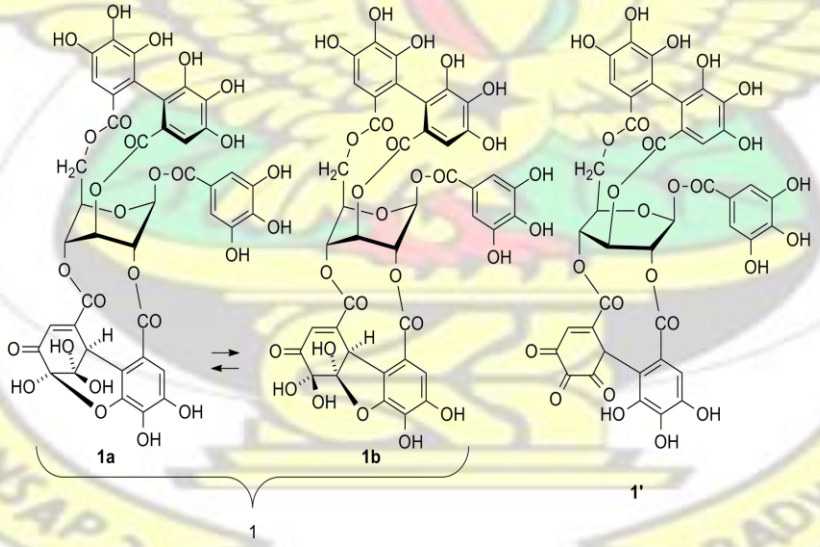


Fig. 2.19. Chemical structure of Geraniin. (Source: www.intechopen.com, accessed on 18th October, 2016).

Geraniin is a pale amorphous compound with a molecular formular $C_{41}H_{28}O_{27}$ and a molecular weight of 952.64 g/mol with density 2.26 g/mL. It is a dehydroellagitannin and occurs in two isomers (a and b) (Fig 2.19) (Agyare *et al.*, 2011). It was first isolated from *Geranium thunbergii* Sieb. et. Zucc. as its major tannin. It has since then been isolated from several medicinal plants found in the tropical and subtropical regions of the Euphorbiaceae family. These are notably of the Phyllanthus and Geranium genus (Luger *et al.*, 1998). The following are some of the sources of the compound Geraniin; the aqueous leaf and aerial extracts of *Phyllanthus muellerianus* (Kuntze) Exell. (Agyare *et al.*, 2011), the aqueous leaf extract of *Phyllanthus niruri* Thonn. and Schum. (Foo, 1993), ethanol leaf and stem extracts of *Phyllanthus sellowianus* Muller Arg. (Miguel *et al.*, 1996), ethanol root extract of *Nephelium lappaceum* L. (Palanisamy *et al.*, 2011), acetone leaf extract of *Phyllanthus urinaria* L. (Yang *et al.*, 2007), acetone-aqueous leaf extract of *Sapium sebiferum* L. (Yang *et al.*, 2007) and butanol root extract of *Thespesia lampas* Dalz and Gibson (Ambrose *et al.*, 2012).

Geraniin is reported to have anti-viral, anti-hypertensive, antinociceptive activity and known to inhibit TNF-α activity (Yang *et al.*, 2007; Velázquez-González *et al.*, 2014). It is also known to possess radio protective effects. It does so by inhibiting apoptosis triggered by gamma radiation in oxidative stress (Bing *et al.*, 2013). Geraniin is also known to possess antimicrobial (Boakye *et al*., 2016a), anti-inflammatory (Boakye *et al*., 2016b), antioxidant and wound healing properties (Boakye, 2015). It is also known to possess antiplasmodial activity (Ndjonka *et al*., 2012). Geranin is also reported to cause the human skin keratinocytes and dermal to undergo cellular activity, differenciation and collagen synthesis (Agyare *et al*., 2011).

2.5.2 Xylopic acid

Xylopic acid (15-acetoxy-(-)-kaur-16-en-19-oic acid) (Fig 2.20), a kaurene diterpine, is the major isolate from the fruit of *Xylopia aethiopica* (Dunal) A. Rich which belongs to the family Annonaceae (Woode *et al.,* 2012). It has molecular formular $C_{22}H_{32}O_4$ and a weight of 360.49 g/mol. The *X. aethiopica* is the only source of xylopic acid and it can be obtained from the ethyl acetate fractions of the petroleum ether extract of the fruits of the plant (Boampong *et al.,* 2013).

Xylopia aethiopica has a lot of traditional uses and some include the treatment of rheumatism, colic pains, headaches and neuralgia. It is also known to possess abortifacient properties (Igwe *et al.,* 2003). Xylopic acid is known to possess antimicrobial, anti-inflammatory and analgesic properties (Ghisalberti, 1997; Garcia *et al.,* 2007; Woode *et al.,* 2012). The ethanol extract is known to possess antimicrobial activity (Boakye-Yiadom *et al.,* 1977). It has been reported that xylopic acid unlike other kaurenes possess no cytotoxic effect against human cells (Cavalcanti *et al.,* 2009). Boampong *et al.,* (2013) also reported of the antimalarial activity of xylopic acid against *Plasmodium berghei* infected rats thus making it an ideal antimalarial candidate and its anti-pyeritc activity. Xylopic acid is also known to possess anti-arthritic effect (Obiri *et al*., 2014).

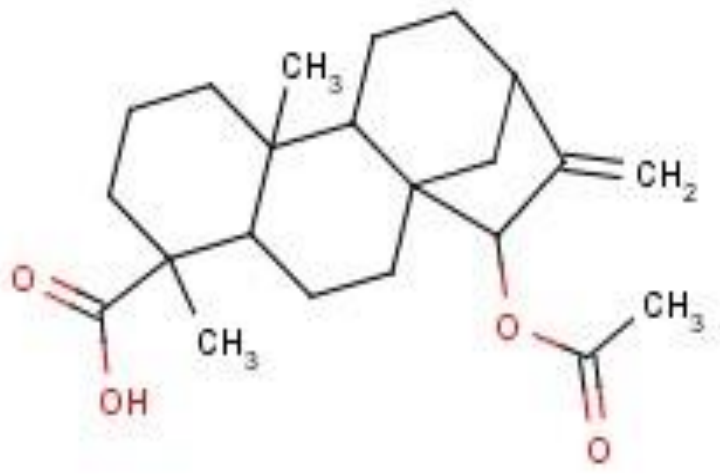


Fig. 2.20. Chemical Structure of xylopic acid

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Table 3.1 Instruments and equipment Instrument/Equipment Source/Manufacturer/Place

25cm^2 tissue culture flasks	Corning, NY, USA
384-, 96- and 24-well assay plate	E&K Scientific, Santa Clara
AxioCam 105 digital camera	Carl Zeiss Microscopy, Thornwood, USA
Axiovert A1 inverted microscope	Carl Zeiss Microscopy, Thornwood, USA

Beakers	Fisher Scientific, Schwerte, Germany
Conical flasks	Fisher Scientific, Schwerte, Germany
Neubauer Counting chamber	Fisher Scientific, Schwerte, Germany
Electronic weighing balance	Ohaus Corporation, PB, USA
Envision plate reader	Bio Tek Instruments, USA
Filter paper	Whatman, London, UK
Freeze dryer	Thermo Scientific, Schwerte, Germany
GasPak EZ	VWR, West Chester, PA, USA
Incubator	Thermo Scientific, Asheville, USA
Lab mill machine	Christy and Norris, England, UK
Laboratory sieve	Retsch, Haan, Germany
Measuring cylinders	Fisher Scientific, Schwerte, Germany
Micropipettes	Fisher Scientific, Schwerte, Germany
Microscope	Carl Zeiss Microscopy, Thornwood, USA
Orbital shaker	New Brunswick, USA
Pipette tips (10, 20, 100, 200, 1000 µL)	Fisher Scientific, Schwerte, Germany
Refrigerators	Sharp Corpration, London, UK
Rotary evaporator	Buchi, Hamburg, Germany
Test tubes	Fisher Scientific, Schwerte, Germany
Thermostatically controlled water bath	New Brunswick, USA
Ultra turrax	Buchi, Hamburg, Germany

Table 3.2 Reagents, Chemicals and Reference Drugs Reagent Source/ Manufacturer/Place

10% heat-inactivated Fetal bovine serum	Gibco, Carlsbad, USA
100U/mL Penicillin/100µg/mL Streptomycin mixture	Gibco, Carlsbad, USA
100X vitamin mix	Gibco, Carlsbad, USA

Table 3.2 continued Reagent Source/ Manufacturer/Place

Acetic anhydride	Sigma-Aldrich, MO, USA
Adult bovine serum	Gibco, Carlsbad, USA
Ammoniacal alcohol	Sigma-Aldrich, MO, USA
Amphotericin B (99.9% w/w)	Sigma-Aldrich, MO, USA
Bacto-casitone medium	Gibco, Carlsbad, USA
Cell titre glo	Promega, Madison, WI, USA
Chloroform (95% v/v)	Sigma-Aldrich, MO, USA
Dimethyl sulfoxide (99.9% v/v)	Sigma-Aldrich, MO, USA
Dragendorf's reagent	Sigma-Aldrich, MO, USA
Ethanol (96% v/v)	Sigma-Aldrich, MO, USA
Fehling's solution A and B	Sigma-Aldrich, MO, USA

Ferric chloride solution	Sigma-Aldrich, MO, USA
Hydrochloric acid (HCl)	Sigma-Aldrich, MO, USA
Lead acetate solution	Sigma-Aldrich, MO, USA
Litmus paper (red and blue)	Whatman, London, UK
Methanol (98% v/v)	Sigma-Aldrich, MO, USA
Metronidazole (99.9% w/w)	Sigma-Aldrich, MO, USA
Pentamidine (99.9% w/w)	Sigma-Aldrich, MO, USA
Phosphate buffered saline	Sigma-Aldrich, MO, USA
Sodium hydroxide (NaOH)	Sigma-Aldrich, MO, USA
Sodium pentobarbital	Sigma-Aldrich, MO, USA
Sulphuric acid (H_2SO_4)	Sigma-Aldrich, MO, USA
SYBR-Green	Invitrogen, Carlsbad, USA
Triton-X 100	Gibco, Carlsbad, USA

Table 3.3 Culture media

Media	Source/Manufacturer/Place
Basch medium	Gibco, Carlsbad, USA
HM1:IMSS medium	Gibco, Carlsbad, USA
HMI-9 medium	Gibco, Carlsbad, USA
Nelson Culture medium	Gibco, Carlsbad, USA
RMPI 1640 medium	Gibco, Carlsbad, USA
TYI-SS-3 medium	Gibco, Carlsbad, USA

Table 3.4 Strains of parasites and vectors

Parasite/Vector	Source/Manufacturer/Place
Biomphalaria glabrata snail M1 line strain	CDIPD, UCSF, CA, USA
Entamoeba histollytica HMI:IMSS strain	CDIPD, SSPPS, CA, USA
Giardia lamblia WB strain	CDIPD, SSPPS, CA, USA
Golden Syrian hamsters	Charles River, San Diego, CA, USA
Naegleria fowleri ATCC 710 strain	CDIPD, SSPPS, CA, USA
Schistosoma mansoni PRI clinical isolate	CDIPD, SSPPS, CA, USA
Trypanosoma brucei brucei Lister 427	CDIPD, UCSF, CA, USA

3.2 Methods

3.2.1 Maintenance of *G. lamblia*

Axenic WB strain of *G. lamblia* (ATCC 30888) trophozoites were maintained in TYI-S-33 medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% v/v heat inactivated fetal bovine serum (FBS) as described by Keister (1983) and Debnath *et al*. (2014). Cells were kept in a non-vented flask in a humidified atmosphere of 5% CO_2 at 37°C. The cells were maintained in the logarithmic phase phase and routine passage performed every 2nd day. The cells were counted using a Neubauer Chamber. The cells were kept for a maximum of 3 subcultured dilution cycles and checked for contamination. Only non-contaminated cells were used in this study.

3.2.2 Maintenance of *E. histolytica*

The *E. histolytica* trophozoites (strain HM1:IMSS) were maintained axenically in TYI-S-33 supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% v/v heat inactivated adult bovine serum as previously described by Diamond *et al*. (1978). Cells were also kept in nonvented airtight flask at 37°C and maintained in log-phase. Cells were subcultured every 48 h and counted using the Neubauer chamber.

3.2.3 Maintenance of *S. mansoni*

The PR1 (Puerto Rica) isolate of *Schistosoma mansoni* was maintained by passage through *Biomphalaria glabrata* (strain M1 line) snails and 3 to 5 week-old, female Golden Syrian hamsters as intermediate and definite hosts, respectively (Long *et al.,* 2016; Abdulla *et al.,* 2009). The infectious cercariae were shed from the snails using the method developed by Colley and Wikel

(1974). The cercariae were transformed to get rid of the tails to obtain the juvenile schistosome known as the Schistosomulae. Vertebrate maintenance and handling at the University of California, San Diego (UCSD) Animal Care Facility were done in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of UCSD. Hamsters were injected with 800 cercariae subcutaneously to begin the process of infection. At week 6 postinfection, hamsters were euthanized with an intraperitoneal injection of 250 to 300 mg/kg sodium pentobarbital containing 1000 U heparin per animal. Adult schistosomes were harvested by reverse perfusion in RPMI 1640 medium (Tucker *et al.*, 2013).

3.2.5 Maintenance of *N. fowleri*

The pathogenic strain of *Naegleria fowleri* (ATCC 710) was axenically cultured in Nelson Culture medium supplemented with 10% heat inactivated FBS and 1x penicillin (100 U/mL), streptomycin (100 µg/mL) (pen/strept) in vented flasks. Cells were kept in a humidified atmosphere of 5% CO_2 at 37°C and subcultured every 48 h covering the nose and mouth using nose mask as a precaution. Cells were harvested during the log-phase of growth for the experiment.

3.2.6 Maintenance of *Trypanosoma brucei brucei*

Culture adapted bloodform of *T. b. brucei* (Lister 427) were cultured in complete HMI medium as described by Hirumi and Hirumi (1989). The medium was supplemented with 10% heat inactivated FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were maintained in a vented flask in humidified incubators at conditions of 5% CO_2 at 37°C and subcultured every 48 h in log-phase.

3.3 Plant collection and extraction

All the plant extracts and fractions were kindly donated by researchers in the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana, except the algae samples incluidng *Ulva fasciata, Sargassum vulgare* and *Hydropuntia dentate* which were collected and prepared by the author.

The *U. fasciata, S. vulgare and H. dentata* which are marine algae were collected from the beaches of Prampram, Osu and Sakumono all of the Ghana in January 2014. The samples were authenticated by Mr. Emmanuel Klubi of the Department of Marine Science and Fisheries, University of Ghana, Legon, Ghana. After collection the samples were washed thoroughly with fresh seawater to rid them of any sand epiphytes and other extraneous matter and dried under shade at a temperature of 25 to 28°C for two weeks. The dried samples were pulverized into fine powder using a laboratory milling machine. Three hundred grams (300 g) of each of the powdered algae were soaked in methanol-chloroform (1:1) in a stoppered container. The samples were extracted using ultra turrax under ice-cooling at a speed of 24000 rpm for 5 min. The extracts were filtered using sieve number 200 and aperture 75 µm and Whatman filter paper number 1. The filtrates were concentrated using a rotary evaporator at 40°C under reduced pressure and the concentrate lyophilized. The lyophilized extracts were weighed, labelled and stored in air tight glass containers at 4°C in the refrigerator until used.

3.4 Phytochemical screening

To perform the phytochemical screening, both the powdered samples and the chloroform/methanol extracts were analysed using the methods described by Trease and Evans (2002), Khandelwal (2010) and Usman *et al*. (2014).

3.4.1 Saponin test

Fifty milligrms (50 mg) of each of the algae extracts and powdered samples were extracted with 5mL of distilled water into a test tube. The filtrate was shaken vigorously to form a froth and made to stand on the bench for about 10 min then 0.5 mL of 2 M HCl was added to the froth and it persistency indicated the presence of saponin (Trease and Evans, 2002).

3.4.2 Tannins test

Fifty milligrms (50 mg) of the powdered samples and extracts were boiled in 25 mL of distilled water for about 5 min. The resulting extract was cooled and filtered. To 1 mL of the filtrate, 10 mL of distilled water was added followed by 5 drops of 1% lead acetate solution were added. A buff precipitate appearing in the test tube indicated the presence of tannins. To distinguish between hydrolysable and condensed tannins, 1% w/v ferric chloride solution was added. The appearance of a dark green or blue black colour indicates hydrolysable or condensed tannin, respectively (Usman *et al*., 2014).

3.4.3 Glycoside test

To 200 mg of the powdered samples and extracts, 5 mL of dilute H_2SO_4 was added and warmed for about 2 min. The two solutions were cooled and filtered using Whatman filter paper 1. Four drops of 20% NaOH was added to each tube to make them alkaline (tested by passing red litmus paper through it to turn blue). One milliliter (1 mL) of Fehling's A and B solution was added and the formation of a brick red precipitate after heating for 3 min on a water bath indicated the presence of glycosides (Usman *et al*., 2014).

3.4.4 Test for Alkaloids

Ten milliliters (10 mL) of ammoniacal-alcohol was added to 50 mg of the powdered samples and the crude extracts to extract. The resulting solution was filtered and evaporated to dryness over water bath. The residue was again extracted with 1% H_2SO_4 and the filtrate was made alkaline by adding dilute NH_3. Chloroform was added to the solution in a separating funnel to obtain two miscible layers. The chloroform portion was allowed to evaporate and the 1% H_2SO_4 added to the residue followed by the addition of two drops of Dragendorf's reagent. Formation of an orange red precipitate indicated the presence of alkaloids (Khandelwal, 2010).

3.4.5 Test for flavonoids

Fifty milligrms (50 mg) of the powdered samples and crude extract each were dissolved in distilled water and filtered. A piece of paper was passed through the filtrate and allowed to dry. The dried filter paper was passed through ammonia solution. A bright intense yellow colouration which turned colourless in the presence of HCl indicated the presence of flavonoids (Khandelwal, 2010).

3.4.6 Test for sterols

To 200 mg of both extracts and powdered samples, 50 mL of chloroform was added and filtered using filter paper. One milliliter (1 mL) of ethanolic acetic anhydride was added to a 2 mL portion of chloroformic filtrate. Concentrated H_2SO_4 was carefully added by the side of the test tube and the formation of bluish-green ring at the interface indicated the presence of sterols (Usman *et al*., 2014).

3.4.7 Triterpenoids test

Two hundred milligram (200 mg) of the powdered material and the extracts were extracted with 10 mL of chloroform. This was shaken vigorously for 5 mins and filtered. To the filtrate in a test

tube, concentrated H_2SO_4 was added carefully to the side of the tube. The formation of a cherryred ring at the chloroform-sulphuric acid interface indicated the presence of triterpenoids (Khandelwal, 2010).

3.5 Screening of plants samples against various selected parasites

3.5.1 First-pass screening of pure compounds and extracts for activity against *G. lamblia* and *E. histolytica*

A first-pass cell viability assay was performed at a concentration of 100 µg/mL of crude extract and fraction and 25 µg/mL of isolated compounds against *G. lamblia* and *E. histolytica* using the method described by Debnath *et al*. (2014). About 0.5 µL of 20 mg/mL and 5 mg/mL of stock extracts and compounds, respectively, were plated into white 96-well flat-bottom plates in duplicate, then 5000 trophozoites in 99.5 µL TYI-S-33 medium were added to the respective wells making a total volume of 100 µL. For positive control, 50 µM of metronidazole (MNZ) was plated with the trophozoites and negative controls contained 0.5 % of dimethyl sulfoxide (DMSO) and the trophozoites. Cell culture plates were incubated at 37°C for 48 h in the GasPak EZ gasgenerating anaerobe pouch system to maintain the anaerobic conditions throughout the incubation period. After two days (48 h) of incubation, the assay plates were equilibrated at room temperature for 30 min and 50 µL of CellTiter-Glo® was added to each well. The CellTiter-Glo® luminescent cell viability assay was employed to quantify the presence of ATP which is proportional to the luminescent signal. The plates were placed on an orbital shaker for 10 min to induce cell lysis. The plates were equilibrated at room temperature (25°C) for another 10 min to stabilize the signal. The luminescent signal, resulting from the lysis of the cells was measured with an EnVision luminometer (Software version 1.13.3009.1401), and converted into the percentage

of inhibition of the cell growth relative to maximum and minimum reference signal controls using the following equation: percentage inhibition = [(mean of maximum signal reference control − experimental value)/(mean of maximum signal reference control − mean of minimum signal reference control)] × 100.

3.5.2 First pass screening of pure compounds and extracts for activity against *N. fowleri*

The modified method described by Debnath *et al*. (2014) was used for the screening of the extracts against *N. fowleri*. The first pass assay was performed at concentration of 100 µg/mL of each extract and 25 µg/mL of the isolated compounds. About 0.5 µL of the stock concentrations was similarly plated in the 96-well assay plate and 99.5 µL (10,000) of trophozoites in Nelson medium added. Fifty micromolar (50 µM) of Amphotericin B and 5% DMSO served as positive and negative controls, respectively. The CellTitre Glo® assay was employed as described for *G. lambia* and *E. histolytica* (Section 3.5.1).

3.5.3 First pass screening of pure compounds and extracts against *T. b. brucei*

The SYBR Green assay was employed for this experiment. The first pass screening was conducted using the method described by Sykes *et al*. (2012) with slight modifications. Five microliters (5 µL) of 2000 µg/mL of each extract and 500 µg/mL of isolated compounds (1 µL of 200 mg/mL and 50 mg/mL stocks in 100 µL PBS) were plated in a black 384-well plate in duplicates. Fortyfive microliters (45µL) of 5000 cells/mL of the trypanosomes in complete HMI-9 medium was added to the plate to make total volume of 50 µL. Ten microliters (10 µL) of Pentamidine and 0.5% DMSO were used as positive and negative controls respectively. Plates were incubated at 37°C and 5% CO_2 for 72 h. After that, the plates were equilibrated at room temperature (25°C) for about 20 min. Then 1,250 nL of SYBR Green in lysis solution (30 mM Tris pH 8, 7.5 mM EDTA,

0.012% saponin, 0.12% Triton X-100 and 3 µL of SYBR Green) was added to each well and the plates agitated at 1700 rpm for 30 min to lyse the cells. The plates were kept in the dark for an hour after which the reading was taken using the Envision plate reader. The activities (% inhibition) of the compounds and exctracts were normalized against the controls from the same plate using the formula: % inhibition = [(negative control-test compound)/negative controlpositive control)] x 100.

3.5.4 *S. mansoni* phenotypic screening

For schistosomulae (somules) and adult worms, transparent u-shaped 96-well plates and flatbottom 24-well plates were used, respectively. The phenotypic screening was carried out as described (Abdulla *et al*., 2009; Long *et al*., 2016). For somules first pass screens, compounds and extracts were added to the wells at a concentration of 25 µg/mL (1 µL of 5 mg/mL stock solution) and 100 µg/mL (1 µL of 20 mg/mL stock solution), respectively. Final concentrations of DMSO in each well were not greater than 0.5 % v/v. The somules were then added at a density of 30 to 40 worms in 200 µL of complete Basch medium (Basch, 1981) supplemented with 5% v/v FBS, 100 U/mL penicillin and 100 mg/mL streptomycin. Parasites were incubated in a humidified atmosphere of 5% CO_2 at 37°C for 72 h. For confirmatory tests, compounds that elicited phenotypic response were selected for dose-response *in vitro* assays using concentrations of 100 or 25 to 0.625 µg/mL (final DMSO 0.5%). Both first-pass and confirmatory dose-response assays were performed in duplicate.

For the adult screening, 4 to 5 male worms were plated in the 24 flat-bottom wells, followed by the addition of 2 mL complete Basch medium per well. The first-pass and confirmatory doseresponse assay were performed once. Phenotypic changes in the somules were recorded at 24,

48 and 72 h intervals while that for the adults werer at 3, 5, 24 and 48 h. To characterize the phenotypic changes observed, an observational system developed by Long *et al*. (2016) which is based upon descriptors and corresponding severity scores, was employed (Table 3.5). The severity score range from 0 (no effect) to a maximum of 4 (severely damaged) and is calculated by addition of the individual single scores per phenotypic change or alteration. Parasites with a damaged surface, or clearly degenerate or dead receive the maximum score of 4 as these phenotypic changes are assumed to be lethal and non-reversible. Images were captured using a Zeiss Axiovert A1 inverted microscope (10X magnification for the somules and 2.5X magnification for the adults) and a Zeiss AxioCam 105 color digital camera controlled by ZEN 2 lite software (Version 2.0.0.0).

Table 3.5 Descriptors and severity score of the effect of bioactive compounds on *S. mansoni*

Descriptor	Key	Severity score
Round (somules)	R	1
On sides (adults)	Os	1
Uncoordinated (adults)	U	1
Tegumental damage (adults)	Td	4
Shrunk (adults)	shrunk	1

Dark color	Da	1
Slow	S	1
Overactive	O	1
Immobile	I	1
Degenerate	Deg/Dg	4
Partial death (%)	PD%	1
Dead	D	4

3.6 Dose response assays

3.6.1 Dose response assay of pure compounds and extracts activity against *G. lamblia, E. histolytica* and *N. fowleri*

For confirmatory screening, extracts, fractions and isolated compounds that exhibited phenotypic effects were picked from the 20 and 5 mg/mL stock concentrations. Serial dilutions were performed to get different concentrations ranging from 100 to 0.78125 µg/mL for the extracts and fractions and 25 to 0.1953 µg/mL for isolated compounds. This was to obtain the 50% effective concentration (EC_{50}). Tests were run in triplicates. Similar to the first pass screening, 99.5 µL of parasites were added to the well containing 0.5 µL of each compound or extractvand incubated for 48 h and CellTitre Glo® added to obtain the luminescence read-out which was analysed to obtain the percentage inhibition.

3.6.2 Dose response screening of compounds and extracts against *T. b. brucei*

In confirmatory counter screening, the compounds that exhibited activity in the first pass screen (known as hits) had their potency (50% effective concentration or inhibitory concentration) determined in dose response curves. This was performed in triplicates and the method for the first pass screen was followed.

CHAPTER FOUR

4.0 RESULTS

4.1 Algae extraction

The maceration method was employed and this method is highly acceptable since plant constituents that are thermolabile are protected. The yields of the extracts were expressed as a percentage in relation to the amount of the dried powdered samples. The yield for the samples were 19.7, 15.2 and 11.8% w/w relative to the dried powdered materials of *U. fasciata, S. vulgare* and *H. dentate*, respectively (Table 4.1).

Table 4.1 Percentage yield of chloroform/methanol extracts of *U. fasciata, S. vulgare* and *H. dentata*

Sample	Extract/Code	Yield (%w/w)
Ulva fasciata	CMUF	19.7
Sargassum vulgare	CMSV	15.2
Hydropuntia dentate	CMHD	11.8

CMUF: chloroform/methanol extract of *Ulva fasciata,* CMSV: chloroform/methanol extract of *Sargassum vulgare,* CMHD: chloroform/methanol extract of *Hydropuntia dentate*.

4.2 Phytochemical screening

The phytochemical screening is done to identify secondary metabolites that are responsible for the biological activity of a said plant. The algae extracts as well as their powdered pulverized materials were screened for their phytochemical constituents such as tannins, alkaloids, glycosides, saponins, sterols and triterpenoids. The *U. fasciata* possessed tannins, saponins, sterols and alkaloids in both the extracts and powdered materials (Table 4.2). There were no glycosides, flavonoids and triterpenoids. The *S. vulgare* showed the presence of glycosides, tannins, flavonoids, saponins and alkaloids in both extract and powdered materials. There were no sterols and triterpenoids present. The extract and powdered material of *H. dentata* possessed all the phytochemical constituents screened for as shown in Table 4.2.

Table 4.2. Phytochemical constituents present in the algae extract and their powdered materials.

Phytochemical	CMUF	UF	CMSV	SV	CMHD	HD

Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Glycosides	-	-	+	+	+	+
Saponins	+	+	+	+	+	+
Sterols	+	+	-	-	+	+
Flavonoids	-	-	+	+	+	+
Triterpenoids	-	-	-	-	+	+

Key: (-): absent, (+): present, CMUF: chloroform/methanol extract of *Ulva fasciata*, CMSV: chloroform/methanol extract of *Sargassum vulgare*, CMHD: chloroform/methanol extract of *Hydfropuntia dentate*.

4.3 First pass activity of compounds and extracts against *E. histolytica.*

All the extracts, isolated compounds (geraniin, xylopic acid and metronidazole) and fractions were screened for activity against *E. histolytica*. The CellTitre Glo® luminescence cell viability assay technique as described by Debnath *et al*. (2012) was employed in this assay. In this technique, the number of viable cells are determined and quantified by quantifying the ATP present. The more the ATP present, the more viable cells and the less effective the compound is and vice versa. As shown (Table 4.3), only xylopic acid, an isolated compound from the dried fruit of *Xylopia aethiopica* exhibited a 100% inhibition of the parasite. Geraniin, an isolated compound from aqueous leaf extract of *Phyllanthus muellerianus* exhibited a 52% inhibition and the rest exhibited inhibition below 50%. The control which was metronidazole at a concentration of 50 µM exhibited a 100% inhibition (Table 4.3).

Table 4.3 First pass activity of compounds and extracts against *E. histolytica.*

Sample	Crude Fractions compound % Inhibition	extract/pure % Inhibition methanol	Ethyl acetate	Petroleum ether	acetone
Extracts/Fraction					
M. nobilis ethanol bark	48	-	28	30	-
E. ivorense methanol leaf	-	38	31	42	48
E. ivorense methanol bark	-	19	14	6	22
M. angolensis ethanol bark	48	-	-	-	-
H. latifolia methanol leaf	41	-	-	-	-
L. ovalifolia methanol leaf	41	-	-	-	-
A. glaberrima ethanol bark	38	-	-	-	-
P. muellerianus aqueous leaf	49	-	-	-	-
U. fasciata (whole alga)	39	-	-	-	-
S. vulgare (whole alga)	26	-	-	-	-
H. dentata (whole alga)	49	-	-	-	-
Compounds					
Geraniin	52	-	-	-	-
Xylopic acid	100	-	-	-	-
Metronidazole	100	-	-	-	-

Key: (-): Not tested

4.4 Activity of pure compounds against *E. histolytica*

After the first pass screening, those compounds and extracts that exhibited activity above 50% were selected for confirmatory screening and IC_{50} (inhibitory concentration of 50%) determined.

Xylopic acid at a concentration of 25 µg/mL and geraniin at a concentration of 50 µg/mL were selected for the IC_{50} determination. Xylopic acid and geraniin had IC_{50} of 4.80 µg/mL (13.30 µM) and 34.71 (36.44 µM) µg/mL. Metronidazole, the positive control had an IC_{50} of 1.287 µM (Fig 4.1).

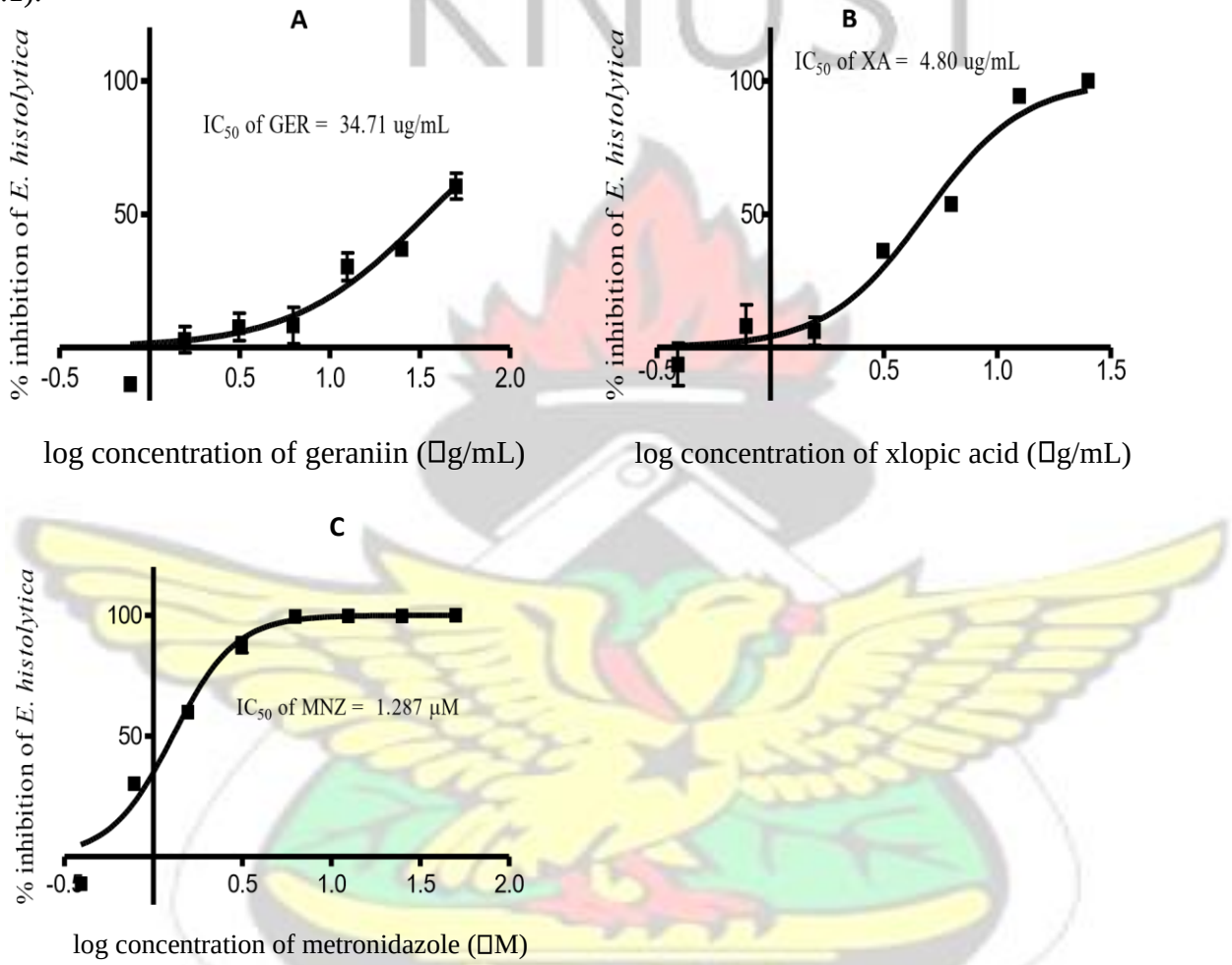


Fig 4.1. Activity of compounds againsts *E. histolytica*. **A**: Activity of geraniin (GER), **B**: Activity of xylopic acid (XA) and **C**: Activity of metronidazole (MNZ) against *E. histolytica*.

4.5 First pass activity of compounds and extracts against *G. lamblia*

The CellTitre Glo® luminescence cell viability assay technique was employed for this assay as described by Debnath *et al*. (2014). The analysed data indicated that ethanol extract of *Albizia*

glaberrima, ethyl acetate fraction of methanol extract of *Erythrophleum ivorense* stem bark, xylopic acid, *Maerua angolensis*, *Sargassum vulgare*, petroleum ether fraction of 70% ethanol bark extract of *Margaritaria nobilis*, *Hydropuntia dentata*, *Ulva fasciata* and *Laportea ovalifolia* exhibited 80% activity or more (Table 4.4). Metronidazole exhibited 99% activity against *G. lamblia*.

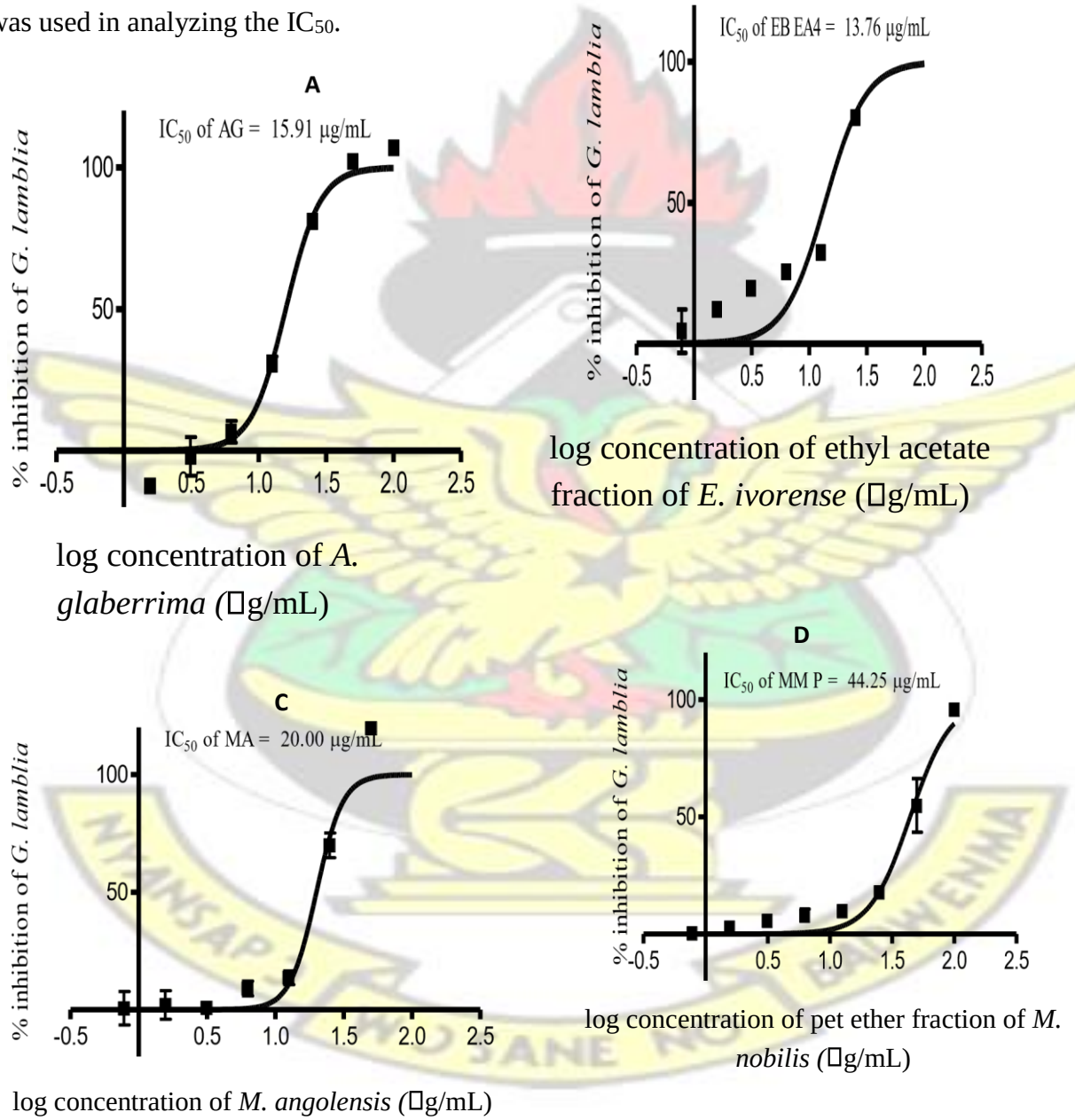
Table 4.4 First pass activity of compounds and extracts against *G. lamblia*

Sample	Crude extract/pure compound % Inhibition	Fractions % Inhibition			
		methanol	Ethyl acetate	Petroleum ether	acetone
Extracts/Fraction					
M. nobilis ethanol bark	36	-	49	80	-
E. ivorense methanol leaf	-	15	20	29	43
E. ivorense methanol bark	-	55	103	76	22
M. angolensis ethanol bark	104	-	-	-	-
H. latifolia methanol leaf	17	-	-	-	-
L. ovalifolia methanol leaf	106	-	-	-	-
A. glaberrima ethanol bark	81	-	-	-	-
P. muellerianus aqueous leaf	22	-	-	-	-
U. fasciata (whole alga)	91	-	-	-	-
S. vulgare (whole alga)	104	-	-	-	-
H. dentata (whole alga)	108	-	-	-	-
Compounds					
Geraniin	72	-	-	-	-
Xylopic acid	105	-	-	-	-
Metronidazole	99	-	-	-	-

Key: (-): Not tested

4.6 Activity of compounds and extracts against *G. lamblia*

The compounds and extracts that exhibited activity above 80% were selected, rescreened and IC_{50} determined. The *A. glaberrima,* ethyl acetate fraction of methanol bark extract of *E. ivorense,* pet ether fraction of *M. nobilis* extract, *U. fasciata, M. angolensis*, and Xylopic acid had confirmatory activities and their IC_{50} were determined. The results are shown in Figure 4.2. The graph prism was used in analyzing the IC_{50}.



Activities of extracts against *G. lamblia*. **A:** Activity of *A. glaberrima*; **B:** Activity o
ate fraction of *E. ivorense*; **C:** Activity of *M. angolensis*; **D:** Activity of pet ether fractio
ilis.

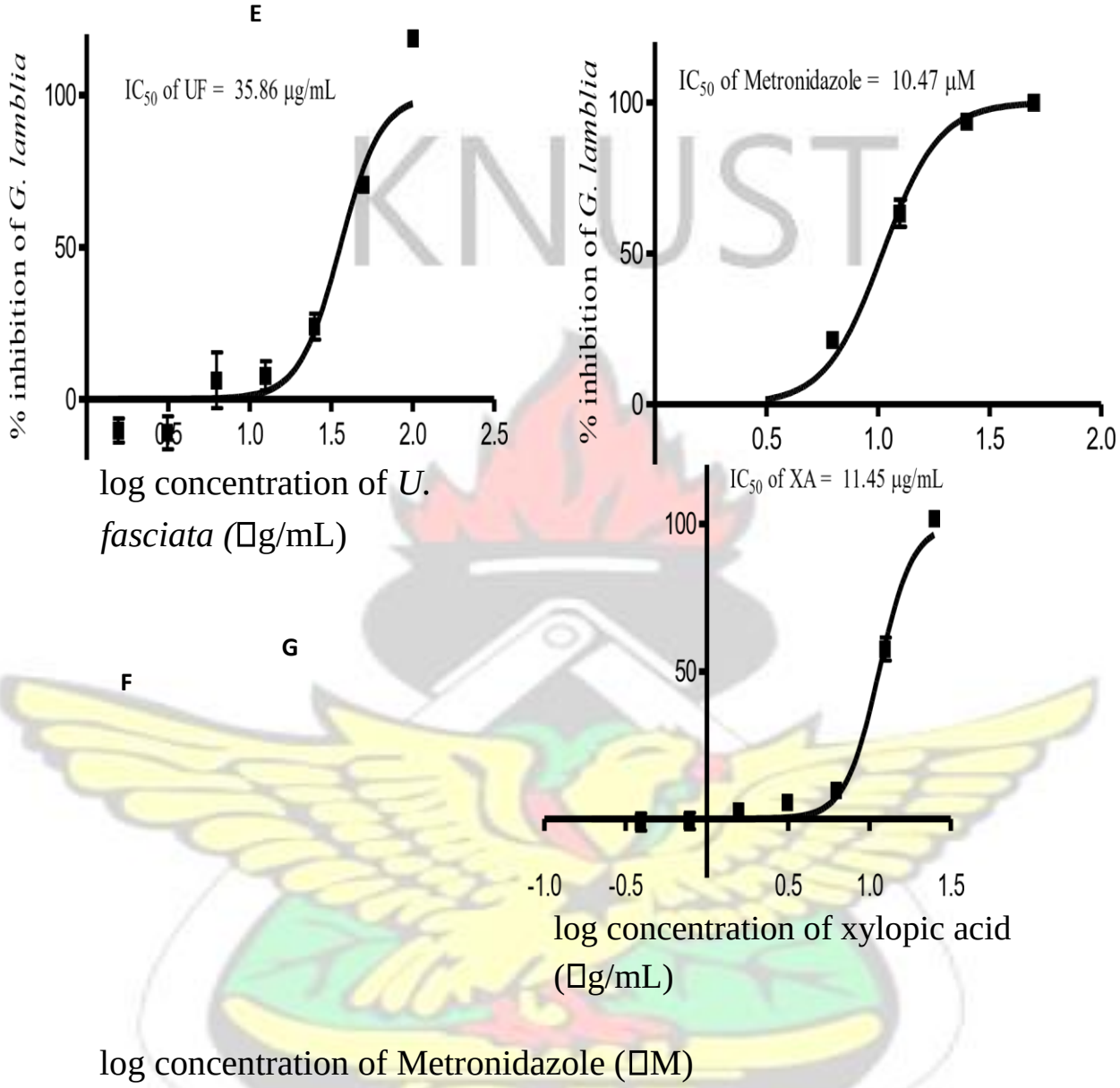


Fig. 4.2b. Activities of compounds and extracts against *G. lamblia*. **E:** Activity of whole extract of *U. fasciata*; **F:** Activity of xylopic acid; **G:** Activity of metronidazole.

4.7 First pass activity of compounds and extracts against *N. fowleri*

The compounds and extracts were screened against the parasite at concentrations similar to that used in the screening against *E. histolytica* and *G. lamlia.* The *A. glaberrima,* xylopic acid, ethyl

acetate fractions of methanol bark and leaf extracts of *E. ivorense* and *M. angolensis* exhibited activities of at least 80%. The positive control amphotericin B exhibited a 100% inhibition (Table 4.5).

Table 4.5 First pass activity of compounds and extracts against *N. fowleri*

Sample	Crude Fractions extract/pure compound % Inhibition	% Inhibition			
		methanol	Ethyl acetate	Petroleum ether	acetone
Extracts/Fraction					
M. nobilis ethanol bark	29	-	47	41	-
E. ivorense methanol leaf	-	32	80	65	28
E. ivorense methanol bark	-	26	87	64	50
M. angolensis ethanol bark	89	-	-	-	-
H. latifolia methanol leaf	51	-	-	-	-
L. ovalifolia methanol leaf	29	-	-	-	-
A. glaberrima ethanol bark	96	-	-	-	-
P. muellerianus aqueous leaf	45	-	-	-	-
U. fasciata (whole alga)	42	-	-	-	-
S. vulgare (whole alga)	62	-	-	-	-
H. dentata (whole alga)	57	-	-	-	-
Compounds					
Geraniin	64	-	-	-	-
Xylopic acid	96	-	-	-	-
Amphotericin B	100	-	-	-	-

Key: (-): Not tested

4.8 Activity of compounds and extracts against *N. fowleri*

The 'compounds' that exhibited activity above 80% were selected and rescreened for confirmatory activity. The *A. glaberrima* and xylopic acid exhibited confirmatory activity at concentrations of 100 and 25 µg/mL respectively. Ethanol bark extract of *A. glaberrima* had an IC_{50} of 38.70 µg/mL and xylopic acid had an IC_{50} of 16.06 µg/mL. The positive control amphotericin B had an IC_{50} of 0.2 µM (Fig 4.3). The graphs were plotted using mean and standard deviation values.

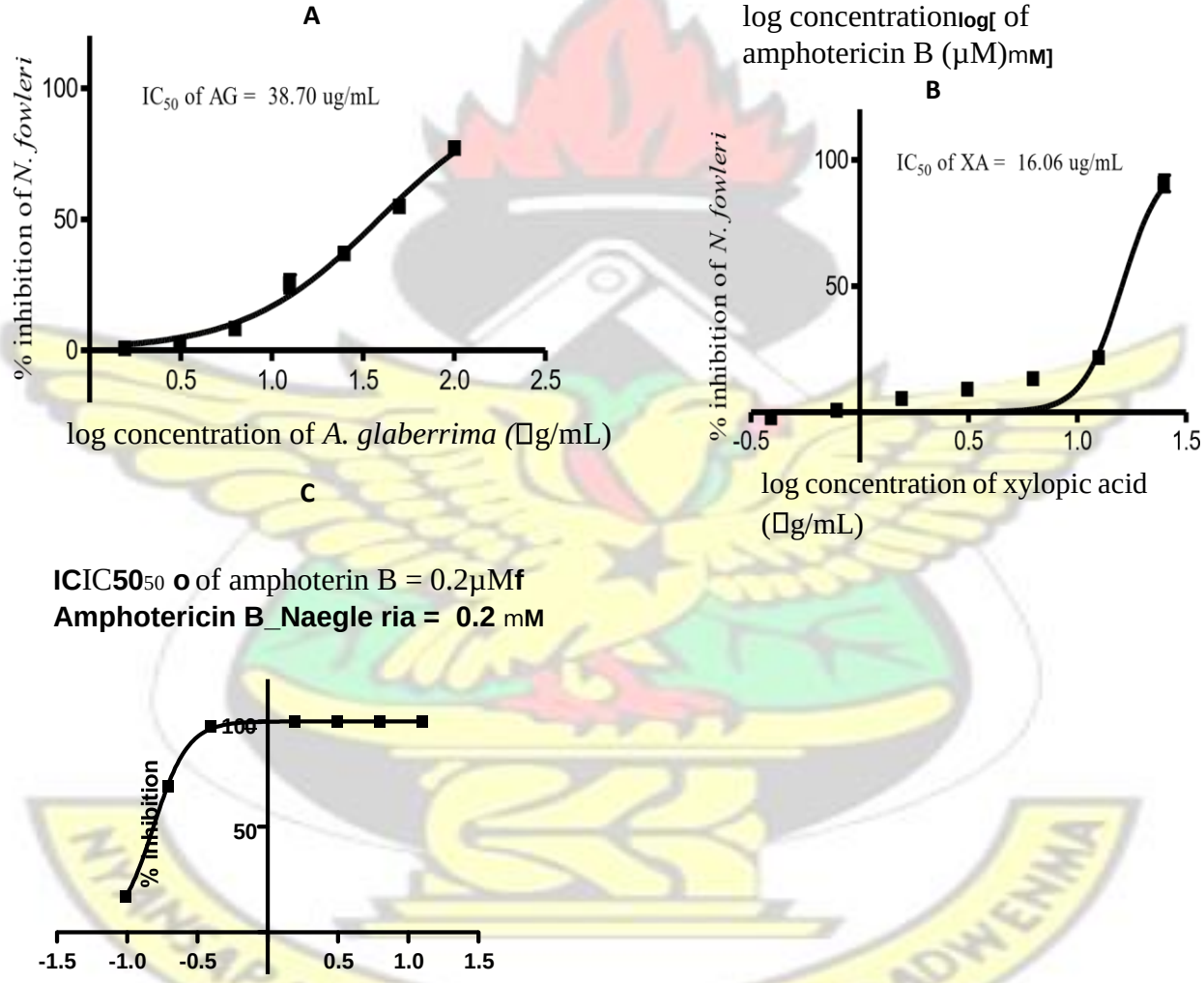


Fig. 4.6 Activities of compounds and extract against *N. fowleri*. **A:** Activity of ethanol bark extract of *A. glaberrima*; **B:** Activity of xylopic acid; **C:** Activity of amphotericin B.

4.9 First pass activity of compounds and extracts against *T. b. brucei*

The compounds and extracts were screened against *T. b. brucei.* The concentrations used were 100 µg/mL for extracts and fractions, and 50 µg/mL for isolated compounds. The SYBR Green assay technique as described by Faria *et al.* (2015) was employed in this assay. The SYBR Green, a cyanide containing dye binds to the double stranded DNA of the cells of the parasite and this provides an assessment of the number of cells alive in a population. The more the live cells, the higher the binding intensity and the less active a compound is to the parasite and vice versa. After the addition of the SYBR Green on the third day of incubation, geraniin, aqueous leaf extract of *P. muellerianus,* xylopic acid, ethyl acetate and acetone fractions of methanol bark and leaf extracts of *E. ivorense* as well as pet ether fraction of 70% ethanol bark extract of *M. nobilis* exhibited activity above 75% inhibition (Table 4.6). The samples then were selected for confirmatory screening and IC_{50} determination.

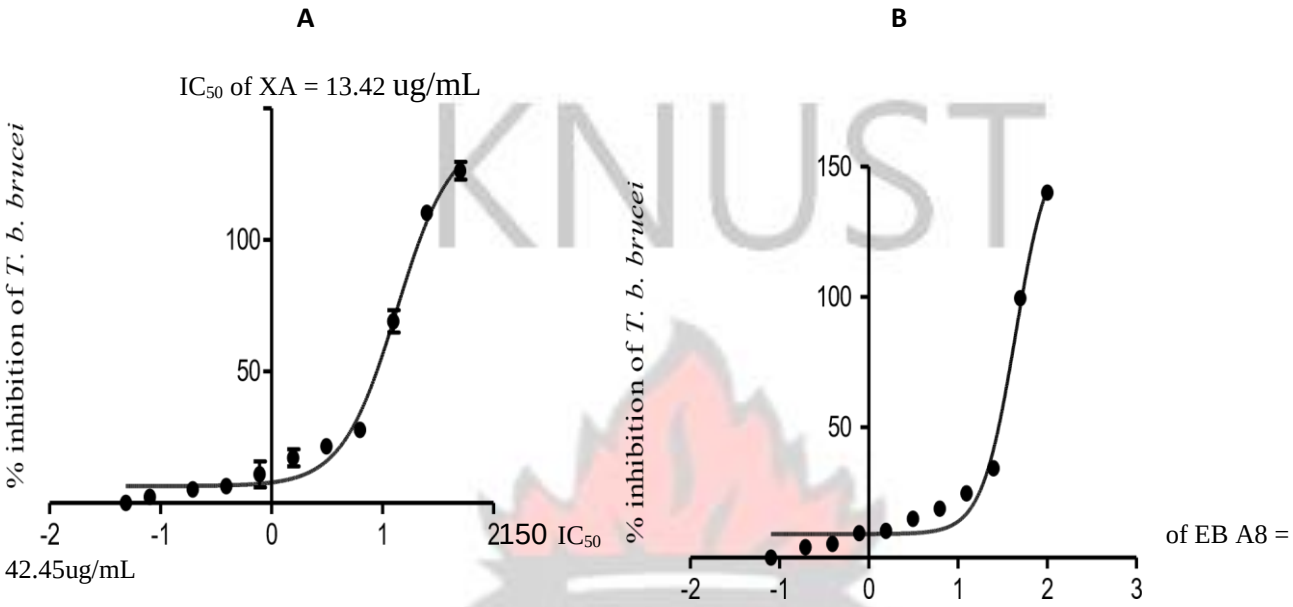
Table 4.6 First pass activity of compounds and extracts against *T. b. brucei*

Sample	Crude extract/pure compound % Inhibition	Fractions % Inhibition		
		methanol	Ethyl acetate ether	Petroleumacetone
Extracts/Fraction				
M. nobilis ethanol bark	59	-	67	81	-
E. ivorense methanol leaf	-	32	76	65	78
E. ivorense methanol bark	-	56	87	64	80
M. angolensis ethanol bark	23	-	-	-	-
H. latifolia methanol leaf	41	-	-	-	-
L. ovalifolia methanol leaf	55	-	-	-	-
A. glaberrima ethanol bark	69	-	-	-	-
P. muellerianus aqueous leaf	88	-	-	-	-
U. fasciata (whole alga)	52	-	-	-	-
S. vulgare (whole alga)	48	-	-	-	-
H. dentate (whole alga)	71	-	-	-	-
Compounds					
Geraniin	84	-	-	-	-
Xylopic acid	95	-	-	-	-

Key: (-): Not tested

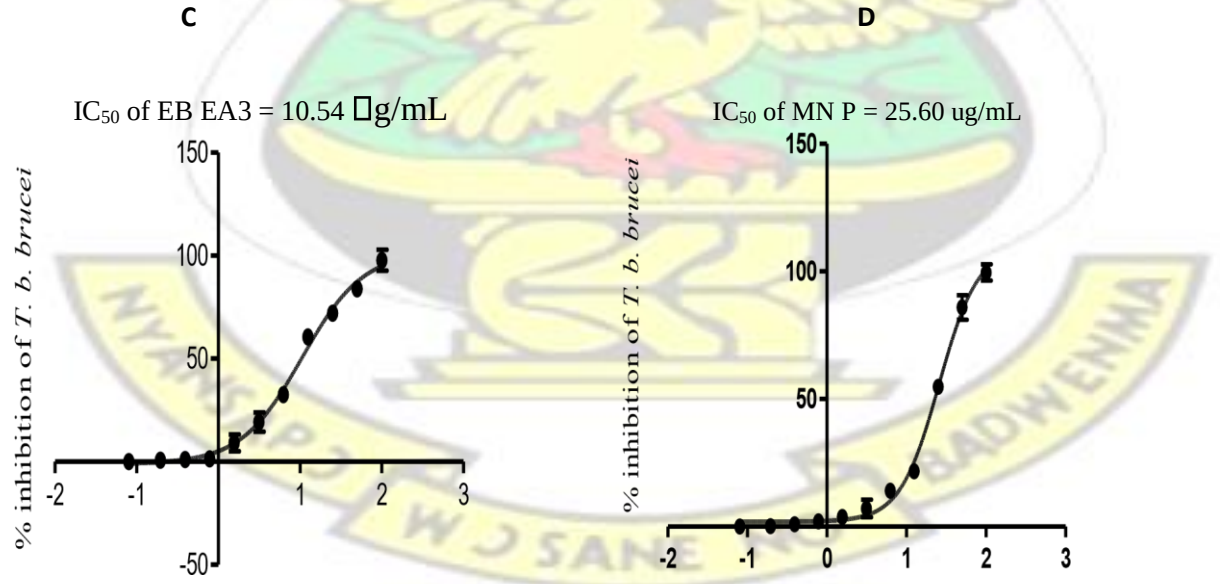
4.10 Activity of compounds and extracts against *T. b. brucei*

Xylopic acid had IC_{50} of 13.42, aqueous leaf extract of *P. muellerianus* had 11.50 while geraniin had 6.41 µg/mL. The pet ether fraction of the ethanol bark of *M. nobilis* had IC_{50} of 25.60 µg/mL. The ethyl acetate and acetone fractions of the methanol bark of *E. ivorense* had IC_{50} 10.54 and 42.45 µg/mL, respectively (Fig 4.8a and b). The ethyl acetate and acetone fractions of methanol leaf extract of *E. ivorense* exhibited no activity in the confirmatory screening.



log concentration of xylopic acid (ug/mL) log concentration of acetone fraction of *E. ivorense* (ug/mL)

Fig 4.8a. Activity of compounds and extracts against *T. b. brucei*. **A**: Activity of xylopic acid **B**: Activity of acetone fraction of methanol bark extract of *E. ivorense* against *T. b. brucei*.



log concentration of ethyl acetate fraction of *E. ivorense*log concentration of pet.ether fraction of *M. nobilis* (ug/mL)

(g/mL)

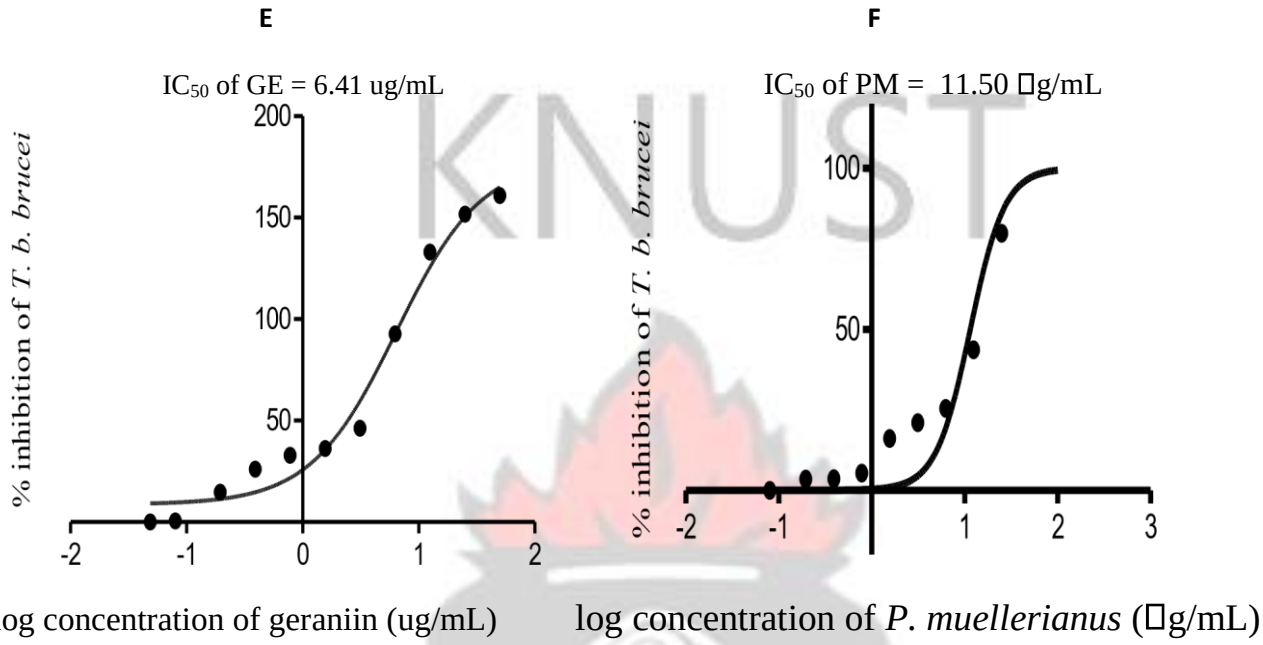


Fig 4.8b. Activity of compound and extracts against *T. b. brucei*. **C**: Activity of ethyl acetate fraction of methanol bark extract of *E. ivorense* **D**: pet ether fraction of ethanol bark extract of *M. nobilis* **E**: geraniin and **F**: aqueous extract of *P. muellerianus*.

4.11 Activity of compounds and extracts against schistosomulae

The anti-schistosomulae activity of the extracts, fractions and isolated compounds were investigated. The starting concentrations were 100 µg/mL for both extracts and fractions and 25 µg/mL for isolated compounds. At 100 µg/mL, the ethyl acetate fraction of the methanol extract of *E. ivorense* stem bark completely killed the worms between 24 and 48 h of incubation yielding a severity score of 4. At concentration of 20 µg/mL, the schistosomulae appeared rounded and degenerated by 24 h and completely dead by 48 h of incubation. This also yielded a severity score of 4. After 20 µg/mL, no effect was observed.

For the acetone fraction of the same extract, it was observed that at 100, 20, 5, 2.5 and 1.25 µg/mL, the worms were completely dead by 48 h of incubation. The minimum concentration that also

exhibited phenotypic effect was 0.625 µg/mL by 48 h of incubation where the worms had become rounded and darkened yielding a score of 2.

The pet. ether fraction of the 70% v/v ethanol extract of *M. nobilis*, after 24 h of incubation, there was no form of effect seen but after 48 h the schistosomulae were dead yielding a severity score of 4. No effect was observed with the remaining lower concentrations.

The least concentration at which the acetone fraction of the methanol leaf extract of *E. ivorense* exhibited a phenotypic effect was 0.3125 µg/mL where the worms had become rounded. Concentrations 100, 20 and 5 µg/mL completely killed the worms yielding a score of 4. At concentration of 2.5 µg/mL, the schistosomulae were overactive and about 50% were partially dead by 24 h and all were degenerated by 48 h of incubation. This gave a severity score of 2 and 4, respectively.

With the methanol fraction of the methanol bark extract of *E. ivorense*, the worms were dead or degenerated by 24 and 48 h of incubation at all the concentrations of 100, 20, 5, 2.5 and 1.25 µg/mL scoring a maximum score of 4. The least concentration that exhibited a phenotypic effect was 0.3125 µg/mL. At this concentration, the worms were observed to be rounded giving a score of 1 by 48 h of incubation. No effect was observed at 24 h of incubation.

The *S. vulgare*, *P. muellerianus*, *A. glaberrima*, and *M. angolensis*, exhibited phenotypic activity on the schistosomulae at concentrations of 100 µg/mL to have a score of 4 for each after which no effect was observed at the lower concentrations.

For *U. fasciata*, the parasites were rounded and dark after 24 h at 100 µg/mL. By 48 h of incubation, the worms were degenerated. This gave a score of 2 and 4, respectively. These effects were seen

at concentrations of 20 µg/mL on 24 and 48 h. The worms were observed to be 50% partial dead and this yielded a score of 1 for each day.

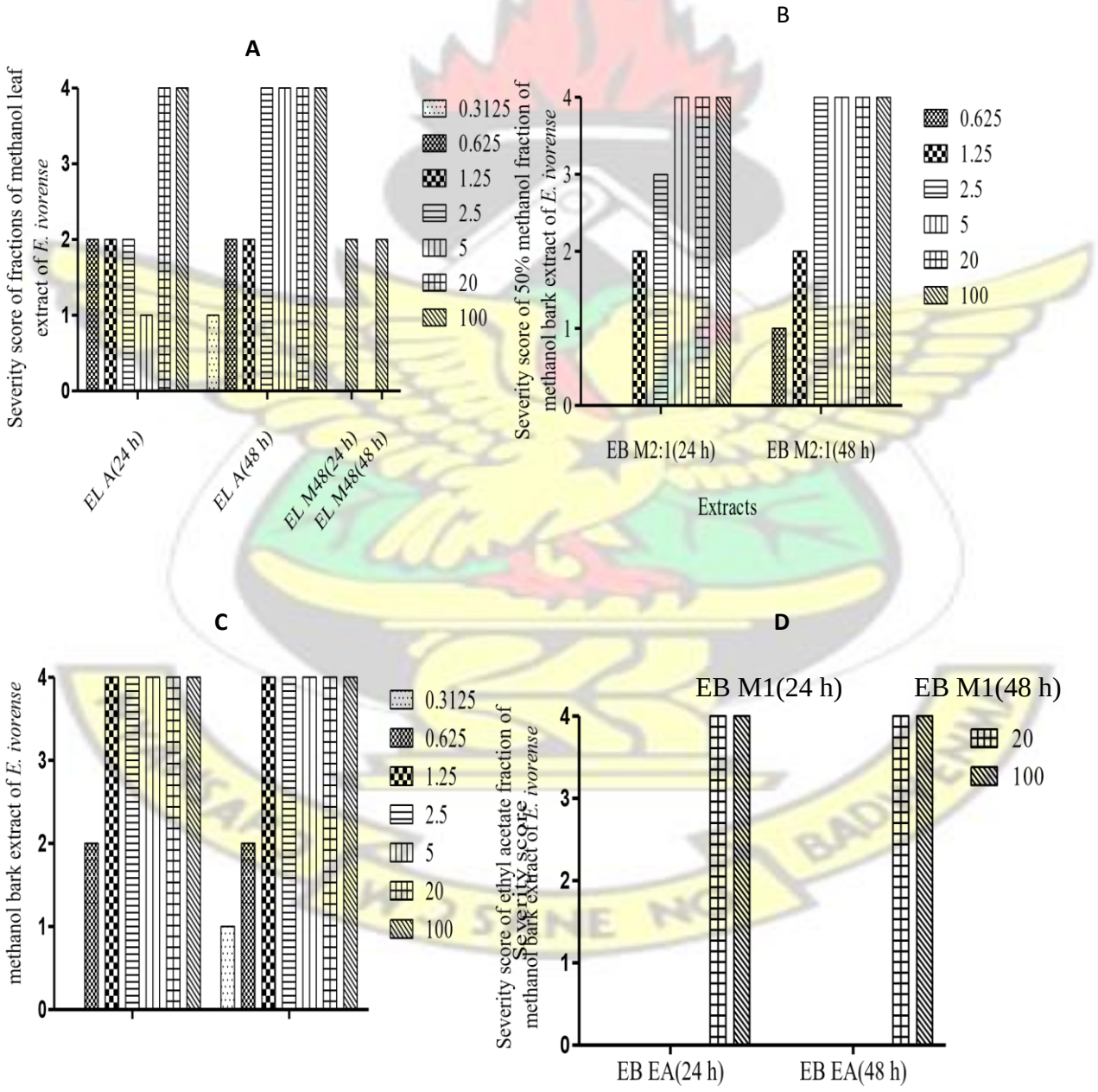
With the compound xylopic acid, the starting concentration was 25 µg/mL. at this concentration, the schistosomulae were dead by 24 and 48 h of incubation giving a score of 4. At 5 µg/mL, the worms were rounded by 24 and 48 h of incubation yielding a score of 1 each (Table 4.7, Fig 4.9, Fig 4.10 and Appendix 5).

Table 4.7 Descriptors showing phenotypic effects of componds and extracts on schistosomule

Conc (µg/mL)	DESCRIPTORS													
	100		20		5 24h		2.5 24h		1.25		0.625		0.3125	
Sample code	24h	2d	24h	2d		2d		2d	24h	2d	24h	2d	24h	2d
EL A36	D	D	D	D	pD 50	D	pD/O	pD/Dg	Da/O	Da/Deg				
EL A40	D	D	Dg	Dg	R/Dg	Dg	Da	Da			Da/R	Da/R		R
EL A34	D	D	D	D	R/Dg	D	pD/O	Da/R/pD		Da/R				
EL M48	R/O	Da												
EB M2:1	D	D	D	D	R/Dg	D	Da/Dg/p	D	Da/R	pD/R		pD		
EB M1	D	D	D	D	R/Dg	D	D	D	R/Deg	D	Da/R	Da/R		R
EB M2	D	D	D	D	R/Dg	D	Da/Dg/p	Da/R/Dg	Da/R/pD	Da/R/PD	Da/R	Da/R		
EB EA	D	D	R/Dg	D										
EB A8	D	D	R/Dg	D	R/Dg	D	Dg/p D	D	Da/Deg	Deg/Pd	Da/R	Da/R		
EB A9	D	D	D	D	R/Dg	D	D	D	Da/pD	D	Da/R	pD/R		
MN PET		D												
MN EA	R/Da	Dg	R	R	R	R	P/Da	S/Da	S/Da	S/Da	Da	Da		
XA	D	D	R	R										
AG		R/S												
UF	R/Da	D	O/pD40	pD50										
SV		R/Dg												

HD		Dg						
PM	D	D						
MA	Deg	D						

Key for Descriptors: **D**: death, **Dg**: degenerate, **R**: round, **O**: overactive, **S**: slow, **Da**: dark and **pD%**: partial death. Extracts: **EL A**: acetone fraction, **EL M**: methanol fraction of methanol leaf extract, **EB A**: acetone fraction, **EB M**: methanol fraction, **EB EA**: ethyl acetate fraction of methanol bark extract of *E. ivorense*. **MN PET** and **MN EA**: pet. ether and ethyl acetate fractions of 70% ethanol bark extract of *M. nobilis*. **XA**: xylopic acid, **AG**: ethanol bark extract of *A. glaberrima*, **UF**: methanol/chloroform extract *U. fasciata*, **SV**: methanol/chloroform extract of *S. vulgare*, **HD**: methanol/chloroform extract of *H. dentata*, **MA**: ethanol extract of *M. angolensis* and **PM**: aqueous extract of *P. muellerianus*.



Extracts Extracts

Fig 4.9a. Severity score of **A:** Activity of acetone and methanol fractions **B:** Activity of 50% v/v methanol fraction **C:** Activity of methanol fraction and **D** etheyl acetate fraction of of methanol bark and leaf of *E. ivorense* against schistosomule.

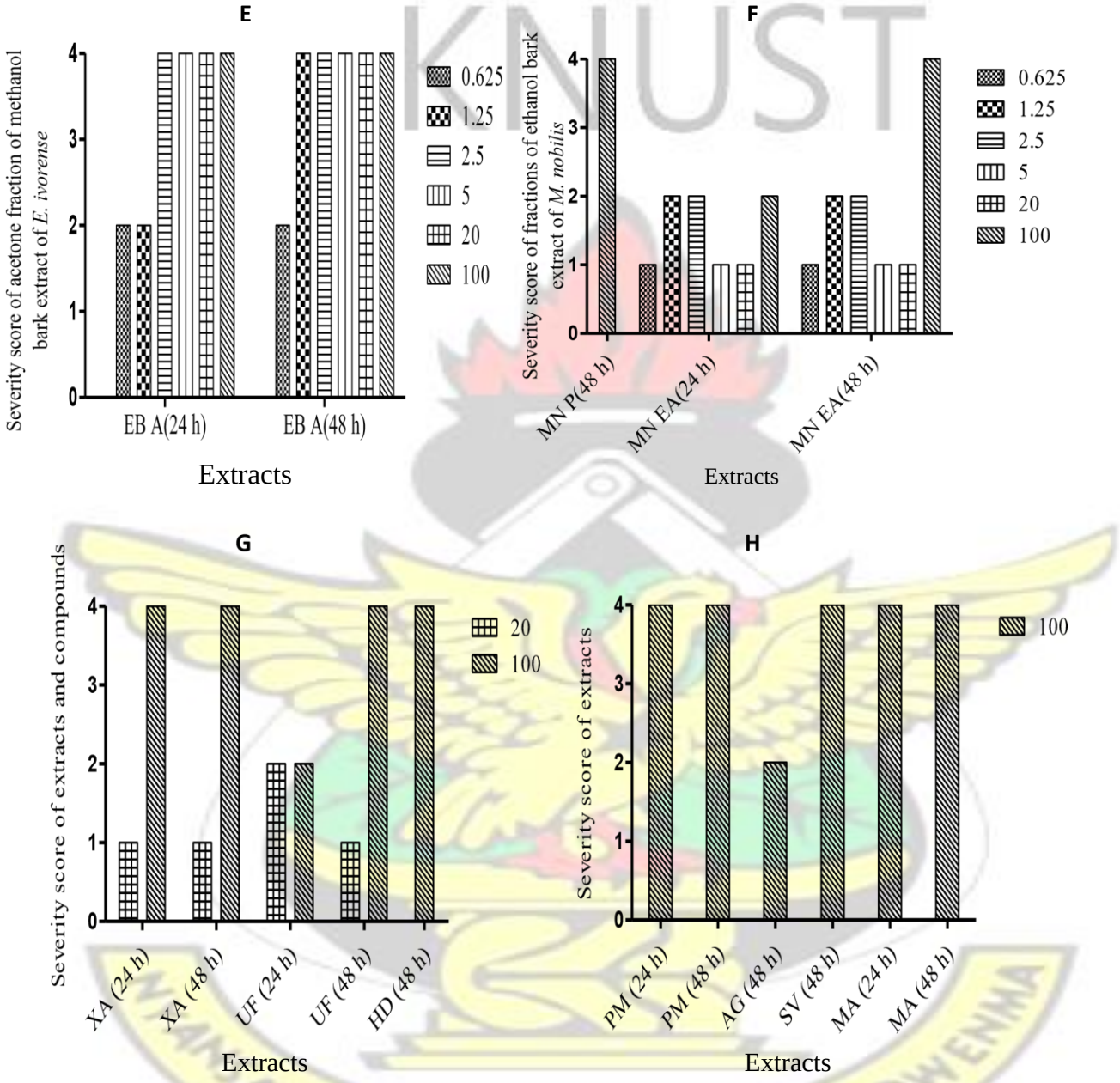
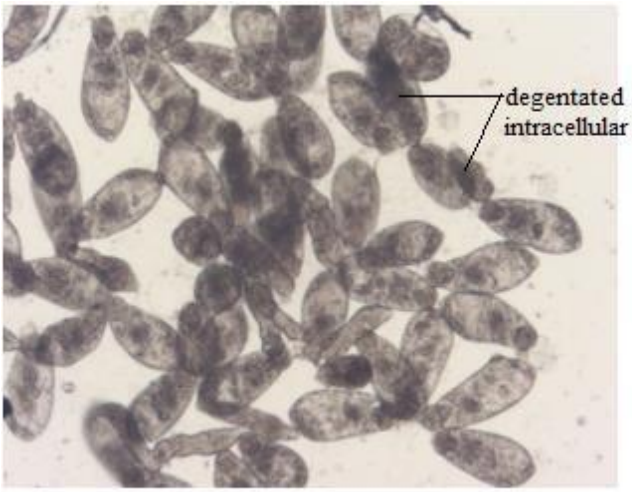
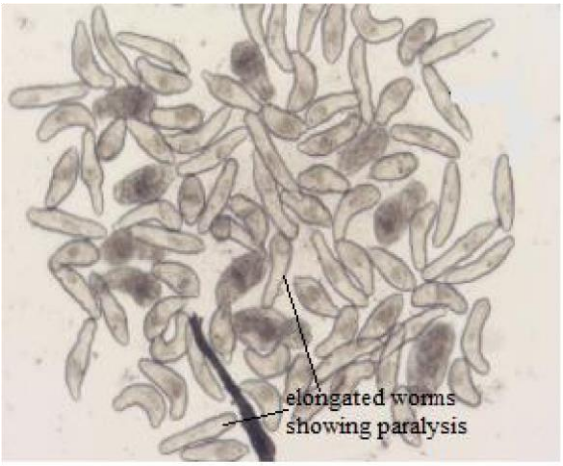


Fig 4.9b. Severity score of **E**: Activity of acetone fraction of methanol bark extract of *E. ivorense* **F**: Activity of fractions of ethanol bark of *M. nobilis* **G:** Activity of xylopic acid, extracts of *U. fasciata* and *H. dentata* and **H:** Activity of extracts of *P. muellerianus, A. glaberrima, S. vulgare* and *M. angolensis* against schistosomule.

A

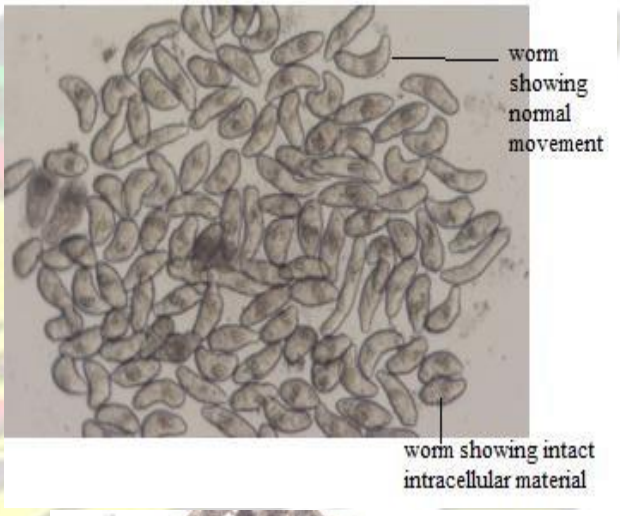

degentated
intracellular matter

B


elongated worms
showing paralysis

C

D


worm
showing
normal
movement
worm showing intact
intracellular material

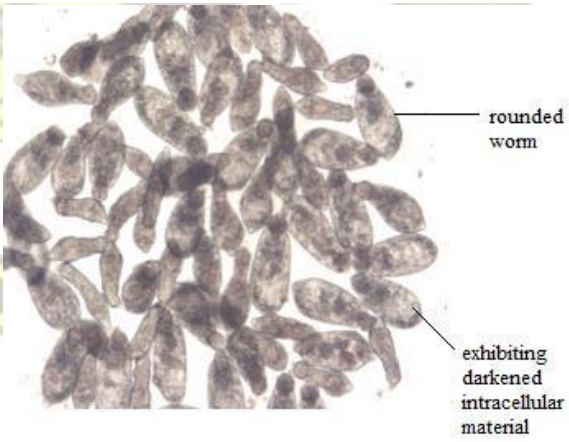

rounded
worm
exhibiting
darkened
intracellular
material

Fig 4.10 Microscopic photograph of worms showing the different phenotypic effects. **A:** degenerated worms. **B:** paralysed worms (slow). **C:** healthy looking worms (control). **D:** rounded and darkened worms

4.12 Screening of compounds and extracts against adult *S. mansoni* worms

After the schistosomule screening, compounds and extracts that exhibited phenotypic changes at lower concentrations (from 5 to 0.3126 µg/mL) were selected and screened at concentration of 1.25 µg/mL against the adult schistosome. This was performed 3, 5, 24 and 48 h and the results presented in Table 4.8. After 3 h of incubation, it was observed that in the 50% v/v methanol fraction of the methanol bark extract of *E. ivorense* the worms were un-coordinated (uncoord) and sticking to the sides of the well yielding a score of 2. By 5 h of incubation, the worms had shrunk in addition to the previous effect giving a score of 3. After 48 h of incubation, all the worms were dead and this gave a score of 4.

The acetone fraction of the methanol leaf extract of *E. ivorense* caused the worms to become uncoordinated by 3 h giving a score of 1. This effect was lost by 5 h and at 24 h of incubation, the worms were sticking to sides of the well, un-coordinated and dark and this yielded a score of 3. By the end of 48 h of incubation, the worms were dead and experienced tegumental damage giving a score of 4.

The methanol fraction of the methanol bark of *E. ivorense* caused no effect at 3 h of incubation. At 5 h of incubation, the worms were un-coordinated and sticking to the sides and the score was 2. At 24 h of incubation, the worms had experienced some tegumental damages and were completely dead by 48 h giving a score of 4.

The acetone fraction of the methanol bark extract also had significant effect on the worms after 3 h of incubation. The worms were sticking to the sides and un-coordinated giving a score of 2. The worms had become slow and shrunk by the 5 h of incubation yielding a score of 4. At 24 h of incubation, the worms were dead with tegumental damage and this gave a score of 4.

With regards to the ethyl acetate fraction of 70% ethanol bark extract of *M. nobilis* no effect was seen at 3, 5 and 24 h of incubation. The worms were slow after 48 h of incubation yielding a score of 1 (Table 4.8, Fig 4.11 and Fig 4.12).

Table 4.8 Phenotypic effects of componds and extracts on adult *S. mansoni*

	DESCRIPTORS			
Sample code	**3 h**	**5 h**	**24 h**	**2 days**
EL A36	O-uncoord		Os, uncoord, Da	D, Td, Da, Os, shrunk
EB M2:1	uncoord, Os	slight shrunk, uncoord, Os	S, Td, on sides, dark	D, Td, Da, Os, shrunk
EB M1		uncoord, Os	Os, uncoord, Td	D, Td, Da, Os, shrunk
EB M2		S-uncoord, Os	uncoord, Os	Slight shrunk, Os, uncoord
EB A9	uncoord, Os	uncoord, Os	D, Td	D, Td, Da, Os, shrunk
EB A6	S-uncoord, Os	S, slight shrunk, Os	Shrunk, D, Td	D, Td, Da, Os, shrunk
MN EA				S

EL A: acetone fraction of methanol leaf extract of *E. ivorense*; **EB M**: methanol fraction of methanol bark extract of *E. ivorense*; **EB A**: acetone fraction of methanol bark extract of *E. ivorense*; **MN EA**: ethyl acetate fraction of 70% v/v ethanol extract of *M. nobilis*. **O**: overactive; **D**: death; **Td**: tegumental damage; **uncoord**: uncoordination; **Os**: on sides; **Da**: dark; **S**: slow.

A **B**

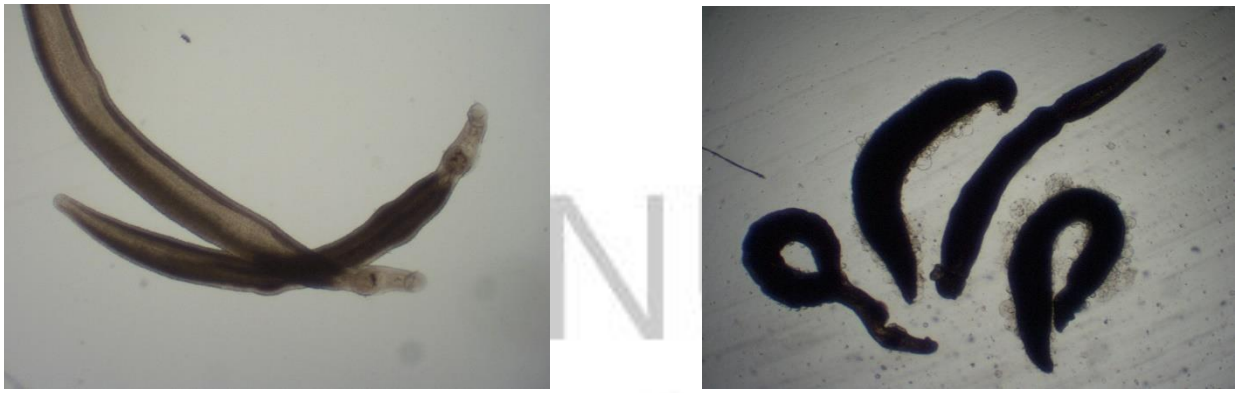
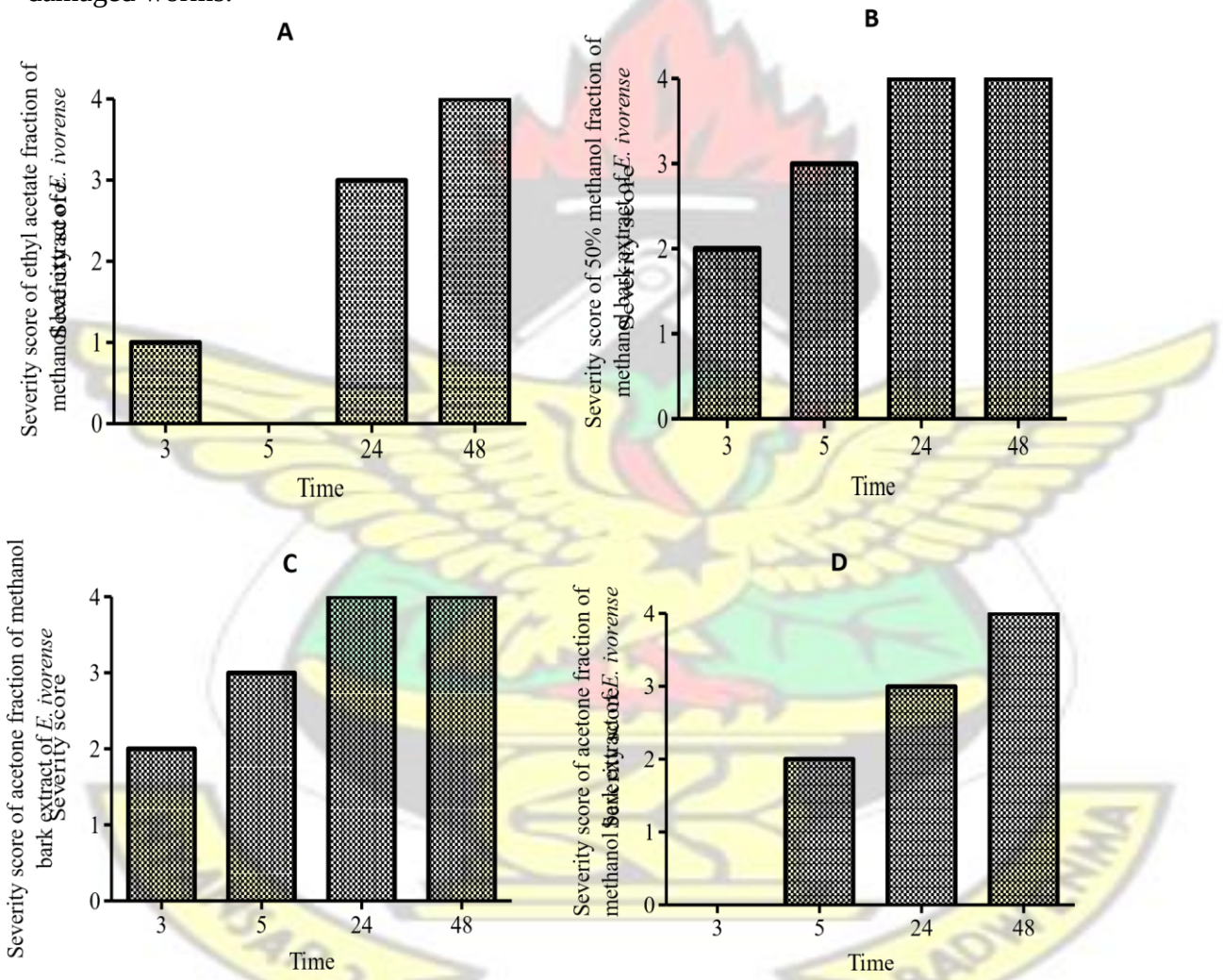


Fig 4.11 Microscopic view (X5) of adult *S. mansoni* worms. **A:** healthy looking worms **B:** damaged worms.



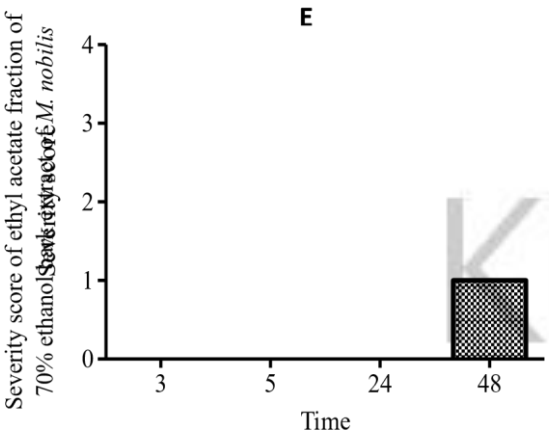


Fig 4.12. Severity score of **A**: acetone fraction methanol leaf extract B: 50% methanol fraction of methanol bark extract **C**: acetone fraction of methanol bark extract **D**: methanol fraction of methanol bark extract of *E. ivorense* and **E**: ethyl acetate fraction of 70% ethanol bark extract of *M. nobilis* against adult *S. mansoni* worms.

CHAPTER FIVE

5.1 DISCUSSION

Neglected tropical diseases (NTDs) affect one tenth of the world's population and represent 1.3% of the world's global burden of diseases and injuries (Mathers *et al*., 2007). They also represent the fourth most important communicable diseases. These diseases may lead to death and disability adjusted life and years (Hotez *et al*., 2006b). The resources for combating this menace of NTDs are lacking hence the inability to adequately control them (Trouiller *et al*., 2002). Medications available for the treatment of these diseases have several untoward side effects such as headaches, metallic taste in the mouth, diarrhea and vomiting. These make them unpleasant to take by patients. The parasites that cause the NTDs have also registered some level of resistannce to most of the medicines rendering them ineffective (Hotez *et al*., 2006b). There is therefore the need to find new compounds that can help curb some of the menace of resistance and also treat these NTDs. The results of the extraction could be indicative that the chloroform/methanol solvent was able to extract more of the constituents in the *U. fasciata* followed by *S. vulgare* and then *H. dentata*. This is evident in percentage yields. It is possible that the difference in the polarity of the two solvents

helped extract compounds of different polarities which contributed to the activity of these algae samples (Jato, 2015).

From the phytochemical screening, *U.* faciata (both powdered material and extract) was found to contain tannins, saponins, alkaloids and sterols. Tannins, flavonoids, saponnins, alkaloids and glycosides were present in both the powdered material and extracts of *S. vulgare* and *H. dentata*. These constituents in higher plants are known to be responsible for their biological activity (Mbagwu *et al.,* 2006; Maganha *et al.,* 2010). Alkaloids, tannins, flavonoids, saponins and sterols have been known to be responsible for activities such as antimicrobial, analgesic, antiinflammatory and antioxidant (Fiori *et al.,* 2013; Murugan *et al.,* 2013; Singh and Kumar, 2012; Xu and Lee, 2001; Akdemir *et al.,* 2011; Irfan *et al.,* 2014). The activity exhibited by the algae extracts against the parasites could be as a result of the phytochemical constituents present in them. Several studies have been carried out over the years and it has been proven that plants and their isolates can be a source of anti-parasitic agents. Asuzu and Chineme (1990) reported that methanol leaf extracts of *Morinda lucida* had activity against *T. brucei brucei*. Barbosa *et al.* (2007) also reported that epicatechin, a flavonoid isolated from the *Geranium mexicanum* exhibited potent activity against *G. lamblia* more than metronidazole which is widely used as the main therapy.

Hoet *et al.* (2004) reported on the antitrypanosomal activity of some selected Beninese plants. de Moraes *et al.* (2011) reported on the antischistosocidal activity of piplartine, an isolate from *Piper tuberculatum* (Piperaceae) against *S. mansoni*. Consistent with literature the plant extracts and compounds screened in this study exhibited activities against the parasites.

Terpenes are well known to be active against protozoan parasites (Phillipson and Wright, 1991). This could be the reason for the activity exhibited by xylopic acid since it is a terpene. McGaw *et al.* (2000) reported that plants extracts and compounds containing tannins and alkaloids possess activity against diarrhoea causing parasites such as *G. lamblia* and *E. histolytica*. The activity of

geraniin may be similar to the reported activities of other tannins against *E. histolytica*. Metronidazole and the nitroimidazoles inhibit the parasite by inhibiting nucleic acid synthesis thereby disrupting DNA of the cells (Debnath *et al.*, 2014).

The activities exhibited by *M. angolensis, U. fasciata, A. glaberrima,* pet ether fraction of 70% v/v ethanol bark extract of *M. nobilis,* ethyl acetate fraction of methanol bark extract of *E. ivorense* and xylopic acid against *G. lamblia* could also be attributed to the phytochemical constituents present. The *M. angolensis* is known to contain tannins (Mothana *et al*., 2009) which have been found to be active against diarrhoea causing parasites. The phytochemical analysis conducted on *U. fasciata* in this study revealed the presence of tannins and alkaloids. *A. glaberrima* is also known to contain alkaloids and tannins (Jato, 2015) as well as *E. ivorense* (Adu-Amoah *et al*., 2014) and *M. nobilis* (Mothana *et al*., 2009). These bioactive constituents maybe responsible for the activity exhibited by these extracts and fractions. Tannins and alkaloids are known to be responsible for anti-inflammatory and antimicrobial activities of some medicinal plants (Xu and Lee, 2001). In diarrhoeal conditions including amoebiasis and giardiasis, inflammation plays a major role by altering the gut sensorimotor function and also compromises the gut walls amking it possible for the parasites to permeate (Wanke *et al*., 1988; Hill, 1993). The anti-inflammatory and antimicrobial properties of these phytochemicals could also be responsible for the avtivity exhibited by the plant extracts and compounds against these parasites. It is possible that the constituents in these extracts worked in synergy to inhibit the growth of the parasite. These active compounds and extracts could be potential anti-giardial and anti-amoebic agents.

The main drug used in managing *N. fowleri* infection is amphotericin B (Kim *et al*., 2008). It works by forming channel like spores that spans the lipid bilayer of the cell membrane of the parasite. This causes destruction to the cell membrane leading to cell death. It is also known to target the mitochondria of the cells (Baginsky *et al.*, 2005). Ethanol bark extract of *A. glaberrima* and xylopic

acid are the extract and compound that exhibited activity against this parasite. The secondary metabolites in the extract could be responsible for the activities observed.

The *T. b. brucei* is not a causative species of the Human African Trypanosomiasis (HAT) but it is very similar to the pathogenic species *T. b. gambiense* and *T. b. rhodesiense* that cause HAT genotypically and this makes it a good experimental model (Faria *et al.*, 2014). Camacho *et al.* (2000) reported that flavonoids found in medicinal plants were responsible for antitrpanosomal activity. All the plant extracts that exhibited activity against the parasite have been found to contain flavonoids and hence the flavonoids may be responsible for the antitrypanosomal of these extracts. Kamanzi *et al*. (2004) also reported that triterpenes and saponins are also responsible for antitrypanosomal activity and most of the extracts were found to contain saponins. For the methanol bark and leaf of *E. ivorense*, it is known that triterpenes are found in the bark and not the leaves (Adu-Amoah *et al*., 2014). This could be the reason for the high activity exhibited by the bark which possessed all the three phytochemicals (flavonoids, saponins and triterpenes) compared to the leaf extract which possessed only two (flavonoids and saponins). Geraniin, an isolate from *P. muellerianus*, may be the compound responsible for the biological activity of the aqueous leaf extract of *P. muellerianus*. The ethyl acetate fraction of methanol bark extract of *E. ivorense* exhibited higher activity than the acetone fraction. It is possible that the ethyl acetate fraction posseesed more of the active metabolites than the acetone fraction. In isolating and characterizing the active compound, the ethyl acetate fraction maybe of more interest. The same could be said of the fraction of the 70% ethanol bark of *M. nobilis.* The pet ether fraction showed a high activity whereas the ethyl acetate fraction of the extract had no activity. The active compounds responsible for the activities identified in the various extracts and fractions could be isolated and further studies performed.

For *S. mansoni* screening, the effects were observed in a dose dependent manner for the schistosomule. It was observed that the higher concentration yielded higher severity scores than the lower concentration for all the extracts and fractions except the ethyl acetate fraction of 70% v/v ethanol bark of *M. nobilis*. de Moraes *et al*. (2011) reported that piplartine exhibited a dose dependent activity against *S. mansoni*. This could mean that higher doses of compounds or medicines are needed to kill these worms.

It was also observed that effects of compounds were proportional to the time the compound had contact with the worms. The longer the time of contact, the more pronounced the effects. This was with respect to the DMSO control group. This was observed with the acetone fraction of methanol bark extract of *E. ivorense*. At 0.625 µg/mL of the acetone fraction of the methanol bark extract of *E. ivorense*, the severity score was 2 at 24 h but the at 48 h of incubation, the score was 4. The worms in the DMSO control looked healthy even after 48 h. Similar observations were made with the ethyl acetate fractions and acetone fraction of the methanol leaf extract of the same plant. Praziquantel is the known drug for treating schistosomiasis and it is known to cause alteration on the surface of the worms known as the tegument and also cause contractions of the muscles of the worms (Pax *et al.*, 1978; Fetterer *et al.*, 1980). This makes the worms vulnerable to host immune mechanism. Praziquantel is unable to exhibit these effects in the juvenile stage of the worm since they lack the tegument (Caffrey and Secor, 2011). It is therefore important that these extracts exhibited activity against the somule. It was observed in the adult screening that the fractions of methanol bark and leaf extracts of *E. ivorense* caused tegumental damage to the worms. de Moraes *et al*. (2011) reported that the worms' death could be as a result of mechanisms other than tegumental damage and this was evident in the result obtained from the schistosomule screening. These plant and algae extracts and isolated compounds have exhibited activity against these parasites and might be sources of new antiparasitic agents. There is a need to perform bioactivity

guided isolation and characterization of the active compounds responsible for the above antiparasitic activities observed.

5.2 Conclusion

The extracts and fraction of *E. ivorense, A. glaberrima, M. nobilis, M. angolensis, P. muellerianus, U. fasciata and S. vulagre* exhibited activity against at least one of the test parasites: the *E. histolytica, G. lamblia, T. b. brucei, N. fowleri* and the schistosomulae and adult stage of *S. mansoni*. Xylopic acid exhibited activity against all the test parasites in varying degrees. The fractions of the methanol bark extract of *E. ivorense* exhibited activity against *S. mansoni, T. b. brucei, E. histolytica and G. lamblia*. Geraniin was active against *T. b. brucei* and *E. histolytica.* The ethanol bark of *A. glaberrima* exhibited activity against *G. lamblia* and *N. fowleri*.

5.3 Recommendations

- Bioactivity guided fractionation and isolation of compounds responsible for the activity against the parasites should be performed.
- The mechanism of action of the bioactive compounds should be studied.
- Toxicology studies should be performed on the active compounds, extracts and fraction of the selected plants.

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APPENDICES

APPENDIX 1: Composition and preparation of culture media

A1.1 TYI-S medium for *E. histolytic*

Table 1.1. Composition of TYI-S medium

Composition	Quantity (g)
Sodium chloride	8
Dibasic Potassium Hydrogen Phosphate	4
Monobasic Potassium Hydrogen Phosphate	2.4
BBL Casein Digest Peptone	80
BBL Yeast	40
L-cysteine	4
Glucose	40
Ascorbic acid	0.8
Ferric nitrate	0.115

These constituents were dissolved in 3 L of deionized water in a beaker and 10 N sodium hydroxide added to make pH 6.8 to 7.0. Sufficient water was added to make 3.5 L. 435 mL of the solution was poured into 500 mL bottles, sterilized at 121 °C for 25 min and stored at -20 °C. Prior to use, 43.5 mL (10%) adult bovine serum, 4.35 mL (1X) penicillin/streptomycin and 4.35 mL 1X vitamin mix were added.

A1.2 HM1:IMSS medium for *G. lamblia*

Table 1.2 Composition of HM1:IMSS medium

Composition	Quantity (g)
Sodium chloride	10
Dibasic Potassium Hydrogen Phosphate	5
Monobasic Potassium Hydrogen Phosphate	3
BBL Tryptone	100
BBL Yeast extract	50
Glucose	50
Ascorbic acid	1
Bile	3.75

A quantity of 4.7 g of the mixture was dissolved in 80 mL of deionized water in a beaker and 10 N sodium hydroxide added to make pH 7-7. 10. Sufficient water was added to make 100 mL. Fifteen (15) mL of the solution was poured into 25 mL bottles, sterilized at 121 °C for 25 min and stored at -20 °C. Prior to use, 10 mL (10%) fetal bovine serum, 1 mL (1X) pen-strep and 1 mL (1X) vitamin mix were added.

A1.3 Nelson Culture medium for *N. fowleri*

Table 1.3 Composition of Nelson Culture medium

Composition	Quantity (mg)
Sodium chloride	12
Magnesium sulphate heptahydrate	4
Calcium chloride dihydrate	4
Sodium hydrogen phosphate	14.2
Glucose	170
Liver infusion	170
Potassium hydrogen phosphate	13.6

All of these were dissolved in 800 mL of deionized water in a beaker. Sufficient water was added to make 1 L. Five (5) mL of the solution was dispensed into 25 mL bottles, sterilized at 121 °C for 25 min and stored at -20 °C. Prior to use, 100 mL (10%) fetal bovine serum and 10 mL (1X) pen-strep were added.

APPENDIX 2: Screening of compounds and extracts against *E. histolytica*

A2.1 First pass activity of compounds and extracts against *E. histolytica*

Table 2.1: Plate map of extracts and compounds in a 96-well plate

	2	3	4	5	6	7	8	9	10
A	EL M1	EL A							MA
B		EB EA	EB M1						
C	EL EA	EL PE				EB M2:1			
D	MN EA		HL			LO	EB A9		
E	EB A1			XA	EB A8		SL	HD	
F	MN PE					AG	GER	SV	
G	EB A								
H	XA	EB PE		UF			EB EA2	PM	

EB M, EB EA, EB PE and EB A: methanol, ethyl acetate, pet ether and acetone fractions of methanol bark extract of *E. ivorense*; EL EA, EL PE, EL A and EL M: ethyl acetate, pet ether, acetone and methanol fractions of methanol leaf extract of *E. ivorense*; MA: *Maerua angolensis*; AG: *Albizia glaberrima*; UF: *Ulva fasciata*; LO: *Laportea ovalifolia*; HL: *Hilleria latifolia*; GER: geraniin; XA: xylopic acid; PM: *Phyllanthus muellerianus*; SV: *Sargassum vulgare*; MN PE and MN EA: pet ether and ethyl acetate fractions of ethanol bark extract of *Margaritaria nobilis*; HD: *Hydropuntia detntata*.

Table 2.2: Activity (luminescence read-out) of compounds and extracts against *E. histolytica*.

Raw data	1	2	3	4	5	6	7	8	9	10	11	12
A	130360	88440	74040	148960	143520	170080	155400	146160	143160	73360	204080	1160
B	140720	156320	121560	114920	164920	152600	161760	148280	152560	190040	221560	1360
C	150080	97920	82840	164720	174760	165680	127680	132920	168040	198640	215800	1200
D	143040	102440	136000	83360	154440	145840	83960	106080	178240	206200	210720	1280
E	127840	110560	174000	147640	154160	106400	148600	138280	72280	224320	205440	1160
F	152760	98720	171520	78640	174160	129840	88040	67760	105280	247880	220720	1160
G	155400	74960	163040	162680	170680	170920	162160	142080	168920	208800	201880	1120
H	136360	960	133160	182120	86360	178760	162840	72880	144720	211880	208160	720

Column 1: DMSO; Column 12: Metronidazole (MNZ)

Table 2.3: Percentage inhibition of compounds and extracts against *E. histolytica.*

% Inh	1	2	3	4	5	6	7	8	9	10	11	12
A	8	38	48	-6	-2	-21	-10	-4	-2	48		100
B	0	-11	14	19	-17	-8	-15	-5	-8	-35		100
C	-6	31	42	-17	-24	-18	10	6	-19	-41		100
D	-1	28	4	41	-10	-3	41	25	-27	-47		100
E	9	22	-24	-5	-9	25	-5	2	49	-60		100
F	-8	30	-22	45	-24	8	38	52	26	-76		100
G	-10	47	-16	-15	-21	-21	-15	-1	-20	-48		100
H	3	100	6	-29	39	-27	-16	49	-3	-51		100

Key: % inh: percentage inhibition; Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%.

A2.2 Dose response activity of compounds and extracts against *E. histolytica*

Table 2.4 Activity (luminescence read-out) of compounds and extracts against *E. histolytica*.

	DMSO	XA (25)			GER (50)			MNZ (50)			MNZ
Conc (µg/mL)	1	2	3	4	5	6	7	8	9	10	12
50	82440	800	760	680	23720	36040	32200	800	880	960	800
25	75640	2000	6640	6600	45160	50080	49800	1360	1200	1040	720
12.5	72520	37440	32960	36880	50840	48400	61200	1000	1160	1080	760
6.25	75080	50240	48000	48360	63600	66560	80360	1240	1240	1120	800
3.125	74760	65000	71760	78800	62800	74360	74640	2000	1840	1480	840
1.5625	73280	64760	63840	82480	79360	67080	76160	17680	25720	22600	1120
0.78125	70600	69400	84680	89560	85040	88560	86880	29960	47000	51800	760
0.390625	78160	100880	84440	98240	103800	112280	118320	84960	94240	100160	800

Key: XA: xylopic acid; GER: geraniin; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

Table 2.5 Percentage inhibition of bioactive compounds and extracts against *E. histolytica.*

	DMSO	XA (25)			GER (50)			MNZ			
Conc (µg/mL)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**
50	-8	100	100	100	70	53	58	100	100	100	100
25	1	98	92	92	41	35	35	99	99	100	100
12.5	5	52	57	52	34	37	20	100	99	100	100
6.25	2	35	38	37	17	13	-5	99	99	100	100
3.125	2	15	6	-3	18	3	2	98	99	99	100
1.5625	4	15	17	-8	-4	12	0	78	67	71	100
0.78125	8	9	-11	-17	-11	-16	-14	61	39	33	100
0.390625	-2	-32	-11	-29	-36	-47	-55	-11	-24	-31	100

Key: Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%; XA: xylopic acid; GER: geraniin; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

APPENDIX 3: Screening of compounds and extracts against *G. lamblia*

A3.1 First pass activity of compounds and extracts against *G. lamblia*

Table 3.1 Plate map of extracts and compounds in a 96-well plate

	3	4	5	6	7	8	9	10
A		HL					MN	
B							EL M	EL EA
C		XA						
D					EL EA	SV		
E						HD	EB A	
F				EL PE	EB M		MN PET	LO
G					MN EA		GER	
H	AG	EA EB		UF		MA	EL A	

EB M, EB EA, EB PE and EB A: methanol, ethyl acetate, pet ether and acetone fractions of methanol bark extract of *E. ivorense*; EL EA, EL PE, EL A and EL M: ethyl acetate, pet ether, acetone and methanol fractions of methanol leaf extract of *E. ivorense*; MA: *Maerua angolensis*; AG: *Albizia glaberrima*; UF: *Ulva fasciata*; LO: *Laportea ovalifolia*; HL: *Hilleria latifolia*; GER: geraniin; XA: xylopic acid; PM: *Phyllanthus muellerianus*; SV: *Sargassum vulgare*; MN PE and MN EA: pet ether and ethyl acetate fractions of ethanol bark extract of *Margaritaria nobilis*; HD: *Hydropuntia detntata*.

Table 3.2 Activity (luminescence read-out) of compounds and extracts against *G. lamblia*

Raw data	1	2	3	4	5	6	7	8	9	10	11	12
A	214000	270160	220880	196800	215080	195640	216640	215840	156480	216880	269000	18160
B	234320	287280	245920	224520	260120	266520	227720	211320	200800	190760	297240	20320
C	237360	304040	253520	7720	220680	220600	223240	243240	8520	62560	314760	18640
D	225840	326120	289160	239680	229720	197520	189960	9720	220520	197600	302160	18200
E	242120	318880	269840	260760	232240	228560	196760	760	186080	212760	273480	19800
F	213680	311040	221480	250080	295000	169720	113920	198680	61920	6480	336200	23840
G	251800	335680	249200	203200	297240	238760	128240	208000	77760	221320	301440	20480
H	239480	297920	60080	11480	277480	38080	1118360	10640	141280	218640	292360	20640

Column 1: DMSO; Column 12: Metronidazole (MNZ)

Table 3.3 Percentage inhibition of compounds and extracts against *G. lamblia*

% Inh	1	2	3	4	5	6	7	8	9	10	11	12
A	9		5	17	8	17	7	8	36	7		100
B	-1		-6	4	-13	-16	2	10	15	20		99
C	-2		-10	105	6	6	4	-5	105	80		100
D	3		-27	-3	1	16	20	104	6	16		100
E	-4		-17	-13	0	2	17	108	22	9		100
F	9		5	-8	-29	29	55	16	80	106		98
G	-9		-8	14	-30	-3	49	11	72	5		99
H	-3		81	103	-21	91	-415	104	43	6		99

Key: % inh: percentage inhibition; Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%.

A3.2: Dose response activity of compounds and extracts against *G. lamblia*

Table 3.4a Activity (luminescence read-out) of bioactive compounds and extracts *G. lamblia*

	DMSO	AG			UF			MNZ
Conc (µg/mL)	1	5	6	7	8	9	10	12
100	149720	25120	29440	24600	14040	13840	11400	33800
50	144200	31520	32080	31160	66560	69480	64280	35040
25	148560	55880	56040	52320	110920	127480	118440	33360
12.5	140000	104560	114520	112720	143960	140560	126440	33520
6.25	142120	134720	145120	130840	150400	147240	118320	28880
3.125	151480	136120	161120	142200	150720	152960	170040	26360
1.5625	140240	155400	162200	156480	157320	164640	149360	32720
0.78125	122320	225840	216720	216880	215360	207800	203160	23000

Key: DMSO: dimethyl sulfoxide; AG: *Albizia glaberrima*; UF: *Ulvs fasciata*; MNZ: metronidazole.

Table 3.4b Activity (luminescence read-out) of bioactive compounds and extracts *G. lamblia*

	DMSO	MA			MN PET			EB EA4			MNZ
Conc (µg/mL)	1	2	3	4	5	6	7	8	9	10	12

100	112280	3200	4360	3600	28920	30320	36000	3440	3560	3560	27400
50	117840	11360	7200	8840	57160	58520	88200	8760	9000	8040	30520
25	115960	56320	42640	55960	102360	99920	100120	41480	45960	48360	27920
12.5	115960	104360	99000	95800	103960	112040	107400	85480	86360	89840	25880
6.25	115960	105800	106720	98320	104720	112080	112000	92760	92600	94440	24640
3.125	115960	114400	106720	110280	107880	114120	112440	98080	96680	100400	29040
1.5625	115960	119520	107040	102120	113720	114480	114280	105680	101240	107800	28200
0.78125	115960	122440	107680	102200	113320	117400	118160	109560	101040	124160	28120

Key: DMSO: dimethyl sulfoxide; MNZ: metronidazole; MA: *Maerua angolensis*; MN PET: pet ether fraction of ethanol bark extract of *Margaritaria nobilis*; EB EA: theyl acetate fraction of methanol bark of *Erythrophleum ivorense*.

Table 3.4 Activity (luminescence read-out) of bioactive compounds and extracts *G. lamblia*

	DMSO	XA			MNZ
Conc (µg/mL)	**1**	**2**	**3**	**4**	**12**
25	576600	7200	6720	5840	19760
12.5	613680	223120	301600	259240	18200
6.25	581720	538960	528440	546200	16440
3.125	582840	555240	587680	541360	13600
1.5625	581520	555320	602720	574360	12280
0.78125	577200	569560	625320	595120	10000
0.390625	584240	571400	629320	595520	10440
0.195313	523880	780160	742920	782120	10320

Key: XA: xylopic acid; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

Table 3.5a Percentage inhibition of compounds and extracts against *G. lamblia*

	DMSO	MA			MN PET			EB EA4			MNZ
Conc (µg/mL)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**
100	4	128	127	128	99	97	91	128	128	128	101
50	-3	119	124	122	67	65	31	122	122	123	97

25	-1	68	83	68	15	18	18	85	79	77	100
12.5	-1	13	19	23	13	4	9	34	33	29	102
6.25	-1	11	10	20	12	4	4	26	26	24	104
3.125	-1	1	10	6	9	2	4	20	22	17	99
1.5625	-1	-5	10	15	2	1	1	11	16	9	100
0.78125	-1	-8	9	15	3	-2	-3	7	17	-10	100

Key: Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%; DMSO: dimethyl sulfoxide; MNZ: metronidazole; MA: *Maerua angolensis*; MN PET: pet ether fraction of ethanol bark extract of *Margaritaria nobilis*; EB EA: theyl acetate fraction of methanol bark of *Erythrophleum ivorense*.

Table 3.5b Percentage inhibition of compounds and extracts against *G. lamblia*

	DMSO	AG			UF			MNZ
Conc (µg/mL)	**1**	**5**	**6**	**7**	**8**	**9**	**10**	**12**
100	-4	108	104	108	118	118	120	100
50	1	102	102	102	71	68	73	99
25	-3	80	80	84	31	16	24	101
12.5	5	37	28	29	1	5	17	100
6.25	3	10	0	13	-4	-1	24	105
3.125	-5	9	-14	3	-5	-7	-22	107
1.5625	5	-9	-15	-10	-10	-17	-3	101
0.78125	21	-72	-64	-64	-62	-56	-52	110

Key: Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%; DMSO: dimethyl sulfoxide; AG: *Albizia glaberrima*; UF: *Ulvs fasciata*; MNZ: metronidazole

Table 3.5c Percentage inhibition of compounds and extracts against *G. lamblia*

	DMSO	XA			MNZ
Conc (µg/mL)	**1**	**2**	**3**	**4**	**12**
25	3	102	102	102	100
12.5	-4	64	51	58	100
6.25	2	9	11	8	100
3.125	2	7	1	9	101
1.5625	2	7	-2	3	101
0.78125	3	4	-6	0	101

0.390625	1	4	-6	0	101
0.195313	12	-33	-26	-33	101

Key: Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; XA: xylopic acid; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

APPENDIX 4: Screening of compounds and extracts against *N. fowleri*

A4.1 First pass screening of compounds and extracts against *N. fowleri*

Table 4.1 Plate map for compounds and extracts in a 96-well plate

	2	3	4	5	6	7	8	9	10
A	HL	UF				MN EA	MN PET	EL A	
B									
C		EL EA			GER	EB M			
D			HD			EB EA			
E	XA	LO	EB A		SV		MN PET		
F							MA	EL M	
G							PM		
H	AG								

EB M, EB EA and EB A: methanol, ethyl acetate and acetone fractions of methanol bark extract of *E. ivorense*; EL EA, EL A and EL M: ethyl acetate, acetone and methanol fractions of methanol leaf extract of *E. ivorense*; MA: *Maerua angolensis*; AG: *Albizia glaberrima*; UF: *Ulva fasciata*; LO: *Laportea ovalifolia*; HL: *Hilleria latifolia*; GER: geraniin; XA: xylopic acid; PM: *Phyllanthus muellerianus*; SV: *Sargassum vulgare*; MN PE and MN EA: pet ether and ethyl acetate fractions of ethanol bark extract of *Margaritaria nobilis*; HD: *Hydropuntia detntata*.

Table 4.2 Activity (luminescence read-out) of bioactive compounds and extracts against *N. fowleri*

Raw data	1	2	3	4	5	6	7	8	9	10	11	12
A	5502720	2623280	3097720	5043440	4978840	4513920	2833120	3130960	3806000	1876200	6596680	3640
B	5388800	4580360	5600080	3920640	4601960	4927920	4715280	4127280	5362280	6448520	5855560	3600
C	5215280	2545120	1078160	5341360	5960640	1903880	3920560	4275320	3664040	6484280	6110480	3200
D	5345080	4539920	5690320	2312080	2603520	4771560	683840	2143520	5418040	6479360	5992760	4440
E	5474880	198800	3795320	2642880	2044160	2045560	3952560	3751320	911680	6706760	6236000	
F	5405040	3063840	5229840	2485280	4779520	3720800	1468520	575280	3612160	6713040	6394800	
G	5771320	2623040	4571840	5454920	5971720	5763520	5480720	2923840	2885920	6491760	6648800	
H	5796920	224680	3936280	5688400	2329080	2626640	5493600	892400	6614560	6441000	6613320	

Column 1: DMSO; Column 12: Metronidazole (MNZ)

Table 4.3 Percentage inhibition of compounds and extracts against *G. lamblia*

% Inh	1	2	3	4	5	6	7	8	9	10	11	12
A	-4	51	42	5	6	15	47	41	28	65		100
B	-1	14	-5	26	13	7	11	22	-1			100
C	2	52	80	0	-12	64	26	20	31			100
D	-1	15	-7	57	51	10	87	60	-2			100
E	-3	96	29	50	62	62	26	29	83			
F	-2	42	2	53	10	30	72	89	32			
G	-9	51	14	-3	-12	-8	-3	45	46			
H	-9	96	26	-7	56	51	-3	83	-24			

Key: % inh: percentage inhibition; Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%.

A4.2 Dose response activity of bioactive compounds and extracts against *N. fowleri*

Table 4.4 Activity (luminescence read-out) of bioactive compounds and extracts against *N. fowleri*

Conc (µg/mL)	DMSO	AG			Conc (µg/mL)	XA			AMP B
	1	2	3	4		5	6	7	12
100	13799760	2957000	3123080	3202960	25	396960	1640960	1757040	3120
50	13030360	6121520	6038000	6109360	12.5	10750880	10347840	10576960	3360
25	13568120	8457920	8581520	8432200	6.25	11619320	11733600	11701360	3000
12.5	13854120	9142760	10555240	10492480	3.125	12238640	12009960	12506840	4840
6.25	13862920	12455760	12109480	12528240	1.5625	12802760	12511920	12965360	
3.125	13683640	13100960	13156640	13167440	0.78125	13454040	13223240	13457160	
1.5625	14161440	13114040	13532800	13523920	0.390625	13649440	13357120	14357840	
0.78125	14636040	14337760	14669400	14454800	0.195313	14366120	13925240	14337720	

Key: AMP B: amphotericin B; XA: xylopic acid; AG: *Albizia glaberrima*; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

Table 4.5 Percentage inhibition of compounds and extracts against *G. lamblia*

	DMSO	AG				XA			MNZ
% Inh	1	2	3	4	conc (µg/mL)	5	6	7	12
A	-2	78	77	76	25	97	88	87	100
B	3	55	55	55	12.5	20	23	22	100
C	-1	37	36	37	6.25	14	13	13	100
D	-3	32	22	22	3.125	9	11	7	100
E	-3	8	10	7	1.5625	5	7	4	100
F	-1	3	2	2	0.78125	0	2	0	100
G	-5	3	0	0	0.390625	-1	1	-6	100
H	-9	-6	-9	-7	0.195313	-7	-3	-6	100

Key: Red highlights: compounds exhibiting activity above 80%; Orange highlights: compounds exhibiting activity above 50%; Yellow highlights: compounds exhibiting activity above 25%; AMP B: amphotericin B; XA: xylopic acid; AG: *Albizia glaberrima*; MNZ: metronidazole; DMSO: dimethyl sulfoxide.

APPENDIX 5: Severity scores of activities of compounds and extracts against *S. mansoni*

A5.1 Severity score of activities of compounds and extracts against schistosomule

Table 5.1 Heat map showing severity score of compounds and extracts against schistosomule

	Severity score of extracts and compounds													
conc (µg/mL)	100		20		5		2.5		1.25		0.625		0.3125	
sample code	24h	2d	24h	2d	24h	2d	24h	2d	24h	2d	24h	2d	24h	2d
EL A	4	4	4	4	1	4	2	4	2	3	2	2	0	0
EL A	4	4	4	4	1	1	0	0	0	0	0	0	0	0
EL A	4	4	4	4	4	4	2	3	0	1	0	0	0	0
EL M	2	1	0	0	0	0	0	0	0	0	0	0	0	0
EB 50M	4	4	4	4	4	4	4	4	2	2	0	1	0	0
EB M	4	4	4	4	4	4	4	4	2	3	2	2	0	1

EB M	4	4	4	4	4	4	4	4	2	2	2	2	0	0
EB EA	4	4	4	4	0	0	0	0	0	0	0	0	0	0
EB A	4	4	4	4	4	4	4	4	2	2	2	2	0	0
EB A	4	4	4	4	4	4	4	4	2	2	2	2	0	0
EB A	4	4	4	4	4	4	4	4	0	0	0	0	0	0
MN PET	0	4	0	0	0	0	0	0	0	0	0	0	0	0
MN EA	2	4	1	1	1	1	1	1	2	2	1	1	0	0
XA	4	4	1	1	0	0	0	0	0	0	0	0	0	0
AG	0	2	0	0	0	0	0	0	0	0	0	0	0	0
UF	2	4	2	1	0	0	0	0	0	0	0	0	0	0
SV	0	4	0	0	0	0	0	0	0	0	0	0	0	0
HD	0	4	0	0	0	0	0	0	0	0	0	0	0	0
PM	4	4	0	0	0	0	0	0	0	0	0	0	0	0
MA	4	4	0	0	0	0	0	0	0	0	0	0	0	0

EB M, EB 50M, EB EA and EB A: methanol, 50% v/v methanol, ethyl acetate and acetone fractions of methanol bark extract of *E. ivorense*; EL EA, EL A and EL M: ethyl acetate, acetone and methanol fractions of methanol leaf extract of *E. ivorense*; MA: *Maerua angolensis*; AG: *Albizia glaberrima*; UF: *Ulva fasciata*; LO: *Laportea ovalifolia*; HL: *Hilleria latifolia*; GER: geraniin; XA: xylopic acid; PM: *Phyllanthus muellerianus*; SV: *Sargassum vulgare*; MN PE and MN EA: pet ether and ethyl acetate fractions of ethanol bark extract of *Margaritaria nobilis*; HD: *Hydropuntia detntata*.

Table 5.2 Heat map showing severity score of compounds and extracts against adult *S. mansoni* worms

Well	**SEVERITY SCORE**			
sample code	**3 h**	**5 h**	**24 h**	**2 days**
EL A	1	0	3	4
EB 50M	2	3	4	4
EB M	0	2	4	4
EB M	0	2	2	3
EB A	2	2	4	4

EB A	2	3	4	4
MN EA	0	0	0	1

EL A: acetone fraction of methanol leaf extract of *E. ivorenses*; EB M, EB 50M and EB A, methanol, 50% v/v metanol and acetone fractions of methanol bark extract of *E. ivorense*; MN EA: ethyl acetate fraction of ethanol bark extract of *M. nobilis*.