STUDIES ON THE TRYPANOCIDAL ACTIVITIES OF SOME ANGIOSPERMS AND FUNGI AGAINST *TRYPANOSOMA BRUCEI BRUCEI* AND *TRYPANOSOMA CONGOLENSE*

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ZARIA, NIGERIA

NOVEMBER, 2017

DECLARATION

I declare that the work in this dissertation entitled **"STUDIES ON THE TRYPANOCIDAL ACTIVITIES OF SOME ANGIOSPERM AND FUNGI AGAINST *TRYPANOSOMA BRUCEI BRUCEI*AND *TRYPANOSOMA CONGOLENSE"*** was carried out by me in the Department of Zoology under the supervision of Prof. H.I. Nock, Prof. A. J. Nok and Prof. I.S. Ndams.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any other university.

Josephine. O. A. Abedo		**29th November, 2017**
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CERTIFICATION

The dissertation entitle **"STUDIES ON THE TRYPANOCIDAL ACTIVITIES OF SOME ANGIOSPERM AND FUNGI AGAINST *TRYPANOSOMA BRUCEI BRUCEI* AND *TRYPANOSOMA CONGOLENSE"*** by Josephine Ohunene Alex-Abedo meets the regulations governing the award of the degree of Doctor of philosophy of Ahmadu Bello University, Zaria, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God the Alpha and Omega, to whom be glory forever and ever. Amen

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ABSTRACT

Plants used in indigenous medicine for the treatment of trypanosomiasis have been reported to have high medicinal activities. A total of 17 plants consisting of 8 angiosperms and 9 fungi were carefully air dried and the powdered extracts were sequentially extracted with petroleum ether, methanol and water; 78 extracts were obtained and tested *in vitro* against *Trypanosoma brucei brucei* and *Trypanosoma brucei congolenses.* The methanolic extracts of *Chrysophyllum albidum* seed, *Sabaflorida* roots, *Tapinantus globiferus* leaves, *Formitopsis pinicola* and aqueous extract of *Chrysophyllum albidum* seed showed highest inhibitory activity at 3.9 µg/ml on both *Trypanosoma brucei brucei* and *Trypanosoma congolense.* The qualitative phytochemical screen of these extract revealed the presence of alkaloids, saponins, tannins, cardiac glycosides and steroids. The methanolic extract of *Chrysophyllum albidum* and *Tapinantus globiferus* were partitioned separately with hexane chloroform and butanol. The butanolic extract of *Chrysophyllum albidum* showed highest activity at 3.9 µg/ml. The bioassay guided studies of the butanolic extract led to the recovery of component A, B and C from preparative thin layer Chromatographic studies. The GC-MS of component C revealed the presence of several compounds which include13-Tetradecynoic acid, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid, methyl,9-Tetradecenal, (Z) and1, 3 ,4-thiadiazol-2-amine,5-ethoxy. The aqueous extract of *Chrysophyllum albidum* and *Tapinantus globiferus* were synthesized using 1Mm of aqueous Silver nitrate. The synthesized silver nano particles of both plants showed minimum inhibitory activity on both *Trypanosoma brucei brucei* and *Trypanosoma congolense* at 0.98 µg/ml. In the *in vivo* experiment 7 groups of mice were infected with *Trypanosoma congolense* at the height of parasiteamia such as 10^7 trypanosomes /ml, groups A1, A2 and A3 were treated with 100, 500 and 1000 mg/kg of butanolic seed extract of *C. albidum* respectively. While group B1, B2 and B3 were administered 100,

500 and 1000 mg/kg of synthesized silver nano particles of seed extract *C. albidum* respectively; group E was treated with standard drug Diminazine; group F was infected but not treated and G was uninfected and untreated group. The animals were treated for five consecutive days. Animals treated with 1000 mg/kg body weight of synthesised nanoparticles of *C. albidum* had reduced parasiteamia such 10^2 and they survived up to the 28^{th} day when the experiment was terminated compared to the untreated group F which culminated in death by day 20 post infection. The prophylactic groups were treated for three consecutive days before begin challenged with the parasites. Animals in group C1and D1 were treated with 50 and 100 mg/kg of butanolic seed extract respectively; animals in group C2 and D2 were treated with 50 and 100 mg/kg of synthesized silver nano particles of seed extract *C. albidum.* The group treated with 100 mg/kg showed highest activity in that parasitemia did not develop up to the time the experiment was concluded. The synthesized silver nano particles of *C. albidum* and *T. globiferus* were characterized. The UV visible spectrum showed that *C. albidum* revealed surface Plasmon resonance at 450 while that of *T. globiferus* were observed to be in the range of 350 nm. The Scanning electron microscopy of *C. albidum* indicate that the silver nanoparticles were predominantly spherical with estimated sizes of between 377 nm-3.97 µm and that of *T. globiferus*are about 1.75-23 µm. This study has shown that most plant have medicinal potential of various degree of activity. Medicinal plant could be used as an excellent and resourceful green material for the rapid and consistent synthesis of silver nanoparticles which could be useful in different applications. Our findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future.

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List of Abbreviations and Symbols

CO_2	Carbon dioxide
^oC	Degree Celsius
DMEM	Dubellco Modified Eagles Medium
FTIR	Fourier Transform Infra-Red microscopy
HCl	Hydrogen Chloride
hr	hour
DEAE	Diethylaminoethyl cellulose
DMSO	Dimethyl Sulphur oxide
gm	grams
HCl	Hydrogen Chloride
H_2SO_4	Sulphuric Acid
Kg	Kilogrammes
PCV	Packed Cell Volume
µl	Microliters
µg/ml	Microgram per mililiters
Mg/ml	Milligrammes per millilitres
$NaHCO_3$	Sodium Hydrogen Carbonate
Mm	Millimolar
Nm	Nanometer
%	Percentage

SNPs	Silver Nano Particles
TLC	Thin Layer Chromatographic Studies
UV	Ultra-Violent
Wt.	Weight
XRD	X-ray Diffractometer

CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction

African trypanosomiasis is an important unicellular blood protozoan parasitic disease of both humans and animals. The disease in animal is commonly called Nagana in Zululand which means powerless, useless, or depressed spirits. It is mainly transmitted cyclically by tsetse fly of *Glossina* species and mechanically by other biting flies(WHO, 2014). Different species of trypanosomes infects cattle and other ruminants. These include *Trypanosoma brucei brucei, Trypanosoma congolense and Trypanosoma vivax.* The two species infective to humans are *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* in humans (Nok and Nock, 2002; WHO, 2014). Nagana has become a major hindrance to livestock production where it thrives in different parts of Sub Saharan Africa restricted to latitude of about 15^0N and 29^0S of the equator which covers over 10 million km^2(Delespaux,2010).

Reports of drug resistance or treatment failure, to limited classes of available trypanocideshas been on the increase (Shaba *et al.,* 2012). There is a resurgence of the disease in the endemic regions of Africa, where millions of human population and cattle are affected with considerable morbidity and mortality (WHO, 2006; Merck, 2010). Estimated losses in agricultural production as a result of the disease are approximately 3 billion pounds annually (Shimelis *et al.,* 2011; Tesfaheywet and Abraham, 2012).

After continued effort especially in the control of the fly vector, there has been decline in number of new cases. The number of Human African Trypanosomiasis (HAT) casereported in 2012 is about 6, 314 new cases(WHO, 2012).

However, the estimated numberof actual human cases is about 20,000 while the estimated population at risk is 65 million people (WHO, 2014). African Trypanosomiasis remains a disease with unsatisfactory medical control to date. The control of Human African Trypanosomiasis (HAT) continues to rely principally on old expensive medicines such as pentamidine, nitrofurans and arsenicals **(**Delespaux *et al.,* 2010; Vitouley *et al.,* 2011). In recent times, drug development has led to the production of eflornithine (DMFO) as the only new trypanocide in the last fifteen years and it is only effective in the last stage of Gambian sleeping sickness and its regimen is complex and difficult toapply (Priotto, 2009).

Other molecules such as Homidium, Isometamidium and Diminazene aceturateare are use in treatment of animal infections.Furthermore, the therapeutic and prophylactic use of trypanocides is beset by numerous limitations, including toxicity, scarcity and the development of resistance by parasites (Delespaux *et al.,* 2010). Similarly, the unlimited antigenic variation exhibited by the parasite has hampered the production of effective vaccine. Also the limited availability and affordability of pharmaceutical medicines, emphasizes the need for research in to a more, effective, affordable sources of trypanocides which will easily be accessibleto the rural populace in Africa, who bear most of the disease burden (Wurochekke and Anyanwu, 2012). These have necessitated the search for alternativemedicines against African trypanosomiasis, hence research into natural products.

Plants as sources of natural products have been the basis for medical and traditional treatment for several diseases through much of human history, and such treatments are still widely practiced today.

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Sherman and Hash 2001; Davis *et al.,* 2004; Moquin, 2009). The most commonly used herbal plants in Africa include *Azadiractha indica* (neem tree) for the treatment of fever; *Magnjfera indica* (mango tree) for the treatment of malaria and thypoid fever; *Allilum sativum* (Garlic) rheumatoid arthritis; *Acacia nilotica* (Acacia) for migraine and pile; *Khaya senegalenses* (African mahogany) for irritable bowel syndrome and cancer; *Carica papaya* (Pawpaw tree) formalaria and typhoid (Sofowora, 1993).A number of African medicinal plants belonging to the angiosperm group have been evaluated for their trypanocidal activity and several reports indicate antitrypanosomal activity exits in some medicinal plants (Hoet *et al*., 2007; Ibrahim *et al.,* 2008; Shuaibu *et al.,* 2008; Abedo *et al.,* 2013 and Abedo *et al.,* 2015) with minimal toxicity.

Fungi are another group of plant which contain compounds, that can make a contribution to the general health of mankind (Pan *et al.,* 2008). As fungi are widely distributed all over the world, some of them have been used in traditional medicine as analgesics, hemostatic, diuretic, nourishment, anti bechic and antitumor agents (Cragg *et al.,*1997; Sullivan *et al.,* 2006). Also fungi present a spectrum of biological compounds with activities against virus, cancer and parasites.

These plants contain compounds mainly secondary metabolites such as alkaloids, glycosides, flavonoids, terpenes and coumarins (Jorgensen, 2007). Furthermore various

bioactive compounds isolated from extracts of Ethiopian higher fungi showed biological properties such as antiprotozoal, anithelmintic, phytotoxic and brine shrimp lethality activities (Hawksworth, 2006).

Similarly, (Davis *et al*., 2004) investigated leishmanicidal and trypanocidal activity of the extracts and secondary metabolites of some class of fungi known as basidiomycetes. A naturally occurring purine nucleoside found in some mushrooms showed high degree of activity against Mycobacterium (Pan *et al.*, 2008). Aqueous extracts of mushroom substrate are used in foliar disease control (Kharwar *et al*., 2011). Based on these facts, this work intends to evaluate extracts of some angiosperms and fungi for anti trypanosomal activity.Research based on this approach can provide both new molecules from promissory secondary metabolites and validation of ethno medical uses of crude extracts or partially fractionated through chemical methods. Natural product chemistry can offer new more molecules which could be used to develop drugs against these diseases (Cravotto *et al.*, 2010; Schmidt *et al.*, 2012; Abbeele and Routureau, 2013).

1.2 Statement of the Research Problem

A large number of angiosperms and fungi are widely used in traditional medications with no scientific evaluation to support claims for potency; whether some of these extract in non-synthesized or synthesized silver nano particles forms, have antitrypanosomal activity is the subject of this investigation.

1.3 Justification

Trypanosomiasis, a disease of major importance in human and animals, has continued to threaten human health and economic development (Priotto, 2009;WHO, 2014). *T. b*.

gambiense and *T. b. rhodesiense*affects hundreds of people in sub-Saharan Africa and are responsible for the death of several hundred per year (Ekanem, 2006).While the animal type causes great economic losses.

Chemotherapy is the most commonly used method of control of the disease. The few existing trypanocides are often associated with severe and toxic side effects (Gehig andEfferth 2008) and require lengthy parenteral administration, lack efficacy and expensive nature of drug(Legros *et al.,* 2002). There is urgent need to source for new, cheap and safe alternative medicines against trypanosomiasis from natural origin.

In African countries where sleeping sickness is endemic, plants have traditionally been used for generations and are still widely used to treat this ailment with possible therapeutic activities, which have not been proved scientifically. Nigeria is greatly influenced by the savannah-forest vegetation which may harbourtsetse fly which is the vector of trypanosomes, thus making the area highly endemic to trypanosomiasis. Traditional medicinein Africa has long been noted for centuries in using medicinal plants in curing human and animal trypanosomal infections (Fang *et al.,* 2011). Natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes (Abedo *et al.,* 2015).

Some Nigerian medicinal plants have been evaluated for their *in vitro* trypanocidal activity (Davis *et al.,* 2004; Ekanem*etal.,*2006); Shuaibu *et al.,* 2008; and *in vivo* anti-trypanosomal efficacies in mice (Abubakar *et al.,* 2008;Atawodi *et al.,* 2011; Abedo *et al.,* 2015) discovery of these potent anti-trypanosomal extracts from plant has increased the great potentials of plant species to provide lead compounds for the development of new natural drugs for effective treatment of sleeping sickness.

1.4 Aim

To evaluate the anti-trypanosomal activities of extracts of some selected angiosperms: *Annona muricata,Chrysophyllum albidum,Saba florida, Tapinanthus Globiferus,Cissus quadrangellum,Vitellaria paradoxa,Ficus capensis,Acanthospermun hispidum* and fungi:*Calvatia cythiforms,Xylaria polymorpia, Trametes versivcolor,Fomitopsispinicola,Lepista sordid,Agaricus bohisii,Polyporus sanguineus,Boletus edulis* and*Cantharellus cilariusin vitro, in viv*o and alsoto synthesize and characterize silver nanoparticles using most active extract.

1.5 Objectives of the Research

i. To determine the anti-trypanosomal activities of crude extracts, fractions and silver nanoparticles of some selected angiosperms and fungi;

ii. To determine the phytochemical properties of crude extract of angiospermsand fungi being investigated in this study.

iii. To determine the bioactive molecule from angiosperms and fungi through bioassay guided fractionation;

iv.To determine the effect of synthesized silver nano particle and non-synthesized extract of angiosperms and fungi on pack cell volume of mice;

v. To characterize synthesized silver nanoparticles using extracts of angiosperm and fungi with the highest trypanocidal activities

1.6Research Hypotheses

i. The extracts of selected angiosperms and fungi have no significant anti-trypanosomal activities.

ii. The extracts of selected angiosperms and fungi are low in phytochemical content.

iii. There are insignificant bioactive molecules in selected angiosperms and fungi.

iv. There are no significant differences in pack cell volume (PCV) of blood before and after the therapeutic application of synthesized silver nano particle and non-synthesized extracts of angiosperms and fungi.

v.Thereare no silvernano particlessynthesized using selected angiosperms and fungiextracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective of African Trypanosomiasis

History has shown that African trypanosomiasis may have been an important selective factor in the evolution of hominids. Ancient history and medieval history indicate that African trypanosomiasis affected the lives of people living in sub-Saharan African at all times (WHO, 2006). African trypanosomiasis is an infectious disease of humans and animals of similar aetiology and epidemiology. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* species) (Njoku *et al.,* 2006). The distribution of trypanosomaisis in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km^2 between 14 degrees North and 20 degrees South latitude (WHO, 2014). Throughout history, African trypanosomiasis has severely repressed the economic and cultural development of Central Africa.

Phylogenetic reconstruction based on the genes coding for the small subunit ribosomal RNA suggested that all Salivarian trypanosomes (to which African trypanosomes belong) separated from other trypanosomes approximately 300 million years ago (Gibson, 2005). Probably soon after their emergence, Salivarian trypanosomes became gut parasites or commensals of early insects, which evolved around 380 million years ago.

With the appearance of tsetse flies some 35 million years ago, trypanosomes have been transmitted to mammals by these bloodsucking insects. The long coexistence of both tsetse flies and game animals may explain why most African wildlife species are tolerant of trypanosomiasis: they become infected by the parasite but show no ill effects (Cox, 2004). In contrast, domestic animals have yet been unable to develop tolerance or resistance to trypanosome infections within the 13000 years of their breeding.

It is likely that trypanosomiasis has played an important role in early hominid evolution. Probably, the disease had an important role in the selection of trypanosome-resistant early terrestrial hominids. This is evident from the observation that arboreal primates are susceptible to trypanosomiasis while humans, with the exception of *T. b. gambiense* and *T. b. rhodesiense*infections, are resistant (Cox, 2004). The fact that humans are resistant to all other African trypanosome species indicates that human African trypanosomiasis is a recent event in human development. Presumably the sustained transmission of trypanosomes between tsetse flies and humans in West Africa has led to the evolution of the less virulent *T. b. gambiense* subspecies (Steverding, 2006). In contrast, the *T. b. rhodesiense* subspecies has remained ill-adapted to humans and is transmitted from game animals to humans (Priotto *et al*., 2006).The infectivity of *T. b. rhodesiense* to humans is due to a serum-resistant-associated (*SRA*) gene (Delespaux and Koning, 2007). It seems that the SRA gene originated in a single event and then spread through *T. brucei* in East Africa by genetic exchange.It was Scottish missionary and explorer David Livingston (1813–1875) who first suggested that nagana is caused by the bite of tsetse flies.

In 1852, he reported the occurrence of a disease in the valleys of the Limpopo and Zambezi rivers as well as at the banks of the lakes Nyasa and Tanganyika from which all the cattle he carried died after they have been bitten by tsetse flies (Lambrecht, 2005).

However, it took another 40–50 years until trypanosomes were identified as the causative agents of nagana and sleeping sickness. In 1895, the Scottish pathologist and microbiologist David Bruce (1855–1931) discovered *T. brucei* as the cause of cattle trypanosomiasis (cattle nagana).

The first unequivocal observation of trypanosomes in human blood was made by the British Colonial surgeon Robert Michael Forde (1861–1948) in 1901 when he examined a steamboat captain in Gambia. He first thought that the organisms he found were worms (Cox, 2004) but the English physician Joseph Everett Dutton (1874–1905) identified them as trypanosomes a few months later and proposed in 1902 the species name *Trypanosoma gambiense* (now *Trypanosoma brucei gambiense*). In the same year, the Italian physician and pathologist Aldo Castellani (1878–1971) found trypanosomes in the cerebrospinal fluid of sleeping sickness patients and suggested that they cause sleeping sickness (Castellani, 1903; Cox, 2004). One year later, Bruce provided conclusive evidence that sleeping sickness is transmitted by tsetse flies (Cox, 2004 and WHO, 2006). At that time, however, he believed that trypanosomes were transmitted mechanically by tsetse flies (Cox, 2004). It was the German military surgeon Friedrich Karl Kleine (1869–1951) who showed in 1909 the cyclical transmission of *Trypanosoma brucei* in tsetse flies. This prompted Bruce to change his original opinion of mechanical transmission of trypanosomes, and instead describe the full developmental cycle of the parasites within their insect host. In the meantime, the two other animal pathogenic trypanosome species *T. congolense* and *T.*

vivax were discovered in 1904 and 1905 by the Belgian physician Alphonse Broden (1875–1929) and the German naval doctor Hans Ziemann (1865–1905),respectively

.

The second human pathogenic trypanosome species, *Trypanosoma rhodesiense* (now *Trypanosoma brucei rhodesiense*), was eventually discovered in 1910 by the Parasitologists John William Watson Stephens (1865–1946) and Harold Benjamin Fantham (1876–1937)(Gibson, 2005).

2.2 Classification: Taxonomic ranks for Protozoa

Kingdom ----------Prostista (unicellular eukaryotes)

Subkingdom----------Protozoa

Phylum----------Sacomastigophora (with pseudopodia and/or flagella)

Subphylum ----------Mastigophora (flagellates)

Class----------Zoomastigophora (zooflagellates, without chloroplasts)

Order----------Kinetplastida (presence ofextra nuclearDNA, kinetoplast)

Family----------Trypanosomatidae

Section ----------Salivaria

Genus----------*Trypanosoma*

Species----------*brucei*

Subspecies----------*Gambiense brucei ,Rhodesiense brucei*

There are four subgenera of the genus *Trypanosoma: Dutonella, Nannomonas, Trypanozoon and Pycnomonas* (Ralston *et al.*, 2009).

The subgenera trypanozoon contains three species:

Trypanosoma brucei, *Trypanosoma evansi*and *Trypanosoma equiperdumTrypanosoma brucei* contains 3 Subspecies *Trypanosoma rhodesience,Trypanosoma gambiense* are twopathology pathogen infective to humans only, while *Trypanosoma brucei brucei* is only infective to animals.

2.3 Aetiology and Morphology trypanosomes

The parasite consists of a single cell varying in size from 8 to over 50 μm (Figure 2.1). All the activities associated with a living organism take place within this unicellular organism nutrition, respiration, excretion and reproduction. The substance of which all living cells consist, the protoplasm, comprises three parts, an outer protective and retaining layer, the pellicle cell envelope cell membrane, within which the cytoplasm forms the bulk of the contents. Suspended in the cytoplasm are various structures, the most prominent being the nucleus, which may be regarded as the command centre of the cell and which also plays a major part in reproduction. It contains DNA (deoxyribonucleic acid), which is arranged in the form of genes and chromosomes; it represents the genetic information and is responsible for the manufacture of enzymes and other proteins of the cell (Odiit *et al.*, 1997).

Trypanosomes are thoroughly adapted to living and moving in the blood plasma or tissue fluid of the host. They are elongated and streamlined, and tapered at both ends.The pellicle, the outer layer of the cytoplasm, is flexible enough to permit a degree of body movement, while retaining a definite shape. Trypanosomes can be differentiated using for morphological features these include the average length and shape of the body; size and position of the kinetoplast; present or absent of undulating membrane and flagellum. A flagellum usually arises near to the posterior end from a parabasal body, and runs the length of the trypanosome; it may be continued beyond the anterior end of the body as a whip-like free flagellum.

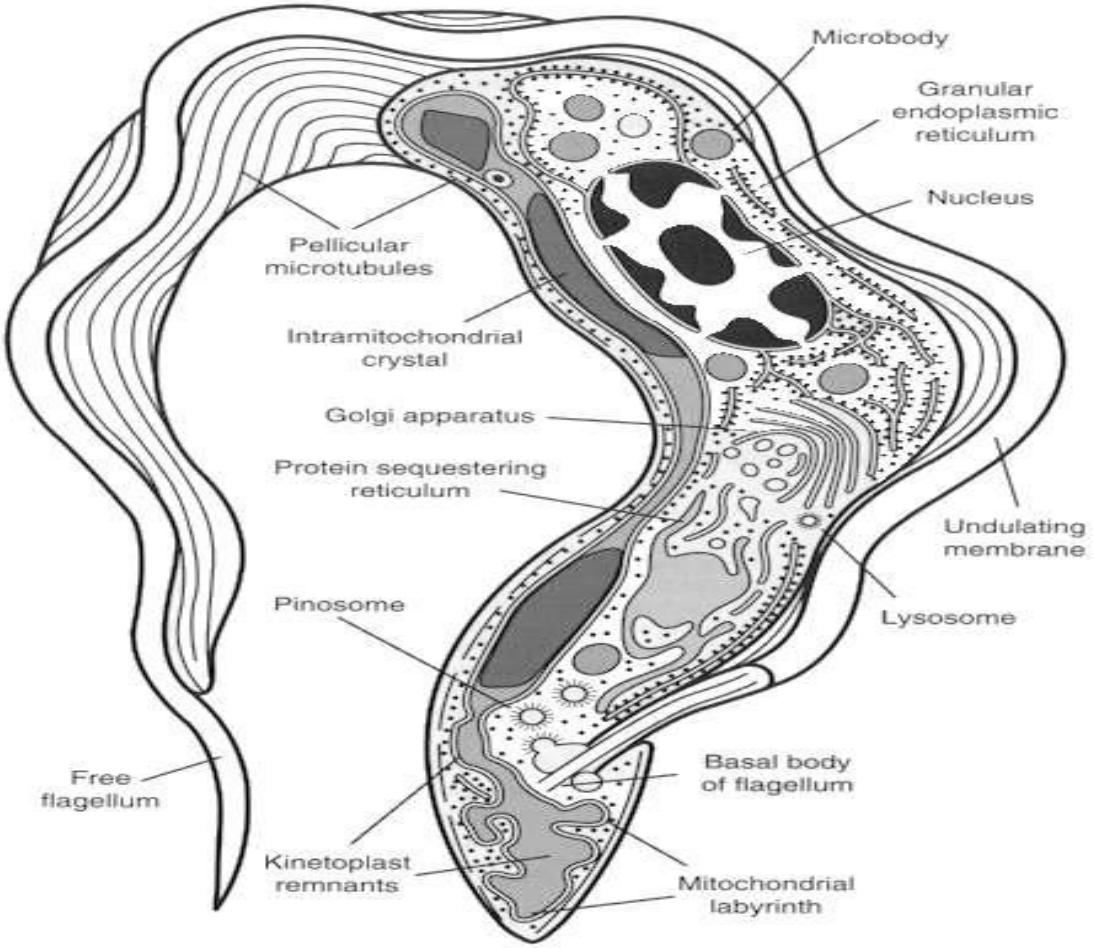


Figure 2.1:1Ultrastructure of Trypanosome as revealed by electron microscope (Vickerman *et al.*, 1992)

Along the length of the body the pellicle and cytoplasm are pinched up into a thin sheet of tissue called the undulating membrane, through the outer margin of which runs the flagellum (Odiit *et al.*, 1997).

Among other basic morphological features, a distinct well-defined body, the kinetoplast, is seen near to the posterior end of the trypanosome and differs in size and position according to the species. It is adjacent to the parabasal body (from which the flagellum arises), and so

close to it that it cannot easily be seen separately with the light microscope. Thekinetoplast has important functions in reproduction and metabolism and is probably essential for cyclical transmission by tsetse flies It is sometimes absent in a proportion of trypanosomes, especially of some strains of *T. evansi*, a species which has lost its ability of being cyclically transmitted). The extent of the undulating membrane and the absence or presences of the free flagellum are also precious in specific identification of trypanosomes.

The various species ofthe parasite forms trypomastigotes in vertebrate hosts and epimastigotes in the insect vector. The trypomastigotes (with posterior kinetoplast and long undulating membrane) are in sizes ranging from 16-42 µm in length by 1-3 µm in width.

They occur as elongate slender dividing forms with long free flagellum or stumpy non-dividing infective (metacyclic) forms (with no free flagellum).

The epimastigotes (with anterior kinetoplast and short undulating membrane) are also variable in size ranging from 10-35 µm in length by 1-3 µm in width (Brun *et al.,* 2010).The *Trypanosoma congolense* are monomorphic in nature, they measure between 10-25µm in length, they are the smallest of the pathogenic trypanosomes. It lacks free flagellum, the kinetoplast is of medium size,and it is posteriorly placed.

The undulating membrane is poorly developed and inconspicuous. The *Trypanosoma brucei brucei*are usually polymorphic in nature exhibiting three main forms: long slender forms, short stumpy forms and intermediate forms. All have kinetoplastwhich is mostly sub- terminally placed with conspicuous undulating membrane.The posterior end is somewhat variable in shape, but usually bluntly pointed. The average length the body is between 17-30µm.

2.4 Geographical Distribution of Trypanosomiasis

Sleeping sickness occurs in 36 sub-Saharan Africa countries where there are tsetse flies that transmit the disease. In the last 10 years, over 70% of reported cases occurred in the Democratic Republic of Congo (DRC). The DRC is the only country that has reported more than 1000 new cases annually and accounts for 89% of the cases reported in 2013. Chad and South Sudan reported about 100 and 200 new cases in 2013. Similar figures were expected in Central African Republic but insecurity hampered the regular case detection activities (Simarro, 2012; Rutto *et al.,* 2013).Countries such as Angola, Cameroon, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Ghana, Guinea, Kenya, Malawi, Nigeria, Uganda, Tanzania, Zambia and Zimbabwe are reporting less than 100 new cases per year. Countries like Benin, Botswana, Burkina Faso, Burundi, Ethiopia, Gambia, Liberia, Mali, Mozambique, Namibia, Rwanda, Senegal, SierraLeone, Swaziland and Togo have not reported any new cases for over a decade (WHO, 2014). Leone, Swaziland and Togo have not reported any new cases for over a decade (WHO, 2014).

Transmission of the disease seems to have stopped but there are still some areas where it is difficult to assess the exact situation because the unstable social circumstances and/or remote accessibility hinder surveillance and diagnostic activities (Onyiah, 1997). Trypanosomes can be found wherever the tsetse fly vector exists. Tsetse flies are endemic in Africa between latitude 15° N and 29° S, from the southern edge of the Sahara desert to Zimbabwe, Angola and Mozambique. Trypanosomes, particularly *T. vivax,* can spread beyond the "tsetse fly belt" by transmission through the aid of mechanical vectors. *T. vivax* is also found in South and Central America and the Caribbean, areas free of the tsetse fly (WHO, 2014).

2.5 Economic Importance of Trypanosomiasis

The economic impacts of trypanosomiasis consist of direct and indirect losses. Direct loses involve decreased in livestock productivity (meat and milk yields), high morbidity and mortality rates, high infertility and inability to work as traction animals (Mersha, 2012; Bizuayehu *et al.*, 2012). The direct cost also includes the detection, treatment of infected animals, fly control and research (Omotainse *et al.,* 2004; Shimelis *et al.,* 2011).

The indirect impact of Trypanosomiasis mostly lies on crop production; through the availability and loss of animals' traction power. Trypanosomiasis is an expensive disease to control and thus, an economic analysis become essential to show the extent of socio-economic losses due to the disease.

African Animal Trypanosomiasis (AAT) costs Africa about US$5 billion a year and Africa spends at least $30 million annually to control cattle trypanosomiasis in terms of curative and prophylactic treatments (Shimelis*et al.,* 2011).

Direct losses due to Trypanosomiasis are estimated to between US$ 1-1.2 billioneach year. The total losses for the total tsetse-infested lands in terms of agricultural gross domestic product are US$4.75 Billion per year (Tesfaheywet and Abraham, 2012;Shaw *et al.,* 2014).

2.6 The Insect Vector

Tsetse flies are bloodsucking flies of the genus *Glossina*. They occur only in tropical Africa and are important as vectors of African trypanosomiasis in both humans and animals. Tsetse flies are robust, 6–15mm in length, they can be distinguished from other biting flies by their forward-pointing mouthparts (proboscis) and characteristic wing venation (Cattand *et al.,* 2001). There are about 30 known species and subspecies of tsetse flies belonging to

the genus *Glossina*. They can be divided into three distinct groups or subgenera: Austenia (*G. fusca* group), Nemorhina (*G. palpalis* group) and *Glossina* (*G. morsitans* group). Only nine species and subspecies, belonging to either the *G. palpalis* or the *G. morsitans* group, are known to transmit sleeping sickness (Cecchi *et al.,* 2014).

The female tsetse fly does not lay eggs but produces larvae, one at a time. The larva develops in the uterus over a period of 10 days and is then deposited fully grown on moist-soil or sand in shaded places, usually under bushes, fallen logs, large stones and buttress roots. It buries itself immediately and turns into a pupa. (De La Rocque *et al.,* 2001). The fly emerges 22–60 days later, depending on the temperature.

Females mate only once in their life and, with optimum availability of food and breeding habitats, can produce a larva every 10 days (Engering *et al.,* 2013). The flies pass most of their rest time in shaded places in forested areas. The preferred sites are the lower woody parts of vegetation, many tsetse flies hide in holes in the trunks of trees and between roots. They search for food only for very short periods during the day and often rest near to food sources.

Common risk areas where people are likely to be bitten by tsetse flies are: on forest trails near water collectionpoints, in forests, at vegetation close to bathing sites and water collection points along the banks of rivers (Dennis *et al.,* 2014).

2.7Life cycles of Trypanosomes

2.7.1 Life cycle in the insect vector (Tsetse fly).

When a tsetse fly hatches from its pupal case it is free from trypanosomes. Until its first blood meal, it is called a Teneralfly. It acquires a trypanosomal infection when feeding on a parasitic (having parasites in the circulating blood) mammalian host (Figure2.2). The trypanosomes undergo a cycle of development and multiplication, they change into long slender forms called epimastigotes, which multiply and finally give rise to the infective metatrypanosomes in the digestive tract of the fly (Jan *et al.,* 2010).

Different trypanosome species develop in different regions of the digestive tract of the fly, and the metatrypanosomes occur either in the biting mouthparts or the salivary glands. The period from ingesting infected blood to the appearance of these infective forms varies from one to three weeks; once infective metatrypanosomes are present the fly remains infective for the remainder of its life. During the act of feeding the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed in the tissues and the fly injects saliva to prevent coagulation. Infection of the host takes place at this stage, with infective metacyclic trypanosomes in the skin tissue (Doudoumis*et al.*, 2013).

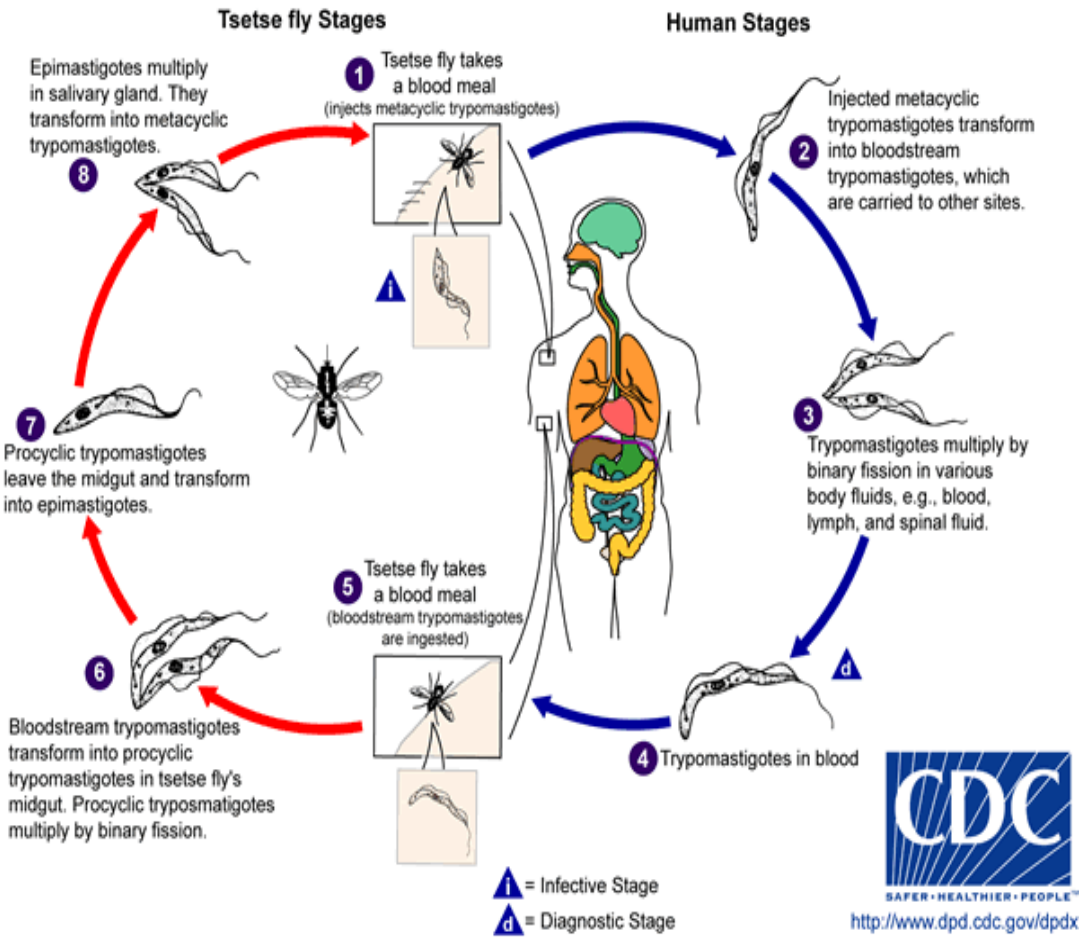


Figure 2. 2: A typical life cycle of trypanosomiasis (CDC http://www.dpd.cdc.govidpdx).

2.7.2 Life cycle in the mammalian host.

The infective meta trypanosomes undergo development and multiplication at the site of infection (Figure 2.2) where a swelling or chancre may be detected in the skin and finally the mature blood trypanosomes (or trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation(Doudoumis *et al.,* 2013).

Reproduction in the mammalian host occurs through a process of binary division, trypanosomes feed by absorbing nutrients through their outer membrane, from the body fluids of the host (Brun *et al.,* 2010). The proteins, carbohydrates and fats are digested by enzyme systems within their protoplasm. Oxygen dissolved in the tissue fluids or blood plasma of their host is absorbed in a similar manner, to generate the energy necessary for the vital processes.Waste products are disposed of by a reverse process, through the outer membrane, into the body fluids of the host. They include carbon dioxide formed during respiration, as well as more complex metabolic products. (Doudoumis *et al*., 2013).

2.7.3 Mechanical Transmission of Trypanosomes.

A biting insect passes the blood forms from an infected animal to a clean susceptible other in the course of interrupted feeding. The time between the two feeds is crucial for effective transmission because the trypanosomes die when the blood dries**.** The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors (Rocha *et al.,* 2004). Tsetse flies can of course also act as mechanical vectors.

This mode of transmission has proved to be sufficiently effective to maintain *Trypanosoma vivax* and *Trypanosoma evansi* in South and Central America, and the latter species in North Africa

and Asia as well. No tsetse flies occur outside tropical Africa, apart from small tsetse pockets in the southwest of the Arabian Peninsula (Cherenet *et al.,* 2004).

2.7.4 By Iatrogenic Means.

Infection can occurwhen using the same needle or surgical instrument on more than one animal, at so sufficiently short intervals that the blood on the needle or instrument does not dry. It is not an uncommon occurrence during animal vaccination, treated by injection, or when blood is collected from several animals in a row, without changing or disinfecting needles.

Infection can also occur when several animals are subjected at short intervals to a surgical intervention (dehorning, castration, etc.) without properly disinfecting the instruments (Franco *et al.,* 2014).

2.7.5 Transmission by Other Means

Research has shown that carnivores can be infected with *T. evansi* and *T. brucei* by ingesting meat or organs from infected animals, as long as these are still sufficiently fresh to contain live trypanosomes. Infection occurs probably through the mucosa of the mouth in which bone splinters make wounds through which parasites can penetrate more easily (Mallewa and Wilmshurst, 2014).

Transmission of *T. evansi* in Latin America by the bites of vampire bats is common. These bats become infected by ingesting blood from infected horses or cattle.

The trypanosomes multiply in the bats and these are thereafterable to transmit the disease to healthy animals. The trypanosomes apparently pass readily through the oral mucosa of the bat in both directions. All trypanosome species are occasionally transmitted congenitally, from the infected mother to the offspring, either through the placenta while the foetus is still in the uterus, or when bleeding occurs during birth. Congenital transmission of *T. vivax,* for example, has been observed in Latin America as well as in Africa, but its real importance is not well known (Mallewa and Wilmshurst, 2014).

Venereal transmission is the normal means by which dourine of equines, caused by *Trypanosoma equiperdum* is propagated.

Because of its presence in the mucous exudate of penis and sheath of the stallion and the vaginal mucus of the mare, *T. equiperdum* is easily transmitted directly during copulation from an infected to a healthy animal and its geographical distribution is not restricted to specific climatic conditions. This species is essentially a tissue parasite and causes at most very low parasitaemia is in the circulating blood of equines.

2.8 African Animal Trypanosomiasis (AAT)

African animal trypanosomiasis (AAT) in domesticated animals is caused by some species of the Trypanosoma genus which are pathogenic to animalswhich also cause this disease in wild animals species. In cattle the disease is called *Nagana,*a Zulu word meaning “to be depressed or powerless” (Mbaya *et al.,* 2010). The disease causes serious economic losses in livestock,common symptoms usually observed include anemia, enlarged lymph nodes, urticaria, exudation, weight loss.Abortions and stillbirths may also occur frequently (Tuntasuvan *et al*., 2003 and Sachs, 2010).

Many untreated cases are fatal. AAT is found mainly in those regions of Africa where its biological vector, the tsetse fly, exists. Animals with acute *Trypanosoma evansi* infection usually have a short clinical course of disease and die within weeks to months of infection. Protecting animals from trypanosomiasis is difficult in endemic areas, as bites from tsetse flies and a variety of other insects are unpreventable. A tsetse fly eradication program being conducted in Africa may help control this disease, as well as other human trypanosomiasis (Raheem, 2014).

2.9Aetiology of AAT

Tsetse-transmitted trypanosomes primarily affect animals and cause African animal trypanosomiasis. The most important species that causes this disease are *Trypanosoma congolense*, *T. vivax* and *T. brucei. Trypanosoma congolense* is classified into three types, which are called the savannah, forest and kilifi types(Ekanem *et al.,* 2006). Other species such as *Trypanosoma simiae* and *Trypanosoma godfreyi* can also cause AAT.

Some trypanosome infections in Africa cannot be identified as any currently recognized species (Cherenet *et al.,* 2004). Concurrent infections can occur with more than one species of trypanosome (Pinchbeck *et al*., 2008).

2.9.1Host range affected by AAT

African Animal Trypanosomiasis can infect most domesticated animals. Clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, pigs, dogs, cats and others (Acha and Szyfres, 2003). In some parts of Africa, cattle are the main species affected, due to the feeding preferences of tsetse flies.In effect, they can shield other domesticated animals such as goats and pigs from the effects of trypanosomiasis(Acha and Szyfres, 2003).

More than 30 animal species in the wild or zoos, including ruminants such as white-tailed deer, duikers, antelope and African buffalo, as well as wild Equidae, lions, leopards, warthogs, capybaras, elephants, nonhuman primates and various rodents are also known to be susceptible to infection. *Trypanosoma vivax* DNA has been found by PCR in crocodiles and monitor lizards (*Varanus ornatus*) in Africa, but whether this organism can become established in reptiles or it is merely inoculated transiently by insect's is yet to be determined.

Experimental infections can be established in laboratory animals including mice, rats, guinea pigs and rabbits. The host preferences of each trypanosome species may differ, but *Trypanosoma congolense, T. vivax* and *T. brucei brucei* have a wide host range among domesticated animals. *Trypanosoma godfreyi* and *Trypanosoma suis*occur in pigs. *Trypanosoma simiae* appears to be most important in pigs, but it has also been reported by PCR in camels, horses and cattle (Picozzi *et al.*, 2008).

2.9.2 Incubation Period of AAT

The incubation period for AAT ranges from 4 days to approximately 8 weeks. Infections with more virulent isolates have a shorter incubation period (WHO, 2008).

2.9.3Clinical signs of AAT

Most cases of trypanosomiasis are chronic, but acute disease which may be fatal within a week, can also occur. The first sign of trypanosomiasis may be a localized swelling (chancre) at the site of the fly bite, but this usually remains unnoticed.

The primary clinical signs are intermittent fever, signs of anemia, lymphadenopathy and weight loss and progressive emaciation(Acha and Szyfres, 2003). Milk yield may be

decreased in dairy animals. Neurological signs, edema, cardiac lesions, diarrhoea, keratitis, lacrimation, appetite loss and other clinical signs have also been reported (Ojok *et al.*, 2002). Effects on reproduction include abortions, premature births and prenatal losses, as well as testicular damage in males.

In an experiment reported by Abenga and Anosa, (2006), they observed that in *T. brucei gambiense* infected vervet monkeys.The late stage occurred eight weeks after infection which was followed by CNS disturbances. Few clinical cases have been described in dogs and cats. A chronic infection in an imported Jack Russell terrier, which may have been exacerbated by prednisone treatment, was characterized by an acute, fatal episode of severe anemia and abdominal distension with hepato-splenomegaly and ascites. The dog is reported to have had mild, fluctuating abdominal distension for six months. In two dogs, the clinical signs included acute hemorrhagic vomiting and diarrhoea, withseizures; the stress of travel may have resulted in the exacerbation of a chronic condition.

Subclinical infections have also been reported in this species (Acha *et al*., 2003).

2.9.4 Post Mortem Lesions of AAT

The gross lesions are nonspecific. In the acute stage petechiae are common on the serosal membranes, particularly in the peritoneal cavity. More chronic cases may have serious atrophy of fat and signs of anemia and the lymph nodes may be enlarged, normal or atrophied. Subcutaneous edema, excessive fluid in the body cavities, pulmonary edema and an enlarged liver may also be seen. Wasting or emaciation is common.

Some trypanosomes can directly damage tissues, resulting in lesions such as keratitis or cardiac damage. Immune complexes also cause inflammation and damage in a variety of tissues, including the kidneys and blood vessels (Raheem, 2014).

2.9.5Susceptibility to Animal Trypanosomes

Morbidity and mortality vary with the breed of the animal, as well as the strain and dose of the infecting organisms. Some breeds of African cattle and small ruminants are genetically-tolerant to the development of clinical trypanosomiasis, a phenomenon known as trypanotolerance. Trypanotolerant breeds of cattle include West African Shorthorn (which may also be known as Muturu, Baoule, Laguna/ Lagune, Namchi, Samba/ Somba or Dahomey cattle) and N'Dama. Although acute cases can be seen, trypanosomiasis is often a chronic disease in susceptible animals. The morbidity rate is high, and many untreated animals infected with *T. vivax*, *T. brucei brucei* or *T. congolens*e eventually die (Ojok *et al.*, 2002). In cattle infected with some virulent strains, the mortality rate can reach 50-100% within months after exposure, particularly when poor nutrition or other factors contribute to debilitation (Pascal *et al.*,2009). 2009).

Other isolates may cause sub-acute disease, followed by spontaneous recovery and the disappearance of trypanosomes from the bloodstream. The roles of different trypanosome species and mixed infections on the severity of disease in each host species are incompletely understood (Magona *et al*., 2008).

2.10Pathogenesis and Pathology of Trypanosomiasisin animals

The disease if untreated may be acute, sub-acute or chronic. Locomotors ataxia is usually one of the earliest signs of the disease and is manifested by a stumbling or wearing gait when walking or trotting and the tendency to fall when turning or when a hoof is lifted for examination (Masocha *et al.,* 2007).

The animal has a dejected look; the ears tend to droop, the eyes become sunken, and the hair coat loses its luster and become harsh and starry. Closer clinical examination reveals the sporadic appearance of urticarial plagues on the body about 10 cm in diameter.

Edema is prominent which usually affects the ventral parts e.g. sternum, belly, legs, etc (Masocha *et al.,* 2007). The mucous membranes of the eye become progressively paler as anemia progresses. Lymphadenitis is usually present but in the horse the superficial lymph nodes are not prominent and careful palpation is required to detect glandular enlargements. Other changes include myocarditis and pericardial effusion in advanced stages accompanied by dilatation of the heart (Dokoet *al.*, 1997; WHO, 1998). While visceral complications had been described for the Gambian trypanosomiasis by Morrison *et al.,* (1981); Vincendeau, (2006), they observed wide spread haemorrhages affecting the diaphragm, other viscera, and the central nervous system in *T. brucei* infected dogs. Olowe, (1975) observed gross haemorrhages into the intestines, mesentery, mediastinum as well as the myocardium in an autopsy of a three week old baby with congenital Gambian trypanosomiasis.Although Poltera, (1985) reported later of no involvement of the gastro intestinal tract in man. This has been implicated by the necropsy findings of Ayub *et al.,* (2011) on a *T.b.gambiense* infected soldier on peace mission. Wellde *et al.,* (1989) observed enlargement of spleen and the liver in patients with *T. rhodesiense* disease. These changes were similar to those earlier observed in *T.brucei* infected rabbits (Vanden Ingh 1976) and dogs (Kaggwa *et al.,* 1983; Morrison *et. al.*, 1981).

Furthermore, intermittent fever with peaks 41.7^{o}C and intervals of normal or even abnormal readings bears a close relationship to the degree of parasitaemia being highest when trypanosomes are most numerous in the peripheral blood.

As aneamia becomes greater, there is usually continuous tachycardia and polypnoea. The heart may be pale and rounded with marked oedema and necrosis of the pericardial fat.

Petechiations and ecchymoses of the myocardium, thus giving the heart a mottled pale haemorrhagic appearance and pericarditis (Morrison *et al.,* 1981; Ayub *et al.*, 2011). These haemorrhagic syndromes mimic those observed earlier in *T. vivax* infected cattle (Wellde *et al.,* 1989). There may also be massive pulmonary edema (Ayub *et al.,* 2011) as well as foci of consolidation (Tailor and Authie, 2004). In an experimental infection, *T. b. brucei* caused corneal opacity 21 days post infection in the dog (Morrison *et al*., 1981). Similar observation was made in a dog by (Omotainse *et al.,* 1989). Wellde *et al*., (1989), reported apparent blindness in cattle infected with *T.b. rhodesiense* without corneal opacity, suggesting this is not a regular finding in natural Sleeping Sickness.

Additional ocular lesions such as unilateral or bilateral conjunctivitis and keratitis have also been reported (Losos and Ikede, 1972). In early stages of the infection, the heart is generally considered to be most at risk in Rhodesian sleeping sickness with death arising mainly from pancarditis (Ormerod, 1970 and Poltera, 1985).

Similar observations were made in monkeys suffering from acute trypanosomiasis (Abenga and Anosa, 2004). In *T.b. gambiense* infection however, cardiac lesions were complicated by bacterial infection (Poltera, 1985).

The involvement of the CNS is associated with chronicity Losos and Ikede (1972); WHO, (2006) and may manifest grossly as oedema of the brain and meninges.

Morrison *et al., (*1981) observed swollen choroid plexus 21 to 25 days post infection as a result of oedema and presence of massive numbers of trypanosomes mixed with cellular infiltrates of the plexus in the *T. brucei* infection.

Other parts of the brain commonly involved include: the thalamus, hypothalamus, hippocampus and basal ganglia (Losos, 1986;Tailor and Authie, 2004).

Acute cases may terminate fatally in a week or two and the animal may show very little loss of condition. The chronic phase may continue for two or three months and the animal will be severely emaciated. In terminal stages the animals is recumbent and sometimes comatose because of muscular weakness and anemia.

2.11 Diagnosis of Trypanosomiasis

Trypanosomiasis may be suspected when a patient from an endemic area presents the following signs; Irregular fever, headache, joint pains and palpable lymph nodes particularly in the post cervical triangular region. Edema is also a common symptom, which usually affects the lower parts of the body (Masocha, 2007). Definitive diagnosis can be made by recovering the trypanosomes from the blood, lymph gland, spinal fluid or bone marrow. Multiple examinations of thick blood films should be made, since the trypanosomes may be few or irregular at the early stage, about 10 to 20 ml of haemolysed blood is centrifuged and the sediment is observed for the characteristic trypanosomes (Kennedy, 2013). Later the parasites may be recovered from the lympnodes by obtaining fluid from them affected individuals (lymph node puncture). The protein count and the cell count of the spinal fluid are used as indices of therapeutic and prognostic value. Cultures and animal inoculations may be employed when direct examination fails to confirm the diagnosis.

2.11.1 Fresh blood wet mount

A very small drop of blood is placed on a glass slide and spread by pacing a cover slip on it. Movement of the parasite causes a characteristic movement of the surrounding blood

cells. Multiple blood examination should be made when trypanosomes are few (WHO, 2008).

2.11.2 Blood concentrations

Blood is collected in 20% citrated physiological sodium chloride solution. Filter the blood sample through a cheese cloth and then centrifuged at or below 300 rpm for 20 min. The cells are separated then haemolysed in 2% acetic acid or distilled water, it is then centrifuged again for 20 min. Smears are prepared from the sediments and stained using Giemsa stain(WHO, 2008).

2.11.3 Lymphatic Node Aspiration

Trypanosomes may be found in the aspirate from the actively enlarged lymphatic glands. The lymph should be examined for motile organisms, stained and observed under a microscope (WHO, 2008).

2.11.4 Bone Marrow

Specimen from bone marrow obtained by sterna or pelvic puncture may be examined if blood and lymphatic gland findings are negative.

2.11.5 Kerandels Sign

When the parasites are in the brain of patients there is a delayed reaction to pain when he is pricked with a pin (WHO, 2008).

2.11.6 Serological Test

Antibodies against trypanosomes can be demonstrated in whole blood, plasma or serum. The principle of the test is the agglutination or binding of whole trypanosomes in the reagent among themselves due to the specific antibodies in the serum of the patient.

The execution on whole blood consists of placing one drop of reconstituted reagent and one drop of heparinized blood on a test card and mixing. The card is then rotated for 4 to 5 min. by a mechanical rotator. Whole blood can be replaced by 5µl of plasma or serum (WHO, 2008).

2.11.7 Immunofluorescence

The principle of the reaction is the formation of an antigen/antibody/anti-antibody complex. The antigens are trypanosomes grown in laboratory rodents. The reaction depends on the antigenic determinants of the trypanosomes being exposed to the specific antibodies, contained in the serum of the patient. If present, the antibodies will bind to the antigenic determinants to form antigen/antibody complexes bind with anti-human antibodies which have been conjugated to a fluorescein dye. Finally, the trypanosomes exposed to invisible ultraviolet rays under the microscope will transform them to visible light (WHO, 2008).

2.11.8 Indirect Hemagglutination

The principle of the test is the fixation or binding of red blood cells to purified trypanosomal antigens. The cells are then fixed and lyophilized, to be rehydrated and suspended just before the test is done. The cell suspension is then mixed with diluted serum.

If the serum contains antibodies, the red blood cells will bind to each other and settle on the rounded bottom of a small button, at the very bottom of the tube to give a negative test (WHO, 2008).

2.11.9 The Microhematocrit Centrifuge Technique (MHCT)

This technique is also called the “Woo” test. It is a relatively simple test to perform but requires a microhematocrit centrifuge, a sophisticated electrical instrument rarely available

to field teams. The test is very sensitive since it concentrates the trypanosomes from a volume of 50 to 60 microliters of blood.

Trypanosomes will appear as small swimmingorganisms close to or sometimes within the "Buffy coat", the accumulated white blood cell layer above the packed red cells (WHO, 2008).

2.11.10Cerebrospinal fluid (CSF) examination

When the trypanosomes have passed the brain barrier and extend to the CSF the patient is said to be in the second stage of the disease. The only way to determine whether thetrypanosomes have invaded the central nervous system is to examine the cerebrospinal fluid.

The lumbar puncture can only be performed by a medical officer or a specially trained nurse under the close supervision of the medical officer. Three tests are commonly done:

- Number of cells present (lymphocytes) per cubic millimeter (mm^3)
- Total protein content (mg/ml)
- Presence or absence of trypanosomes (WHO, 2008).

2.11.11 Mini anion-exchange centrifugation technique

This involves separation of trypanosomes from venous blood by anion-exchange chromatography and concentrating them at the bottom of a sealed glass tube by low speed centrifugation (WHO, 2008). Other tests include culture, PCR Assays, Imaging techniques; such as computed tomography and magnetic resonance imaging (WHO, 2014).

2.12 Chemotherapy of Human African Trypanosomiasis

Antitrypanosomal treatment is indicated for all persons diagnosed with African trypanosomiasis. The type of treatment depends on the infecting subspecies of the parasite and on the disease stage. The first drugs for both first and second stage disease are highly effective. Pentamidine is a water-soluble aromatic diamidine that has been in use since the last 80 yrs. It is effective against early-stage *T. b. gambiense* infection, but is less effective against *T. b. rhodesiense* infection, and is ineffective against late stage *T. b. gambiense* infection (Priotto., *et al.,* 2006 ; Barret *et al.,* 2007).Dosing for early-stage disease consists of a series of 7–10 intramuscular injections.However, lower dose regimens are being experimented (Chappuis, 2005). Suramin is a sulfonated naphthylamine, which has been used for many years to treat first stage *T. b. rhodesiense.*

Suramin is also effective against *T. b. gambiense,* it does not penetrate the blood-brain barrier and is not used for CNS-stage (late stage disease). It is not often used because severe reactions occur in persons who are co-infected with *Onchocerca volvulus*. Adverse reactions to suramin are frequent, but usually mild and reversible.

These include drug rash, nephrotoxicity, and peripheral neuropathy. In rare instances, suramin administration results in a hypersensitivity reaction, and, for this reason, a small test dose is usually given prior to the full first dose (Pépin and Milord, 1994). Melarsoprol is an arsenical, produced by Ernst Freidheim in the late 1940s. His initial compounds, melarsenoxide, *p*-(4,6-diamino-*s*-triazinyl-2-*yl*) aminophenylarsenoxide was complexed with dimercaptopropanol (British anti-Lewisite) to form a less-toxic complex, merlarsoprol. It is the only drug available for treatment of late-stage CNS disease of both *T. b. gambiense* and *T. b. rhodesiense*.

It is usually given as two to four series of three daily I.V. injections, or a single daily injection for 10 days (Chappuis, 2005). It is insoluble in water and must be dissolved in propylene glycol, given intravenously.

Difluoromethylornithine (eflornithine, DFMO, OrnidylR) is the most recently developed agent for late-stage *T. b. gambiense* sleeping sickness, which came into use by 1990. DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), the initial enzyme in the polyamine synthetic pathway.

It is given intravenously at 400 mg/kg I.V, every 6 hrs for 14 days DFMO is effective in children, adults and patients with melarsoprol-refractory. Also DFMO was curative in combination with many new agents as well as clinically-used trypanocides, including suramin and melarsoprol (Priotto *et al.,* 2009 and Chappuis *et al.*, 2007). DFMO in combination with Nifurtimox has cure rate of 100%.

In another related study, total doses of DFMO were reduced to 14 (two/day for 7 days) and a 94% cure rate resulted with a DFMO-nifurtimox regimen (Priotto *et al*., 2009). All of the DFMO-nifurtimox regimens were associated with significantly reduced adverse side effects as compared to melarsoprol-based therapy (Chappuis, 2005). However major adverse effects of eflornithine include bone marrow suppression, gastrointestinal symptoms, and seizures. Eflornithine is highly effective, but the major setback is the difficulty in administering 4 infusions daily especially in rural areas, it is very expensive, it is not readily available in combination with nifurtimox. Eflornithine is not effective against *T. b. rhodesiense* (East African form of the disease).

Melarsoprol, an organoarsenic compound, is the only drug available for treating second stage *T. b. rhodesiense* because of its ability to penetrate the blood-brain barrier.

Adverse reactions to melarsoprol can be severe and life-threatening. An encephalopathic reaction occurs in 5-10% of patients with a case-fatality rate of approximately 50% when it occurs. Prednisolone is often given to patients who are being treated with melarsoprol to reduce the risk of encephalopathy. Unfortunately, even when administered under careful medical attention, the treatment has a mortality rate as high as 12% (Chappuis, 2005). Other adverse reactions observed with melarsoprol include skin reactions, gastrointestinal upset and peripheral neuropathy. Intravenous injections of melarsoprol are painful and can cause phlebitis. The drug is administered by use of lengthy and complicated dosing schedules; however, an abbreviated 10-day regimen appears promising (Chappuis, 2005).

Parafuramidine (DB289).

A new drug candidate currently in use for treatment of sleeping sickness is the diamidine pafuramidine (DB289). In January 2007, parafuramidine had completed enrolment for Phase III clinical trials in the Democratic Republic of Congo and Angola (Wenzler, 2009 and Kotthaus, 2011).

Which is the final step before the compound can be registered as a drug against human African trypanosomiasis. If successful, parafuramidine would be the first orally available treatment for early stage sleeping sickness. Another approach to improve the treatment of sleeping sickness is the development of a combination therapy. Currently, the anti-Chagas disease drug, nifurtimox, is being tested in combination with melarsoproloreflornithine in a randomized clinical trial in Uganda (Delespaux and Koning, 2007 and Wenzler, 2009).

2.13 Chemotherapy of Animal Trypanosomiasis

Little progress has been made to the control of animal trypanasomiasis using drugs becausethe susceptibility of trypanosomes to a drug within the infested host depend on the intrinsic sensitivity of the parasite population, which is determined by the drug-parasite interactions (Williamson, 1976; Onyeyili and Onwualu, 1999; WHO, 2014). The lack of drug development along with the drug resistance and relapse in parasitaemia has led to an impasse in the chemotherapy of trypanosomosis.

The Trypanocides in use include;

2.13.1 Diminazine aceturate (Berenil) ®

According to Mulugeta *et al.,* (1997), 2-3 mg/kg of this drug can cure clinical manifestation of trypanosomosis in cattle. While Singyangwe, *et al*., (2004) also reported that 3.5 mg/kg body weight dose given intramuscularly (I.M) could cure naturally infected *T. evansi* in camels. Dose rates of 10 and 16 mg/kg body weight were utilized by in the treatment of *T. evansi* infections in Buffalo calves.

It was observed that at these dose rates, all the calves were cleared of parasite and no relapse occurred after splenectomy. It was observed that at these dose rates, all the calves were cleared of parasite and no relapse occurred after splenectomy. They were of the opinion that this drug brings about the rapid cure of *T.congolense* and *T. vivax* infections. Studies on diminazine treatment of experimental *T. brucei* and *T. congolense* infections have shown that the drugs become less effective as the time interval between infection and treatment increases (Onyeyili, 1999; Delespaux *et al*.,2010). The effect was not confined to diminazine alone but was also exhibited by ethidium, prothidium and isometamidium. This explanation was also advanced by P Mamoudou *et al.* (2008) who suggested that only

drugs with a high interstitial concentration will kill the parasites. While also working with vervet monkeys infected with *T. b.rhodensiense* or *T. b. brucei* in the same year he contended that diminazine reduced concentrations in patient CNS infection leads to a protracted less virulent disease course with terminal meningoencephalitis and intra cerebral persister trypanosomes.

Other investigators have also reported relapse infections following the treatment of animals with diminazine aceturate, (Egbe-Nwiyi *et al*., 2005; Clausen *et al.,* 2010). Resistant strains however may be sensitive to homidium and Isometamidium. Resistance to drugs by trypanosomes has been reported since (Arowolo and Ikede, 1980, Clausen *et al.,* 2010). The drug resistance has been ascribed to the wide spread abuse and misuse of the drug making it progressively less effective (Onyeyili and Onwualu, 1990; Desalegn, 2010).

2.13.2 Mechanism of action of Diminazine aceturate

Diminazine is one of the derivatives of diamidines. Diamidines' antiparasitic activity may be related to their interference with aerobic glycolysis as well as with DNA synthesis in the parasite (Wang,1995). All the aromatic diamidines are known to cause hypoglycemia in treated animals. Blood parasites such as trypanosomesdepend on the host's glucose for aerobic glycolysis. Apart from preventing glucose utilization by the parasites, diminazine aceturate has been known to selectively block kinetoplast DNA replication and to induce dyskinetoplasty (De Koning, 2001). Diminazine is rapidly and irreversibly bound by the trypanosome DNA-containing organelles, first in the kinetoplast and subsequently nucleus. The drug does not bind to the DNA itself, rather across the small groove between the two complementary strands, resulting in distortion of the helical structure (Delespaux and Koning, 2007).

The accumulation of replicating forms indicates that diminazine aceturate does not block the replication process at its initiation but at different specific points along the cycles. It was suggested that Diminazine does not kill trypanosomes directly, but makes them accessible to other defence mechanism e.g. macrophage system (De Koning *et al.,* 2004; Priotto and Sevscik, 2008).

2.14 Limitation of chemotherapy

The attention paid to developing chemotherapy for HAT has lagged behind compared to other tropical diseases no significant development has been made over the last 2 decades (Gehrig and Efferth, 2008). The current agents in routine clinical use have been available for more than 50 yrs. and are not ideal drugsGehrig and Efferth, 2008). Pentamidine which is very effective is generally well tolerated, but adverse reactions of hypoglycemia, injection site pain, diarrhoea, nausea and vomiting is usually observed.

Suramin is a sulfonated naphthylamine, which has been used successfully against early-stage sleeping sickness caused chiefly by *T. b. rhodesiense*. Suramin does not penetrate the blood-brain barrier and is not used for late CNS-stage disease.

Melarsoprol is an arsenical resulting from the efforts of Ernst Freidheim in the late 1940s, it is toxic, painful to administer and destroys veins after several applications, it also takes the form of reactive arsenical-induced encephalopathy in 10% of treated patients, which is often followed by pulmonary edema and death in more than half these cases within 48 hour (Babokhov *et al.,* 2013). Eflornithine is the only new agent in 58 years for clinical use of second stage HAT.

Eflornithine (DFMO) necessitates constant dosing when given as an I.V. drip. The most frequent toxic reaction was reversible bone marrow suppression, which was alleviated upon reduction of the doses (Baker *et al.,* 2013).

The major drawbacks with respect to DFMO are its cost, the duration of treatment, and its availability. However as a result of advocacy campaigns by Medicines Sans Frontiers and other organizations, coupled with financial support by Sanofi-Aventis, DFMO has been increasingly available in the field since 2001 (Pritto *et al.,* 2008).

The surface of the trypanosome is covered by variant surface glycoprotein (VSG) which is the main antigenic determinant to the human immune system. The genome contains over 1000 genes capable of coding for VSG genes which are randomly switched on and off at each generation. This immune evasion mechanism makes it unlikely that a vaccine could be developed for HAT (Engering *et al.,* 2013). However, little has been achieved so far in terms of controlling the disease, all the trypanocidal drugs used are from Europe to Africa, and are bought in foreign currency which is hard to get. The presence of uncontrolled environment containing tsetse fly and wild reservoir hosts is another limitation. Engering *et al.,* (2013) postulated that, farms thought to be cleared of the disease following treatment with the trypanocides may be re-infected from such uncontrolled environments. Also only a small percentage of infected animals when diagnosed and treated would show that eradication by chemotherapy is impossible.

Therefore, the control of African trypanosomiasis will undoubtedly involve large-scale use of anti-trypanosomiasis to achieve effectiveness (Rochon, 2011). Nevertheless, this is clearly a disease for which new and better drugs are needed. The main disadvantage of the conventional trypanocides is thattrypanosomes had developed resistance to most of them.

Nevertheless with all the challenges pose my present trypanocides, it has become necessary to research into the field of medicinal plants as chemotherapeutic source may lead to the discovery of better and less toxic drugs or even prophylactic drugs in conjunction with anti-tsetse measures (Abedo *et al.*, 2013).

2.15 Prevention and control of the insect vector

In the absence of vaccines and effective and affordable drugs, disease control relies on vector control, especially for control of animal diseases. Several different approaches have being used with eventual impacts ranging from reduction of fly populations to total eradication. Most trypanosomes are transmitted by tsetse flies, and can only become established in areas where these vectors exist. Controlling arthropod vectors is important in preventing new infections (Couston *et al.*, 2015). In endemic areas of Africa, African animal trypanosomiasis can be controlled by a tsetse fly eradication campaign, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), is being conducted in Africa.

Its goal is to eliminate tsetse flies from the continent and, with them, to eliminate most animal trypanosomes, by reducing or eliminating tsetse fly populations with traps, insecticides and other means, and by treating infected animals with antiparasitic drugs and the selection of trypano tolerant breeds of cattle to lessen the impact of trypanosomiasis (Malele, 2011). Animals given good nutrition and rested are more likely to recover rapidly than undernourished and stressed animals.

In the absence of a vaccine for trypanosomosis and with the looming threat of further trypanocidal drug resistance, the most theoretically desirable means of controlling the

disease is through controlling the vector population (Shaw *et al.*, 2014). Although complete eradication of the vector is impossible, the most successful attempts at controlling tsetse flies are likely to be at the extreme limits for survival of the fly, where both the density of the fly is low and "personal" contact with humans may be highest.

2.15.1 Chemical Control

There are several different control techniques available today, but the use of chemicals in controlling tsetse populations is still the most common method. Aerial or ground, residual insecticides such as organochlorines (DDT, Dieldrin, Endosulfan), pyrethroids (deltamethrin, permethrin, and alphamethrin), and avermectins (ivermectin) are used to target areas where human-to-fly contact are likely (Adam *et al.,* 2013). Pyrethroids are preferred because they are rapidly degraded in soil and are environmentally safe, unlike organochlorines, carbamates and organophosphates that bio accumulate in the food chain and are highly toxic to mammals and other vertebrates. Despite being effective, the use of organochlorines and organophosphates are now banned for widespread outdoor spraying. Susceptibility to insecticides varies from one species to another, and between the different classes of species. The most common form of administering insecticides is through the use of pressurized knapsacks. Over 200,000 km^2 of tsetse-infested land has been cleared by ground-spraying in West Africa, mainly in Nigeria, and this has proved to be successful and cost-effective (Barrett, 1997).Although the process is labour intensive and limited in geographical scope, the spraying is administered discriminatively to day and night resting sites during the dry season and is much more effective than indiscriminate spraying from the air or from vehicles.

2.15.2 Targets and Traps

Traps and targets are mechanical devices used to kill or weaken tsetse flies through insecticides or various trapping methods. Targets and traps are usually deployed in and around areas where human-fly contacts are greatest, such as streams frequented by villagers, or fringes of cultivated fields. All aspects of these targets and traps, from its design and color to their strategic placement, rely on understanding the biology and behavioural ecology of the various tsetse fly species. The use of traps and targets to control tsetse populations have been successful primarily because tsetse flies are k-strategists with a low rate of reproduction, and require very little sustained mortality pressure to bring about a reduction in population or even eradication from an area (De Garine-Wichatitsky, 2001; Dennis *et al.,* 2014).

Muse *et al.*, (2015) estimated that an additional mortality of 4% per day imposed on female flies was enough to cause extinction, in the absence of immigration. The traps and targets attract tsetse flies by taking advantage of their primary host-seeking behaviours, visual and olfactory stimulation. The developments of potent attractants in the last 20 years as well as the production of second-generation synthetic pyrethroid insecticides are making this form of control technique highly successful (Muse *et al.,* 2015).

There are many prototypes of traps and targets customized to attract as many tsetse flies as possible in different environments, with a strong emphasis on designs that are easy to duplicate and maintain locally. Although most traps strongly rely on chemical attractants and insecticides, some have recently been designed to attract tsetse flies based on visual stimulation alone and to kill tsetse flies through a trapping mechanism (NGU and

NG2Btraps). Although these traps may not be as efficient in attracting and killing tsetse flies, they are far more affordable and feasible to implement in resource poor settings.

2.15.3 Bush Clearing

Bush-clearing methods have been used all over west and East Africa to drastically alter tsetse fly habitation. Discriminative bush-clearing was used in Uganda to control for *G. m. centralis* by clearing taller Acacia trees in the Ankole district (FAO, 2003). In Tanzania, between 1923 and 1930, bush-clearing methods were also widely employed to stop the spread of sleeping sickness epidemic in Maswa district, where *G. swynnertoni* was prevalent (Bouyer, 2014). Similar tactics were used in Ghana to control sleeping sickness around villages where human-fly contacts were high. Despite the apparent success of these methods, it is unsuitable as a long term control measure due to the expense and speed of reinvasion, as well as the environmental damage it causes through soil erosion, decreased soil fertility, and its adverse effects on water supplies.

2.15.4 Sterile Male Insect Technique

In 2001, the Organisation of African Unity (OAU) launched a new initiative, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) to eliminate the tsetse fly from Africa.

It was planned to employ an area-wide approach using odour-baited traps, insecticide-treated targets and ultra-low volume aerial spraying of insecticides to reduce the tsetse fly population, and finally the sterile male technique to ensure total elimination of the target *Glossina* species (Muse *et al.,* 2015). The sterile male technique was successfully used in the eradication of tsetse flies and thus trypanosomiasis on the island of Zanzibar in 1997

(Muse *et al.,* 2015). This technique relies on the mating of wild females with sterile male flies.

Physiologically, female tsetse flies are only required to mate once to store sperm in its spermathecae in sufficient quantity such that fertilization can occur over its entire reproductive life. Mating with a sterile male would thus result in no offspring. However, SIT was considered to be impractical for control of high-density tsetse populations above 1,000 males per square mile due to the large number of sterilized males that would be required (De La Rocque *et al.,* 2001). For SIT to be effective, it has been estimated that 10% of the females in the population need to be inseminated, and in order to achieve that, the number of sterile males released must constitute 80% of the male population (courtin *et al.,* 2015).However, in contrast to the Zanzibar project, which worked because it was on an island (isolated area of 1,651 km^2) infested with only one tsetse fly species, the PATTEC initiative has to deal with a vast area of Sub-Saharan Africa (10 million km^2) inhabited by at least 7 different *Glossina* species are recognised as vectors for transmission of sleeping sickness. Therefore, many scientists are sceptical that the PATTEC project will succeed as similar eradication campaigns failed in the past because the tsetse fly infested areas could not be isolated. The huge costs associated with the eradication project are also a concern as most of the countries involved belong to the most heavily indebted poor countries in the world.

2.15.5 The Future of Trypanosomiasis Control

Public awareness of the dangers associated with insecticides is increasingly changing the way we treat our environment, and the way we institute environmental controls.

Consequently, efforts to introduce more environmentally friendly methods of vector control, such as the use of traps without insecticides, challenges us to understand more about the vectors that transmit the disease, as well as the ecological balance that we - as humans - strike with them. We live in a world where various technical means of control are available to address the spread of the disease. However, sleeping sickness is a disease of the developing world, where despite the multitude of control strategies, the issues have widely been neglected and abandoned.

One of the key components required to bring about effective change is to consider the sustainability of the control strategy, and to encourage local communities to take ownership over the process, thereby empowering people to take an active role in an environmentally conscious solution. Increasing knowledge through culturally sensitive education, providing technical support, and a long-term commitment of basic resources to beneficiary communities is essential for large-scale tsetse control (Simo and Rayaisse, 2015).

Alongside efforts to reduce the spread of the disease through environmental controls, there is also an urgent need to improve current surveillance and diagnostic procedures. Mortality can be drastically reduced when cases can be diagnosed early enough to prevent the progression of late-stage sleeping sickness. Training and resources are desperately needed in endemic areas for proper diagnostics and sero-surveillance.

2.16 Herbal Medicine

Herbal medicine also called botanical medicine or phytomedicine refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.Herbalism has a long tradition of use outside of conventional medicine.

It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease (Gratus *et al.,* 2009; Chen *et al* ., 2014).

2.16.1 History of herbal medicine

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC (Hassan *et al.,* 2009). Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used (Soerjato, 1996; Hassan *et al.*, 2009).

Researchers found that people in different parts of the world tend to use the same or similar plants for the same purposes. In the past 30 years, the use of herbal medicine supplements has increased dramatically. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act (DSHEA) of 1994. That means herbal supplements unlike prescription drugs can be sold without being tested to prove they are safe and effective. However, herbal supplements must be made according to good manufacturing practices.

The most commonly used herbal supplements in the U.S. include echinacea (*Echinacea purpurea* and related species), St. John's wort (*Hypericum perforatum*), ginkgo (*Ginkgo biloba),* garlic (*Allium sativum*), saw palmetto (*Serenoa repens*), ginseng (*Panax ginseng)* (Roberts *et al.,* 2007).

Plants are used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, cancer and Trypanosomiasis (Sugimachi *et al.,* 1997; Dans *et al.,* 2009; Gratus *et al.,* 2009; Fang *et al.,* 2011 and Damery *et al.,* 2011). Several plants have been reported to be traditionally used for the treatment of trypanosomiasis in Uganda and Tanzania.Someof these plants have been scientificallyevaluated such as the works of Freiburghaus *et al.*(1997; 1998;Anita *et al.*,2009 ;Abdullahi andEmmanuel, (2012). evaluated some plants used in Nupeland in Nigeria use for the treatmenttrypanosomiasis. Ethno medicine and ethno botany have long been of interest to medical researchers, physicians, the pharmaceutical industry, anthropologists and botanists (Mann *et al.,* 2010; Ugwu *et al.,* 2011). Furthermore as greater importance is now being attached to the use of locally available medicines as a means of reducing reliance on expensive imported drugs (Yusuf*et al.,* 2012).The need to investigate unexplored natural products for their medicinal properties with some urgency cannot be over-emphasized. Other reports that indicate anti-trypanosomal activities and potentials in some medicinal plants includes the works of (Talakal*et al*., 1995; Tchinda *et al.,* 2002; Wurochekke and Nok, 2004; Shaba *et al.,* 2011; Ibrahim *et al.,* 2008; Atawodi *et al.*,2011; Habila *et al*., 2011; Maas *et al.,* 2011; Gressler *et al*., 2012 and Eze*et al.,* 2012). Also strong anti-trypanosomal efficacy of some isolated compounds undergoing trials are also on the pipeline (Otoguro *et al*., 2011; Wink *et al.,* 2012; Ngulde *et al.,* 2013 ; Feyera *et al.,* 2014).

Trypanosomiasis, is a disease of major importance in human and animals, has continued to threaten human health and economic development (Priotto, 2009;WHO, 2014). *Trypanosoma bruceigambiense* and *Trypanosoma brucei rhodesiense*affects hundreds of people in sub-Saharan Africa and are responsible for the death of several hundred per year

(Ekanem, 2006).While the animal type causes great economic losses,chemotherapy is the most commonly used method of control of the disease. The few existing trypanocides are often associated with severe and toxic side effects; require lengthy parenteral administration, lack efficacy and expensive nature of drug(Legros *et al.,* 2002; Gehig andEfferth, 2008). There is urgent need to source for new, cheap and safe alternative medicines against trypanosomiasis from natural origin.

In African countries where sleeping sickness is endemic, plants have traditionally been used for generations and are still widely used to treat this ailment with possible therapeutic activities, which have not been proved scientifically. Nigeria is greatly influenced by the savannah-forest vegetation which may harbour tsetse fly which are the vector of trypanosomes, thus making the area highly endemic to trypanosomiasis. Traditional medicine in Africa has long been noted for centuries in using medicinal plants in curing human and animal trypanosomal infections (Fang *et al.,* 2011). Natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes (Abedo *et al.,* 2015).Some Nigerian medicinal plants have been evaluated for their *in vitro* trypanocidal activity (Davis *et al.,* 2004; Ekanem*etal.*,2006); Shuaibu *et al.*, 2008; and *in vivo* anti-trypanosomal efficacies in mice (Abubakar *et al.,* 2008;Atawodi *et al.,* 2011; Abedo *et al.,* 2015).

The discovery of these potent anti-trypanosomal extracts from plant has increased the great potentials of plant species to provide lead compounds for the development of new natural drugs for effective treatment of sleeping sickness.

2.17 Angiosperm

The flowering plants, also known as angiosperms the most diverse group of land plants, with 295, 383 known species classified under 416 families, they produce seeds, flowers and fruits (Hochuli and Feist-Burkhardt, 2013).Angiosperms constitute the largest vegetation of the earth's surface. Angiosperms are found on land-area from the poles to the equator, where plant-life is possible. They also occur abundantly in the shallows of rivers and fresh-water lakes, and to some extent, in salt lakes and in the sea (Christenhusz and Byng, 2016). Such aquatic angiosperms are not however, considered primitive forms, but rather derived from the enclosed condition of the seeds.

The angiosperms provide valuable pharmaceuticals, almost all medicinal are either derived directly from compounds produced by angiosperms or, if synthesized, were originally discovered in angiosperms (Fabricant and Farnsworth, 2001). This includes some vitamins for example vitamin C, originally extracted from fruits; aspirin, originally from the bark of willows (*Salix*; Salicaceae); narcotics (e.g., opium and its derivatives from the opium poppy, *Papaver somniferum* (Papaveraceae); and quinine from *Cinchona* (Rubiaceae) bark.

Some angiosperm compounds that are highly toxic to humans have proved to be effective in the treatment of certain forms of cancer, such as acute leukaemia. Vincristine from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), areused for the treatment of heart problems. Also digitalis from foxglove, (*Digitalis purpurea*;) is used as muscle relaxants while curare derived from (*Strychnos toxifera*; Loganiaceae) are used during open-heart surgery (Soltis *et al.,* 2016).

218 Fungi

Fungi are among the most widely distributed organisms on Earth and are of great environmental and medical importance (Hibbett, 2007). There are about 99,000 known species belonging to this group include micro-organisms such as yeasts, rusts, smute, mildew, molds and mushrooms (Desjardin*et al.,* 2010). Their cell walls are made of chitinDesjardin *et al.,* 2010).

Fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesise they perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange in the environment (Bhagobaty and Joshi, 2011). They have long been used as a direct source of human food, in the form of mushrooms and truffles; as a leavening agent for bread; and in the fermentation of various food products, such as wine, beer, and soy sauce (Verma *et al.,* 2009). Since the 1940s.

Fungi have been used for the production of antibiotics and more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases and insect pests (Bhagobaty *et al.,* 2010).

Endophytic fungi produce a number of substances such as antioxidants, novel antibiotics, antimycotics, immune suppressants and anticancer compounds and thus providing rich source of biologically active metabolites that are useful in medicine, agriculture, and industry (Ren *et al.,* 2006; Bhagobaty *et al.,* 2010)**.**

2.19 Silver Nanoparticles (SNPs)

Rapid industrialization and urbanization, has ledto research into nanoparticles. The field of nanotechnology is one of the new areas of research field of material science; it deals with the biotic and abiotic materials (Huang *et al.,* 2007). Nanoparticles have unique magnetic, electrical and optical properties, these depend on specific characteristics such as size, morphology and distribution (Sharma *et al.,* 2009). Nanoparticles play an important role in all fields.Due to the emergence of antibiotic resistant pathogens, there is a pressing need to search for new antimicrobial agents from natural and inorganic substances.

There are different types of nanoparticles these include metals, fibres, etc. among these silver nanoparticles have been found to have various important applications. Historically, silver is known and has been widely use to fight infections. Silver nano particles act as an antimicrobial, anti-bacterial,anti-parasitic anti-viral, anti-biotic and anti-fungal agent. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and animals at low concentrations without any side effects (Gilaki, 2010). Silver nanoparticles are also used for deactivating HIV at low concentrations with less toxicity.It is also use in the treatment of skin cancer and for reducing bleeding in trauma patients (Makarov *et al.,* 2014). There have been several reports on the use of AgNPs in the field of medicine.

The AgNPs have been used as glyconano sensors for disease diagnosis (Sastry *et al.,*1997) and as nano carriers for drugs delivery (Cheng *et al.,* 2014). Reports are also available on the use of AgNPs in water purification system, in radiation therapy (Lu *et al.,* 2012), in H2O2 sensor (Tagad *et al.,* 2013), as heavy metal ion sensors and as catalyst for reduction of dyes such as methylene blue (Edison and Sethuraman, 2012).

There are various methods for SNPs synthesis, for example; sol-gel process,chemical-precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapor deposition and biological methods etc. (Sharma *et al.,* 2009; Murthy *et al.,* 2010). Most

commonly use is the chemical method, which may be toxic, costly and difficult to procure. Hence green methods of synthesis are currently envisaged.

Plant extracts are rich sources of secondary metabolites that can easily reduce silver nitrate to silver nanoparticles. The plant molecules undergo highly controlled assembly because of their exclusive properties making them suitable for the metal nanoparticle synthesis which was found to be reliable, eco-friendly,does not require the use of toxic chemicals, cost effective and less laborious (Nalwa, 2005; Gurunathan *et al.,* 2009). Various medicinal plants have been synthesized and used for pharmaceutical and biological applications these include the works of (Huang *et al.,* 2007;Prabhu *et al.,* 2010; Gilaki, 2010; Linga and Somthromma, 2011;Ahmad *et al.,* 2011; Roy *et al.,* 2013; Benelli, 2016).

2. 20 Angiosperms and fungi used for the experiment

2. 20.1 *Annona muricata* (Linn.)General Description

Botanical Name: *Annona muricata* (Linn.)

Family: Annonaceae

Common name: Sour sop

Hausa Chop -chop

This is a small erect evergreen tropical fruit tree plant (Plate I) belonging to the family Annonaceae, growing 5 to 6 m in height. It is under-utilizedand is grown in South America and some parts of West Africa mainly for ornamental purposes and for its fruits. The leaves of *A.* contain several groups of substances collectively called annonaceous acetogenins including murihexocin and annocuricin, annopentocin A, B and C, (2,4-cis).



Plate I: *Annona muricata* (Linn)

Others include annomuricin-D-one, murihexocin A and B, (2,4-trans)-annomuricin-D-one, 4-acetyl gigantetrocin and cis gigantrionin , muricatocin A, B and C , and annohexocin . The high potency, selectivity, wide chemical with biological diversity, and effectiveness of these compounds against microbial resistance could well make them the next class of useful natural antitumor, pesticidal agents and other pharmacological effects (Jaramillo*et al.*, 2000; Ragasa, 2012). The leaves of *A. muricata* have essential oils with parasiticidal, anti-diarrhoeal, rheumatological and anti-neuralgic properties (Mishra, 2013). Also the boiled water infusion of the leaves possess: anti-plasmodic, astringent, used in the treatment of diabetes, gastric upset, jaundice and for treating kidney problems. The leaves are also hepatoprotective against carbon tetrachloride, acetaminophen induced liver damage and instreptozotocin-treated diabetic rats (Mishra, 2013).

2. 20. 2 *Chrysophyllum albidum* Linn(General Description)

Botanical Name:*Chrysophyllum albidum*(G.Don)

Family: Sapotaceae.

Common name: Africanstar apple

Hausa: Agwaluma

Chrysophyllum albidum is primarily a forest tree species with its natural occurrences in diverse ecozones in Uganda, Nigeria and Niger Republic (Nwadinigwe, 1982).

Across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa *et al.,* 2003). The plant is known as ‘Agbalumo’ in Yoruba. Several components of the tree including the roots and leaves are used for medicinal purposes (Iwalewa *et al.*, 2003).

The bark is used as a remedy for yellow fever and malaria while the leaves are used as emollients for the treatment of skin eruption, diarrhoea and stomach ache (Iwalewa *et al.*, 2005). The fruit of African star apple has been found to have a very high content of ascorbic acid with 1000 to 3,300 mg of ascorbic acid per 100 g.

It is about 100 times that of oranges and 10 times that of guava or cashew (Amusa *et al.*, 2003). Eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has been reported to have anti-nociceptive, anti- inflammatory and antioxidant activities (Idowu *et al.*, 2006)

2. 20. 3*Saba florida*(G. Don) (General Description)

Botanical Name:*Saba florida*(G. Don)

Family: Apocynaceae.

Common name: Saba (English)

Hausa Ciwo

Saba florida grows in the riparian equatorial rain forest of Africa. The plant is very abundant in undisturbed forest (Plate II), coastal areas and around Great Lake regions of Africa (Omale and Omajali, 2010) but rare in open areas. The plant is found in Ibaji and other parts of Kogi state, Nigeria. The fruit pulp is edible and it makes a refreshing sour drink. The fruit does not abscise and must be harvested when it turns yellow. The stem yields latex that is an inferior rubber.Traditionally, bark decoction is used to treat rheumatism. The leaves are utilized in Senegal to prepare sauces and condiments as salty appetizer. In Coted'Ivoire, the latex is prescribed as an adhesive for poison preparation for arrows as it hardens upon exposure. The inferior rubber produced from the latex it sometimes used to adulterate genuine rubber (Omale and Omajali 2010).



Plate II: *Saba florida* (G.Don)

In Kogi State, the latex is used as trap for birds, rats and smaller rodents. The leavesare eaten as antidote against vomiting and the bark decoctions are administered for diarrhoea and food poison. The leaves are also efficacious in the treatment of skin ulcer. The fruit is a special delicacy for the monkeys in the forest and humans are beginning to compete with monkeys for the fruit. It appears in the local market during fruiting season (Pandey *et al.*, 2006).

2.20.4*Tapinanthus globiferus* (A. Rich.) (General Description)

Botanical Name:*Tapinanthus globiferus* (A. Rich)

Family: Loranthaceae

Common name: Mistletoe (English)

Hausa Kauchi Dauruwa

Tapinanthus globiferus is a parasitic plant growing on a large number of tree species such as Kola, Citrus, Combretum, Acacia, Aloe and Terminalia as host plants (Waterberg *et al.*, 1989). It is widely spread and has been known to be very common in North Central Namibia and the tropical rainforest of Nigeria (Waterberg *et al.*, 1989). The leaves of *Tapinanthus globiferus* are generally ovate, rounded at the apex and is about 7cm long and 3cm broad with irregular pinnately arranged lateral nerves.It has many medicinal uses (Ouedraogo *et al.*, 2005; Aina *et al.*, 2010).

In Niger State, the leaves of this plant are extensively used locally for the treatment of several human and animal ailments that include stomach ache, diarrhoea, dysentery and wound.

In Tangayika, the root decoction of this plant is used to treat hard abscesses while in Congo; its dried bark powder is used for the treatment of colds and sinusitis (Katsyal and

Lamai, 2009). In Ayurvedic medicine, *Tapinanthus dodoneifolius* is used for the treatment of various diseases such as sciatica, chronic fever, rheumatism, internal worm infections, asthma, inflammations, dyspepsia, dermatitis, bronchitis, cough, constipation, greyness of hair and baldness (Spencer, 2008).

2.20.5*Cissus quadrangularis*(Linn.(General Description)

Botanical Name: *Cissus quadrangularis*(Linn.)

Family:Vitaceae.

Common name: Veldt Grape, Devil's Backbone (English)

Hausa Da'ddori.

Cissus quadrangularis (Plate III), was originally native to Bangladesh, India or Sri Lanka, but now found in South America, Africa, Arabia, and Southeast Asia. It normally reaches a height of 1.5 m and has quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide; along each angle is a leathery edge (Greenway and Bray, 2010). Toothed trilobe leaves 2 to 5 cm wide appear at the nodes, each has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red when ripe.*C. quadrangularis* has been investigated for its effects in rat model for osteoporosis and also bone fracture (Lobb, 2010). Its bactericidal effects on *Helicobacter pylori* indicate a potential use for treating gastric ulcer.Its chemical constituent includes:carotenoids, triterpenoids, and ascorbic acid. The plant also produces the resveratrol dimer quadrangularin A.



Plate III: *Cissus quadrangularis* (Linn)

2.20.6 *Ficus capensis* Fossk (General Description)

Botanical Name:*Ficus capensis* (Fossk)

Family: Moraceae.

Common name: Cape fig

Hausa: Farin bauree

The Cape fig or Broom cluster fig (Plate IV) is a widespread Afrotropical species of cauliflorousfig. This fast-growing, deciduous or evergreen tree usually reaches 5 to 12 m, but may attain a height of 35 m to 40 m (Feleke *et al.,* 2005). Large specimens develop The Cape fig or Broom cluster fig (Plate IV) is a widespread Afrotropical species of cauliflorousfig. This fast-growing, deciduous or evergreen tree usually reaches 5 to 12 m, but may attain a height of 35 m to 40 m (Feleke *et al.,* 2005). Large specimens develop a massive spreading crown,fluted trunks and buttress roots. The large, alternate and spirally arranged leaves are ovate to elliptic with irregularly serrated margins (Setshogo, 2005). Fresh foliage is a conspicuous red colour and papery.The bark of younger trees is smooth and pale greyish-white in colour. With increasing age the bark becomes darker and rough. The wood is light and soft and is not much used commercially.

All parts may exude latex, which has some traditional medicinal applications. The latex has been shown to contain ursene and oleanane triterpenoids, of which the latter may be effective in cancer treatment, while a methanolic extract from the roots is potentially effective against chloroquine-resistant malaria.



Plate IV: *Ficus capensis* (Fossk)

2.20.4*Vitellaria paradoxa* (Fossk) (General Description)

Botanical Name: *Vitellaria paradoxa* (Fossk)

Family: sapoteceae.

Common name: Cape fig or shea tree

Hausa Kadanyaa

Vitellaria paradoxa (formerly *Butyrospermum parkii*), commonly known as shea tree (Plate V) It is the only species in genus *Vitellaria* and is indigenous to Africa(Bayala *et al.*, 2009). The shea fruit consists of a thin, tart, nutritious pulp that surrounds a relatively large, oil-rich seed from which shea butter is extracted. The shea tree is a traditional African food plant.It has been claimed to have potential to improve nutrition, boost food supply in the annual hungry season, foster rural development, and support sustainable land care (Maranz, 2004).The fruits resemble large plums and take 4 to 6 months to ripen. The average yield is 15 to 20 kg of fresh fruit per tree, with optimum yields up to 45kg. Each kilogram of fruit gives approximately 400 g of dry seeds.

2.20.8 *Calvatia cyathiformis*(Batsch) (General Description)

Botanical Name: *Calvatia cyathiformis* (Batsch)

Family: Lycoperdaceae

Common name: Puff ball

Calvatia cyathiformis is distinguished from similar sized puffballs by the purple brown spore mass (Plate VI). *Calvatia cyathiformis* has a yellow-brown spore mass (Kerrigan, 2005) edible when white and firm. *Calvatia cyathiformis* looks more like round loaves of bread than a pear-shaped mushroom, found exclusively in ground Fruiting Body: 5-20 cm high.



Plate V: ***Vitellaria paradoxa*** **(Fossk)**



Plate VI: ***Calvatia cyathiforms*****(**Batsch)

It is mostly round or flattened when young becoming pear-shaped or round with a flattened top and narrowed base; white, tan or pinkish gray to light brown and smooth. When aged, the skin cracks and flaks. It has a sterile base prominent chambered white to dingy yellow or darker, persisting as a deep purplish to purple-brown cuplike structure after the spores have dispersed (Barrows *et al.*, 2008).

2.20.9*Lepista sordida* (Fr.) Singer. General Description

Botanical Name: *Lepista sordid*(Fr.) Singer

Family Tricholomataceae

Common names: Goblet

Lepista sordida(Plate VII) is a small mushroom with deeper violet colours and a thinner cap margin when mature; it is commonly pitcher or goblet. Commonly found and widely spread in Britain and Ireland. The mushroom is edible only if it is well cooked, however it is always wise to try a small portion because it is known to disagree with some people (Bas,1991).

2.20.10 *Fomitopsis pinicola* (Swartz , Fr.) (General Description)

Botanical Name:*Fomitopsis pinicola* (Swartz , Fr.)

Family: Fomitopsidaceae

Common name: Red –belt conk

Fomitopsis pinicola, is a stem decay fungi (Plate VIII),commonly known as the Red-Belt Conk.



Plate VII: *Lepista sordida*(Fr.) Singer



Plate VIII: ***Fomitopsis pinicola*** **(Swartz, Fr.)**

It is a polypore mushroom of the genus *Fomitopsis* (Bas, 1991). The species is common throughout the temperate Northern hemisphere. It is a decay fungus that serves as a small-scale disturbance agent in coastal rainforest ecosystems. It influences stand structure and succession in temperate rainforests. It performs essential nutrient cycling functions in forests. Cap is hoof-shaped or triangular, hard and tough texture (Challen *et al.*, 2003). Surface is more or less smooth, at first orange-yellow with a white margin, later dark reddish to brown and 5then frequently with orange margin. Pore surface is pale yellow to leather-brown, 3-4 pores per mm. Grows as thick shelves on live and dead coniferous or (less common) deciduous trees. Most of the stem decay (heart rot) in mature forests that results from this fungus does not interfere with the normal growth and physiological processes of live trees since the vascular system is not affected. It is classified as a brown rot, which primarily degrades cellulose in tree stands (Keller and Snell, 2002).

2. 20. 11 *Xylaria polymorpha* (Pers.) (General Description)

Botanical Name:*Xylaria polymorpha* (Pers.)

Family: Xylariaceae

Common: Dead Man's Finger

This mushroom belongs to the class of fungus knowas Ascomycetes (division Mycota known as the sac fungi (Plate IX), they are characterized by a saclike structure, the ascus, which contains anything from four to eight ascospores.

It is a common inhabitant of forest and woodland areas, usually growing from the bases of rotting or injured tree stumps and decaying wood. It has also been known to colonizesubstrates like woody legume pods, petioles, and herbaceous stems (Kerrigan *et al.*, 2005).



Plate IX*: Xylaria polymorpia* (Pers.)

Xylaria polymorpha is characterized by its elongated upright clavate or strap-like stromata, poking up through the ground much like fingers. Fruiting Body is about 3-10 cm tall about 2 cm across tough shaped more or less like a club or a finger but occasionally flattened; usually with a rounded tip at first coated with a pale to bluish or purplish dust of conidia (asexual spores), which eventually turns black on its overall surface and minutely pimpled and wrinkled with maturity. They are inedible variety (Hall *et al.,* 2003).

2.20.12 *Trametes versicolor* (Lloyd) (General Description)

Botanical Name: *Trametes versicolor* ((Lloyd)

Family: *Polyporaceae*

Common name: Turkey tail.

Trametes versicolor also known as *Coriolus versicolor* and *Polyporus versicolor* is a common polypore mushroom found throughout the world. Meaning of several colours', *versicolor* reliably describes this mushroom found in different colours. By example, due to its resembling multiple colours in the tail of wild turkey, *T. versicolor* is commonly called turkey tail. The top surface of the cap shows typical concentric zones of different colours. The flesh is 1–3 mm thick and has a leathery texture. Commonly grows in tiled layers.

The cap is rust-brown or darker brown, sometimes with blackish zones (Oba *et al.,* 2007).The cap is flat, up to 8 x 5 x 0.5–1 cm in area. It is often triangular or round, with zones of fine hairs. *T. versicolor* contains polysaccharides under basic research, including the protein-bound PSP and B-1,3 and B-1,4 glucans.

The lipid fraction contains the lanostane-type tetracyclic triterpenoid sterol ergosta-7, 22, dien-3B-ol as well as fungisterol and B-sitosterol (Yamasaki, *et al.,* 2009*)*.Polysaccharide-K (PSK) displays anticancer activity in laboratory studies and in preliminary human research (Oba *et al.,* 2007). PSK is useful as an adjuvant in the treatment of gastric, esophageal, colorectal, breast and lung cancers(Fisher, 2002). PSK adjuvants are under study for their potential to affect cancer recurrence.

2.20.13*Polyporus sangunelis*(Pers.) (General Description)

Botanical Name: *Polyporus sangunelis*(Pers)

Family: *Polyporaceae*

Common name:

Polyporus sangunelis (Plate X)is saprophobic on decaying hardwood logs and stumps, and parasitic on living hardwoods. In the Midwest and eastern North America, it is found on a wide variety of hardwoods, but it is especially found in silver maple and box elder in western North America Arora, 2008). It appears primarily on quaking aspen causing a white heart -rot growing alone or more often in clusters of two or three. Typically found in spring, but also sometimes found in summer and occasionally fall during winter and warm spells. Widely distributed in North America but much more common at east of the Rocky Mountains (Arora, 2008). It is generally semi-circular, kidney-shaped, or fan-shaped, broadly convex, becoming flat, shallowly depressed, or deeply depressed, dry, pale tan to creamy yellowish, with an overlay of large, flattened, brown to blackish scales that are

vaguely radially arranged. In old age it is sometimes whitish with reddish to black scale or develops a black area over the centre.



Plate X: ***Polyporus sangunelis*** **(Pers.)**

The thin margin initially incurved, later even.The stem is usually about 2-8 cm long, 1-4 cm thick, usually off-centre or lateral whitish above, but soon becoming covered from the base up with a velvety dark brown to black tomentum solid. The Flesh is thick and soft when young but becomes corky and tough when sliced (Hibbett, 2007).

2.20.14 *Boletus edulis*(Bull.) (General Description)

Botanical Name: *Boletus edulis* (Bull.)

Family: *Boletaceae*

Common names: penny bun or king bolete.

Boletus edulis (Plate XI) is a basidiomycete fungus, belonging to the genus *Boletus*. Widely distributed in the Northern Hemisphere across Europe, Asia, and North America, it does not occur naturally in the Southern Hemisphere (Alexopoulos *et al.,* 1996).Although it has been introduced to southern Africa, Australia and New Zealand. The fungus grows indeciduous andconiferous forests and tree plantations, forming symbiotic ectomycorrhizal associations with living trees by enveloping the tree's underground roots with sheaths of fungal tissue (Lian *et al.,* 2008). The fruit body has a large brown cap which occasionally can reach 35 cm in diameter and 3 kg in weight. Like other boletes, it has tubes extending downward from the underside of the cap, rather than gills (Rolfe and Rolfe, 1974). The stout stipe, or stem, is white or yellowish in colour, up to 25 cm tall and 10 cm thick and partially covered with a raised network pattern, or reticulations.

Boletus edulis fruit bodies contain about 500 mg of ergosterol per 100 g of dried mushroom (Brothwell and Brothwell, 1998).



Plate XI: ***Boletus edulis*** **(Bull.)**

Ergosterol is a sterol compound common in fungi. Additionally, the fruit bodies have about 30 mg of ergosterol peroxide per 100 g of dried mushroom. Ergosterol peroxide is a steroid derivative with a wide spectrum of biological activity, including antimicrobial, anti-inflammatory activity, and cytotoxicity to various tumor cell lines grown in laboratory culture(Mello *et al.,* 2006; Dentinger and Bryn 2010.

2.20.15 *Cantharellus cilarius*(Fr.) (General Description)

Botanical Name: *Cantharellus cilarius* (Fr.)

Family: *Cantharellaceae*

Common Name: golden chanterelle or girolle.

Cantharellus cilarius(Plate XII) is a fungus and probably the best known species of the genus *Cantharellus*. It is orange or yellowish in colour, meaty and funnel-shaped. Underneath the smooth cap, it has gill-like ridges that run almost all the way down its stipe. It emits a fruity aroma, reminiscent of apricots and a mildly peppery taste.Chanterelles are common in northern parts of Europe, North America, Mexico, in Turkey, Himalayas including Congo and Uganda (Dar *et al.,* 2002). Chanterelles tend to grow in clusters in mossy coniferous forests, but are also often found in mountainous birch forests and among grasses and low-growing herbs.

In central Europe, including Ukraine, the golden chanterelle is often found in beech forests among similar species and forms (Ren *et al.,* 2006). In the UK, they may be found from July through to December. Chanterelles are relatively high in vitamin C (0.4 mg/g fresh weight), (Barrows *et al.*,2008) as well as potassium about 0.5%,fresh weight (Callac *et al.*, 2005).



Plate XII: ***Cantharellus cilarius*** **(Fr.)**

It is among the richest sources of vitamin D known, with ergocalciferol (vitamin D2) as high as 212 IU/100 grams fresh weight(Rochon *et al.,* 2011). Scientific research has suggested that the golden chanterelle may have potent insecticidal properties that are harmless to humans and yet protect themushroom body against insects and other potentially harmful organisms (Cieniecka, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Unguwan Rimi in Kaduna metropolis and Samaru in Zaria

with the following coordinates (10□ 31□ 39. 263□ N; 7□ 27□ 49. 435□□and 11□ 9□ 40□ N; 39□ 21□ E

respectively.

3.2 Collection of angiosperms and fungi material

Eight angiosperms (leaves, stem, bark, fruit and root) (Table3.1) and nine fungi (mushrooms materials)(Table 3.2) were collected from the wild environs of Zaria and Kaduna local government areas at different time of the year. The angiosperms wereidentified and authenticated by Mallam Musa at the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria andvoucher number was issued to each of the plants (Table 3.1). Similarly, the fungi were identified by Dr Hannatu Musa also in Botany department.

The plant parts of *Vitellaria paradoxa* was collected in February. Plant parts of *Annona muricata* and fungi material of *Calvatia cythiforms* were collected in May. Most of the fungi materials were collected in June these include:*Trametes versivcolor, Fomitopsis pinicola, Agaricus bohisii, Boletus edulis* and *Cantharellus cibarius.* Also plant parts of *Tapinanthus globiferus* and *Acanthospermun hispidum* were collected in July.

Table 3.1: List of angiosperms collected screened for anti trypanosomal activities

Voucher Number	Plant	Family	Common name	Month of collection	Part used
900167	*Annona muricata*	Annonace	Sour sop	May	Leaves, Stem bark, Fruit bark, and Seed
7182	*Chrysophyllum albidum*	Sapotaceae	Africa star apple	November	Leaves, Stem bark, Fruit bark and Seeds
65719	*Saba florida*	*Apocy*naceae	Ciwo	August	Leaves, Stem bark Root, Fruit bark andSeeds
900107	*Tapinanthus Globiferus*	Loranthaceae	Mistletoe	July	Leaves and Stem bark
900387	*Cissus quadrangellum*	Vitaceae	Veldt Grape	August	Entire plant
900072	*Vitellaria paradoxa*	Sapotacae	Shea butter	February	Stem bark
2368	*Ficus capensis*	Moraceae	Cape fig	August	Stem bark
900051	*Acanthospermun hispidum*	Asteraceae	Bristle starbur	July	Entire plant

Fungi	Common names	Family	Monthof collection	Part used
Calvatia cythiforms	Pufff ball	Lycoperdaceae	May	Whole part
Xylaria polymorpia	Dead man's finger	Xyariaceae	August	Whole part
Trametes versivcolor	Turkey tail	Polyporaceae	June	Whole part
Fomitopsis pinicola	Red –belt conk	Fomitopsidaceae	June	Whole part
Lepista sordid	Globlet	Tricholomataceae	May	Whole part
Agaricus bohisii	----------	Agaricaceae	June	Whole part
Polyporus sangunelis,	----------	Polyporaceae	September	Whole part
Boletus edulis	King bolete	Boletaceae	June	Whole part
Cantharellus cilarius	Golden chanterelle	Cantharellaceae	June	Whole part

Table3.2: List of fungi collected screened for anti trypanosomal activities

Saba florida and *Cissus quadrangellum* plant parts were collected in August, while fungi material of*Polyporus sanguineus* was collected in September and *Chrysophyllum albidum* was collected in November.

3. 2. 2 Preparation of crude plant extract

The fungi were air dried in the oven at 40^{o}C while the angiosperm materials were air dried at ambient temperature and both were pulverized into powder using laboratory blender; pestle and mortar. The powdered extracts were separately weighed and stored in air tight container (Shaba*et al.*,2012; Umar *et al.*, 2013).

Fifty grams (50 g) of each driedpowdered plant material were sequentially extracted using Petroleum ether, methanol and distilled water as follows: 200 ml was dispensed in a round bottom flask with reflux condenser. The powdered extract was added to it andmounted on a shaker and allowed to macerate for 72 hrs. The extract was filtered and concentrated by distillation in a rotary evaporator (Perkin, UK) under reduced temperature of below 50 ^{o}C. The Mac recovered from the petroleum extracts were air dried at room temperature (27^{o}C) and refluxed exhaustively in 200 ml methanol for 72 hr(Shaba *et al.*,2012 ; Umar *et al.*2013).

Five millilitres (5 ml)of glacial acetic acid was added to the methanolic filtrate before evaporation to prevent the loss of active component (Eldahshan and Abdel-Daim, (2015). The same procedure was adopted for the aqueous extract using 200 ml of distilled water, after 24 hr the filtrate was concentrated on water bath at 50^{o}C.

All the resultant fractions were collected separately and stored in glass sample bottles in a refrigerator at 4^{o}C until required (Ugwu *et al.*,2011).

3. 3 Laboratory Animal

Albino mice were used throughout the studies. They were obtained from the laboratory animal colonies of the Nigerian Institute for Trypanosomiasis Research, Kaduna. They comprised male and female white mice of about 8 - 10 weeks old, weighing between 22 – 27 g. They were fed with pelletized growers' mash (from Ladokun Feeds Ltd.) throughout the course of the experiment. Feed and water were given *adlibitum* as previously described by Fang *et al.*(2011)and Feyera *et al.*(2014).The experiments were conducted in compliance with the internationally accepted principles for laboratory animal use and care as contained in the ABU Zaria Committee on Animal Use and Care guidelines and protocol.

3. 4 Trypanosomes

Trypanosoma brucei brucei (Federe strain) and *Trypanosome congolense* (savanna strain) were obtained from stabilates maintained in the cryogenic Tanks at the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna State, Nigeria. The parasites were maintained in the laboratory by continuous passaging in rats and mice three tines to give parasitaemia of approximately $\log10^6$ parasites/ml at days 4–8 and 7-11 for *Trypanosoma brucei brucei* and *Trypanosome congolense* respectively post inoculation.

3. 5 Daily Assessments of Parasitaemia in Infected Animals

The daily parasitaemia in infected animals was assessed using the rapid matching method of Herbert and Lumsden (1976). The tail of each infected animal was disinfected with methylated spirit before it was punctured with a sterile needle. A drop of blood was expressed on a glass slide and covered with cover slip.

The parasitemia was determined by examination of the wet mount microscopically at X40 magnification using the "rapid matching" method which involves the counting of parasite per field of the microscope. The counted trypanosome number was then compared with the standard table provided by Herbert and Lumsden (1976) from where the corresponding number of trypanosomes per ml of blood was extrapolated.

3.6Harvesting blood stream Trypanosomes using ion Exchange Chromatography.

At peak of each parasitaemia such as 10^8/ml, the mouse was sacrificed and blood recovered cardiac puncturing with a heparinised needle and syringe. The recovered blood was carefully dispensed into a heparinised tube. Parasites free of heamoglobin were isolated using ion exchange chromatographic method as described by lanham and Godfery (1970). The DEAE cellulose was pre swollen in 10% glucose phosphate buffer saline and transferred into a column of 5x1cm whose outlet was blocked with cotton wool. The column was mounted on a retort stand and a clean beaker was placed under it, equilibrated with Phosphate buffer saline glucose.The recovered blood with trypanosomes was dispensed into the column and the trypanosomes were eluted with glucose phosphate buffer saline, while the red cells stock to the resin.

While the parasites came down in the eluent as a thick white suspension. The column was continually washed until the eluent was transparent again.

3. 7Preparation of Culture Medium and Determination of*In vitro* Activity of Crude Extract

The culture medium for the cultivation of the bloodstream forms consist of 70% of Dubellco Modified Eagles Medium (DMEM), 20% of fetal calf serum, 2% of D-glucose, (1g $NaHCO_3$, and 0.5 mg non-essential amino acids) stirred gently on a magnetic stirrer. The stock medium was further supplemented with 0.2 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.1 mM hypoxanthine, 100 µl/ml of antibiotics i.e. gentamicin tetracycline or streptomycin. The whole mixture was further stirred thoroughly and the PH of the medium was adjusted to 7.8 by gradual addition of HCl to the medium until the desired PH is met (Freiburghaus *et al.*, 1997).

Before performing an assay, all solutions of crude plant extracts were freshly prepared. The lipophilic extracts were first dissolved in 10% Dimethyl Sulphur Oxide (DMSO). One milligram of each extract was dissolved in 1ml of the constituted trypanosome culture medium to give a concentration of 1mg/ml (stock solution). Each plant or fungal extracts was tested twice in duplicate in 96 well microtiter plate (Costar, USA) in four fold serial dilution using stock media of DMEMto get various concentrations of 1000, 250, 62.5, 15.6, 3.9, 0.98 and 0.2 µg/ml. fifty microliters of the prepared medium above containing each extract were dispensed into a well of the microtiter plate in duplicate using a micropipette, followed by inoculation of 50 µlcontaining approximately 10^6 trypanosome per well to give a final volume of 100 µl. Positive and negative control wells were also included as previously done by Rosas *et al.* (2007).

After incubation at 27^oC for 12 hr in the presence of 5% CO_2 generated using 5CG-Desicator as described by Bulus *et al*. (2012) as follows: About 5 g of calcium carbonate was transferred into a glass bottle, after which 2M hydrochloric acid was pumped from a wash bottle into the glass bottle (A) into a reaction glass unit (B).

The gaseous carbon dioxide released was passed through rubber tubing into a polythene gas reservoir (C) of 1liter capacity, until it was fully inflated. The micro titre plate was carefully kept in a desiccator of 5, 500 litre capacity. The Co_2inflated bag was carefully released into the desiccator and covered tightly.

The Minimum Inhibitory Concentration (MIC), which is defined as the lowest concentration of crude plant extract, in which no trypanosomes with normal morphology or motility could be found, was determined microscopically by viewing several field of a drop of each concentration under X 40 objectives (Kuypers *et al*. 2006 ; Rosas *et al.*,2007).

3. 8 Phytochemical screening of crude extracts

Chemical tests were carried out on *Chrysophyllum albidum, Tapinanthus globiferus,Formtopsis pinicola, Saba florida and Cantharellus cibarius* as described by Trease and Evans (1989); Sofowora (1993) and Sayeed (2007).

3. 8. 1 Test for Tannins

a. Test with Iron salts: One gram of each extract sample was separately boiled with 20 ml of distilled water for five minutes 50^{o}C in a water bath and filtered, while hot. 1ml of the cool filtrate was distilled to 5 ml with distilled water and 3 drops of 10% ferric chloride were added. Bluish-black or brownish-green precipitate indicated the presence of tannins.

b. Phenazone test: Aqueous extract of (5 ml) was mixed with 0.5 gm of sodium hydrogen phosphate, the solution was boiled at 50^{o}C, cooled and filtered. The filtrate was treated with 2% solution of phenazone, drop wise to form bulky precipitate which indicate presence of tannins.

3. 8. 2 Tests for Saponins

a. Frothing test:One gram of extract was boiled at 50^{o}C with 10 ml of distilled water in a water bath for 10 mins. The mixture was then filtered allowed to cool and shaken vigorously for 2 mins, frothing indicated the presence of saponin in the filtrate.

b. Emulsifying test: Two drops of olive oil and 10 ml distilled water was added to another 2.5 ml of the filtrate, it was shaken vigorously for a few minutes and formation of a fairly stable emulsion indicated the presence of saponins.

3. 8. 3 Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of each material sample was boiled with1% aqueous hydrochloric acid was taken as evidence for the phlobatannins.

3. 8. 4 Tests for Steroid and Terpenoids

a. Salkowavaski Test: 5 ml of each extract was mixed with 2 ml of chloroform; 3 ml of Conc. H_2SO_4 was then added to form a layer. A reddish brown precipitate colouration at the interface indicates the presence of terpenoids.

b. Antimony trichloride test: 1gram of the dry extract was added to 2 ml of chloroform followed saturated solution of antimony trichloride in 20% acetic anhydride. Formation of pink color on heating indicates presence of steroids and triterpenoids.

3. 8. 5 Tests for Flavonoids

a. Ammonia test:Filter paper was dipped in methanolic extract solution of each plant extract and exposed to ammonia vapour. Formation of yellow spot on filter paper indicates the presence of flavonoid**.**

b. Shinoda test:To 2 ml of the methanolic extract Magnesium turning and 0.5 ml dilute Hydrochloric acid was added, formation of red colour indicates the presence of flavonoids.

3. 8. 6 Tests for Cardiac Glycosides

a. Keller Killiani test:5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, 1ml of concentrated sulphuric acid was also added in drops. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed indicating the presence of cardiac glycosides.

b. Legal test:To 2 ml of extract solution, equal volume of water and 0.5 ml of strong lead acetate solution was added, shaken and filtered. Filtrate was extracted with equal volume of chloroform and the chloroform extract was evaporated to dryness. The residue was dissolved in 2 ml of pyridine and sodium nitropruside 2 ml was added followed by addition 2 ml sodium hydroxide solution to make alkaline. Formation of pink colour indicates the presence of glycosides or aglycon moiety.

3. 8. 7 Testsfor Anthraquinones

a. Borntragor's Test: 1gram of each extract was boiled with 2 ml of 10% hydrochloric acid for 5 mins. The mixture was filtered while hot and allowed to cool. The cooled filtrate was partition against equal volume of chloroform. The chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour. A change in colour to bright rose pink indicates the presence of an anthraquinone.

3. 8. 8 Test for free Anthraquinones

5 ml of Chloroform was added to 0.5 g of each extract. The resulting mixture was shaken for 5 mins and filtered; equal volume of 10% ammonia solution was added to the filtrate. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

3. 8. 9 Test for Steroids

Liebermann Bruchard test: One gram of the powdered extract was treated with three drops of acetic anhydride in a test-tube and Concentrated H_2SO_4 was carefully added to the side of the test tube. The presence of a brown ring at the boundary of the mixture was taken as a positive result.

3. 8. 10 Test for Alkaloids

One gram of each sample of was separately boiled with water followed by addition of 10 ml hydrochloric acid, added on a water bath and filtered after boiling. The pH of the filtrate was adjusted with ammonia to about 6-7. Few drops of the following reagents Picric acid, Mayer's reagent and Wagner's reagent were added separately to about 0.5 ml of the filtrate in three different test tubes. Formation of turbidity, creamy white and reddish brown precipitate observed indicates the presence of alkaloids.

3.8.11 Test for Carbohydrates

Molish test: Aqueous solution of extract carbohydrate mixed with few drops of Molish reagent (alpha naphthol) and Concentrated H_2SO_4 was added from side wall of test tube. Formation of purple coloured ring at junction indicates presence of carbohydrates.

3.8.12 Test for Proteins

Millons test: The Millon reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. White precipitate is produced, which turns red after heating for 5 minutes on water bath.

3. 9Biosynthesis of Silver Nanoparticles Using Extracts of *C. Albidum*and *T. globiferus*

The aqueous seed extract of *Chrysophyllum albidum* and leave extract of *Tapinantus globiferus* were used for the bioreduction` process as follows: 20 ml of each the extract was treated separately with 80 ml of aqueous 1mM AgNO3.

The solution was boiled in a conical flask on water bath at 80^{o}C for 15 min. Appearance of brownish yellow colored solution indicates the formation of silver nano particles(John *et al.*, 2014).

3. 10 Bioassay Guided Chromatographic Studies

3. 10.1 Liquidliquid Extraction

The methanolic extract of *Chrysophyllumalbidum* and *Tapinantus globiferus* was partitioned with water against solvents in order of increasing polarity as follows i.e. hexane: water 1:1 (50:50 ml) chloroform: water1:1(50:50 ml), and butanol: water 1:1(50:50 ml) as described previously by Sulsen*et al.*(2011). The recovered partitioned extracts were tested for trypanocidal activity.

3. 10. 2 Thin-layer Chromatography studies (TLC) (Izumi *et al.,* 2011).

This technique is used for the initial screening of the plant for extract, column fractions and checking the purity of compounds. In this study, pre coated silica gel plates were used.

The plates were always kept in desiccators and activated in the oven at 110 ^{o}C for one hour when needed. Some of the plates were also prepared manually by dissolving 10 grams of silica gel is mixed with 1 gram of calcium sulphate binder distilled water is carefully added until the desired consistency was reached. The slurry was carefully used to coat microscopic glass slides by using a class rod to roll the mixture over the glass plate and were air dried for about 45 minutes. The procedure was as follows: A small spot of the solution was applied on the activated silica plate with a capillary tube just 1 cm above the lower edge of the plate.

The spot was allowed to dry and a straight line was drawn 2 cm below the upper edge of the activated plate which marks the upper limit of the solvent flow. The solvent system was mixed and poured into the tank, covered and allow to saturate the plates was carefully placed in the tank taking care the sample spots were above the solvent system. The lid of the tank was placed again. The plates were left to develop after which the Rf value was determined. The Rf (retention factor) is defined as the distance travelled by the compound divided by the distance travelled by the solvent. The plates were viewed under UV light of various wavelengths as well as treated with suitable reagents to detect the compounds.

$$Rf = \frac{\text{distance travelled by compound}}{\text{Distance travelled by the solvent front}}$$

3. 10. 3 ColumnChromatography of Butanolic Extract of *Chrysophylum albidum*

A slurry was first prepared by dissolving 120 g of Silica gel of mesh size 200-300 in 200 ml methanol and was packed into a column size (2 cm x 50).Eighteen grams of the butanolic extract of *Chrysophyllum albidum* was dissolved in 50 ml of methanol and was loaded in the column, the tap at the end of the column was opened and was use to adjust the flow rates previously described by Ali *et al.*(2011). Based on the result obtained from the Thin Layer Chromatography of the butanolic fraction, the gradient solvent system used to elute the eluents from the column was chloroform and methanol of various ratio of increasing polarityas follows:

(a) 100 : 0 ml, (b)95 : 5 ml, (c) 90 : 10 ml, (d) 80 : 20 ml, (e) 70 : 30 ml, (f) 50 : 50 ml, (g) 0 : 100 ml.

The mobile phase passes through the column under the force of gravity. The eluents were collected at 0.5 ml per minute in a 20ml beaker and followed by TLC screening.

Similar fractions were pooled together, concentrated under pressure by rotary evaporator (Beer *et al.,* 2016). Each fraction was also constituted and tested for trypanocidal activity *in vitro* as described earlier in (3.7) above.

3. 10. 4 Column Chromatography of Butanolic Extract of *Tapinantus globiferus*

The sameprocedures in 3. 9. 3 were adopted. Twenty grams of the butanolic extract of*Tapinantus globiferus* was dissolve in 20 ml of methanol and applied on silica gel column as follows: the “dry packing” method was used this involves coating the dissolve extract with 5 g of the stationary phase (silica gel) introduced into a porcelain dish and

mixed with a spatula until even dispersion was formed. The methanol evaporates quickly and the sample becomes dry again. The sample was carefully introduced into the column. Gradient elution procedure was used to elute the fractions, starting with chloroform followed by mixtures of chloroform: methanol of increasing polarity. Chloroform : Methanol (a) 100: 0 ml, (b) 95 : 5 ml (c) 90 : 10 ml, (d) 80 : 20 ml, (e) 70 : 30, (f) 50 : 50 and 0 : 100%. The choice of solvents was based on the result obtained from the Thin Layer Chromatographic of butanolic fraction.

3. 10. 5 Preparative Thin layer Chromatography Studies (PTLC) (Rosas *et al.*,2007).

Preparative Thin Layer Chromatographic technique is routinely used in separating compounds and for final purification.PTLC were made on glass plates coated with silica gel (kiesel gel 60 PF2540) as described for Thin Layer Chromatography, except that glass plates measuring 20 x 20 cm diameter plates were used. PTLC was carried out on fraction S2 obtained from Sephadex LH-20 column in order to isolate the active compound from each of the plant extract.

The plates were thoroughly washed and dried in the oven. Each was swabbed with acetone and allowed to dry. Forty grams of Silica gel was mixed with 80 ml of water in a conical flask, and the flask was gently shaking to form homogenous slurry which was then evenly spread over the plates with the help of a spreader (wet thickness 1 mm) 1t was allow to dry used. The spotted plates were developed in the saturated tank for an hour. The plates were then removed and air dried. It was visualised in daylight and UV light at various wavelength (2.54-3.54). The different spots or separations and colours were noted, scooped and dissolved in methanol and the desired isolate were collected separately using a fritter

funnel and dried. The resulting soluble substance was prepared and tested for trypanocidal activity *in vitro*.

3. 10. 6 Sephadex LH-20 Column

Fraction was passed through Sephadex LH-20 column as follows:

10 gm of the Sephadex powder was soaked in 20 ml methanol for 3 hrs. The media slurry was poured into a clean glass column of size 0.5 x 30 cm in one continuously motion taking care not to introduce air bubbles.

The out let was open and methanol was used to elute the fractions which Were collected in 2 ml and the eluent were monitored with Thin Layer Chromatography similar fractions were pulled together, evaporated and tested for trypanocidal activity.

3.11 GC-MS Analysis

GC-MS analysis was carried out on a GC system 7890D, MSD59775977A, Agilent technology made in USA. Comprising a AOC-20i auto sampler and gaschromatograph interfaced to a mass spectrometer instrumentemploying the following conditions: column Elite-1 fusedsilica capillary column (30 x 0.25mm ID x 1μMdf, composedof 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gasat a constant flow of 1 ml /min and an injection volume of 0.5μI was employed (split ratio of 10:1) injector temperature 2ºC; ion-source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with anincrease of 10ºC/min, to 20ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Softwareadopted to handle mass spectra and chromatograms was aTurbo Mass Ver 5.2.0.

3.11.1 Identification of Components

Interpretation on mass spectrum GC-MS was conducted usingthe databaseof Multi-User Science Research Laboratory having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of theknown components stored in the National Institute Standard and Technologylibrary (NIST). The name,molecular weight and structure of the components of the testmaterials were ascertained.

3. 12*In vivo* Studies

3. 12. 1 Experimental Animal

Laboratory bred Swiss albino mice of both sexes weighing between 20-25 g each (8-12 weeks old) were obtained from the animal colonies department, of Nigerian Institute for Trypanosomiasis,Kaduna. They were housed in group of 5 in each clean cage in a well-ventilated room.They were acclimatized for the period of 7 days before conductingany experimental procedure during which they were fed with rat pellets and water *ad libidum*.

3. 12. 2 Toxicity studies

Based on the *in vitro* result obtained, the butanolic extract of *Chrysophyllum albidum*and *Tapinantus globiferus* were tested for toxicity studies in albino mice using the method of (Lorke *et al.,* 1983) as follows: three groups (A B C) of four mice each were administered 100, 500 and 1000 mg/kgintraperitoneally with butanolic extracts of*Chrysophyllum*

*albidum*and *Tapinantus globiferus* intraperitoneally. The fifth group (D) served as control and was only administered phosphate buffer saline. Physical activities like grooming, excitation, fatigue, diarrhoea,hyperactivity, sedation, respiratory arrest, convulsions, motor activity, mortality and other signs of toxicity manifestations. The animals were observed initially after every one hour for 4 hrs, then 12, 24, 48, 36 and 72 hrs after administration of different doses of the extract.

The lethal dose (LD50) was determined using the method of Lorke *et al*.,(1983)as follows:

LD_{50}= Sum (dose of difference x dead mean) (Lorke *et al*., 1983).
2

3. 12. 3 Infection of experimental animal and *in vivo*anti-trypanosomal activity of the plant extracts.

The butanolic extract and synthesised nano particles of *Chrysophyllum albidum* seedwere tested for *in vivo* trypanosomal activity.

The choice of concentrations was computed based on the results obtained from the toxicity studies. Experimental animals were infected with approximately $7x10^6$ trypanosome /ml and were divided into 13 groups of 5 animals each; parasiteamia was monitored daily as described in 3.4 above. At the peak of parasiteamia ($31x10^6$–$63x10^6$trypanosome/ml) treatment commenced intraperitoneally with different doses as follows: Group (A1- A3) treated with butanolic extract with 100, 500 and 1000 mg/kg/day respectively for seven consecutive days. (B1- B3) were treated with 100, 500 and 1000 mg/kg/day of synthesized silver nano particles.The prophylactic groups (C1- C2) were treated with 50 and 100 mg/kg body weight with butanolic seed extract of *C. albidum* while (D1-D2) were treated with 50 and 100 mg/kg body weight withsynthesised nano particles, of aqueous seed extract of *C. albidum,*before they were infected with the parasite.

Parasiteamia was also monitored daily. Group (E) were treated with Diaminazine while group (F) serve as negative control and (G) serve as positive control group. All Infected animals were closely monitored by counting the number of parasites daily (Nok *et al.*, 1993).

3.13 Determination of packed cell volume (PCV)

The PCV of each mouse treated in groups A1-A3 and B1-B3 was monitored on days 0,7,14 and 28 blood was collected from the tail of each mouse in heparinized micro haematocrit capillary tubes.

The tubes were immediately sealed and placed in a micro hematocrit centrifuge and were centrifuge for 5 min at 10000 rpm. After centrifugation, the height of the red blood cell column was determined by use of haematocrit reader and compared to the total height of the column of the whole blood (Ajibade *et al.*, 2012).

3. 14 Characterization of Silver nanoparticles from *Chrysophyllum albidum* and *Tapinantus globiferus*

3. 14.1 UV-visible adsorbance spectroscopy analysis

The characterization of the synthesizednanoparticles were carried out using UV- visible spectroscopy by Unicam Helios (λ) spectrophotometer as follows: the aliquot of the sample was diluted with distilled water and the reduction of silver ions was monitored from 200 nm -800 nm. The spectral data recorded was then plotted (Perni *et al*., 2014).

3. 14.2Fourier-Transform infrared spectroscopy (FTIR)

Five milligrams of each dried synthesized silver nanoparticle powder was added to 10 ml of deionized water separately at 10, 000 rpm for 15 min and the pellet was washed three times with 20 ml of deionized water.

The resulting purified suspension was completely dried and analyzed by Fourier-Transform Infrared,In order to obtain good signal/noise ratio) (Khan*et al.,* 2013).

3. 14.3 Scanning Electron Microscopy (SEM) Analysis

Five drops of the synthesized silver nano particle of *C. albidum*in colloidal form was samples were prepare on a separate glass plates and allow drying completely at room temperature.

The morphology and size of the synthesized silver nanoparticles were characterized by using high resolution Scanning Electron Microscope (SEM) (Ortega *et al.,* 2013), (Model 6390 LV JEOL Asia PTE Ltd., Singapore/JEOL JSM).

3. 14. 4 X-Ray diffraction studies (XRD)

The crystallinity and the lattice properties of the silver nanoparticles, was carried out using 5 mg of each dried silver particles coated separately on to carbon film and tested on X-ray defractometer (P Analytical, Philips PW 1830, The Netherlands). It was operated at 40 kV and at a current of 30mA with Cu Kα radiation (λ= 1.5404 Å). Theaverage particle size was calculated using Debye – Scherrer's Equation $D = K\lambda / \beta \cos\theta$ where K= constant, λ = wavelength of the X-rays, β = full width halfMaximumof the XRD.

CHAPTER FOUR

4.0 RESULTS

4.1 *In vitro*trypanocidal Activities of Crude Extracts

Table4.1 showed the *in vitro* results after 12 hr of incubation of 78 fragments of petroleum ether, methanol andaqueous extracts from 27 plant materials obtained from angiosperm (leaves, stem bark, root and seed) and fungi that were tested for trypanocidal activity against*T. brucei brucei* and *T.congolense*.Seven methanolic extracts (8.97%): of *Chrysophyllum albidum* seed, *Saba florida* roots, *Tapinantusglobiferus* leaves, *Formitopsis pinicola,Boletus edulis* and *Cantharellus cilarius* had minimum-inhibitory concentration at 15.6 µg/ml on *Trypanosoma congolense* and *Trypanosoma bruceibrucei*. Petroleum ether extracts of *Boletus edulis* (1.15%) and *Cantharellus cibarius* (1.15%) had minimum inhibitory concentration at 15.6µg/ml on *Trypanosoma congolense* and *Trypanosoma brucei brucei*respectively.

While Figure 4.1 showed summary of percentage inhibitory activities of extracts. Nine extracts (11.53%): Petroleum ether extract of *Saba florida* leaf, *Tapinanthus globiferus* leaves, *Xylaria polymorpia,* fruit bark of *C. albidum*, *Polyporus sangunelis* others include methanolic extract of *Cissus quadrangellum*,fruit bark of *Annona muricata, Vitellaria paradoxa* and *Boletus edulis* had activity on *T. brucei* only at 62.5 µg/ml. Furthermore, seven extracts (8.97%): Petroleum ether extracts of: fruit bark, seed, of *Saba florida*; *Acanthospermun hispidum; Formitopsis pinicola*; aqueous extracts of fruit bark of *Annona muricata; Saba florida* leaves and *Formitopsis pinicola* had activity at 250 µg/ml on both *T. congolense* and *T. brucei.*

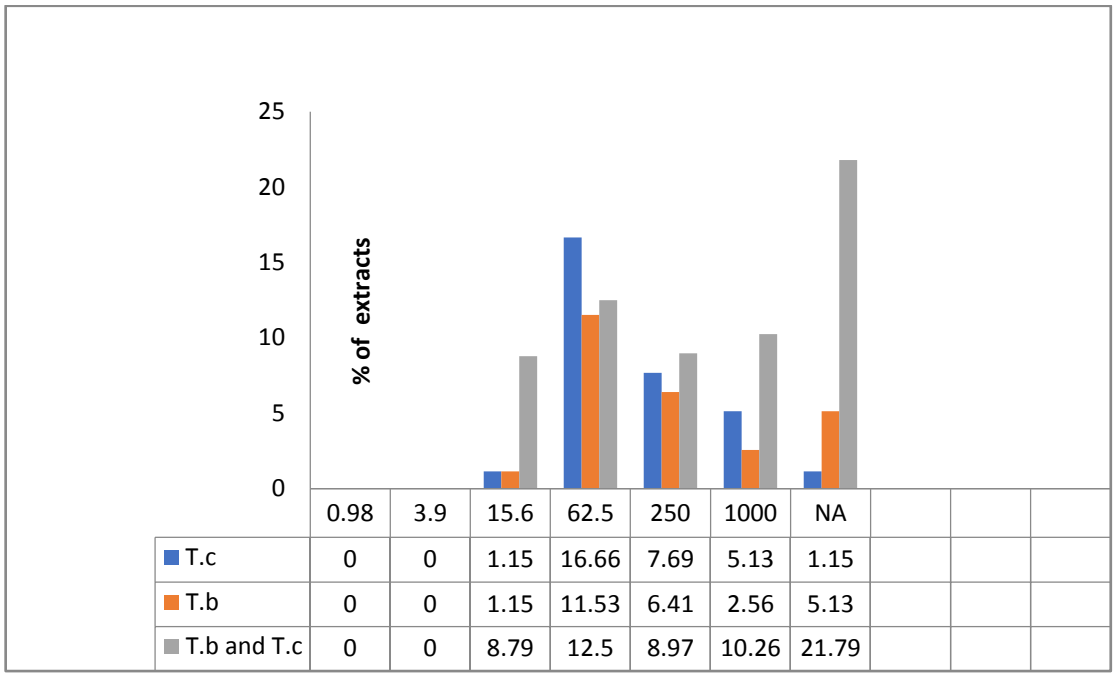
Table 4.1: Minimum Inhibition Concentration (MIC) of all extracts recovered from Angiosperm and fungi, tested against *Typanosoma brucei brucei* and *Trypanosoma congolensein vitro*

Plant name	Part of plant	Petroleum(µg) extract		Methanolic(µg) Extract		Aqueous(µg) Extract	
Angiosperms		*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*
Annona muricata	Leaf	NA	NA	62.5	62.5	62.5	62.5
	Stem bark	NA	NA	62.5	62.5	250	62.5
	Fruit bark	1000	1000	250	62.5	250	250
Chrysophyllum albidum	Leave	62.5	62.5	62.5	1000	62.5	NA
	Stem bark	1000	1000	62.5	62.5	62.5	62.5
	Fruit bark	250	250	62.5	62.5	NA	250
	Seed	250	250	3.9	3.9	3.9	3.9
Saba florida	Leaf	250	62.5	62.5	62.5	250	250
	Stem bark	NA	NA	62.5	62.5	62.5	62.5
	Root	NA	NA	15.6	15.6	62.5	62.5
	Fruit bark	NA	NA	1000	NA	NA	NA
	Seeds	1000	1000	15.6	15.6	1000	1000
Tapinanthus globiferus	Leaf	250	6 2.5	15.6	15.6	62.5	250
	Stem bark	NA	NA	62.5	62.5	250	1000
Cissus quadrangellum	Entire plant	1000	NA	250	62.5	NA	NA
Vitellaria paradoxa	Stem bark	NA	NA	62.5	62.5	62.5	62.5
Ficus capensis	Stem bark	NA	NA	1000	NA	1000	1000
Acanthospermun hispidum	Entire plant	250	250	62.5	62.5	62.5	62.5

Table 4.1
continued

Fungi	Part of Plant	Petroleum) Extract (µg)		Methanolic(µg) Extract		Aqueous(µg) Extract	
Calvatia-cythiforms	Entire plant	1000	1000	1000	250	NA	NA
Xylaria-polymorpia	Entire plant	62.5	62.5	NA	NA	NA	NA
Trametes-versivcolor	Entire plant	62.5	62.5	NA	NA	1000	1000
Fomitopsis -pinicola	Entire plant	250	250	15.6	15.6	250	250
Lepista sordid	Entire plant	NA	NA	62.5	62.5	NA	NA
Agaricus bohisii	Entire Plant	NA	NA	NA	NA	1000	1000
Polyporus-sangunelis	Entire Plant	62.5	62.5	62.5	62.5	62.5	250
Boletus edulis	Entire Plant	15.6	250	15.6	15.6	62.5	250
Cantharellus cibarius	Entire Plant	62.5	15.6	15.6	3.9	62.5	250

Various concentrations of extract use: 1000, 250, 62.5, 15.6, 3.9, 0.98 and 0.2 µg/ml
Key-**NA=no active in all concentrations use.**



	0.98	3.9	15.6	62.5	250	1000	NA			
T.c	0	0	1.15	16.66	7.69	5.13	1.15			
T.b	0	0	1.15	11.53	6.41	2.56	5.13			
T.b and T.c	0	0	8.79	12.5	8.97	10.26	21.79			

Figure4.1: Minimum Inhibitory Concentration (MIC) of extracts at various concentrations on *T.brucei*and *T.congolense*

Keys

T.c-*Trypanosoma congolense*
T.b-*Trypanosoma brucei brucei*

Six extracts (7.69%):Petroleum ether extracts of leaf; methanolic extract of fruit bark of *A. muricata; C. quadrangellum;* aqueous extracts of fruit bark of *A. muricata*; stem bark of *T. globiferus* had activity on *T. congolense* only. Also five extract (6.41%): petroleum ether extract of *Boletus edulis,* methanolic extract of *Calvatia cythiforms,* aqueous extracts of *Polporus sangunelis, Boletus edulis* and *C. cilarius* had activity only on *T. brucei*only at 250 µg/ml.Furthermore, extracts that had minimum inhibitory anti-trypanosomal activity at 1000 µg/ml on both *T. congolense* and *T. brucei* were observed with eight extract (10.26%): Petroleum ether extracts of fruit bark of *Annona muricata*, stem bark of *Chrysophyllum albidum, Saba florida* seed, *Calvatia cythiforms,* aqueous extracts of *Saba florida* seed and *Ficus sur*.Ten extracts (12.8%): Petroleum ether extract of *Trametes versivcolor;* methanolic extracts of leaves, stembark of *Annona muricata*; leaves, stembark, of *Saba florida;* stembark of *Tapinanthus globiferus; Lepista sordida;* stem bark of *C.albidum;* others include aqueous stem bark, root, of *Saba florida* and stem bark of *Vitellaria paradoxa*which had activity on both *T. congolense* and *T. brucei* at 62.5 mg/ml.

Thirteen extracts (16.66%): Petroleum ether extracts of *Xylaria polymorpia*, of *C. albidum* leaves, *Xylaria polymorpia, Polyporus sangunelis, Boletus edulis,* methanolic extracts, of*Vitellaria paradoxa, Acanthospermun hispidum, Cantharellus cilarius,* aqueous leaf extract of *T.globiferus, Acanthospermun hispidum,C. albidum* leaves,*Polyporus sangunelis* and *Boletus edulis* had activity at 62.5 µg/ml on *T. congolense* only. Other extracts include aqueous extract of *Trametes versicolor* and aqueous extract of *Agaricus bohisii*. Also, two extract (2.56%); methanolic leaf extract of *Chrysophyllum albidum*, and aqueous stem bark extract of *Tapinantus globiferus* had trypanocidal activity at 1000 µg/ml on *Trypanosoma brucei brucei* only.

Similarlyfour extract (5.13%); Petroleum ether of *Cissus quadrangellum*, methanolic extracts of fruit bark extract of *Saba florida,* stem bark of *Ficus capensis* and extract of *Calvatia cythiforms* had activity on *Trypanosoma congolense* only at 1000 µg/ml. Seventeen extracts (21.79%) showed no antitrypanosomal activities on both *Trypanosoma brucei brucei* and *Trypanosoma congolense* these include: petroleum ether extracts of leaf, stem bark of *Annona muricata, Vitellaria paradoxa, Lepista sordid,Ficus capensis, Agaricus bohisii.* Also methanolic extracts of *Ficus capensis*, *Xylaria polymorpia,Trametes versicolor* and *Agaricus bohisii* showed no activities on bothparasites.Other extracts that did not exhibit any activity on both parasites include aqueous extracts of *Xylaria polymorpia, Cissus quadrangellum, Calvatia cythiforms* and *Lepista sordid*.

One extract (1.15%) did not show activity on *Trypanosoma congolense* this was fruit bark of *Chrysophyllum albidum*. Finally four extract (5.13%); petroleum ether extract of *Cissus quadrangellum,*methanolic extracts of fruit bark of *Saba florida,* stem barkextract *of Ficuscapensis,*and aqueous leave extract of *Chrysophyllum albidum,* had no activity on *Trypanosoma brucei brucei.*

4.2 Phytochemical Profile of the most Active methanolic Extracts*In vitro*

Various concentration of phyto-chemical level of chemical composition were exhibited by both plant materials. *Chrysophyllum albidum* showed highest content level of alkaloids (++), while *Tapinantus globiferus, Saba florida, Formitopsis pinicola and Cantharellus cilarius* showed low content of alkaloids (+). Also *Chrysophyllum albidum, Tapinanthus globiferus, and Saba florida*exhibited highest concentration of saponin followed by *Formitopsis pinicola* which showed moderate level (+), while *Cantharellus cilarius* did not show the presence of saponins, cardiac glycoside was moderately present in *Chrysophyllum*

albidum, Formitopsis pinicola and *Cantharellus cilarius* while it was absent in *Tapinanthus globiferus* and *Saba florida*.

Anthroquinones was absent in all the screened plants but moderately present in *Cantharellus cilarius*. Flavonoid was moderately present in *Tapinanthus globiferus* and *Cantharellus cibarius* while it was absent in *Chrysophyllum albidum, Saba florida* and *Formitopsis pinicola*. Terpenoides was moderately present in *Chrysophyllum albidum, Saba florida* and *Cantharellus cilarius* while it was absent in *Tapinanthus globiferus, Formitopsis pinicola* and *Tapinantus globiferus*. Phlobatanins was present in four of the plant extract except in *Tapinanthus globiferus*. Furthermore, tannins were present in all the plant materials but absent in *Formitopsis pinicola*. Also steroids were present in *Chrysophyllum albidum, Formitopsis pinicola* and *Cantharellus cilairus* but absent in *Tapinantus globiferus* and *Saba florida*. (Table 4.2).

4.3 Formation of Silver Nano Particles using Aqueous Extracts of*Chrysophyllum Albidum* (seeds) and *Tapinanthus globiferus* (leaves)

The aqueous seed extract of *Chrysophyllum albidum* was light brown in colour before the addition of 1mM (80 ml) of silver nitrate solution and this changed to dark brown in colour within one hour after boiling at 50^{o}C for 30 min.This suggests the formation of silver ions. The dried sample weighed about 10 gm. Similarly, the formation of silver nanoparticles of *T. globiferus* involved the addition of*T. globiferus* Leaf extract (20 ml) to 1 mM silver nitrate solution (80 ml). The plant extracts were pale green in color before addition of Ag+ ions and this changed to brownish color within 20 min. after boiling at 50^{o}C for 30 min., suggested the formation of silver nanoparticles.

Table 4.2: Phytochemical screening of Methanolic extract of *Chrysophyllum albidum*, *Tapinanthus globiferus*, *Saba florida*, *Formitopsis pinicola* and *Cantharellus cilarius*

Chemical Tested	*Chrysophyllum albidum* (seed)	*Tapinanthus globiferus* (leaves)	*Saba florida* (root)	*Formitopsis Pinicola* (whole)	*Cantharellus cilarius* (whole)
Alkaloids	++	+	+	+	+
Saponins	++	++	++	+	–
Cardiac Glycosides	+	–	–	+	+
Anthraquinones	–	–	–	–	+
Flavonoids	–	+	–	–	+
Terpenoides	+	–	+	–	+
Phlobatanins	+	–	+	+	+
Tannins	+	+	+	–	+
Steroids	+	–	–	+	+
Carbohydrate	+	+	+	-	+
protein	+	+	+	-	+

Highly present ++; moderately present + and Absent

4.4 *Invitro*trypanocidal Activities of butanolic extracts and synthesized silvernano particles of seed extract of *C. albidum*and leave extract of *T. globiferus*

The butanolic extracts from the partitioned methanolic extracts of both plants Showed highest minimum inhibitory activity and was used for further studies. The *in vitro* anti-trypanosomal activity of 5 components: butanolic extracts of *C. albidum* and *T. globiferus,*synthesized nano particle of *C. albidum,* synthesized nano particle of *T. globiferus* and Diaminazine were tested against *Trypanosoma bruceibrucei* and *Trypanosoma congolense* at concentration ranging from 1000 µg/ml to 0.24 µg/ml. Observations were taken at four time intervalsof: 3, 6, 9 and 12 hours incubation period, respectively.The result showed a varying percentage of anti trypanosomalactivity which was dose dependant, the higher the dose of plant extractthe greater the level of trypanocidal activity.

All the extracts showed inhibitory activity from dose range of 1000-15.6 µg/ml by the third hour(Figure 4.2). At the sixth hour of incubation, onlyC3(synthesized silver nanoparticles of *C. albidum)* had 100% inhibitory activity on both *T.congo* and *T. brucei* at concentration of 0.98 µg/ml.while C1 (butanolicseed extract of *C. albidum* on *T. brucei brucei*), C2(butanolic leaf extract*T. globiferus*on *T. brucei brucei*), C4 (synthesized nano particle of *T.globiferus*on *T. brucei brucei*).Also, C5 (butanolic seed extract of *C. albidum*on *T. congolense*), C6, (butanolic leaf extract of *T. globiferus* on *T. congolense*) C8 (synthesized nano particle of *T. globiferus on T. congolense*), C9 Diaminazine on *T. brucei brucei*) and C10 (Diaminazine on *T. congolense*)5had percentage surviving parasite of 50, 40, 4.4, 33.3, 26.7, 3.2, 34.8 and 30.4% respectively.

Figure 4.3 showed trypanocidal activities of the test components over 12 hrs of incubation at different concentration: 1000, 250, 62.5,15.6, 3.90 and 0.24 µg/ml.

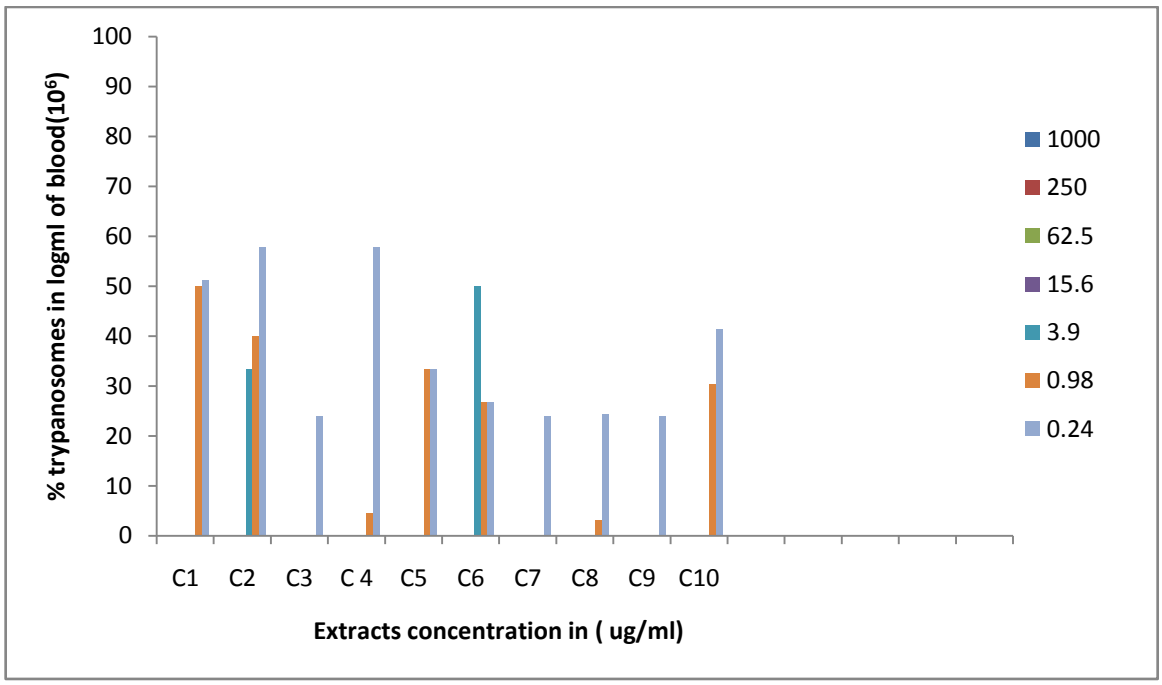


Figure 4.2*: In vitro* trypanocidal activity of extract at various concentrations at 6 hr of incubation

Keys

C1*: C. albidum* butanolic seed extract on *T. brucei brucei*
C2: *T. globiferus* butanolic leaf extract on *T. brucei brucei*
C3: synthesised nano particle of *C. albidum* on *T.brucei brucei*
C4: synthesized nano particle of *T.globiferus* on *T.brucei brucei*
C5: *C. albidum* butanolic seed extract on *T. congolense*
C6: *T. globiferus* butanolic leaf extract on *T. congolense*
C7: synthesised nano particle of *C. albidum* on *T. congolense*
C8: synthesized nano particle of *T. globiferuson T. congolense*
C9: Diaminazine on *T.brucei brucei*
C10: Diaminazine on *T. congolense*

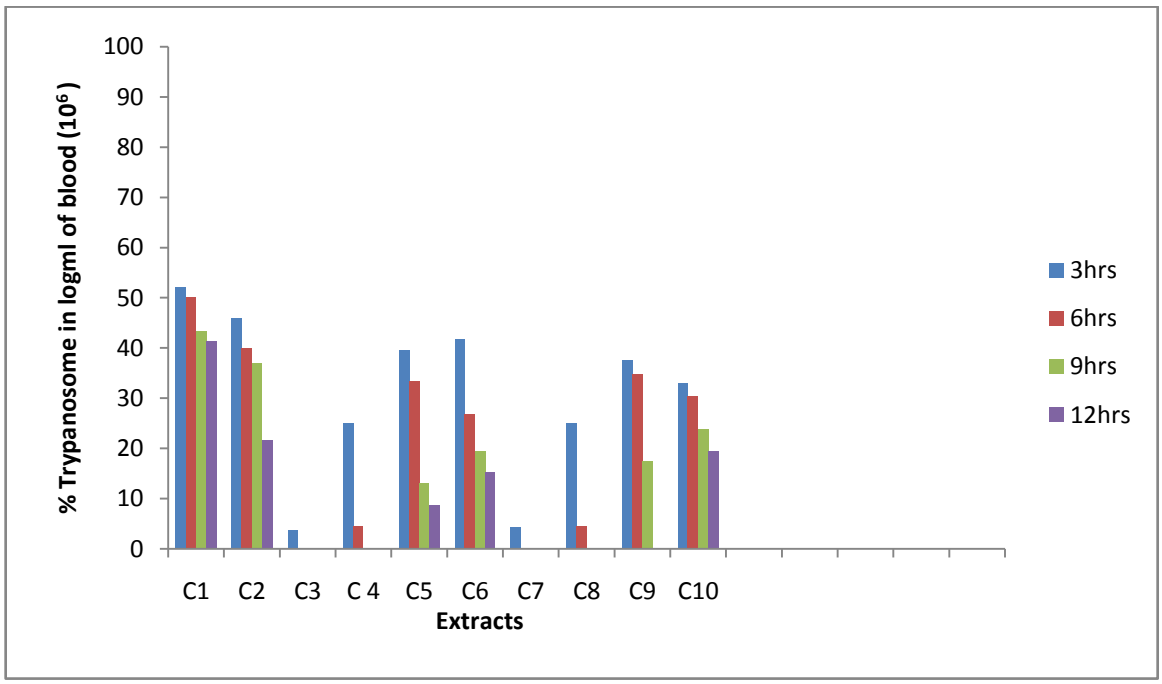


Figure4.3: *In vitro* trypanocidal activity of extracts and synthesized extract on *Trypanosoma brucei brucei* and *Trypanosoma congolense* at a concentration 0.98 µg/ml for a period of 12 hrs incubation

Keys
C1*: C. albidum* methanolic seed extract on *T. brucei brucei*
C2: *T. globiferus* extract on *T. brucei brucei*
C3: synthesised nano particle of *C. albidum* on *T.brucei brucei*
C4: synthesized nano particle of *T.globiferus* on *T.brucei brucei*
C5: *C. albidum* methanolic seed extract on *T. congolense*
C6: *T. globiferus* extract on *T. congolense*
C7: synthesised nano particle of *C. albidum* on *T. congolense*
C8: synthesized nano particle of *T. globiferus son T. congolense*
C9: Diaminazine on *T.brucei brucei*
C10: Diaminazine on *T. congolense*

ll the components showed 100% inhibitory activity at concentrations ranging from 1000-3.90 µg/ml. However, component C3, C4, C7, C8 and C9 also had 100% inhibitory activity at 0.98 µg/ml while extracts C1, C2 and C5 had percentage of trypanosomes survival after 12 hrs of incubation as 41.3, 21.7, 8.7, 15.2 and 19.5% at 0.98 µg/ml. None of the extracts showed complete inhibitory activity at 0.24. However, a varying percentage of anti-trypanosomal activity was observed.

The multiple comparism tests showed there was significant difference in number of trypanosomes species in wells with test substances and the control extracts on trypanosomes at 0.98 µg/ml after 6 hr of incubation (Table 4.3). The result also showed that there was no significant difference ($P<0.05$) in the sensitivity of Trypanosoma sp to the synthesized silver nanoparticle of extracts of *C. albidum* and *T. globiferus* at 0.98 µg/ml, six hours post-incubation. However, there were significant differences ($p < 0.05$) between the effects of the synthesized silver nanoparticles of extracts and their corresponding non-synthesized solvent extracts, with the synthesized nanoparticles of *C. albidum* exhibiting greater anti activity.When the sensitivity of the two Trypanosoma spp. to the standard drug (Diaminazine aceturate) and the synthesized silver nanoparticles of extracts were compared, the nanoparticles had significantly ($p < 0.05$) greater effect on the parasite an the drug.At 12 hours post-incubation, there was a statistically significant difference ($p < 0.05$) in the trypanosome count between wells containing the test substances and the control wells. Multiple comparison tests indicated that at this time interval, there were significant differences ($p< 0.05$), between the effects of the synthesized silver nanoparticles of extracts and their corresponding non-synthesized solvent extracts on *Trypanosoma brucei brucei* and *Trypanosoma congolense* with the synthesized nanoparticles exhibiting greater trypanocidal activity.

Table4.3: *In vitro* trypanocidal activitiesof butanolic and synthesized silver nano particlesof *C. albidum*(seed) and *T. globiferus*(leaves) extractsat 0.98 mg/ml on *T. brucei* after the 6th and 12th hours of incubation.

Extracts of *C.albidum* (Seed) and *T.globiferus* (leaves)	6 hr incubation duration	12 hr incubation duration
Non synthesized extract of *C. albidum*	11.0 ± 1.0^{bc}	8.5 ± 0.5^{c}
Non synthesized extract of *T.globiferus*	14.0 ± 1.0^{c}	5.0 ± 2.0^{c}
Synthesized extract of *C. albidum* synthesized	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Synthesized extract of *T.globiferus*	1.0 ± 1.0^{a}	0.0 ± 0.0^{a}
Diaminazine (standard drug)	8.0 ± 1.0^{b}	1.0 ± 1.0^{ab}
Control (no extract)	22.5 ± 1.5^{d}	23.0 ± 2.0^{d}

The values are given as mean ± (SEM). In each column, values with different superscripts have statisticallysignificant differences ($p< 0.05$) compared with control.

Also, at this time interval, there was no significant difference between the effect of Diaminazine and the synthesized silver nanoparticles of extracts *C. albidum and T. globiferus* on *Trypanosoma brucei brucei* and *Trypanosoma congolense.* However there were significant difference in trypanocidal activities in all the test component, standard drug and control wells without extract (Table 4.4).

4.5. *In vitro*trypanocidal Activities of Butanolic Fraction of*Chrysophyllum albidum* seed on *Trypanosoma congolense* and *Trypanosoma brucei brucei*

The butanolic fraction from the partitioned extract of methanolic extract of *Chrysophyllumalbidum* weighed 33.6g. It showed highest *in vitro* anti trypanosomal activity against both *Trypanosoma congolense* and *Trypanosoma brucei brucei*at 15 µg/ml compared to hexane and chloroform extract which gave activity at 250µg/ml (Figure 4.4) and was further fractionated on silica gel column, 308 fractions were collected and were further pulled together based on TLC identification of similar fractions to give eleven fractions (B1, B2, B3, B4, B5, B6, B7, B8, B9, B10 and B11). The *in vitro* result test showed that there was a significant difference ($P< 0.05$) in activity of fraction and substances of butanolic fraction of *C. albidum* and synthesized silver nano seed extract of *Chrysophyllum albidum* compared with the standard drug Diminazine aceturate (Figure 4. 4) the synthesized nano particle exhibited highest minimum inhibitory activity (MIC) at 0.98 µg/ml while Diminazine and fractions B4 had activity at 3.9 µg/ml, followed by fractions B11 with minimum inhibitory concentration at 15.6 µg/ml. Fractions that exhibited Minimum inhibitory concentration at 62.5 µg/ml are B6 and B8 and only on *T.congolense*. Furthermore, fractions B3, B5 and B7 showed activity at 250 µg/ml. while the lowest activity was exhibited with fraction B1, B2, B9 and B10 at 1000 µg/ml.

Table 4.4: *In vitro* trypanosomal activities of butanolic and synthesized silver nanoparticles of *C.albidum*(Seed) and *T. globiferus*(leaves) extracts at 0.98 mg/ml on *T. congolense* at the 6th and 12th hours

Extracts of *C.albidum* (Seed) and *T.globiferus* (leaves)	6hr incubation duration	12hr incubation duration
Non synthesized extract of *C. albidum* extract	7.5 ± 0.5^{b}	2.0 ± 0.0^{b}
Non synthesized *T.globiferus* extract	6.0 ± 0.0^{b}	3.5 ± 0.5^{b}
Synthesized extract of *C. albidum*	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Synthesized extract *T.globiferus*	1.0 ± 1.0^{a}	0.0 ± 0.0^{a}
Diaminazine	7.0 ± 1.0^{b}	4.5 ± 2.5^{b}
Control	22.5 ± 1.5^{c}	23.0 ± 2.0^{c}

The values are given as mean ± (SEM). In each column, values with different superscripts have statistically significant differences ($p< 0.05$) compared with control.

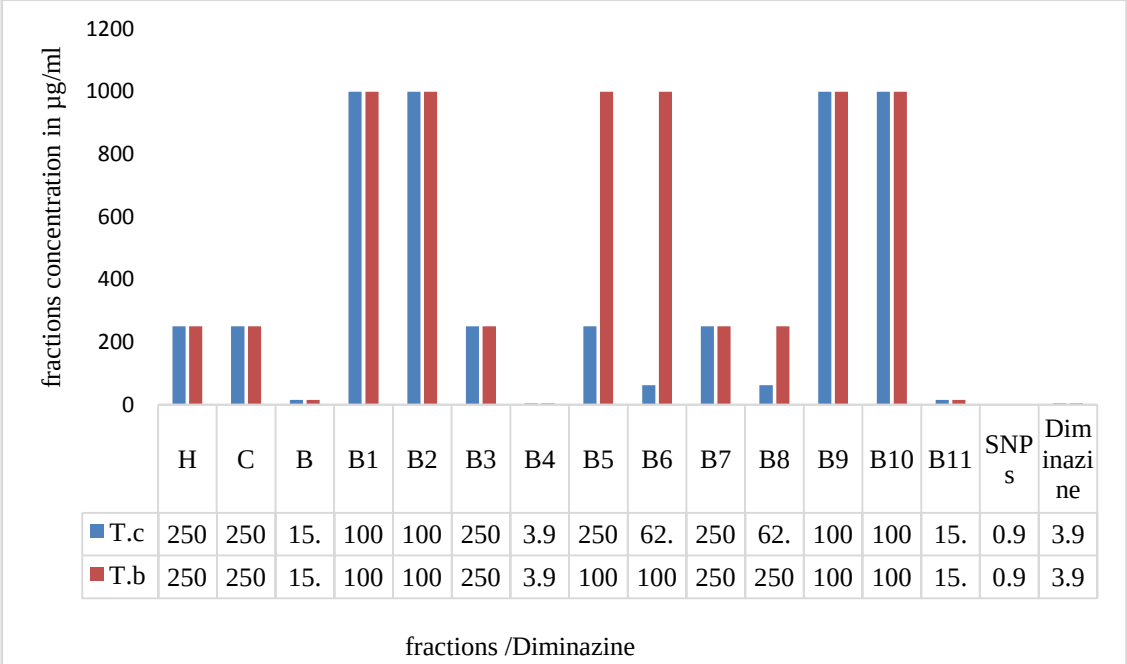


Figure 4.4: Minimum Inhibitory Concentration (MIC) of hexane, chloroform and butanolic fractions obtained from methanolic fraction of *C. albidum* on *Trypanosoma congolense*

Keys:

T.c-*Trypanosoma congolense*

T.b-*Trypanosoma brucei brucei*

H -Hexane fraction B5-fraction136-175

C -Chloroform fractionB6-fraction 176-205

B-Butanolic fraction B7- fraction206-220

B1-fraction 1-35 B8- fraction 221-250

B2-fraction 36-56 B9-fraction 251-263

B3-fraction 57-90 B10-fraction 264-287

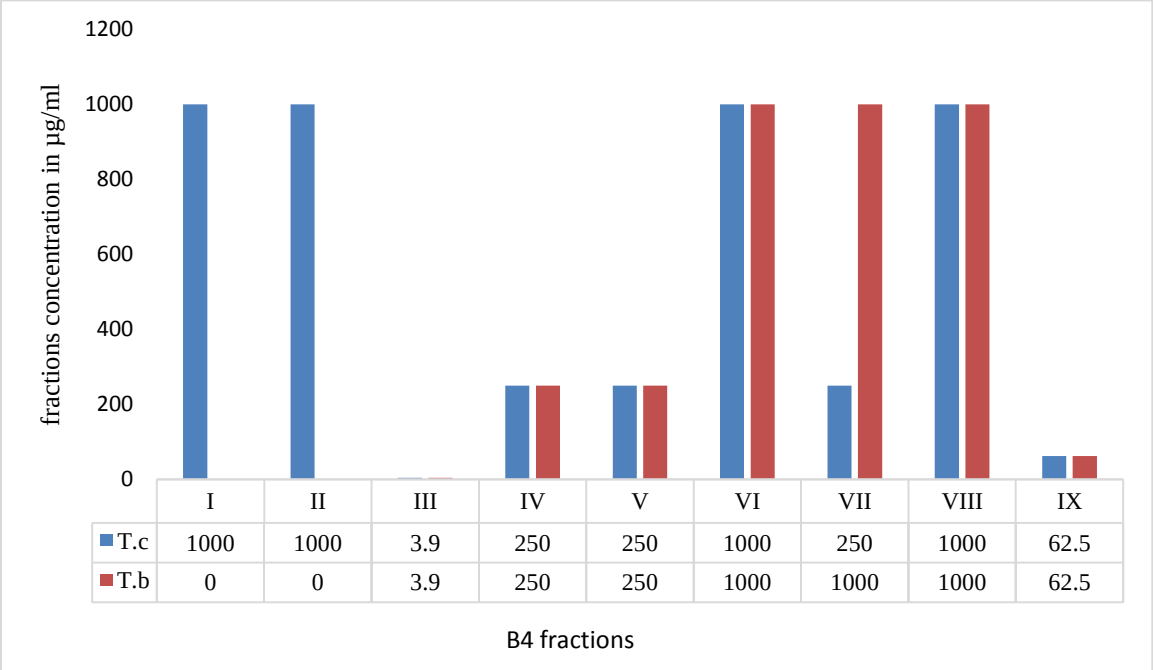
B4-fraction 91-135 B11-fraction 288-308

SNPs-synthesized Silver nanoparticle of*C. albidum*

Fraction B4 was yellowish in colour and weighed 8.3g. It was fractionated on silica gel column and one hundred and ninety eight (198) fractions were collected and were further pulled together to obtain nine fractions denoted as (I, II, III, IV, V,VI, and IX) based on similar fraction observed on TLC. The result in Figure 4.5 showed that there was significant difference ($P<0.05$) in activity of fractions compared tosynthesized nano particle and standard drug (Diaminazine). The synthesized *C.albidum* extract gave highest *invitro* activity at 0.98 µg/ml, while the standard drug and Fraction III showed MIC at 3.90 µg/ml gave the highest *in vitro* anti trypanosomal activity at 3.9 µg/ml on both *Trypanosoma congolense* and *Trypanosoma brucei brucei* followed by fraction IX which showed activity at 62.5 µg/ml also on both parasites. While fractions that exhibited activity at 250 µg/ml were; IV and V on both parasites while VII had activity on *T.congolense* alone at 250 µg/ml and the lowest activity was exhibited with fractions I, II, VI and VIII at 1000 µg/ml. Fraction I and II had no activity on *T. brucei brucei* in all concentrations.

4.6 Minimum Inhibitory Concentration (MIC) of Fractions obtained from Sephadex LH- 20 Column of fraction (III) on *Trypanosoma brucei congolense* and *Trypanosoma brucei brucei*

Based on the result obtained from 4.5, fraction III was separated on Sephadex LH -20 column and twenty one fractions which were pulled together to give four fractions based similar fractions identified on TLC and were represented as follows: S1, S2, S3 and S4). The result showed there was significant difference($P<0.05$) in *in vitro* activity test of fractions, synthesized nano particles and Diaminazine. Fraction S2 showed minimum inhibitory activity at 15.6 µg/ml (Figure 4.6) followed by fraction S1 which showed activity at 62.5 µg/ml while fraction S4 showed activity at 250 µg/ml lowest activity was exhibited by fraction S3 at 1000 µg/ml. Highest MIC activity was observed with synthesized extract of *C. albidum* at 0.98µg/ml.



	I	II	III	IV	V	VI	VII	VIII	IX
T.c	1000	1000	3.9	250	250	1000	250	1000	62.5
T.b	0	0	3.9	250	250	1000	1000	1000	62.5

Figure 4.5: Minimum Inhibitory Concentration (MIC) of fractions obtained from fraction B4 on *Trypanosoma congolense* and *Trypanosoma brucei brucei*

Keys

T.c -*Trypanosoma congolense*
T.b-*Trypanosoma brucei brucei*

I- fraction 1-18
II- fraction 19-27
III- fraction 28-48
IV- fraction 49-52
V- fraction 53-83
VI- fraction 84-125
VII- fraction 126-151
VIII- fraction 152-174
IX- fraction 175-198

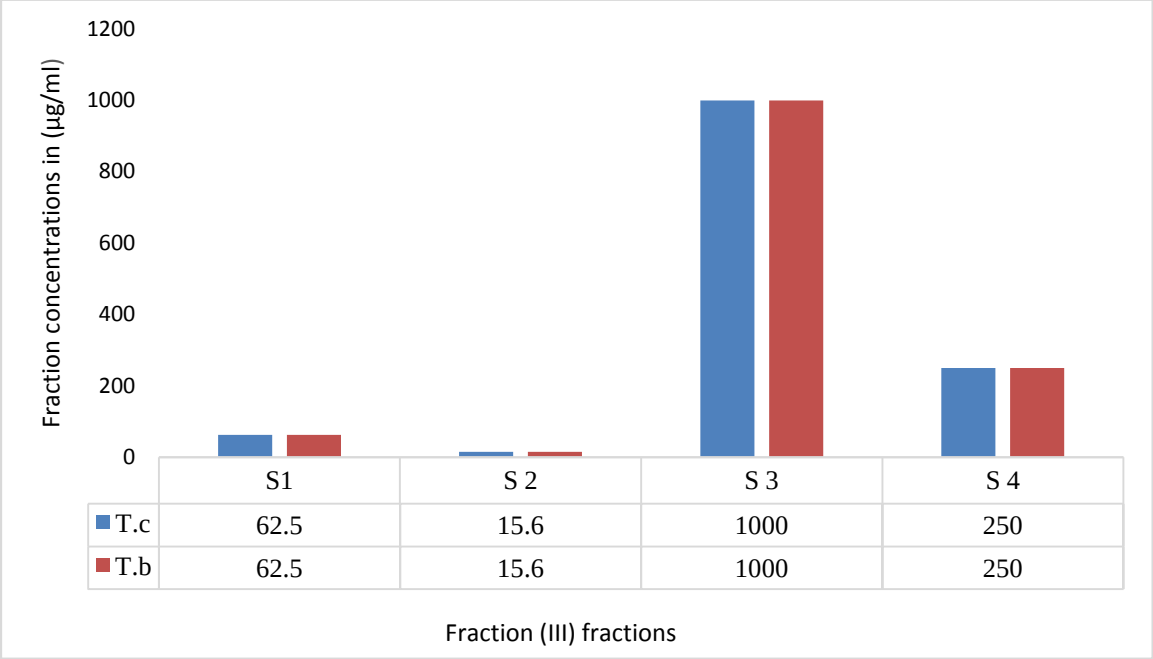


Figure 4.6: Minimum inhibitory concentration of fractions obtained from sephadex LH-20 of Fraction (III) on*Trypanosoma brucei congolense* and *Trypanosoma brucei brucei*

Keys

S1-fractions 1-4; S2-fraction 5-10; S3-fraction 11-16; S4-fraction 17-21 ; T. b-*Trypanosoma congolense* and T. b-*Trypanosoma brucei brucei*

4.7 Minimum Inhibitory Concentration (MIC) of Fractions obtained from Preparative Thin Layer Chromatographic of fraction S2 Studies on *Trypanosomacongolense and Trypanosoma brucei brucei.*

Based on the result obtain above, fraction S2 was fractionated on Preparative Thin Layer Chromatographic plate. Three (3) bands were recovered from the PTLC (P1, P2 and P3). P1 was pinkish, while P2 was greenish and P3 was brownish in colour. The anti trypanosomal activity (Figure 4.7) showed that there was significant difference ($P<0.05$) in activity of fractions and synthesized silver nano particle of seed extract of*C. albidum* fraction P3 exhibited MIC activity at 62.5 µg/ml on *T. congolense* only, while on *T.brucei brucei* was at 250 µg/ml. Followed by fraction P1 and P2 which- showed MIC activity at 250 and 1000 µg/ml on both *Trypanosoma congolenses* and *Trypanosoma brucei brucei*.The synthesized extract of *C. albidum* showed highest activity at 0.98 µg/ml, while Diaminazine exhibit activity at 3.9 µg/ml.

4.8 Minimum Inhibitory Concentration (MIC) of Hexane, Chloroform and Fractions obtained from Butanolic Fraction of *Tapinantus globiferus* on *Trypanosoma congolense* and*Trypanosomabrucei brucei*

The butanolic fraction was found to have highest activity at 15.6 µg/ml and was further fractionated on silica gel column chromatography to give 203 fractions which were pulled together to give 14 fractions (TG 1, TG 2, TG 3, TG4, TG 5, TG 6, TG 7, TG8, TG 9, TG10, TG11, TG12, TG 13 and TG14. Figure 4.8, showed that there was significant difference ($P<0.05$) in activity of fractions compared with synthesized leave extract of *Tapinantus globiferus* and Diaminazine. Synthesized leave extract of *Tapinantus globiferus* showed highest activity at 0.98, while Diaminazine, fraction TG5 and TG10 showed highest activity at 3.9 µg/ml on both *Trypanosoma congolense*and *Trypanosoma brucei brucei,* followed by fraction TG2 and TG14 which showed activity at 15.6 µg/ml.

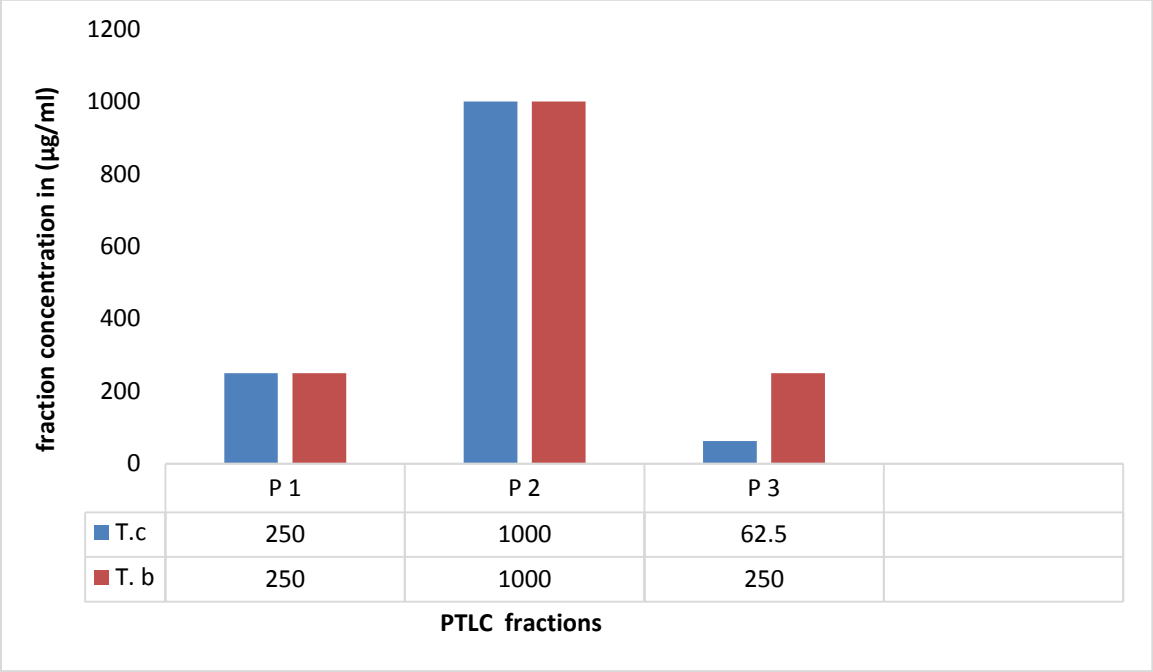


Figure4.7: Minimum Inhibitory Concentration (MIC) of fractions obtained from Preparative Thin layer Chromatography of fraction S2 on *Trypanosoma congolense* and *Trypanosoma brucei brucei*.

Keys : T.b-*Trypanosoma congolense* and T.b-*Trypanosoma brucei brucei*

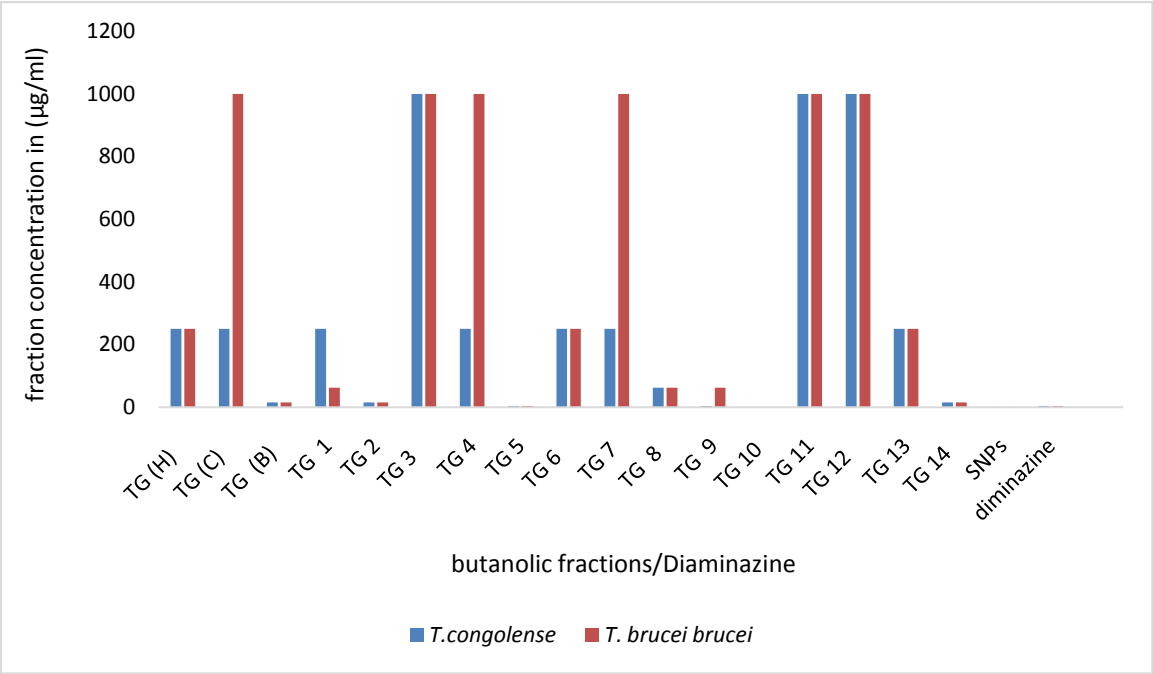


Figure4.8: Minimum Inhibitory concentration (MIC) Hexane, chloroform and fractions obtained from butanolic fraction of *T. globiferus on Trypanosoma congolense* and *Trypanosoma brucei brucei*

Keys

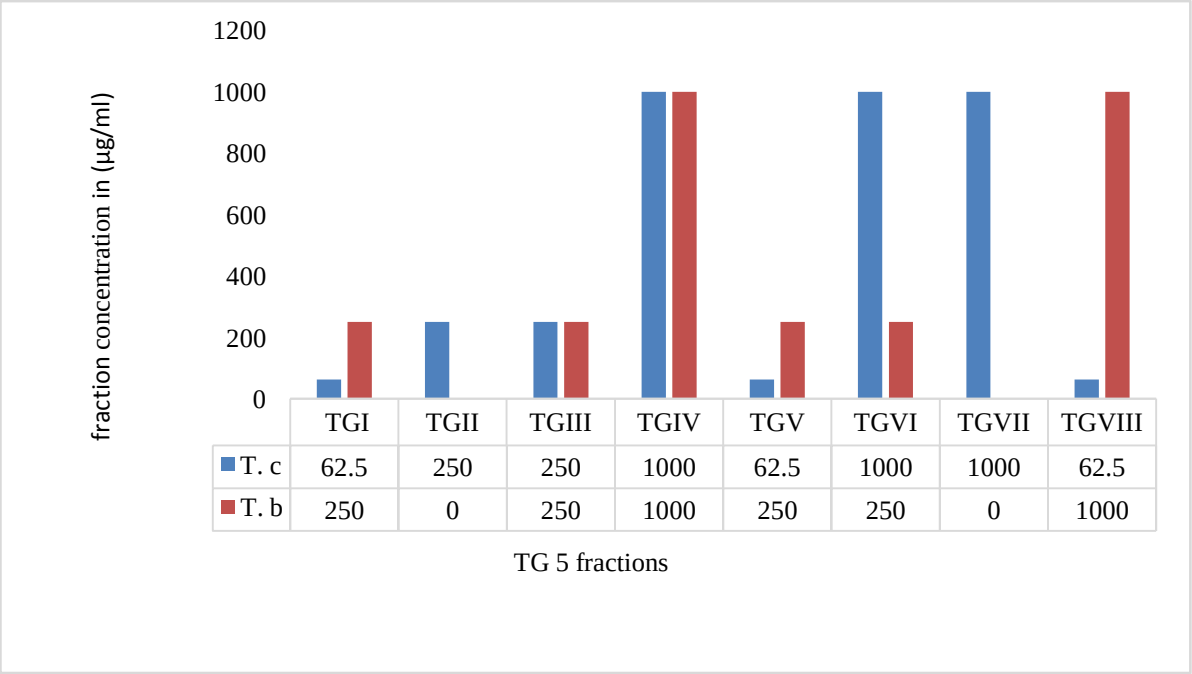
TG (H)-*Tapinanthus globiferus*
TG (C)-*Tapinanthus globiferus*
TG (B)-*Tapinanthus globiferus*
TG1- fraction 1-12
TG2-fraction 13-27
TG3-fraction 28-44
TG4-fraction 45-53
TG5-fracton 54-64
TG6-fraction 65-72
TG7-fraction 73-86
TG8 fraction-87--103
TG9 fraction 104-118
TG10 fraction 119-141
TG11 fraction 142-168
TG12 fraction 169-182
TG13-fraction 183-191
TG14-fraction 192-203
SNPs-Silver nanoparticle of *T. globiferus*
Diaminazine

Further activity at 62.5µg/ml was exhibited with fraction TG8, similarly activity at 250 µg/ml was exhibited with fraction TG1, TG4, TG6 and TG7 on *T. congolense* only. while TG4 and TG7 exhibited activity on *T. brucei brucei* at 1000 µg/ml Fractions TG3, TG11, and TG12 showed activity at 1000 µg/ml. only fraction TG10 did not exhibit *in vitro* activity against *Trypanosoma congolense*.Based on the result obtained, TG5 was fractionated on silica gel column. One hundred and twelve fractions were obtained and were also pulled together to give eightfractions (Figure 4.9).

TGI, TGII, TGIII, TG IV, TGV, TGVI, TGVII and TGVIII. TGI, TGV and TGVIII showed that there was significant difference ($P<0.05$) in activity of fractions compared with synthesized leave extract of *Tapinantus globiferus* and Diaminazine. Synthesized leave extract of *Tapinantus globiferus* showed highest activity at 0.98 followed by Diaminazine which showed activity at 3.9 µg/ml. TGI, TGV and TGVIII showed activity at62.5 µg/ml on*T. congolense*. Also fraction TG I, TG III, TGV and TG VI exhibited MIC activity at250 µg/mlon*T.brucei brucei* only. While fractions TG IV, TG VI and TG VII had lowest activity at 1000 µg/ml. TGII and TG VII did not exhibit any activity against *T.brucei brucei*.

4.9 Gas Chromatography Analysis of fraction P3

The GC-MS analysis belonging to different chemical classes such as terpenoids, aldehydes, ketones, fatty acid estersand carboxylic acids were found to be present in fraction P3 obtained from preparative thin layer chromatographic studies of butanolic extract of *C. albidum*. Tencompounds were identified to be in abundance and were mostly fatty acids. Theactive principles with their retentiontime (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 4.5).



	TGI	TGII	TGIII	TGIV	TGV	TGVI	TGVII	TGVIII
T. c	62.5	250	250	1000	62.5	1000	1000	62.5
T. b	250	0	250	1000	250	250	0	1000

Figure 4.9: Minimum Inhibitory concentration (MIC) of fractions obtained from TG5 (fractions54-64) *Trypanosoma congolense* and *Trypanosoma brucei brucei*

Keys

T.c-*Trypanosoma congolense*
T.b-*Trypanosoma brucei brucei*
TGI-fractions 1-22
TGII-fraction 23-38
TGIII-fractions39-45
TGIV-fractions 46-63
TGV-fractions 64-80
TGVI-fraction 81-95
TGVII-fractions 96-102
TGVIII-fractions 103-112

Table 4.5: GC-MS Analysis and Mass Spectral Data of fraction P3

Peak	Time rate	Compounds	Area %	Molecular formula	Molecular weight	Molecular weight
1	12.131	Diaziridine,3-ethyl-3-methyl	0.43	CH_4N_{25}	44.057 g/mol	
2	24.444	2,4Dimethylhexanedioic acid	0.24	$C_8H_{16}O_2$	144.214g/mol	
3	29.279	13-Tetradecynoic acid,	1.29	$C_{15}H_{30}O_2$	242.395g/mol	Used to induce sustaintial dilation of human coronary micro vessels.
4	33.668	Hexadecanoic acid, methyl ester	34.45	$C_{17}H_{34}O_2$	270.724g/mol	Anti- bacteria and anti-fungal.
5	37.084	9,12-Octadecadienoic acid, methyl	5.68	$C_{19}H_{34}O_2$	294.223g/mol	Anti-cancer
6	37.193	11-Octadecenoic acid, methyl ester	44.56	$C_{19}H_{36}O_2$	296.487g/mol	
7	37.662	Methyl stearate	4.86	C19H38O2	298.53g/mol	
8	38.566	9-Tetradecenal, (Z)	0.23	C14H26O	210.361g/mol	Anti-oxidant, cancer preventive, Hyper cholesterolemic, nematicide, lubricant, cosmetic.
9	40.821	12-Hydroxydodecanoic acid	2.81	C12H24O3	216.321g/mol	
10	46.628	1,3,4-thiadiazol-2-amine, 5-ethoxy	5.47	C2H3N35	102.127g/mol	Anti-neoplastic agent

The identified compounds are listed with their peak areas (or percentage composition so the metabolites shown in brackets). Diaziridine,3-ethyl-3-methyl (0.43%), 2,4Dimethylhexanedioic acid (0.24%), 13-Tetradecynoic acid(1.29%), 13-Tetradecynoic acid (1.29%), Hexadecanoic acid, methyl ester (34.45%), 9,12-Octadecadienoic acid, methyl (5.68%), 11-Octadecenoic acid, methyl ester (44.56%), Methyl stearate(4.86%), 9-Tetradecenal, (Z)(0.23), 12-Hydroxydodecanoic acid (0.23)and1,3,4-thiadiazol-2-amine, 5-ethoxy. Most of these constituents have been found to show interesting biological activities against several diseases and pathogens.The GC-MS Chromatogram confirmed the presence of various components with different retention times as illustrated in (Figure 4.10). The observed peaks are as a result of large compound fragmenting into small compounds at different m/z ratios. These mass spectra are fingerprints of compounds which can be identified from the data library.The biological activitieslisted in (Table4.5) are based on Dr Jim Duke's Phytochemical and Ethnobotanical Databases by Agricultural Research Service/USDA.

4. 10 Acute toxicological studies of Butanolic extracts of *Chrysophyllum albidum* And*Tapinantus globiferus*on mice

Table 4.6 showed that animals were initially treated with 100, 500 and 1000 mg/kg body weight with *Chrysophyllum albidum* and *Tapinantus globiferus* had no mortality, when observed after every one hour for 4 hrs, then 12, 24, 48, 36 and 72 hrs after administration. However the groups treated with 3000 and 5000 mg/kg/wt., with *Chrysophyllum albidum* showed high physiological activities such as tremor, diarrhoea and convulsion leading to 100 percent mortality within 12 hrs of administration of extracts.

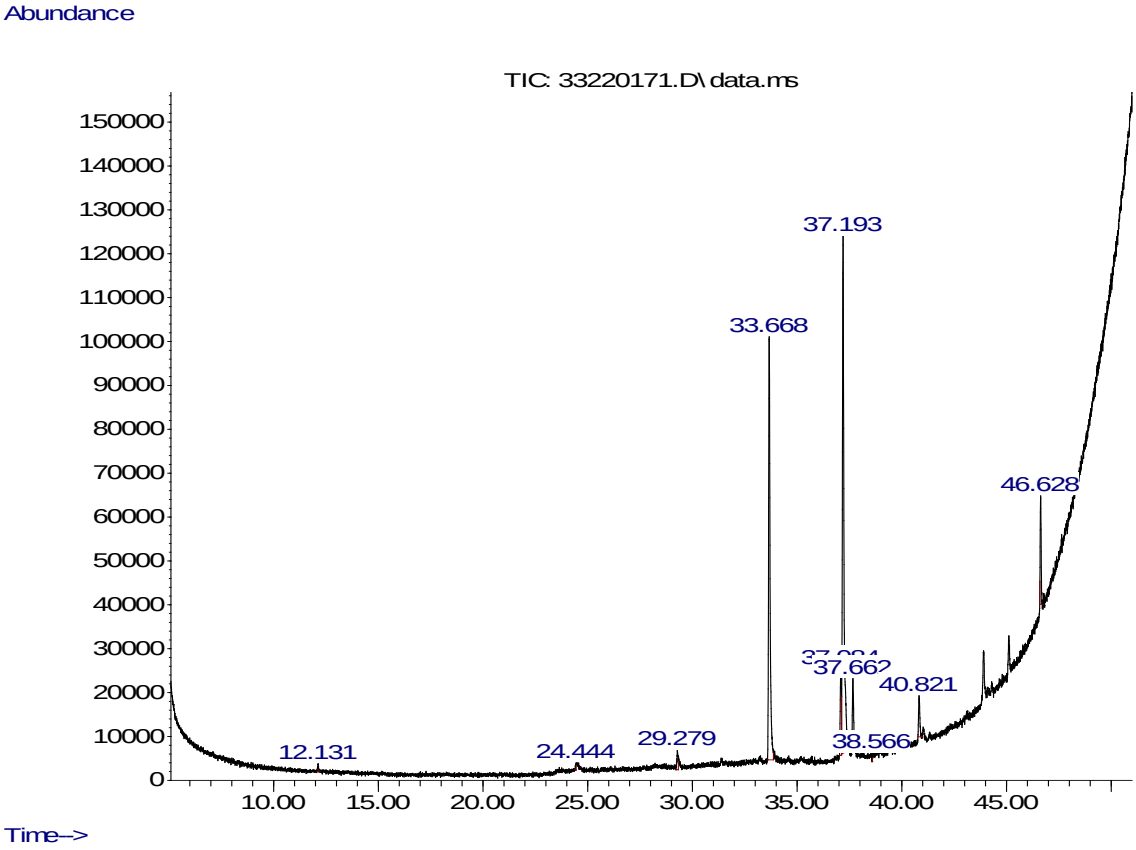

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110000
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10000
0
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33.668
46.628
37.662
40.821
12.131
24.444
29.279
38.566
10.00
15.00
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25.00
30.00
35.00
40.00
45.00
Time-->
Figure 4.10:GC-MS chromatogram of fraction P3 obtained from preparative thin layer chromatographic studies of butanolic extract of C. albidum.

Table 4.6: Acute toxicity of butanolic extract of *Chrysophyllum albidum* seed and *Tapinanthus globiferus* leaves on Mice.

Plant extract	First phase of investigation		Second phase of investigation	
	Doses	Mortality	Doses	Mortality
C. albidum	100 mg/kg	0/3	2000 mg/kg	0/1
,,	500 mg/kg	0/3	3000 mg/kg	1/1
,,	1000 mg/kg	0/3	5000 mg/kg	1/1
T. globiferus	100 mg/kg	0/3	2000 mg/kg	0/1
,,	500 mg/kg	0/3	3000 mg/kg	0/1
,,	1000 mg/kg	0/3	5000 mg/kg	0/1
Controldistilled H_2O	0/1		distilled H_2O	0/1

The groups treated with extract of *Tapinantus globiferus* at 2000, 3000 and 5000 mg/kg body weight did not record any mortality or serious adverse effect. The LD_{50} of

Chrysophyllum albidum was deduced from probit log dose graph in figure 4.11 and was calculated to be 1258.93 mg/kg.

4. 11. *In vivo*Anti- trypanosomal Activities of butanolic and synthesized Silver Nano Particles of Seed Extract of *Chrysophyllum albidum* in Mice Infectedwith*Trypanosoma congolense.*

The result presented in Figure 4. 12, showed that treatment of infected mice commenced on day nine post infection when parasitaemia was about $5x10^6$ trypanosomes/ml. Parasitemia in group B1 treated with 100 mg/kg/wt. of non-synthesized extract, continue to increase progressively, but from days 14-17 there was significant reduction in parasiteamia to $3x10^6$ trypanosomes/ml compared to the negative control group which was about $15x10^7$ trypanosomes/ml. However by day 18, the level of parasiteamia began to increase, the animals started dying and the last animal in this group died by day 21 post infection. Similarly group (B1) treated with 100 mg /kg/wt. of synthesized nanoparticle of *Chrysophyllum albidum* showed reduction in mean parasite count from 42.5±6.4 to 34.4±5.2 by day 15-19. However, there was relapse and the last animal died by day 23 post infection. While animals in Group (E) treated with Diaminazine were completely cured by day 15 post infections. The infected not treated group (F), parasitemia increased uncontrollably until they culminated in death by day 20 post infection. Animals in group (G) were not infected not treated (Negative control) and survived throughout the course of experiment.

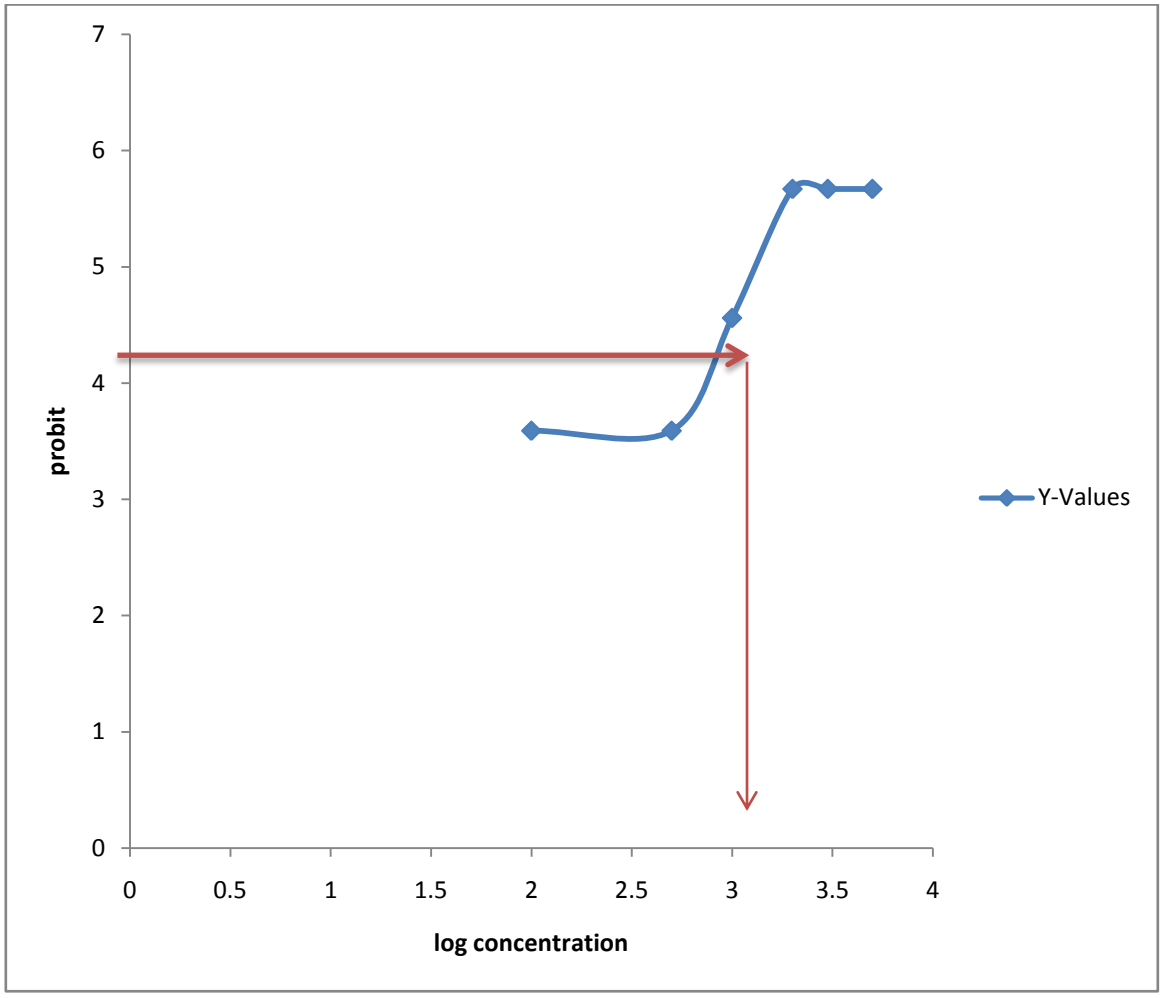


Figure 4.11: Probit LD50 = 1258.9 mg/kg for *Chrysophyllum albidum*

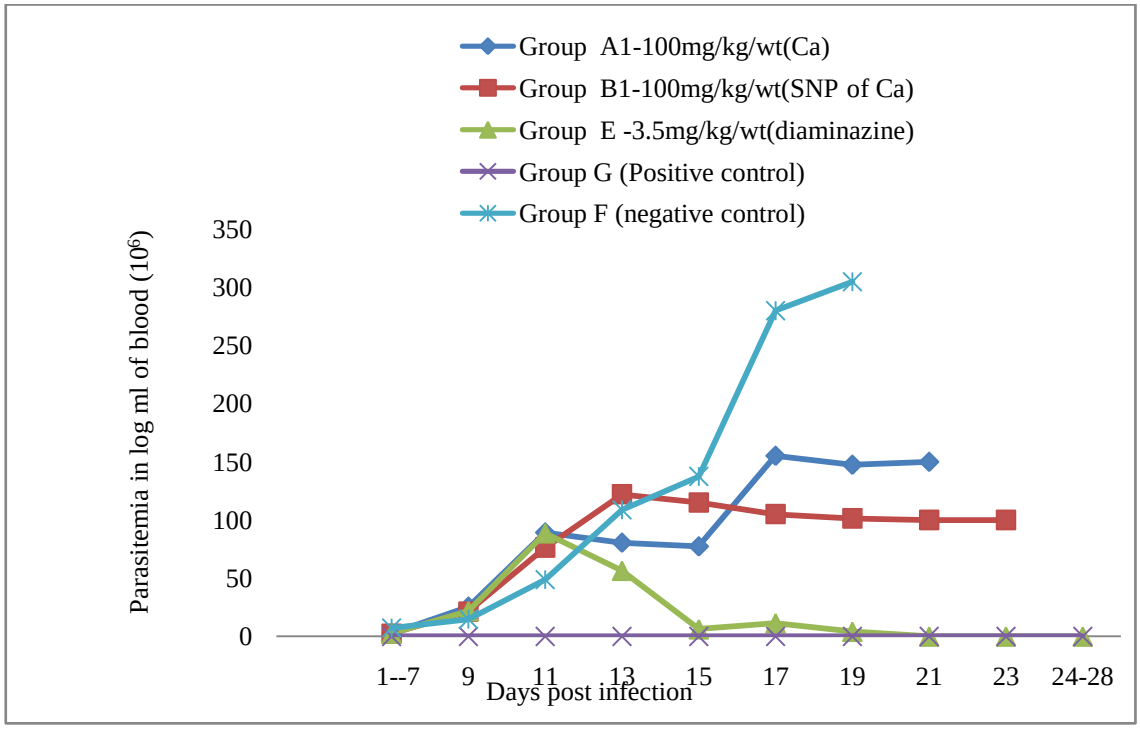


Figure 4.12: Course of parasitemia in five groups of animals infected with *Trypanosoma congolense* and treated with butanolic and synthesised silver nano particles of seed extract *C. albidum*.

Key*: Ca=Chrysophyllumalbidum*, SNP =Silver nano particle

The result in (Figure 4.13) showed that treatment commenced on day 9 post infection when average mean trypanosome count was about 28 ±1.6 of trypanosomes. Group (A2) treated with 500 mg/kg/wt. with butanolic extract had reduction in Parasitemia from 54 ±5.4 to 20.5 ±0.9 by days 13-17. However there was trypanosoma infection relapse at day 21exhibited by parasitemia increase to 58.2 ± 3.3 and by day 24 post infection, all the animals were dead. Also Group (B2) treated with 500 mg/kg synthesized silver nano particle had reduced parasitemia from days 19 and their lives were prolonged up to day 25th post infection. Similarly animals treated with 1000 mg/kg/wt. (A3) of butanolic extract had reduction by day 19 and fluctuation in parasiteamia but survived up to day 26 when the last animal died. However the group treated with synthesized aqueous seed extract of *Chrysophyllum albidum* at 1000 mg/kg (B3) also showed reduced parasitemia by days 11 post infection. Fluctuations in parasitemia level were not observed and the animals survived with very low level of parasitemia (6.94 ±1.0)up to the 28 day when the experiment was terminated.While in the infected not treated group (F), The parasitemia increased uncontrollably until they culminated in death by day 21 post infection with average mean parasiteamia of (316 ± 0.2). Animals in group (G) were not infected not treated (negative control) and survived throughout the course of experiment. The multiple comparism tests showed that there was a statistically significant difference ($p < 0.05$) in the mean trypanosome count per millilitre of blood from day 9 to day 27 post-inoculation across all the treatment groups.

In mice treated with Diminazine aceturate, there was a decline in trypanosome count from day 13post inoculation after which infection remained below detectable limits and the survived up to the time the experiment was terminated. In the untreated control group, there was a progressive raise in trypanosome until they culminated in by day 21 post infection.

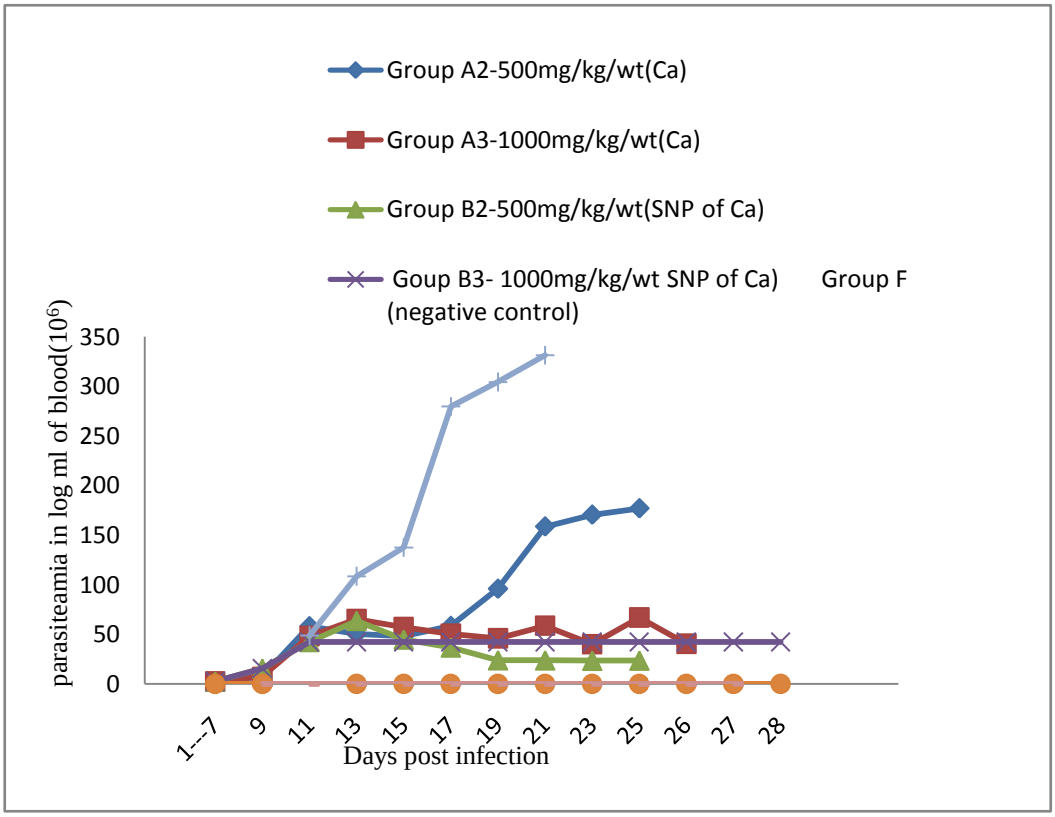


Figure4.13: Course of parasitemia in groups of animals infected with *Trypanosoma congolense* andtreated with butanolic and synthesized silver nano particle of seed extract of *C. albidum*

Key: *Ca=Chrysophyllum albidum,* SNP=Silver nano particles

Figure 4.14showed the result of prophylactic treatment on mice with synthesised and non-synthesized seed extract of *Chrysophyllum albidum*. There was significant difference in all the treated groups compared with the control (infected not treated group). Group D1 treated with 50 mg/kg of butanolic extract of *C. albidum* for three days before being challenged with the parasite on the third day did not develop parasitemia until the 12th day post infection. The number of trypanosome did not exceed average mean count of 10.8 ±71 and the animals survived up to the 28th day when the experiment was terminated. On the other hand the group D2 treated with 50 mg of synthesized silver nanoparticle of seed extract of *Chrysophyllum albidum*developed parasiteamia on the 18^{th} while the experiment lasted.

Also the group treated with 100 mg/kg of butanolic seed extract of *C. albidum* group C2 for three consecutive days before being challenged with the parasite on the third day, did not come up with parasites until the 16th day post infection. The level of parasiteamia did not rise above 3.28 ±1.43 and the animals survived up to the 28th day when the experiment was terminated. Conversely, group D2 treated with 100 mg/kg of synthesized silver nanoparticle of seed extract of *Chrysophyllum albidum* before been infected with the parasite did not develop parasitemia up to day 28 when the experiment was terminated. Furthermore, the infected not treated group F, parasitemia increased uncontrollably until they culminated in death by day 18th post infection. Animals in group G were notinfected not treated, negative control and survived throughout the course of experiment.

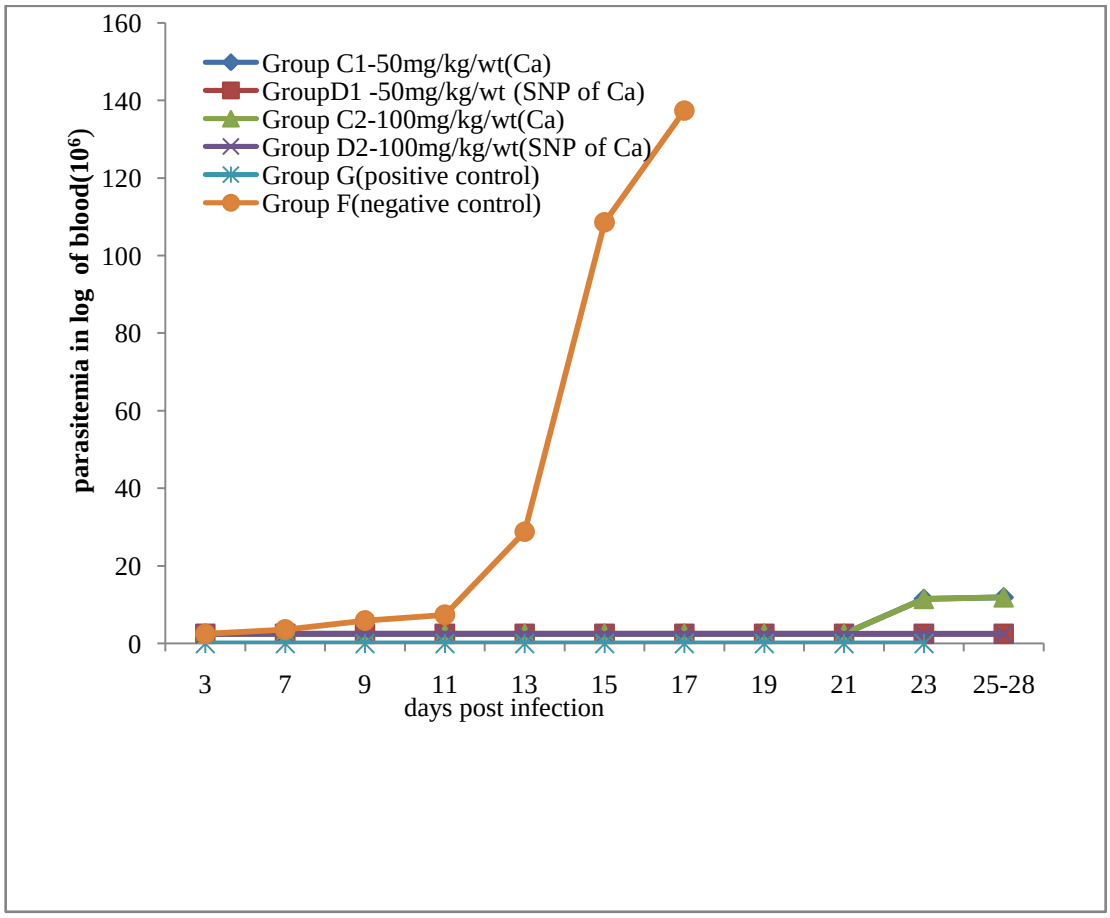


Figure 4.14: Course of parasitemia in animals treated with butanolic and synthesized silver nano particles of seed extract of *Chrysophylum albidum* beforeand after infection (prophylactic group)

4.12 Effect of extract on packed cell volume of experimental mice

The mean PCV pattern for the different experimental groups prior and after the initiation of the study period is shown in Figure 4.15. The result showed that the initial mean PCV levels of all infected groups before commencement of treatment was 36.15 ± 0.12 compared to uninfected untreated group with mean PCV levels of 39.51 ± 0.10. by day 15 post infection (days 6 post treatment). The groups treated with 100 mg, 500 mg non synthesized extract and negative control groups showed significant reduction in average mean PCV levels such as 34.13±1.05 before they culminated in death between day 20-23 post infection. Mean PCV level of mice treated with synthesized extract at (1000 mg/kg) showed steady rise up to the period the experiment was terminated with final value of 41.53±0.12 which is comparable to the positive control. Conversely the prophylactic group treated with 50 and 100 mg/kg have maintained theirmean PCV value of 39.6 ± 2.10 from the initial to the end of the experiment, with slight increase in those treated with 100 mg/kg synthesized silver nano particles of *C.albidum*.

4.13 Characterization of silver nanoparticles of *Chrysophyllum albidum*and *Tapinantus globiferus*

4.13.1 UV-visible spectroscopy of synthesized silver nano particles extracts of *Chrysophyllum albidum* and *Tapinantusglobiferus*

The UV visible spectrum of colloidal solution of silver nano particles synthesised from *C. albidum* seed extract (Figure 4.16) showed surface Plasmon resonance at 450 nm which conforms to the characteristic UV-Visible spectrum of metallic silver. The broadening of the peak indicates that the particles are poly dispersed.

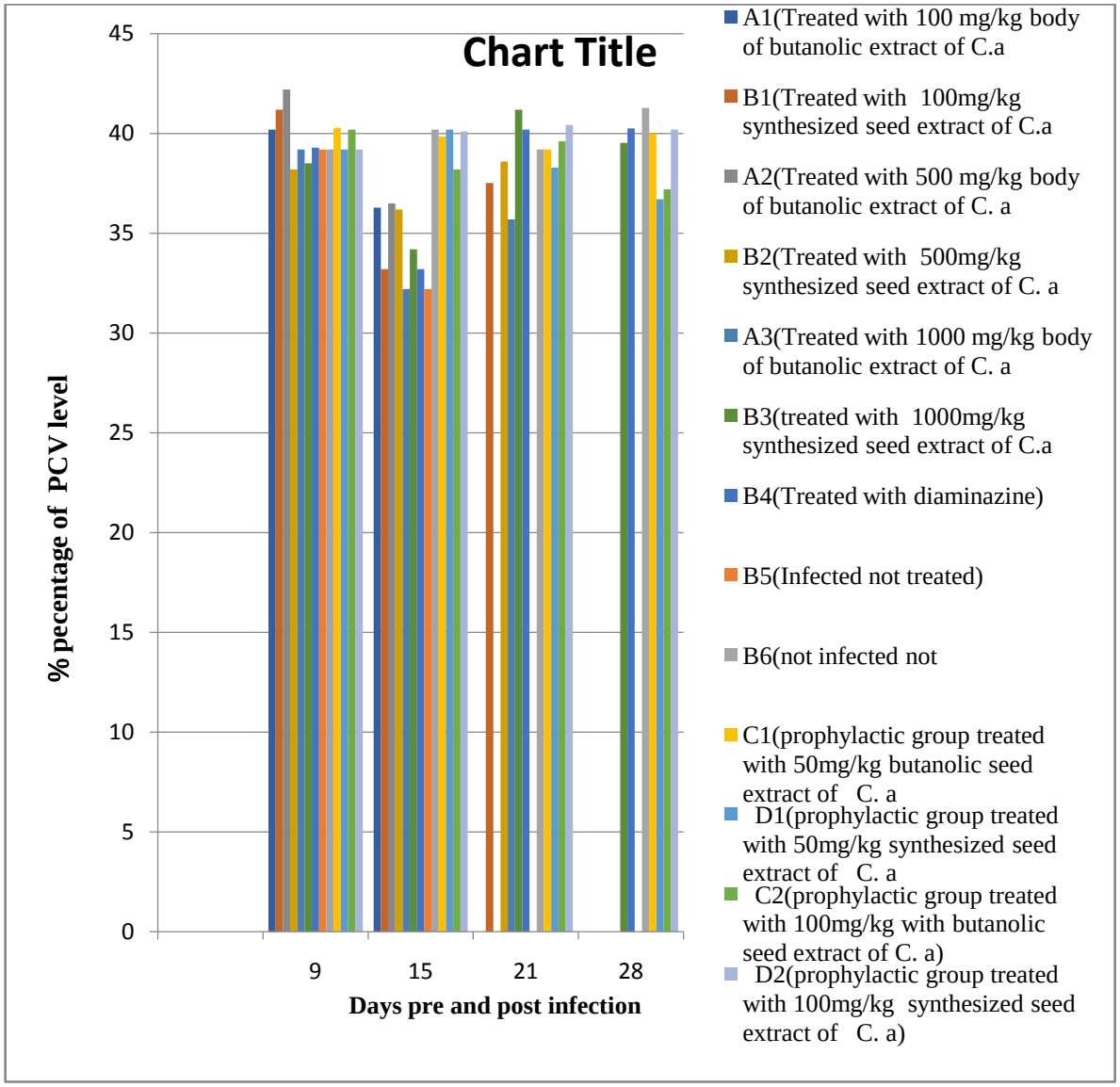


Figure 4.15: Effect of butanolic seed extract and synthesized extract of *C albidum*on Packed Cell Volume on mice infected with *Trypanosoma congolenses*

Key: C.a –*Chrysophyllumalbidum*

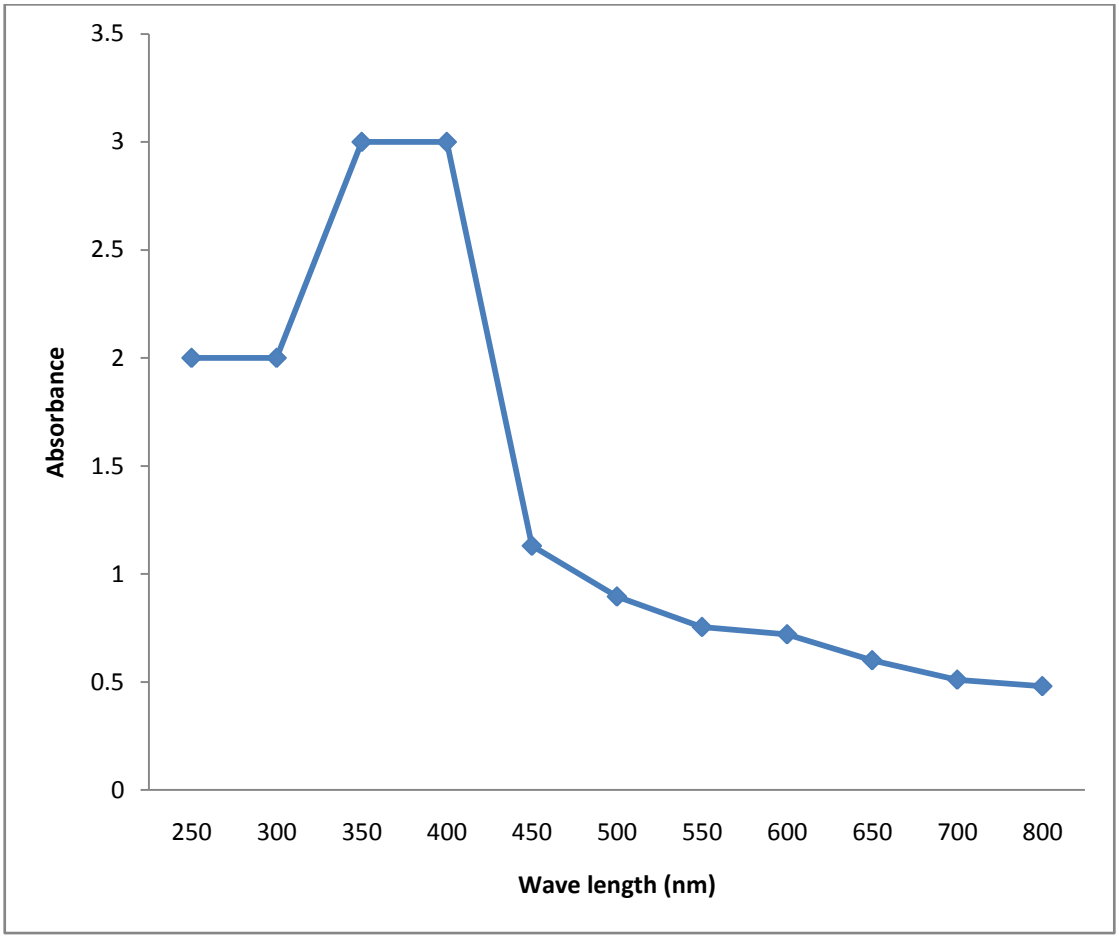


Figure 4.16: The UV- Visible Spectra of silver nanoparticles synthesised using aqueous extract of *C. albidum*

Figure 4.17, shows the UV -visible spectroscopy of the synthesized nano particles of *T. globiferus*leaves were observed to be in the range of 350 nm. By the indication of suitable surface Plasmon resonance at 350 nm; this conforms to the characteristic UV-Visible spectrum of metallic silver. The week adsorption peak at shorter wave length was due to the presence of several organic compounds which are known to interact with silver ions.

4.13.2 Fourier-Transform Infrared spectroscopy (FTIR) of butanolicseed extracts of *C. albidum*

Fourier Transform IR bands of butanolic seed extract of *C. albidum* revealed strong bands of alcohol group at 3268.9. Also observed were strong amide bands at, 2105.9, 1718.3 and 1617.7. At 1233.7 and 1021.3 strong bands belonging to the C-O group were observed (Table 4.7). The spectrum also revealed weak broad band of C-H at 292.60. The peaks at 864.7 and 816.3 are of the alkene group. The strong peaks observed at 1401.5 and 1148.0 were attributed to coo-and NH groups respectively. Furthermore the FTIR spectrum result of synthesized silver nanoparticles of seed extract of *C. albidum* reveal the nature of capping ligands that stabilises the silver nano particles formed, by bio reduction process and also identify the possible biomolecules responsible for the reduction of the silver ions into silver nanoparticles(Table 4.8). The bands at 524.13, 532.05 and 601.09 indicate the presence of alcohols and phenols, while at 668.30, 782.43, 8382 were bands of alkene group.

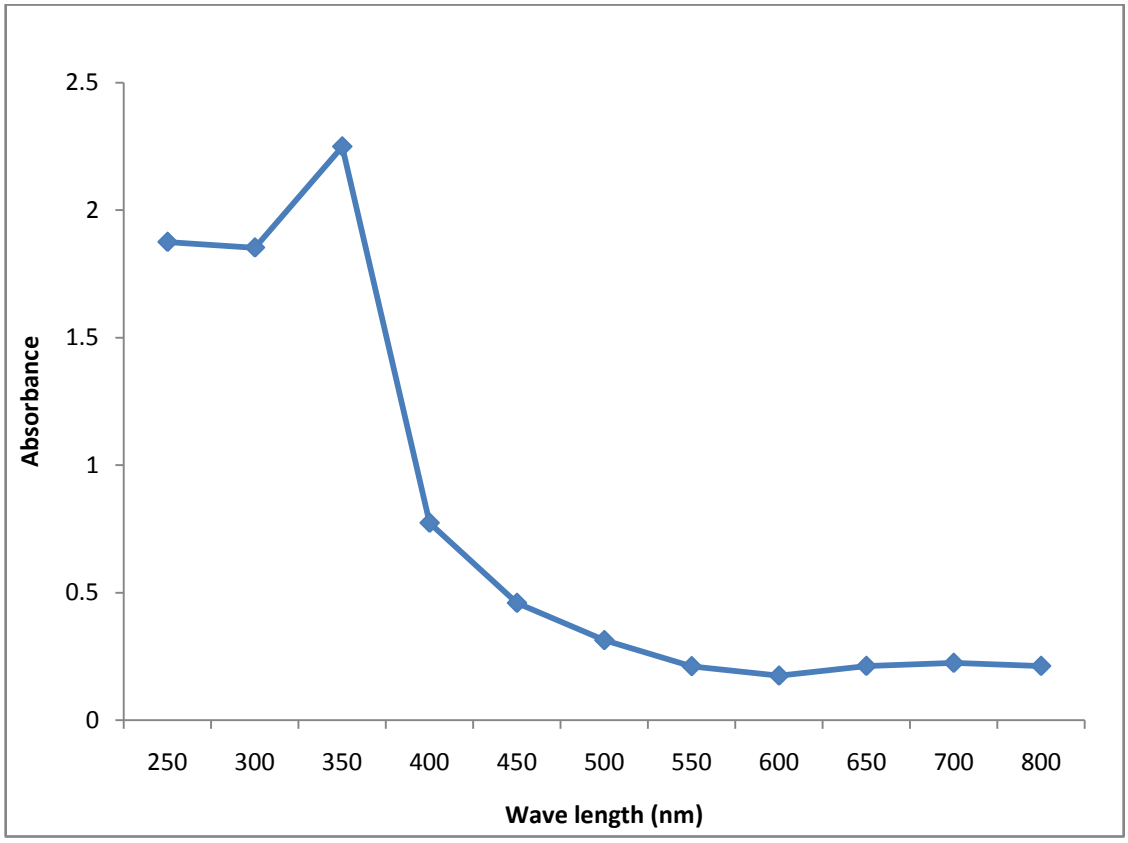


Figure 4. 17: The UV- Visible Spectra of silver nanoparticles synthesized using aqueous extract of *T. globiferus*

Table 4.7: Fourier Transform infrared spectroscopy (FTIR) of bands and functional groups of butanolic seed extract of *Chrysophyllum albidum*

Bands(cm^{-1})	Functional group	Intensity
3268.9	Alcohol O-H	Strong broad
2926.0	C-H	Weak broad
2105.9	Alkynes C-H	Strong
1718.3	Amide C=O	Strong
1617.7	Amide C=O	Strong
1233.7	C-O	Strong
864.7	Alkenes=C-H	Strong
1401.5	Asymmetric(COO-)	Strong
1148.0	NH	strong
816.3	Alkene=C-H	strong
1021.3	C-O	strong

Table 4.8:Fourier Transform Infra-Red spectroscopy (FTIR) bands and Functional groups of synthesized silver nanoparticles of seed extract of *Chrysophylum albidum*

Bands(cm^{1})	Functionalgroup	Intensity
668.30	Alkenes	strong
783.43	Alkyl halide C-Cl	Strong
832.99	Alkene=C-H	Strong
970.71	Alkene =C-H	Strong
1099.06	Aliphatic amines	weak, multiple bond
1384.89	Alkyl halide C-F	Strong
1648.09	Amide C=O	Strong
1745.63	Amide C=O	Strong
2921.91	Alkenes	Strong
3410.06	Alcohol OH	Strong
3734.42	Alcohol OH	Strong
3904.24	Alcohol OH	Strong

4.13. 3 Fourier Transform Infra-Red Spectroscopy (FTIR) of butanolic leave extractof *Tapinantusglobiferus***.**

Fourier Transform Infra-Red Spectroscopy analysis was used to identify functional groups in butanolic leave extract of*Tapinantus globiferus*. The spectrum revealed strong broad band of the hydroxyl group which was observed at 3257.7 (Table 4.9) also strong bonds of carboxylate ion and amide were observed at 2929.7 and 1599.0 respectively. While at 1304.6, 1379.1 and 764.1 strong bonds of alkyl halide were observed. Alkene band was observed at 864.7 and that of aliphatic amines was observed at 1043.7.

Similarly Table 4.10 showed FTIR analysis of synthesized silver nano particle of leave extract of *T. globiferus.* Alcohol bands were observed at 3753.4, 3852.8, 3712.4 and 3369.5. Bands of alkenes were observed at 2922.2 and 2109.7, while bands of amide and aromatic amines were observed at 1751.8 and 1271.0.

4. 13. 4 Scannedelectron microscope of synthesized silver nano particles of seed extract of *Chrysophyllum albidum and Tapinantus globiferus*

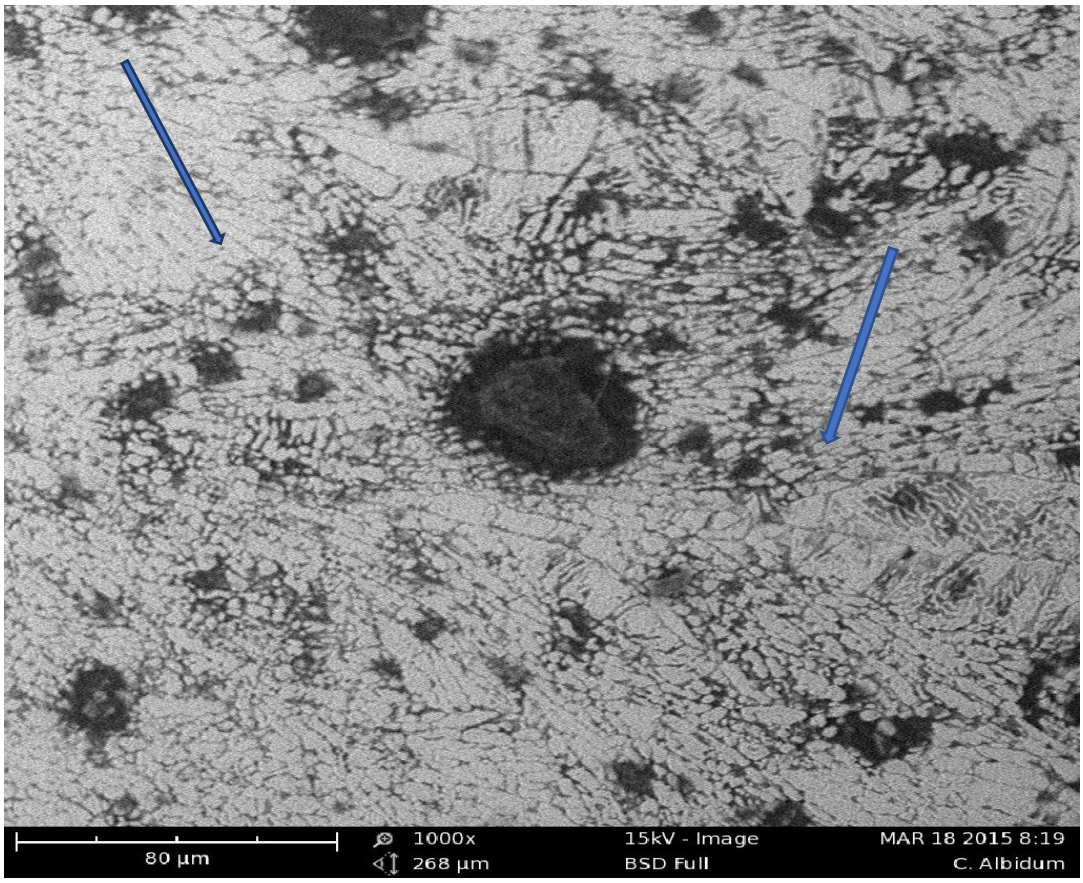
Plate XIII and XIV shows representative scanned electron microscopic 5 (SEM) images recorded at magnifications of X1000 and X3500 from drop-coated films of the silver nanoparticles synthesized by treating $AgNO_3$ solution with *Chrysophyllum albidum*. The resulting silver nanoparticles were predominantly spherical and of various sizes. The fibre frequency histogram taken from a large number of micrographs (Figure 4.18) showed variation in nano particle size estimated to be between 377 nm to 3, 970 nm with average size of 1.200 nm. Similarly, Plate XV shows the morphology of the formed synthesized silver nanoparticles of*Tapinantus globiferus* at X3500 magnification.

Table4.9: Fourier Transform Infra-Red Spectroscopy (FTIR) bands and functional groups of butanolic leave extract *T. globiferus*

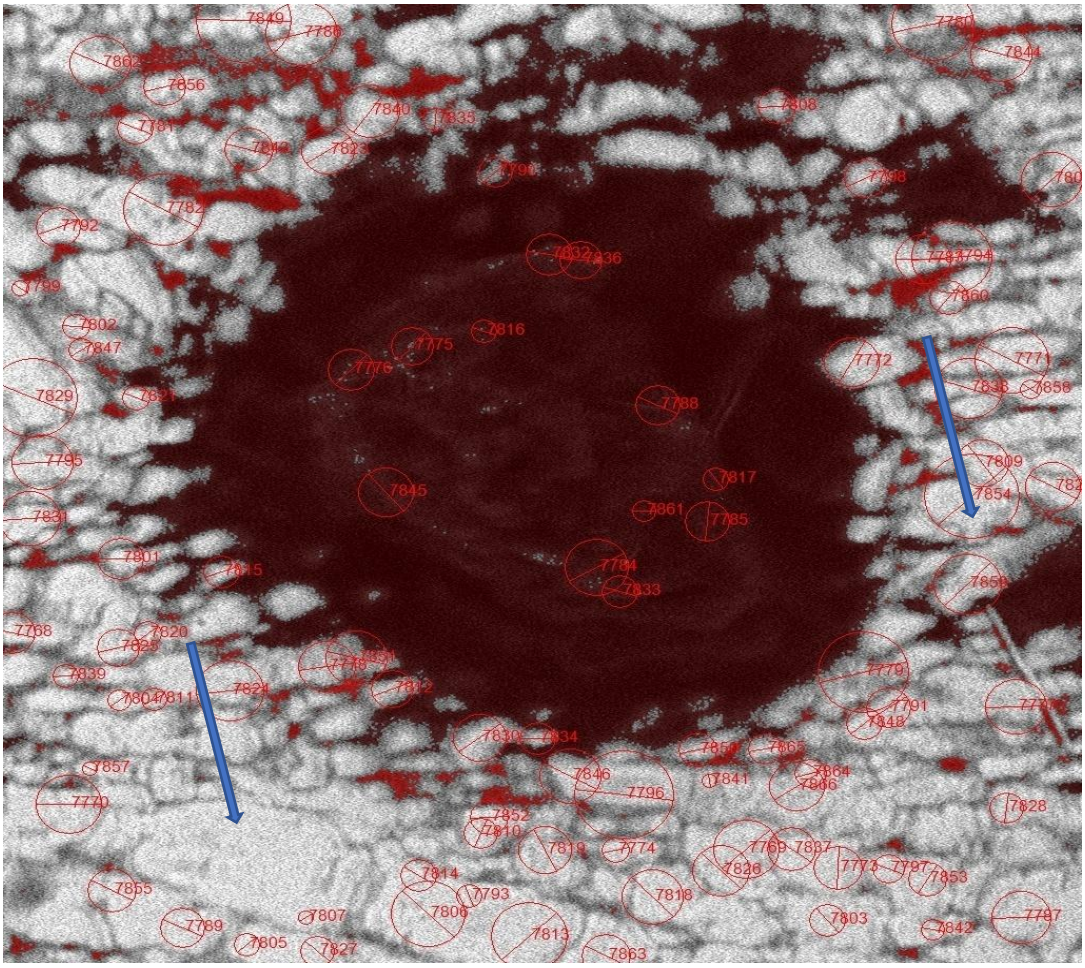
Bands (cm^{-})	Functional group	Intensity
3257.7	Hydroxyl OH	Strong broad
2929.7	Carboxylate ions	Strong
1304.6	Alkyl halide	Strong
1379.1	Alkyl halide	Strong
1599.0	Amide C=O	Strong
1222.6	1222.6	Strong
764.1	Alkyl halide C-Cl	Strong
864.7	Alkene =C-H	Strong
1043.7	Aliphatic amines	Strong

Table4.10: Fourier Transform Infra-Red Spectroscopy (FTIR) bands and functional groups of synthesized silver nanoparticles of leave extract of *T. globiferus*

Bands(cm^{-1})	Functional group	Intensity
3753.4	Alcohol OH	Strong
3852.8	Alcohol OH	Strong
3712.4	Alcohol OH	Strong
3369.5	Alcohol OH	Strong
2922.2	Alkenes	Strong
2359.4	Amide C=O	Strong
2109.7	Alkenes	Weak Bond
1751.8	Amide C=O	Strong
1271.0	Aromatic amines	Strong



PlateXIII: Scanning Electron Microscope image of the synthesized silver nanoparticles (agglomerated nano particles arrowed) of *C. albidum* seed (X1000)



PlateXIV: Scanning Electron Microscope image of the synthesized silver nanoparticle (agglomerated nano particles arrowed) of *C. albidum* seed: (X3500)

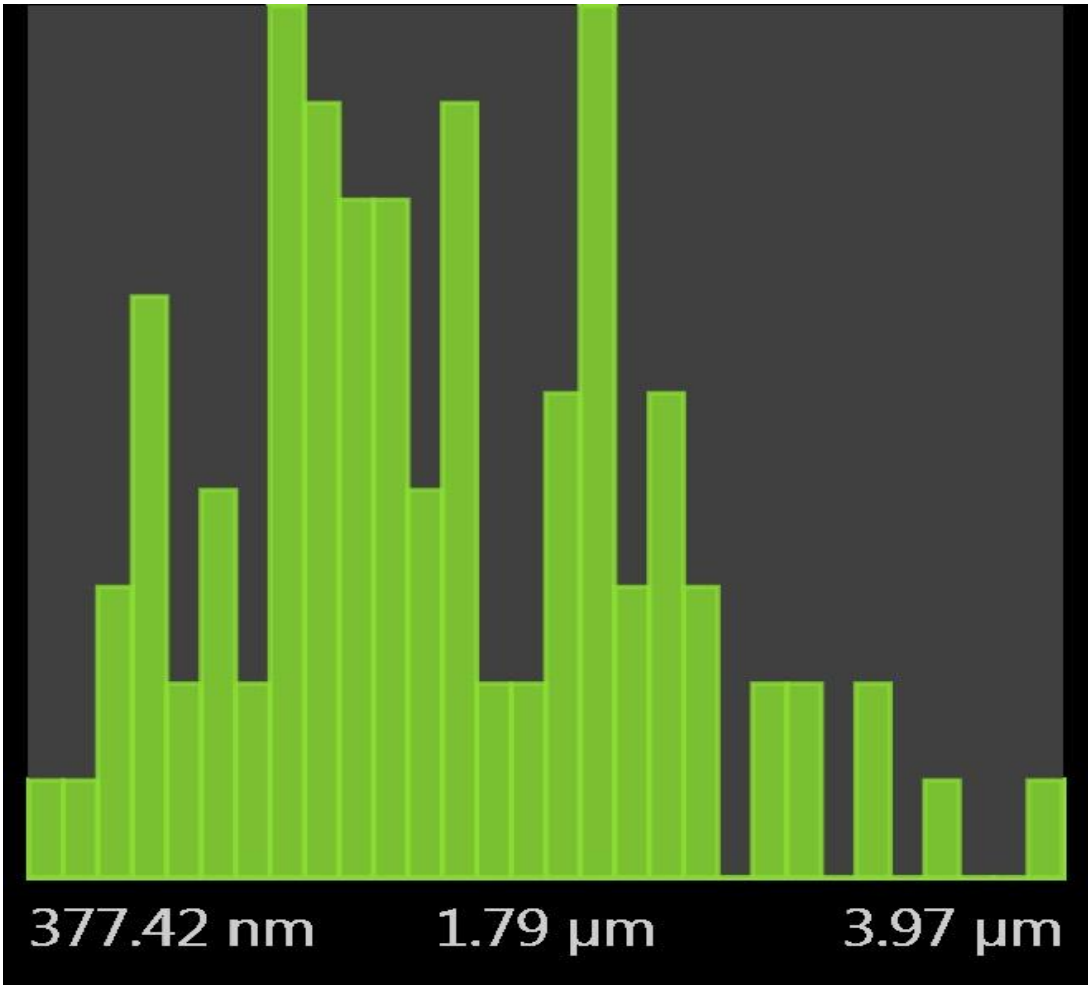


Figure 4.18: Fibre histogram showing average particle size of synthesized silver nano particles of seed extracts of *Chrysophyllum albidum*

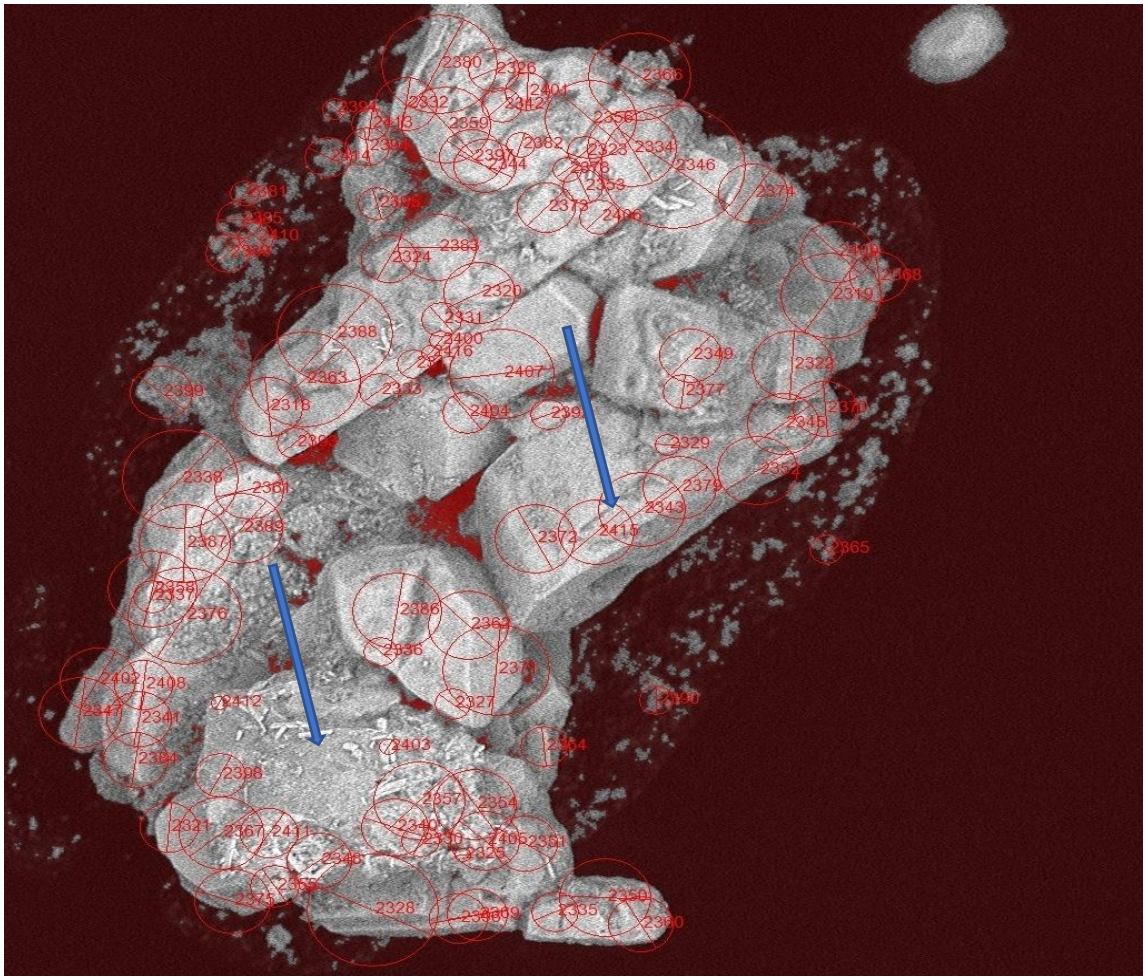


Plate XV: Scanning Electron Microscopic image of the synthesized silver nanoparticles(agglomerated nano particles arrowed)of *T. globiferus* (X3500)

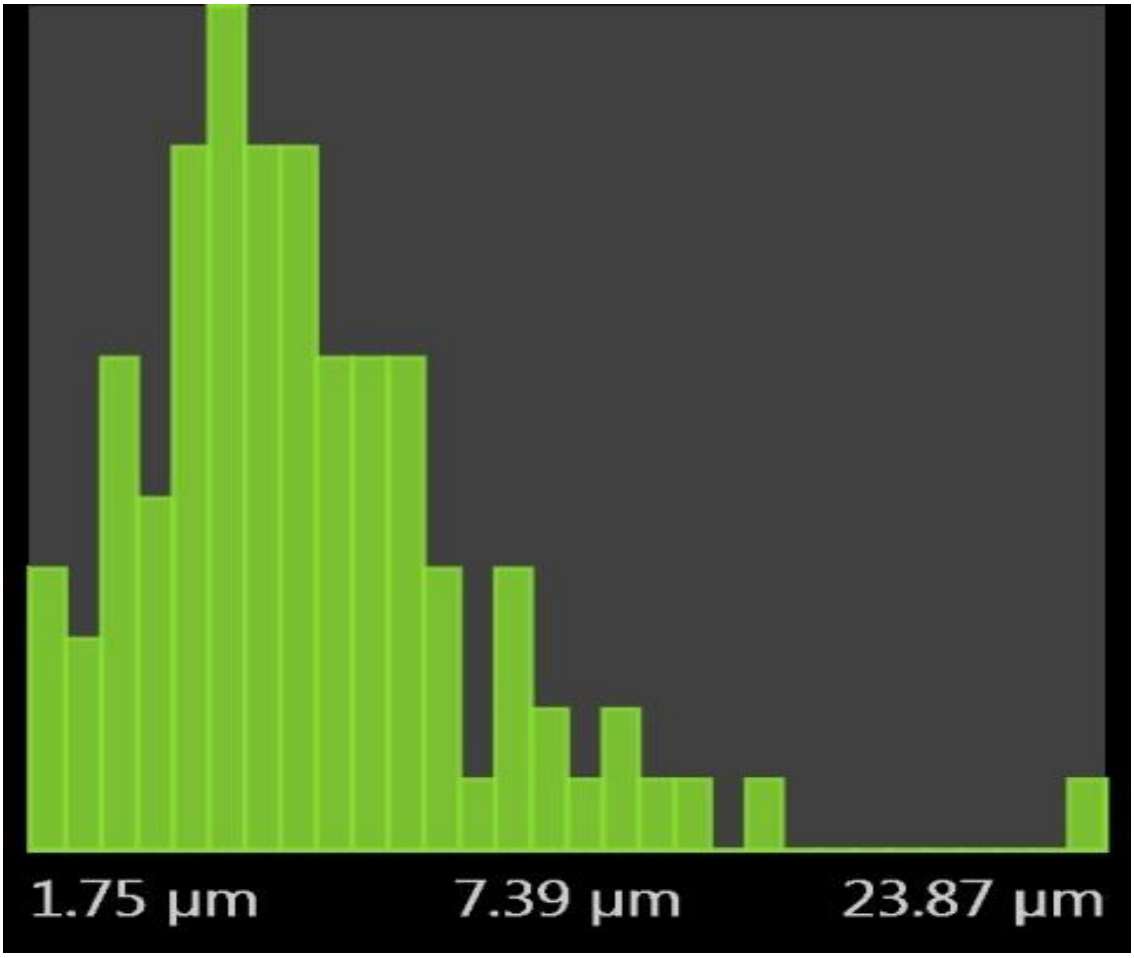


Figure 4. 19: Fibre histogram showing average particle size of synthesized silver nano particles of seed extracts of *T. globiferus*

Most of the formed silver nanoparticles have agglomerated. Most of the particles formed were square and rectangular in shape with various sizes. The average diameter of these silver nanoparticles estimated from the fibrehistogram are about 1.75-23 µm, with an average size of 12.81 µm.

4. 13.5X-ray defractometer of synthesized silver nanoparticles of seed Extract*Chrysophyllum albidum* and *Tapinantus globiferus* leave extract

The structure of the biosynthesized silver nanoparticles was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Table 11). The XRD pattern showed six intense peaks in synthesized silver nano particle of *C. albidum* the whole spectrum of 2θ value ranging from 10 to 80. Average size of the particles synthesized was from 7-31 nm with cubic and hexagonal shapes and were crystalline in nature. The X-ray diffraction of silver nanoparticle formed from aqueous *T.globiferus* extract showed four characteristic diffraction peaks as shown in the table and the average particle size was from 0.1- 201nm.

Table 4. 11: X-ray diffraction of *Chrysophyllum albidum* and *Tapinanthus globiferus*

Synthesizedsilvernano particle	Strongest peak	d spacing in nm	2 value	Plane FWHM	Element Particle size
C.albidum	7	6.11014	14.4850	111 1.0100	7.8 nm
	6	7.13245	12.4000	200 0.76000	10.4 nm
	13	4.15651	21.3600	220 0.60000	13.0 nm
	39	1.45200	64.0800	222 0.0000	0.10 nm
	42	1.26737	74.8600	311 0.52000	12.07 nm
	44	1.11730	87.1700	400 0.1800	13.8 nm
T. globiferus	9	5.13943	17.2400	111 0.0400	201 nm
	16	4.74636	18.6800	200 0.0000	0.13 nm
	41	1.49998	61.800	220 0.0000	0.16 nm
	43	1.21307	87.1700	222 0.18000	32.0 nm

FWHM=Full width at half maximum

CHAPTER FIVE

5.0 DISCUSSIONS

This current study has identified methanolic extracts from seeds, leaves, roots, of some angiosperm and fungi, the angiosperm are*Chrysophyllum albidum* (seed), *Tapinantus globiferus* (leaves), *Saba florida* (roots) and two fungi (*Formitopsis pinicola* and *Cantharellus cibarius*) which exhibited various activities at different concentrations against *Trypanosoma brucei* and *Trypanosoma congolense*. The results reject the hypothesis which says there is no trypanocidal activity from the selected angiosperms and fungi. The high activity observed with the methanolic extracts could be associated with high dielectric constant of methanol in comparison with petroleum ether and water, which exhibited lower activities, which could be due to their low dielectric constant. The unique properties of methanol enable it to extract very polar, neutral, basic and acidic compounds, amino acids, nucleotides, sugar and polysaccharides (Venugopal and Liu, 2012).

The high antitrypanosomal activities observed with the extracts might be as a result of addictive or synergistic action of several chemical compounds acting at a single or multiple target sites associated with a physiological process (Tyler, 1999; Feyera, 2014). The low activity observed with petroleum ether extracts could be as a result of main active extraction products such as alkaloids saponins flavonoids etc are low or containing non-polar hydrophobic compounds with extremely high lipophilicity, of which low polar neutral compounds, steroids and high carbon fatty acids are usually present (Venugopal and Liu, 2012).

Similarly aqueous extraction product mainly contains metals, ions, high hydrophilic compounds, and water soluble proteins/enzymes, glycoproteins, peptides, amino acids, nucleotides, sugars, and polysaccharides which may not have trypanocidal properties.

Many plant extracts owe their potency to the presence of one or more secondary metabolites, which may exert action through addictive or synergistic action of several chemical compounds acting at a single or multiple target sites. These metabolites are usually found in various parts of the plants like roots, leaves, shoots and bark (Tyler, 1999; Ngulde *et al*., 2013; Feyera*et al.,* 2014).

The Phytochemical screening of these 5 extracts from 5 plants revealed the presence of high concentrations of alkaloids, saponins flavonoids among others. Several of these compounds were reported to exert antitrypanosomal activities, antioxidant and anticancer effect (Camacho *et al*., 2000; Tasdemir *et al*., 2006, Sultanbawa, 1980, Elfita *et al*., 2009, Saputri and Jantan, 2012, Aisha *et al*., 2012, Chitchumroonchokchai *et al*., 2012). These results also reject the hypothesis which says that the extracts of selected angiosperms and fungi are low in phytochemical constituents.

The choice of *Chrysophyllum albidum* (seed) and *Tapinantus globiferus* (leave) extracts for further studies was as a result of high *in vitro* trypanocidal activity displayed by these plants and also due to availability of the plants. The partitioned butanolic extract of *C. albidum seed* and *T. globiferus* leaves produced the highest *in vitro* activity which was the highest polar fraction. Hence anti trypanosomal principle of the extracts appears to reside in the high polar solvents.

Which suggest natural products such as alkaloids, terpenes, quinones, and polyphenols. Alkaloids as one of the major active ingredient as observed from the phytochemical screening, has the ability to inhibit protein biosynthesis, to intercalate DNA, interrupt membrane fluidity, to inhibit microtubule formation or induce programmed cell death in blood stream forms of *Trypanosomes* (Omar and Khan, 2007). Although isolation of pure compounds, characterization and classification of various compounds that could be responsible for the observed activity was not carried out in this work, reports have shown, a class of alkaloid known as spermidine alkaloids present in some plants reported to exert high antitrypanosomal activity (Nibret and Wink, 2011). The mechanism of action of this compound could involve the presence of aryl moiety in the spermidine molecule which interacts with the hydrophobic region of trypanothione reductase, so that the spermidine would adopt a non- extended bound conformation. This could be responsible for the observed effect of the alkaloid. (Omar and Khan, 2007). Spermicidine alkaloid resembles the chemical structure of pentamidine, which binds to nucleotides in DNA and RNA, by promotion of cleavage of the parasite's circular DNA in a manner similar to that of topoisomerarase II inhibitors, which may have been responsible for the trypanocidal activity (Nibret and Wink, 2011).

Flavonoids as another important group of compounds also found present in the extracts, probably have the ability to cause significant reduction of parasiteamia when administered *in vivo.* The mode of action could be interference with replicating forms of trypanosomes which are totally dependent on glycolysis for energy production and membranes of the parasites (Colgate and Molyneux, 1993).

Similarly, sterols such as vernoguinosterol and vernoguinoside, have been reported with soapy characteristics which enables it to precipitate and coagulate red blood cells (George *et al.,* 2002).

Saponins with detergent properties can dissolve in biomembranes and disturb their fluidity and the function of membrane proteins of parasites (Mann *et al.,* 2009).

Saponins are also known to inhibit the development of protozoa by interaction with the cholesterol present on the parasite cell membrane, thus leading to parasite death (Wang *et al.,* 2008). Several studies with saponins have demonstrated their positive effect on improve nutrient absorption by increasing intestinal permeability via membrane depolarization (Efterpi *et al.,* 2012). Similarly Tannins are a group of compounds with high medicinal activity, they are water soluble plant polyphenols that precipitate proteins have the ability to form chelates with metal ions, particularly iron (Muhammed *et al.,* 2014). This could have led to high activity observed with extracts of *Chrysophyllum albidum*. Tannins have also been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Min and hart, 2003).

The growth of many fungi, bacteria, yeast, virus and protozoa was inhibited by tannins (Newbold *et al.,* 1997).Further purification of the butanolic extract of *C. albidum* and *T. globiferus* on column chromatography led to the collection of 308 and 203 fractions respectively. Only fraction B4 (91-135) from *C. albidum* and TG5 (54-64) from *T. globiferus* had activity on both *T.b.brucei* and *T.congolense.* TG9 (104-118) and had MinimumInhibitory Concentration at 3.9 µg/ml *on T.congolense* only.

It is obvious that the active components were largely pooled and concentrated in these fractions (chloroform/methanol) resulting in very active *in vitro*trypanocidal activities against both *Trypanosoma brucei brucei* and *Trypanosoma congolense*. Similar studies showed that fractions of extract exhibit potent growth inhibitors of trypanosomes (Mbaya *et al.,* 2007; Umar *et al.,* 2010 and Yusuf *et al.,* 2012).

Further purification of B4 and TG5 led to the collection of 198 and 112 fractions respectively. However, only fraction III (28-48) from B4 had MIC at 3.9 µg/ml on both species of parasite. Compared to the fractions obtained from TG5 which had highest MIC at 64 and 250 µg/ml with most fractions on *T.congolense* and *T. brucei*respectively. These suggest that the active ingredient in TG5 may not be sufficient to cause inhibition. Consequently high concentration was needed to cause inhibition. Based on this results further fractionation was carried out on fraction III (28-48). However the antitrypanosomal activity after performing two separations on sephadex LH-20 and Preparative Thin layer Chromatographydecrease in activity. A possible explanation is that disintegration of unstable bioactive compounds might have occurred due to combined action of the various compounds present.

The green synthesis of silver nanoparticles with plant extracts were successfully carried out in this project. Silver nitrate was used as reducing agent owing to the fact that silver has distinctive properties such as good conductivity, catalytic and chemical stability (Ahmad *et al.,* 2010; Jagtap and Bapat, 2013; Suresh *et al.,* 2014). The addition of aqueous silver nitrate solution, the metal plant extract interaction was confirmed.

The extract of *C. albidum* seed changed from yellowish brown to dark brown while that of *T. globiferus* leave extract changed from pale green to dark brown colour within 1 hr. and

20 min. respectively due to excitation of surface plasmon vibrations leading to formation of silver nanoparticles (Rao *et al.,* 2010).

The time duration taken to change in colour can vary from species to species. Report showed that *Boswellia ovalifoliolata* took 10 min. while*Shorea tumbuggaia* took 15 min to synthesize silver nanoparticles.

These results therefore reject the hypothesis which says that silver nano particle was not synthesized from the plants tested. The findings obtained are useful in terms of selection of medicinal plants for the synthesis of silver nanoparticles (Gilaki, 2010).

There are various ways in which reduction of silver takes place in plant extracts. This could be achieved due to presence of secondary metabolites present in plant systems. Another biogenic route is the energy from electron released during Glycolysis (photosynthesis) for conversion of NAD to NADH which may lead to the transformation of Ag(NO3)2 to form nanoparticles . Another mechanism in the formation of nanoparticle, involves the release of an electron during the formation of ascorbate radicals from ascorbate (Pal *et al.,* 2007; Arya, (2010). It is a well-known fact, that silver ions exhibit higher activity than there bulk properties (Khan *et al.,* 2013).

The synthesized silver nanoparticles of *C.albidum* and *T. globiferus* showed very high *in vitro* trypanocidal activity at 0.98 µg/ml compared to the non-synthesized which had MIC at 15.6 µg/ml. The silver nano particle have been found to be effective larvicidal agents against dengue vector *Aedes aegypt* (Suresh *et al.,* 2014), *Culex quinquefasciatus*, filariasis vector *C. quinquefasciatus* (Santoshkumar *et al.,* 2011); malarial vector *A. subpictus, Aedes aegypti*, and other parasites (Marimuthu *et al.,* 2011). High activity of nano particles on microbial pathogens such *as Escherichia coli, Pseudomonas aeroginosa, Bacillus subtilis,*

Aspergillus, *Candida* and Saccharomyces, *Klebsiella pneumonia* have also been reported (Ramya and Sylvia, 2012).

One of the unique properties possess by nano particles which is responsible for the high lethal activity, is that they exhibit larger surface area to volume ratio (Kaviya *et al*., 2011). When surface area of the nanoparticles gets increased, their surface energy gets increased and hence their biological effectiveness also increases. Other studies have suggested that because of their magnetic properties, nanoparticles can affect the membrane permeability and respiratory function when they attach to cell surface (Vijayaraghavan *et al.*, 2012). Another possible reason is that silver nano particle have relatively higher anti- bacteria activity against gram negative bacteria than gram positive bacteria, which may be due to thinner peptidoglycan layer and presence of beta barrel proteins called purines in the former.The molecular basis for the biosynthesis of these silver crystals is speculated that the organic matrix contains silver binding proteins that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al.*, 2010).

The FTIR spectroscopy of synthesized nanoparticles of plants extract was able to identify secondary metabolites which may have been acting as reducing and capping agents such as terpenoids, flavonoids and proteins. Terpenoids have been suggested to play a key role in the transformation of silver ions into nanoparticles (Shafaghat, 2014). Terpenoids are a class of diverse organic polymers synthesized in plants from five-carbon isoprene units, which display strong antioxidant activity (Shankar *et al*., 2005).

Based on the FTIR spectroscopy data, Shafaghat, (2014) suggested that dissociation of a proton of the eugenol OH-group results in the formation of resonance structures capable of further oxidation. This process is accompanied by the active reduction of metal ions, followed by nanoparticle formation.

Flavonoids are a large group of polyphenolic compounds that comprise several classes: anthocyanins, isoflavonoids, flavonols, chalcones, flavones, and flavanones, which can actively chelate and reduce metal ions into nanoparticles (Spencer, 2008). Flavonoids contain various functional groups capable of nanoparticle formation. It has been postulated that the tautomeric transformations of flavonoids from the enol-form to the keto-form may release a reactive hydrogen atom that can reduce metal ions to form nanoparticles. For example, it is believed that in the case of *Ocimum basilicum* (sweet basil) extracts, it is the transformation of flavonoids luteolin and rosmarinic acid from the enol- to the keto-form that plays a key role in the formation of silver nanoparticles from Ag ions (Ahmad *et al.*, 2010).

The LD_{50} value of methanolic seed extract of *C. albidum*given intraperitoneally to mice was 1065.99 mg/kg. Tremors, convulsionand mortality which were observed in most of the treated mice, especially those given high doses of 3000-5000mg /kg of *C.albidum* could be associated with the high level of alkaloids present. Alkaloids are known to be toxic when ingested or administered in large doses and could lead to having central nervous system stimulant effects (Brunton *et al.*, 2005). The result of the acute toxicity test of leaf extract of *T. globiferus* may be safe as the LD_{50} could not be determined at the doses given (100-5000) mg/kg. The Organization for Economic Corporation and Development document on Acute Toxicity Testing, (OECD, 2001), recommended that the maximum dose levels for any chemical compounds should not exceed 5000 mg/kg of the animal body weight.

The results of the present study suggest that the butanolic extract of *T. globiferus* is not acutely toxic to the mice thereby providing a support to the use of the leaves in indigenous

system of medicine. However, further long-term toxicological studies (chronic toxicity), are needed in order to establish it as safe medicine.

Though the phytochemical screening revealed many chemical constituents, which could affect the animal positively or negatively as a result of prolong usage.

Three doses 100, 500 and 1000 mg/kg were used for the *in vivo* experiment. The result showed that the effect was dose dependent, as animals treated with 100 mg/kg/wt. of butanolic and synthesized silver nano particle of the seed extract of *C. albidum* had steady rise in parasiteamia and culminated in death by days 19and 21 post infection (Figure 11). However mice treated with 500 mg/kg/wt. also died by day 14and 16 post infections due to relapse. The possible explanation for these could be based onthe peculiarities in metabolic pathways in *in vitro* and *in vivo*system.Shaba *et al.,* (2012), reported in a similar experiment were the methanolic extract of *Calotropis gigantean* had very high trypanocidal activity but could not establish any activity *in vivo*. They suggested that plant with *in vitro* activity may not have *in vivo* activity because the extract may exert some static action where by it affects the growth and multiplication of trypanosomes but it is not able to eliminate the parasites. Also, several researchers reported that such actions may be as a result of loss in extract activity which could be due to pharmacokinetic reasons (Abubakar *et al.,* 2008; Antia *et al.,* 2009). Another explanation for relapse in treatment, may be due to inaccessibility of the extract to other tissue where the flagellates are known to hide, as a way of evading trypanolytic action of drugs.

The groups treated with 1000 mg/kg with butanolic seed extract of *C. albidum* showed better result in that parasitemia was reduced to 16.3±2.2 and their lives were prolonged to day 26th post infection. The death of mice even when parasitemia was low may suggest the

release of extra cellular factors other than direct effect of the parasites or may be due to the release of trypanosomes from the tissues which can occur when treatment is delayed or the dose rate is inadequate (Abubakar *et al.,* 2008 ; Antia *et al.,* 2009). It is also possible that the active component may be of short half-life making it unable to stay in the blood medium in sufficient amount long enough to exert its properties on the parasites, completely. Other speculation includes that of Umar *et al.,* (2013), who suggested that the antibodies produced by the host against the parasite are not effective because of the ability of the parasite to produce a large repertoire of antigens. The host defense mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen antibody interaction, eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system leading tocoma and death. The possibility that the active component is only trypanostatic rather than trypanocidal, needs, also be seriously considered.

Similarly, parsiteamia was greatly reduced in the group treated with 1000 mg/kg/wt. with synthesized silver nano particle of seed extract of *C. albidum*($p<0.05$), compared to the groups treated with 100 and 500 mg/kg/wt.

Although parasiteamia was not cleared from the blood stream of the animals, but they survived with low parasiteamia up to the 28^{th} day when the experiment was terminated.

The high trypanocidal activity observed with silver nano treated groups, may agree with similar observation by other researchers who suggested that Ag-NPs have different mechanisms for killing bacteria cells (Song and Kim, 2009). One of such speculations is that ionic silver strongly interacts with thiol group of vital enzymes and inactivates their activities (Lee *et al.,* 2007).

Also, Silver nanoparticles have the ability to bind strongly to sulfur- and phosphor-containing compounds (Zheng *et al.,* 2008) and because of this property, they can damage the bacterial cell membrane by impairing sulfur-containing proteins.

It also hasthe ability to penetrate the bacteria cell wall, and can damage sulfur-containing enzymes and phosphor-containing DNA. Silver nanoparticles are also known to accumulate heavily within mitochondria and are reported to impair mitochondrial function via oxidative stress (Xia *et al.,* 2006; Geoprincy *et al.,* 2013). Adenosine triphosphate (ATP) synthesis ceases when mitochondrial enzymes are damaged. Therefore, it is thought that their antibacterial effects are due to the ability to inhibit ATP synthesis (Song *et al.,* 2006). Another antimicrobial mechanism of Ag-NPs is the releasing of silver ions these ions contribute to cell death by producing high amounts of reactive oxygen species (Ratte, 1999; Sambhy, 2006).

Other school of thought suggested that pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth. Also silver nano particles may form complexes with cysteine groups of enzymes, these complexes prevent the enzymatic functions of proteins. According to this mechanism, silver ions exhibit antibacterial effects (Matsumura *et al*., 2003 and Kim *et al.,* 2008).

Parasiteamia in the untreated group increase uncontrollably until they culminated in death by day 21 post infection.

Trypanosomiasis is a serious disease and if not treated it can be very fatal. It is well known that trypanosome infection generally occur in three phases, the early phase of parasiteamia which is characterized by death or may be followed by chronic crisis of variable duration with less intense parasiteamia but with persistent of anemia, which often led to death in

infected animals (Pentreath, 1995). Furthermore, trypanosomiasis infection in its mammalian host is controlled by five intrinsic processes, four of which are intrinsic to the parasite, Replication; differentiation from dividing to non-dividing form; antigenic variation; dispersion from blood stream into extra vascular sites and the fifth process involves the trypanosome specific responses of the host (Pentreath, 1995). The interaction between the processes provides a complex mechanism for controlling the infection (Antoine and Desmecht, 2009).

Furthermore, the prophylactic groups treated with 50 and 100 mg/kg wt. of butanolic seed extract of *C. albidum* developed infection on day 9^{th} and 11^{th} respectively post infection. The level of parasiteamia in both groups did not exceed 10.8±71 and they survived up to the 28^{th} day. However the prophylactic groups treated with 50 and 100 mg/kg/wt. of synthesized silver nano particle of butanolic seed extract of *C. albidum* did not develop parasiteamia. This result shows that nano particles could be usedfor both curative and prophylactic applications.

A similar result was also reported by Singh *et al,* (2015) on antileshmanial effect of silver nano particles, they suggested that silver nano particle inhibit growth, metabolic activity, and infectivity of promastigotes by preventing survival of amastigotes inside host cells. The authors also reported that live parasites lose their infection abilities following exposure to nanoparticles.

This appears more significantly in parasites exposed to both Ag-NPs and UV light. The authors also went further to suggest the reason for this, could be the interaction of Ag-NPs with the surface of parasites leading to the impairment of the structure of lipophosphoglycan and glycoprotein 63 molecules that are found on the surface of parasite.

Furthermore, Rogers *et al.,* (2008) suggested the enhanced cytotoxic effects of SNPs observed in their work could be that SNPs interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry. Zolghadri *et al.,* (2009) demonstrated that SNPs provide a relatively high hydrophobicity inside bovine haemoglobin which causes a transition from alpha helixes to beta sheets and leads to partial unfolding and aggregation of the protein.

Packed Cell Volume (PCV) was measured to predict the effectiveness of the test extracts in preventing haemolytic anaemia which is one of the main causes of death resulting from increasing parasitemia of trypanosomiasis. Low PCV levels is usually experienced when parasiteamia increases probably due to trypanosome generated reactive oxygen which may lead to destruction of red blood cells membranes, by induced oxidation and subsequently haemolysis (Ekanem *et al.,* 2006;Bizuayehu *et al.,* 2012). The slightly lower PCV observed in all the infected groups before the commencement of the treatment may be as a result of acute haemolysis caused by increasing parasitemia.

These findings are in agreement with a report of Ekanem *et al. (*2006). However animals treated with 1000 mg/kg synthesized nano particle of *C. albidum* extract, led to remarkable improvement of anemia. So also, were the prophylactic groups that showed stability in there PCV, evident from the significant difference in the levels of the PCV of animal treated with lower doses of the extract and the infected not treated group (Table 8).

The result is in agreement with similar observations reported by Ekanem *et al.* (2006) and Nwodo *et al.* (2007) that the increase in PCV may be due to mechanisms associated with reduction in trypanolytic crisis which enhances red blood cells damage and destruction leading to anemia.

This, in turn, may be mediated by extracts ability to eliminate parasites from the blood, probably by reaching the site of action or rapid metabolization or neutralization of the toxic metabolites produced by trypanosomes. Also the plant extract may have prevented the onset of leucocytosis due to lymphocytosis usually observed at the outset of leucopenia at the terminal stage of the infection. This is a phenomenon associated with waves of parasiteamia (Atawodi, 2011). Also Maikai (2011) and Feyera *et al.* (2014), speculated that certain components of the plant might have helped stabilize the membrane of erythrocytes. Specifically; the anti-oxidant or free radical scavenging properties of phenolic compounds and flavonoids may play vital roles in this regard.

The FTIR results also showed organic of microbes that contain silver binding proteins that provide amino acids moieties which may have contributed to nano particle formation. Tan *et al.,* (2010), speculated that amino acids such as lysine; cysteine, arginine, and methionine are capable of binding silver ions. Other studies have shown that aspartate can reduce tetrachloroauric acid to form nanoparticles, although valine and lysine do not possess this ability (Elumalai*et al.,* 2014). The mechanism of action involves the conversion of amino acids to metal ions through the amino and carbonyl groups of the main chain or through side chains, such as the carboxyl groups of aspartic and glutamic acid or a nitrogen atom of the imidazole ring of histidine.

Other side chains binding metal ions include the thiol (cysteine), thioether (methionine), hydroxyl (serine, threonine, and tyrosine), and carbonyl groups (asparagine and glutamine) (Sadeghi *et al.,* 2015).

The SEM analysis is used to provide information about the morphology and size of the synthesised silver nanoparticles.SEM images of *C.albidum* show that the particles are monodispersed and mostly hexagonal in shape, the particle size is between 377 nm to 3.97 µm. The average size of the particle shape and size of nanoparticles synthesized by biological systems is common (Champa *et al* 2012). While on the other hand, the SEM images of *Tapinantus globiferus* shows that the diameter ranges of the particles size are between 1.75-23 µm of various shapes and were polydispersed. Most of the particles had agglomerated, this is not unusual. Similar observations have been reported by several authors, this includes the works of Arumugam *et al.* (2013). They reported SEM results for synthesized nano particle of *Nyctanthes arbor-tristis* to be of various shapes, sizes and to have agglomerate.

Furthermore, it can be said that variation in shape, sizes and agglomeration of nano particles is mainly due to some physical and chemical factors which include: increase in temperature of reaction beyond 75^{o}C, when PH of reaction is above 8, when the duration of "time" of reaction increases. Other factors include concentration of silver nitrate solution, which should not exceed 1mM and extract composition and reaction period largely affect the size, shape and morphology of the AgNPs (Das *et al.,* 2012; Khalid *et al.,* 2014).

This experiment took in to consideration all these factors. However, the larger silver particles observed with our nano particles may have been as a result of aggregation of smaller ones, due to the delay in taken SEM measurements, these were carried out after six months of synthesis due to lack of function facility (Ravichandran *et al.,* 2010; Sougata and Submersing, 2012).

Nevertheless further characterization of the structure of the crystalline nanoparticle material using X-ray defractometer showed that silver nano particles were actually formed.

The defraction peak at2θ values of 21.3, 64.0 and 74.8 of *C. albidum* and that of *T. globiferus* was 61.8 and 87.1degree, conforms to the crystalline planes of the face centred cubic structure of metallic silver standards of XRD of silver nanoparticles. The observed peak broadening and noise were probably related to the effect of Nano sized particles and the presence of various crystalline biological macromolecules in the plant extracts. This result is similar to that of Mehrdad and Khalil (2010), in which XRD of aqueous extract of *Acanthephylumbracteatum*with spherical structured nano particles of high purity was reported.

These results clearly suggest that, by using safe non-toxic amounts of silver ions with a convenient, eco-friendly and cheap method using *C.albidum* aqueous extract, silver nano particles can be synthesized with promising anti trypanosomal activities.This may eventually lead to standard pharmaceutical formulations using nanoparticles. The taking into consideration of the movement of SNPs into cells and their effect in a bioprocess or even in the environment should be extensively researched into and encouraged in future studies.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMMENDATIONS

6.1 Summary

This study has shown the importance of medicinal plants in the treatment of disease afflicting both humans and animals.

Twenty seven plant materials (stem, roots, leaves and fruits) were obtained from 18 plants. Eighty one (78) extracts of petroleum, methanolic and water extracts were recovered the plant materials. 62 extracts (78%) had activity on both *Trypanosoma congolense* and *Trypanosoma brucei brucei*. 17 extracts (23%) had no activity on both parasite. Phytochemical screening of five of these plants revealed the presence of secondary metabolites with bioactive properties.

Synthesized silver nano particles of *Chrysophyllum albidum* and *Tapinantus globiferus* showed highest activity at 0.98 µg/ml.

The UV absorbance of *C. albidum* seed extract, showed surface Plasmon resonance at 450 nm while that of *Tapinantus globiferus* was at 350 nm. Functional group of the hydroxyl group, amine, alkyl halide, aliphatic amines were observed from FTIR of both *Chrysophyllum albidum* and *Tapinantus globiferus.* The SEM result showed the average particle size of *Chrysophyllum albidum* and *Tapinantus globiferus* to be377 nm to 3,970 nm and 1.75-23.87 µm respectively. The *in vivo* result showed that the groups treated with 1000 mg/kg synthesized nano particle of *Chrysophylum albidum* and 100 mg/kg for prophylactic activities presented remarkable antitrypanosomal activities.

6.2 Conclusions

- This study has been able to show that 5 extracts (methanolic extracts of *Chrysophyllum albidum*, *Tapinantus globiferus, Sabaflorida, Formitopsis pinicola* and *Cantharellus cilarius*) out of the 78 extracts tested,had highest*in vitro*trypanocidal activity at 15.6 µg/ml. There by leading to the credence to the usage of the plants in pharmacological and ethno medicine.
- The phytochemical test reveal the presence of Alkaloids and saponins only in all the 5 extractsscreened(*Chrysophyllum albidum*, *Tapinantus globiferus, Sabaflorida, Formitopsis pinicola* and *Cantharellus cilarius*).
- The GS-MS of butanolic seed extract of *C. albidum* identified 10 prominentcompounds which include: Diaziridine,3-ethyl-3-methyl,; 2,4Dimethylhexanedioic acid,;13-Tetradecynoic acid,;Hexadecanoic acid, methyl ester,;9,12-Octadecadienoic acid,methyl, ;11-Octadecenoicacid, methylester;Methylstearate;9-Tetradecenal,(Z),; 12-Hydroxydodecanoic acid and1,3,4-thiadiazol-2-amine, 5-ethoxy.
- There was significant increase in pack cell volume (PVC) of animals treated with the synthesized silver nano particle of *C. albidum* compared with other groups.
- This study synthesized silver nanoparticles using aqueous extracts of *Chrysophyllum albidum* and *Tapinantus globiferus*. The synthesized nano particles were mostly spherical and rectangular in shape with sizes ranging from 377-23000 nm.The particles were surrounded by thin layer of proteins and metabolites such as terpenoids with major functional groups as amines, alcohols, ketones and aldehydes.

6.3 Recommendation

- Medicinal plants may be explored as an option for decreasing the pathogenicpotential of infectious parasitic species.
- It is important to put in place adequate measures to preserve natural resources toensure continuous availability of plants.
- The medicinal plant could be used to produce stable silver nanoparticles in bulk. These particles could be further used in the field of medicine and animal health care industry.Formulations based on nanotechnologies have been proposed to replace conventional dosage forms (Olowokudejo *et al.*, 2008).
- Further purification should be carried out on active fraction in other to identify active compounds that could be used to formulate new and more potent anti-parasitic agents of natural origin for the treatment of parasitic infections in both humans and animals.
- Silver nano particles also exhibit higher activities when use as a carrier drug as in photo dynamics therapy, because they possess selectivity, specificity and solubility which are limitations to most existing trypanocides. This therapy should be encouraged, in order to reduce resistance and drug toxicity.

6.4 Contributions to Knowledge

- The study established that the methanolic extracts of some Angiosperms (*Chrysophyllum albidum* seed, *Saba florida* roots, and*Tapinantus globiferus* leaves) and fungi(*Formitopsis pinicola*,*Boletus edulis* and *Cantharellus cilarius*) showed highest inhibitory activity against *T. brucei brucei and T. congolense* at 15.6 µg/ml.

- Synthesized silver nanoparticles of *C. albidum* had 100% inhibitory activity at 0.98 µg/ml on both *T.congo*and *T. brucei* within 6 hr of incubation.
- The butanolic extract and the synthesized silver nanoparticle of *C. albidum*have anti-anaemic properties.
- The Gas Chromatography Mass Spectrometry (GC-MS) of Fraction P3 obtained from Preparative thin layer chromatographic studies ofbutanolic fraction of *C.albidum* seed revealed the presentences of these compounds: Diaziridine, 3-ethyl-3-methyl,; 2,4 Dimethylhexanedioic acid,;13-Tetradecynoic acid,; Hexadecanoic acid, methyl ester,; 9,12-Octadecadienoic acid, methyl,;11-Octadecenoic acid, methyl ester; Methyl stearate; 9-Tetradecenal, (Z),;12-Hydroxydodecanoic acid and 1,3,4-thiadiazol-2-amine, 5-ethoxy.
- Characterized silver nanoparticles of *C.albidum*and *T. globiferus* had sizes ranging between 377 nm – 3, 970 nm and 1.75-23 µm respectively.

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Appendix I:*Invitro* trypanocidal activites of butanolic extracts of *C. albidum*seed and *T.globiferus*leaves on *Trypanosoma brucei brucei* after 12 hr of incubation

		3 hrs			6 hrs			9 hrs			12 hrs		
extracts	Conc. (µg/ml)	Mean parasite /field	No of parasites/ ml of blood	Percentage (%)	Mean parasite	No of parasites /ml of blood	Percentage (%)	Mean parasite	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites/ ml of blood	Percentage (%)
C. albidum butanolic seedextract	1000	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	3.90	8	$31x10^6$	33	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.98	12.5	$45x10^6$	52	11	$42x10^6$	50	10	$39x10^6$	43.4	9.5	$36x10^6$	41.3
	0.24	12.5	$45x10^6$	52	11.5	$43x10^6$	51.1	11.5	$43x10^6$	50	9	$35x10^6$	39.1
NA	0.0	24	$97x10^6$	100	22.5	$80x10^6$	100	23	$83x10^6$	100	23		100
butanolic extractof *T.globiferu*	1000	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	3.90	12	$44x10^6$	50	7.5	$27x10^6$	33.3	0.0	$< 2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.98	11	$42x10^6$	45.8	9	$35x10^6$	40	8.5	$32x10^6$	37	5	$19x10^6$	21.7
	0.24	14	$56x10^6$	58.3	13	$50x10^6$	57.7	12	$44x10^6$	52	8	$31x10^6$	34.8

NA--No activity (control wells with trypanosomes **)**

Appendix II: *In vitro* trypanocial activities of synthesized silver nano particles of aqueous *C. albidum* and *T. globiferus* extracts on *Trypanosoma brucei brucei* after 12 hr of incubation

		3hrs			6hrs			9hrs			12hrs		
Extracts	Conc. (µg/ml)	Mean parasite /field	No of parasites/ ml of blood	Percentage (%)	Mean parasite	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites/ ml of blood	Percentage (%)
synthesized extract of *C. albidum*	1000	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0
	3.90	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0
	0.98	1	$3x10^6$	3.8	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 2.5*10^4$	0
	0.24	10.5	$40x10^6$	43.75	5.5	$20x10^6$	23.9	5	$19x10^6$	21.7	4.5	$16x10^6$	19.6
NA	0.0	24	$97x10^6$	100	22.5	$80x10^6$	100	23	$83x10^6$	100	23	$83x10^6$	100
synthesized extract of *T.globiferus*	1000	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	3.90	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.98	6	$22x10^6$	25	1	$3x10^6$	4.4	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.24	14	$42x10^6$	58.3	13	$50x10^6$	57.8	13	$50x10^6$	56.5	8	$31x10^6$	34.8

Appendix III*: In vitro* trypanocidal activities of butanolic extract of *C. albidum* seed and *T. globiferus* leaves on

***Trypanosoma congolense* after 12 hr of incubation**

		3 hrs			6 hrs			9 hrs			12 hrs		
extracts	Conc. (µg/l)	Mean parasite /field	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites /ml of blood	Percentage (%)	Mean parasite	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites/ ml of blood	Percentage (%)
C.albidum lic extract	1000	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	3.90	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.98	9.5	$36x10^6$	39.6	7.5	$27x10^6$	33.3	3	$12x10^6$	13	2	$7x10^6$	8.7
	0.24	8	$31x10^6$	33.3	7.5	$27x10^6$	33.3	5.5	$20x10^6$	23.9	4	$15x10^6$	17.4
NA	0.0	24	$97x10^6$	100	22.5	$80x10^6$	100	22.5	$80x10^6$	100	23	$83x10^6$	100
T.globifeu butanolic extracts	1000	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0	0.0	$<25*10^4$	0
	250	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<25*10^4$	0
	62.5	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<25*10^4$	0
	15.6	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<25*10^4$	0
	3.90	12	$44x10^6$	50	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<25*10^4$	0
	0.98	10	$39x10^6$	41.7	6.0	$22x10^6$	26.7	4.5	$16x10^6$	19.5	3.5	$13x10^6$	15.2
	0.24	8.5	$32x10^6$	35.4	6.0	$22x10^6$	26.7	4.0	$15x10^6$	17.4	2.5	$8x10^6$	10.9

NA--No activity (control wells with trypanosomes)

Appendix IV:*In vitro* trypanocidal activities of synthesized silver nano particles of aqueous extracts of *C. albidum*seed and *T.globiferus*leaves on *Trypanosoma congolense* after 12 hr of incubation

		3 hrs			6 hrs			9 hrs			12 hrs		
extracts Fractions	Conc. (µg/ml)	Mean parasite /field	No of parasites/ ml of blood	Percentage (%)	Mean parasite	No of parasites /ml of blood	Percentage (%)	Mean parasite	No of parasites/ ml of blood	Percentage (%)	Mean Parasite	No of parasites/ ml of blood	Percentage (%)
synthesized extract of *C. albidum*	1000	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 25*10^4$	0	0.0	$<25*10^4$	0
	62.5	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 25*10^4$	0	0.0	$<25*10^4$	0
	15.6	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 25*10^4$	0	0.0	$<25*10^4$	0
	3.90	0.0	$<25*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$< 25*10^4$	0	0.0	$<25*10^4$	0
	0.98	1	$3x10^6$	4.2	0.0	$<2.5*10^4$	0	0.0	$< 25*10^4$	0	0.0	$<25*10^4$	0
	0.24	10.5	$40x10^6$	43.75	5.5	$20x10^6$	23.9	4	$15x10^6$	17.4	4.5	$16x10^6$	19.6
NA	0.0	24		100	22.5	$80x10^6$	100	23	$83x10^6$	100	23	$83x10^6$	100
synthesized extract *T.globiferu*	1000	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	250	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	62.5	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	15.6	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	3.90	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.98	6	$22x10^6$	25	1	$3x10^6$	4.4	0.0	$<2.5*10^4$	0	0.0	$<2.5*10^4$	0
	0.24	6.5	23x106	27.1	5.5	$20x10^6$	24.4	4	$15x10^6$	16.6	3	$12x10^6$	12.5

NA--No activity (control wells with trypanosomes)

Appendix V: Minimum Inhibitory Concentration of fractions obtained from butanolic extract (B1-B 11) of *Chrysophyllum*)*albidum*

and fraction B4 (1-IX) Concentration (µg/ml)

	T.congoT.brucei	*T.congo T.brucei*	*T.congo T.brucei*	*T.congo T.brucei*	*T.congo T.brucei*	*T.congo T.brucei*
H					250 250	
C					250 250	
B			15.6 15.6			
B1						1000 1000
B2						1000 1000
B3					250 250	
B4		3.90 3.90				
B5					250 ----	1000
B6				62.5 -------		1000
B7					250 250	
B 8				62.5 ------	----- 250	
B 9						1000 1000
B10						1000 1000
B11			15.6 15.6			
SNPs	0.98 0.98					
Diminazine		3.90 3.90				
I						1000 0
II						1000 0
III		3.90 3.90				
IV					250 250	
V					250 250	
VI						1000 1000
VII					250 ------	------ 1000
VII						1000 1000
IX				62.5 62.5		

Appendix VI: Minimum Inhibitory Concentration (MIC) of fractions obtained from Sephadex LH- 20 (S1-S4) sand Preparative Thin layer Chromathograpic fractions (P1-P3) on *Trypanosoma brucei congolense* and *Trypanosoma brucei brucei*

Fractions	0.98 µg/ml		3.90 µg/ml		15.6 µg/ml		62.5 µg/ml		250 µg/ml		1000 µg/ml	
	T.congo	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*
S1							62.5	62.5				
S2					15.6	15.6						
S3											1000	1000
S4									250	250		
P1									250	250		
P2											1000	1000
P3							62.5	-----	---	250		

Keys

S1- Sephadex LH- 20 fraction I; S2- Sephadex LH- 20 fraction 2; S3- Sephadex LH- 20 fraction 3; S4- Sephadex LH- 20 fraction 4; P1, Prep.TLC fraction 1; P2, Prep.TLC fraction 2; P3, Prep.TLC fraction 3.

Fractions	0.98 µg/ml		3.9 µg/ml		15.6 µg/ml		62.5 µg/ml		250 µg/ml		1000 µg/ml		
	T.congo	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	*T.congo*	*T.brucei*	
TG(H)									250	250			
TG(C)									250	-----	----	1000	
TG(B)					15.6	15.6							
TG1							---	62.5	250	-----			
TG2					15.6	15.6							
TG3											1000	1000	
TG4									250		------	1000	
TG5													
TG6			3.90	3.90					250	250			
TG7									250		-----	1000	
TG8							62.5	62.5					
TG9			3.90					62.5					
TG10													
TG11											1000	1000	
TG12											1000	1000	
TG13									250	250			
TG14					15.6	15.6							
SNPs Diminazin			3.90	3.90									

e							

AppendixVII: Minimum Inhibitory Concentration (MIC) of fractions obtained from butanolic fraction of *Tapinantusglobiferus*leaves on *Trypanosoma congolense* and *Trypanosoma brucei brucei*

TG(H),*Tapinanthus globiferus* leaves hexane extract; TG(C), *Tapinanthus globiferus* leaves chloroform extract; TG(B),

Tapinanthus globiferus leaves butanolic extract,TGI-TG14,various fractions of butanolic extracts.

Appendix VIII: Minimum Inhibitory Concentrations (MIC) of fractions obtained from TG5 on *Trypanosoma congolense* and *Trypanosoma brucei brucei*

Fractions	0.98 µg/ml	3.90 µg/ml	15.6 µg/ml	62.5 µg/ml	250 µg/ml	1000 µg/ml
	T.congoT.brucei	*T.congo T.brucei*	*T.congoT.brucei*	*T.congo T.brucei*	*T.congo T.brucei*	*T.congo T.brucei*
TGI				62.5 ------	---- 250	
TGII					250 ----	
TGIII					250 ----	
TGIV						1000 1000
TGV				62.5 ----	---- 250	
TGVI					--- 250	1000
TGVII					----- ----	1000
TGVIII				62.5 -----		1000

-------No activity

Keys

TG5, *Tapinantus globiferus* fraction

TGI, *Tapinantus globiferus* fraction ITGII; *Tapinantus globiferus* fraction II TGIII; *Tapinantus globiferus* fractionII I:

TGI, *Tapinantus globiferus* fraction IV; TGV, *Tapinantus globiferus* fraction V; TGVI, *Tapinantus globiferus* fraction VI

TGVII,*Tapinantus globiferus* fraction VII; TGVIII, *Tapinantus globiferus* fraction VIII.

AppendixIX: Project Analysis for Synthesized Nano Particles of *Chrysophyllum albidum* seed extract

7768

F

5212e999-c83e-44fa-843e-400c7e958c52

1.3318443248429727E-06

8.3327675849086057E-07

0.21112866818834047

7769

F

5212e999-c83e-44fa-843e-400c7e958c52

2.4257323679563592E-06

1.0117410306033542E-06

-0.75517210606384577

7770

F

5212e999-c83e-44fa-843e-400c7e958c52

2.3194729534057267E-06

6.6327923454178972E-07

-0.0048543337901327446

7771

F

5212e999-c83e-44fa-843e-400c7e958c52

Appendix IX Continued :

2.5397448402273763E-06

7.91409712865014E-07

0.50620329991717994

7772

F

5212e999-c83e-44fa-843e-400c7e958c52

2.3195898714353258E-06

7.0111833805434465E-07

-1.0528563948902039

Appendix X: Project analysis for synthesized silver nano particles of *Tapinantus globiferus* leaves extract

2317

F

aea10a87-dae8-4ad7-bda4-2601449eccc0

4.7654373058726272E-06

7.32394874516728E-07

-0.76506565586676156

2318

F

aea10a87-dae8-4ad7-bda4-2601449eccc0

9.7409558192895747E-06

2.2450543576154E-06

1.4688638958962232

2319

F

aea10a87-dae8-4ad7-bda4-2601449eccc0

1.3794947641315617E-05

4.2913169041043665E-06

-1.0087262540743385

Appendix XContinued :

2320

F

aea10a87-dae8-4ad7-bda4-2601449eccc0

8.1787222093700142E-06

3.1740577070811913E-06

-0.47709949863412954

2321

Appendix XI: The Course of Parasitemia in Six Groups of Mice Infected with *Trypanosoma congolense* treated with butanolic and synthesized silver nano particle of seed Extract of *C. albidum*

Group/doses In mg/kg/wt.	Mean trypanosome count ($x10^6$) per ml of blood Days post infection										
	1-7	9	11	13	15	17	19	21	23	25	28
(A1) 100	0.25±0.0^{a}	25.4±4.1^{d}	43.3±1.0^{b}	69.5±4.5^{cd}	42.5±6.4^{c}	41.4±8.3^{c}	62.5±0.6^{d}	105.9±0.0^{e}	D	D	D
(B1)100sn	0.25±0.0^{a}	21.3±7.5^{d}	43.3±9.1^{b}	69.3±9.8^{cd}	72.9±6.9^{d}	59.8±1.2^{d}	34.4±5.2^{c}	D	D	D	D
(A2)500	0.25±0.0^{a}	11.2±1.5a	54.2±5.4^{c}	56.4±1.0^{c}	48.9±4.5^{c}	34.5±0.9^{c}	29.1±7.3^{c}	16.4±2.3^{b}	D	D	D
(B2)500sn	0.25±0.0^{a}	7.85±5.4a	42.7±1.1^{b}	63.7±7.0^{cd}	68.6±2.6^{d}	38.9±3.3^{c}	18.7±1.7^{b}	60.2±3.4^{d}	28.2±3.3^{c}	16.3±2.2^{c}	D
(A3)1000	0.25±0.0^{a}	7.03±0.7^{a}	46.4±3.8^{b}	58.3±4.6^{c}	24.0±2.4^{b}	46.0±6.2^{c}	37.9±1.8^{a}	33.0±3.5^{c}	23.8±1.9^{c}	10.0±2.3^{b}	6.94±1^{b}
(B3)1000sn	0.25±0.0^{a}	38.8±4.1^{e}	25.9±5.2^{a}	29.2±3.2^{b}	53.9±8.8^{cd}	22.3±2.4^{b}	36.4±1.7^{c}	16.8±2.2^{b}	15.3±1.8^{b}	0.25±0.0^{a}	0.25±0.0^{a}
(E)Diminazine	0.25±0.0^{a}	6.7±1.6^{a}	23.5±3.5^{a}	18.3±1.9^{a}	13.9±8.4^{a}	6.87±4.4^{a}	1.79±1.5^{a}	1.79±1.5^{a}	0.25±0.0^{a}	D	
(F) (Infected) not treatd	0.25±0.0^{a}	16.1±5.0^{b}	42.7±6.7^{b}	79.5±7.2^{d}	124.6±2.6^{d}	153.6±3.2^{e}	230.5±3.7^{e}	D	D		

The values are given as mean ± standard error of mean (SEM). In each column, values with different superscripts have statistically significant differences ($p<$ 0.05)sn=synthesized nano particle.

Key: Group A1, A2 and A3 were treated with butanolic seed extract of*C.albidum*; Group B1, B2 and B3 were treated with synthesized aqueous seed extract of *C. albidum*.

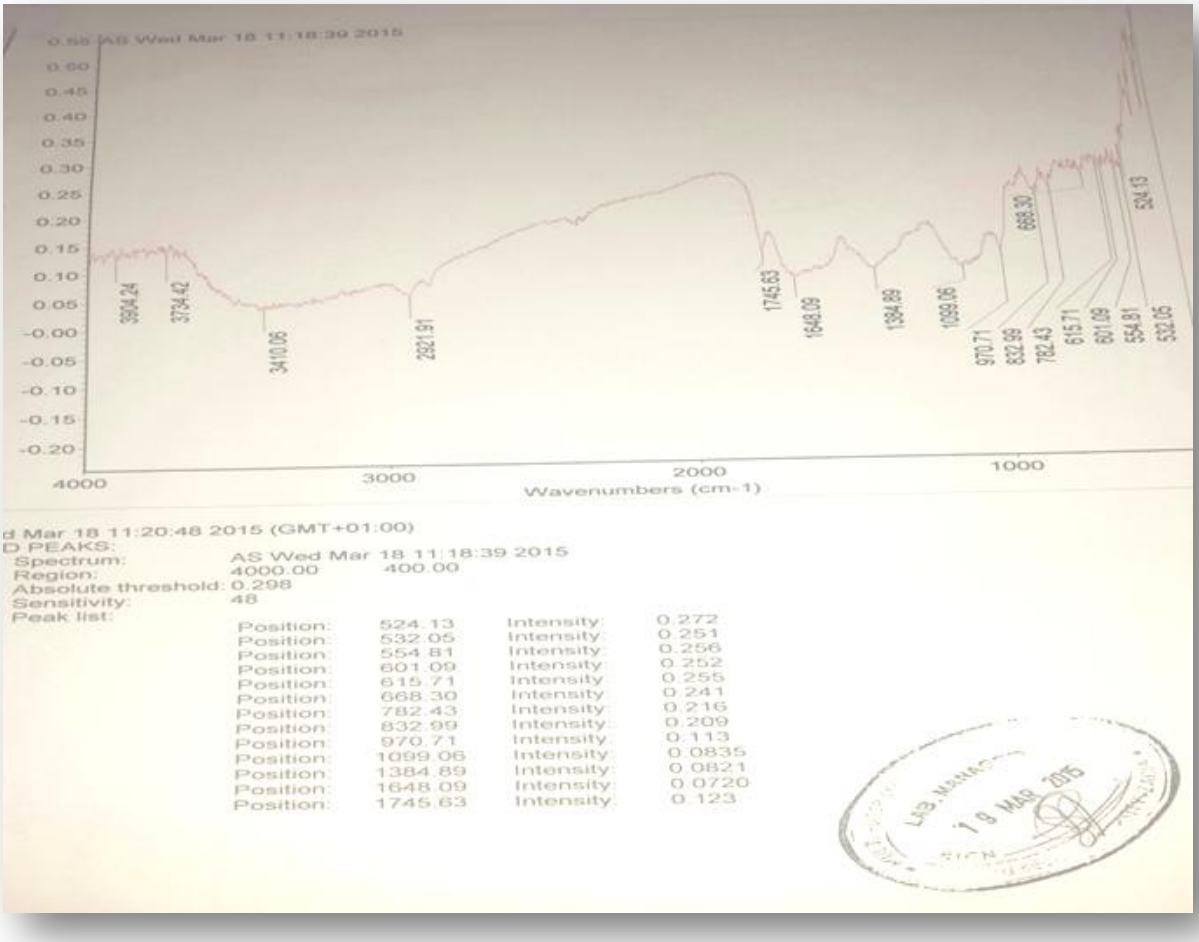
Appendix XII: The Course of Parasitemia in Six Groups of Mice Infected with *Trypanosoma congolense* treated with *C. albidum* (prophylactic group)

Groups/Doses In mg/kg/wt	Mean trypanosome count per ml of blood										
	Days post infection										
	1-3	4	9	11	13	15	17	19	21	23	25-28
(C1) 50	0.00	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	4.07±1.72[b]	7.07±1.42[b]	7.86±1.89[c]	6.36±0.97[c]	5.8±0.71[c]	3.96±1.14[c]
(D1)50sn	0.00	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	1.18±0.93[a]	1.97±0.99[b]	2.00±0.36
(C2)100	0.00	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00a	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	2.49±0.91[b]	3.28±1.40[b]	3.28±1.43[b]	2.49±0.91[b]
(D2)100 sn	0.00	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]	0.25±0.00[a]
(F) Control	0.00	0.25±0.00[a]	42.8±6.79[b]	87.5±10.9[b]	124.6±26.9[b]	153.6±31.9[c]	230.5±37.8[c]	316.2±0.00[d]	D	D	D

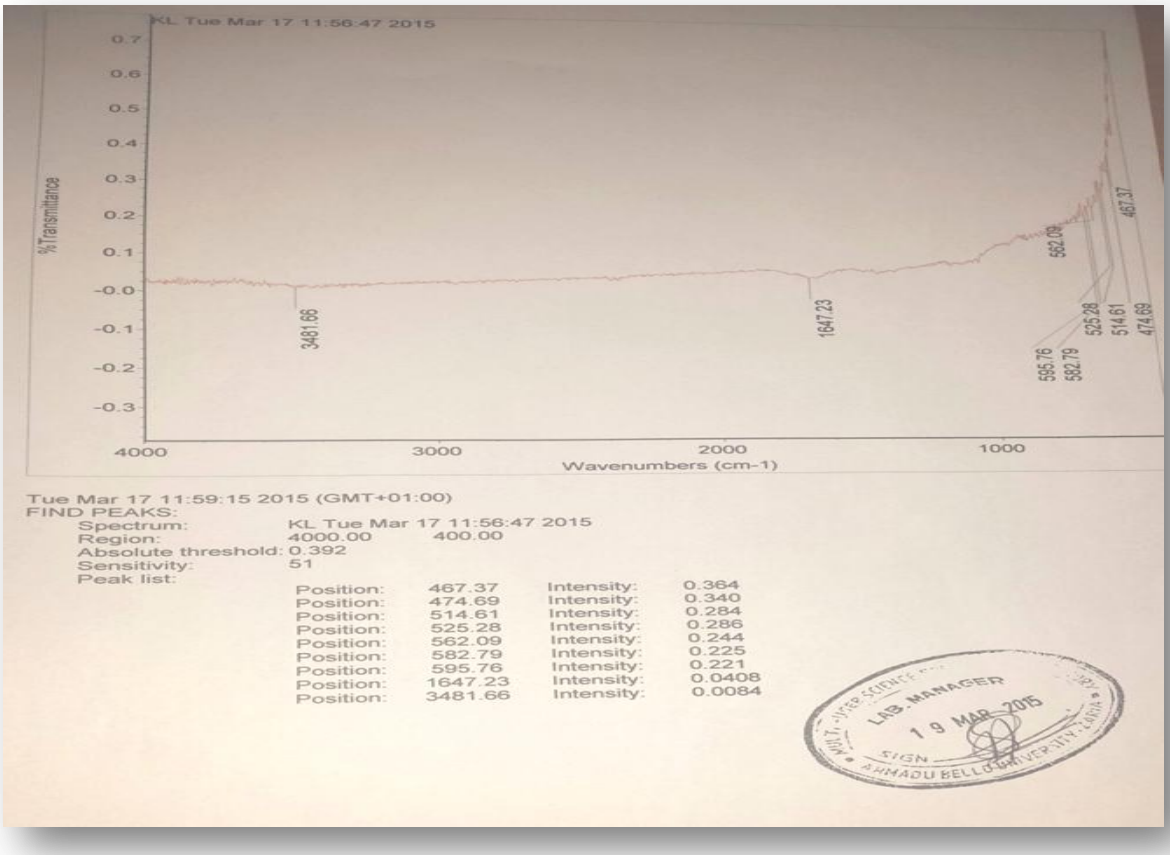
The values are given as mean ± standard error of mean (SEM). In each column, values with different superscripts have statistically significant differences ($p<0.05$) sn= synthesized nano particle

Key: Group C1 and C2 were treated with butanolic seed extract of*C.albidum*; Group D1and D2 were treated with synthesized aqueous seed extract of *C. albidum*.

AppendixXIII: FTIR spectra of Ag nanoparticles synthesized by reduction of Ag+ ion by seed extract of*C. albidum*



Appendix XIV: FTIR spectra of Ag nanoparticles synthesized by reduction of Ag+ ion by aqueous *T. globiferus*



Appendix XV: X-ray diffraction of *Chrysophllum albidum*

