ANTITRYPANOSOMAL ACTIVITY OF *HAEMATOSTAPHIS BARTERI* STEM BARK EXTRACT AGAINST *TRYPANOSOMA BRUCEI BRUCEI* AND *TRYPANOSOMA CONGOLENSE*

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A THESIS BEING SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF PURE AND APPLIED SCIENCES,
MODIBBO ADAMA UNIVERSITY OF TECHNOLOGY YOLA,
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF
THE MASTER DEGREE OF TECHNOLOGY IN BIOCHEMISTRY
THE RESEARCH WAS CONDUCTED AT NIGERIAN INSTITUTE FOR
TRYPANOSOMIASIS RESEARCH (NITR) VOM, JOS PLATEAU

FEBRUARY, 2013

Declaration

I hereby declare that this thesis was written by me and it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All references cited have been duly acknowledged.

Dedication

This thesis is dedicated to my wife and my children.

Approval

This thesis entitled "Antitrypanosomal activity of *Haematosterphis barteri* stem bark extract against *Trypanosoma brucei brucei* and *Trypanosoma congolense*" meets the regulations governing the award of Masters of Biochemistry of the Modibbo Adama University of Technology, Yola and is approved for its contribution to knowledge and literary presentation.

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Acknowledgement

First and foremost, glory to God Almighty for merciful guidance throughout the programme. My gratitude and appreciation goes to my supervisor in the person of Dr A.U. Wurochekke for taking his time to go through my work and make the necessary suggestions at all time despite his tight schedules. Also Miss Hajarah Yusuf Balaraba at National Veterinary Research Institute (NVRI) Vom Jos for her kindness, punctuality and promising in addition to determination throughout the period of this research work, I say thank you. I acknowledge all the entire staff of Nigerian Institute for Trypanosomiasis Research (NITR) Vom Jos who contributed a lot of knowledge in this work and even provide a work space for me in the Institute. To the Head of Department, Dr (Mrs.) M.S Nadro and the entire lecturers of the Department of Biochemistry, there is no word to express my sincere gratitude to you all.

I am also indebted to the following persons Mrs. Ahmed M. U. and Angama Pwano for their contributions toward the success of this work; indeed I am very grateful for understanding some of my needs.

Finally, my sincere thanks go to all my well wishers, I wish all of you success in life. Thanks for every assistance.

ABSTRACT

Preliminary phytochemical analysis and trypanocidal activities of stem bark aqueous extract of *Haematostaphis barteri* against *Trypanosoma brucei brucei* and *Trypanosoma congolense* were investigated. The *in vitro* and *in vivo* activity of crude aqueous extract was evaluated against both parasites. The crude aqueous extract was separated using different solvents on chromatographic column. The methanolic extract found to possess the highest *in vitro* activity among the four fractions, was used for *in vivo* against *T. brucei brucei*. The crude extract has found to contain alkaloids, flavonoids, terpenoids, tannins, saponins, anthraquinone, cardiac glycoside and carbohydrate. Aqueous and fractionated fractions were all found to be effective *in vitro* and their effects were dose dependent. Parasites motility ceased within 30-35 minutes after incubation with 1mg of crude aqueous extract against *T. brucei brucei* and *T. congolense* infected blood respectively, while the motility ceased within 40 minutes for methanolic fraction against *T. brucei brucei*. Both aqueous extract and methanolic extract fraction did not completely eliminate the parasites from the bloodstream of infected rats. Treatment with aqueous extract on either of the parasite did not significantly ($p<0.05$) lower the parasitaemia level when compared to infected control. The parasitaemia continue to increase until the death of all infected rats. The methanolic fraction, significantly ($p<0.05$) kept the parasitaemia lower than that of infected control. Parasites were completely eliminated from the bloodstream of the Diminazene-treated animals. All infected rats developed anaemia which was not affected by the extract treatment. The packed cell volume (PCV) of the diminazene-treated rats returns to normal. It was therefore concluded that the stem bark extract of *H. barteri* possessed *in vitro* antitrypanosomal activity, with little amelioration of the disease condition *in vivo*. The present study is a step towards validation of the folkloric use of this medicinal plant as antitrypanosomal agents.

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CHAPTER ONE

INTRODUCTION

Trypanosomes belong to kingdom *Excarvata*, phylum *Euglenozoa*, subphylum *Mastigophora*, class *Kinetoplastea*, order *Trypanasomatida* and genera *Trypanosoma* with many sub-species. Trypanosomes are group of kinetoplastid protozoa distinguished by having only single flagellum. All members are exclusively parasitic, found primarily in insects (Podlipaer, 2001).They have life-cycle involving a secondary host, which may be a vertebrate or a plant. These include several species that cause major diseases in humans (Simpson *et al* 2006). These are responsible for the important trypanosomal diseases "trypanosomiasis".

The most important trypanosomiasis diseases in human (African sleeping sickness and South American Chagas disease); these are caused by species of *trypanosoma*. Trypanosomiasis is parasitic disease that caused serious economic losses in livestock (Coustou *et al*, 2010). Human health is also impacted indirectly by the parasite, as animals used for food are also subject to infection. An infected animal experiences fever, listlessness, emaciation and paralysis, leading the animal to be unfit for use, hence the term "nagana"which is a *Zulu* word that means "powerless/useless". Many untreated cases are fatal. Trypanosomiasis is found mainly on the region of Africa where its biological vector, (tsetse fly) exists. One organism, *trypanosoma vivax*, has become established in South America, where it is transmitted by biting flies apart from tsetse fly acting as mechanical vectors. 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.*Trypanosoma vivax* can spread beyond the tsetse fly belt through transition of mechanical vectors up to South and Central America and the Caribbean areas free from tsetse fly.

The host preferences of each trypanosome species may defers, but *trypanosoma congolense*, *trypanosoma vivax* and *trypanosoma brucei brucei* have a wide host range among the domesticated animals. Also the tsetse-transmitted parasites, *trypanosoma brucei gambiense* and *trypanosoma brucei rhodesiense*, cause sleeping sickness in human. *Trypanosoma congolense* and *Trypanosoma brucei brucei* are parasites responsible for severe disease of African livestock. Trypanosomes life cycle is complex and divided into two phases, one in the insect vector and the other in the bloodstream of mammalian host (Coustou *et al*, 2010). Many of *trypanosoma* family are heteroxenous i.e. they live in more than one host species over their life cycle (the bloodstream and/or fixed tissues in vertebrates and in the intestine of blood sucking invertebrates). The infection sign start with swelling (chancre) at the site of the fly bite, but remain unnoticed. The primary clinical sign are intermittent fever, sign of anaemia, lymphadenopathy weight and decrease in milk yield. Neurological signs include; Gardiac lesions, diarrhea, keratitis, lacrimation, lost of appetite. Some of the effect includes; premature birth, abortion, prenatal losses, mucosal hemorrhages, particularly in gastrointestinal tract. Trypanosomes can infect all domestic animals. Clinical cases have been described in cattle, water buffalo, sheep, goat, camels, horses, donkeys, pig, dog, cats and many other species in part 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. More than 30 species in the wild or zoos, including ruminates such as, white tail deer, antelope and African buffalo as well as equidae, lions, leopards, warhogs, capybaras, elephants and various rodents are also known to be susceptible to its infection.

Chemotherapy, the main means of controlling the disease is face with some challenging problems as comparison parasites resistance and toxicity of trypanocidal drugs (Amaechi, 2001: Maser *et. al*., 2003). Poor prospect for vaccine due to antigenic variation of parasites (Nantylya and Maloo, 1998) is further compounded by unwillingness of pharmaceutical industry to develop new compounds because of uncertain and unprofitable market or perhaps the localized nature of the disease. The few commercial trypanocides (diminazene aceturate, isometamamadium, homidium) among others have been in use for well over 40 years.Thus, the search for medicinal plants with trypanocidal activities continues to generate a lot of research interest (Hoet *et.al*. 2004; Samson, 2005).

Medicinal plants generally are those plants that are helpful in the process of healing any illness or infection. Some example of the African medicinal plants are; *Cassia sieboriana*, *Khaya sengalenses*, *Accacia polyacantha*, *Pericopsis laxiflora*, *Lophira lanceolata*, *Cussonia arborea*, *Uapaca togoensis*, *Hymenocardia acida*, *Xenemia africana*, *Ocimum gratissimum* just to mention few among numerous that has been reputed for their medicinal uses. Medicinal plants have been used ethno medically as remedy for several human and animal ailments like helminthiasis, antifungal, antiviral, antitrypanocidal and bactericidal activities. Some are taken against diarrhea, gynecological disturbances, digestive disorders and nervous confulsions.

Haematostaphis barteri (Hook) "Blood plum" (English), "Jinnin kaafuri" (Hausa) belongs to the family *Anacardiaceae* class *Equisetopsida*, order *Spindales* and family genus. The stem bark is claimed to be effective locally in treating hepatitis, used as laxative, emetic and in treating trypanosomiasis, but there is no much literature as regards efficacy to its antitrypanosomal activities. It was on this account that this work was designed to document this ethno-veterinary practice, and ascertain the efficacy *in vitro* and *in vivo* against *Trypanosoma brucei brucei* and *Trypanosoma congolense*.

Objectives

To determine the phytochemicals constituents of the plant extracts.

To determine the *invitro* trypanocidal activity of the extract on *trypanosoma congolense* and *trypanosoma brucei brucei*.

To evaluate the *invivo* trypanocidal effect of the plant extract.

To compare the trypanocidal effect of the extract with diminizene aceturate.

To partially fractionate the crude extract to determine the most effective component.

CHAPTER TWO

MATERIAL AND METHODS

Sample collection

The stem bark of a matured *Haematostaphis barteri* was collected from Gashala Pubba, in Hong Local Government Adamawa State Nigeria and was identified in the department of Forestry, Modibbo Adama University of Technology, Yola.The stem bark of the plant will be washed thoroughly and shade-dried for a week. The stem bark will be pounded to fine powder with wood mortar and pestle then stored in dry container until it is needed.

Experimental Animals

Healthy white albino male rats weighing between 90-110g was used for this work and was purchased from Nigerian Institute for Trypanosomiasis (NITR) Vom, Jos, Nigeria. The rats were kept in well ventilated laboratory cages at a room temperature. They were maintained on a commercial poultry feeds (ECWA feeds Jos, Nigeria) and drinking water. The experiment was conducted in compliance with international accepted principles for laboratory animals used. Animals care guidelines on animal used protocol review (CCAC; 1997). The animal were allowed a 14-days period to rich the proposed weight.

Inoculation of the donor rats

Blood was collected by cardiac puncture with an EDTA coated syringe from a heavily infected rat and immediately diluted with physiological saline. The diluted infected blood contain 1-2 parasite per field (microscopic field), 0.1ml of this blood was inoculated into two clean healthy rats intraperitoneally to serve as the donor for each of the parasite (T. b. brucei and T.congolense). Infection was monitored every morning by microscopic examination of blood samples taken from the tail of the infected rats.

Infection of the animals

Blood was collected by cardiac puncture with an EDTA coated syringe from the heavily infected rat and immediately diluted with physiological saline to serve as inoculums. Healthy rats were infected intraperitoneally with 0.25ml of the inoculums containing about 2 trypanosomes per field to each of the rats in the infected groups. The Parasitemia was monitored every day by the microscopic examination.

Extracts preparation

One hundred grams of the fine powdered stem bark was soaked in a 400 ml distilled water and shake for sometimes to mix properly and left to stand for further 48 hours, followed by filtration through whatman No 1 filter paper. The filtrate of the extract will concentrated to dryness over water bath at 40-50°C. The solid extract is packaged in a clean water-proof polythene bag and stored in the refrigerator until time usage.

Extract administration

In order to determine the effective dose, six groups of 5 rats each for both T.b. brucei and T. congolense distributed into cages. Three groups (1-3) of the rats were intraperitoneally treated with the extract at dose of 20, 40 and 80mg/kg body weight per day. Group IV consist of the negative control which were infected with the parasite but were not treated with the extract. Group v consists of the standard control which was infected with the parasite and treated with deminazene aceturate nozomil® (standard drug). Group VI consist of the positive control were neither infected nor treated with the extract. Treatment commence immediately the parasite were seen daily for at least 7-10 days. Parasitemia was monitored every day under the microscopic examination.

Therapeutic monitoring of the extract

Development of Parasitemia in these rats was checked daily by wet blood film prepared from tail blood at × 40 magnification. Treatment commence immediately with the extract. The numbers of parasite seen per field under the microscope field was counted as described by Herbert and Lumsden (1976)

Challenging Trypanosomes

T*rypanosoma brucei brucei* and *trypanosoma congolense* will be obtained from Nigeria Institute for Trypanosomiasis Research (NITR) Vom, Jos, Nigeria. The parasites are maintained in a laboratory by passage in rat blood harvested from donor animal at peak Parasitaemia 10- parasites/ml of blood will be used for invitro anti-trypanosomal assay.

Trypanocidal Drug

Commercial diminazene diaceturate (Nozomil® animal care) KEPRO B.V. Maagdenburgstraat 7421 Deventer Holland was used for in vitro anti-trypanosomal assay as standard control likewise in standard control in one of the infected group.

Fractionation of the crude water extract

Four different solvent (methanol, water, ethyl acetate and acetic acid) are used in column chromatographic technique with coarse silica gel to fractionate the crude water extract of *H. barteri* stem bark

Phytochemical screening

The plant stem bark part were extracted after shade dried and pounded in to fine powder on the same day to prevent, as far as possible, physiological changes taking place before extraction. An extracts of each plant part was prepared by macerating a known weight of the fresh plant in any of the selected solvent (water in this study). Each Extract was then filtered. The extract from the stem bark was evaporated to dryness *in vacuo* at about 45°C and further dried to constant weight at the same temperature in a hot-air oven. The yield of residue was noted and a portion of it was used to test for the following plant constituents: alkaloids, saponins, tannins, phlobatannnins, anthraquiunones, terpenes and cardiac glycosides. The screening procedures from those of Wall *et al*., (1952 and 1954),Soforawa(1990); Trease and Evans(2002)and Harbone (1973) to test for their presences.

Basic phytochemical screening consist of performing simple chemical tests to detect the presence of alkaloids,tannins,saponins,anthraquinones,etc.in the

plant extract. Simple, standard chemical tests have been devised for such screening though person carrying out the test should be aware of possible false-positives Harbone, (1973). For example, the Dragendorff´s test for alkaloids (and its various modifications), anthraquinone (Birntrager´s test),ferric chloride test ;cardiac glycoside (Salkowaski test and Keller-Kiliani test) ;carbohydrate (Fehling´s solution test) ;flavonoids (lead acetate test, ferric chloride test) ; saponins (froth test and legal test) ; tannins (ferric chloride solution test, bromine water test and formaldehyde test) ; terpenes (Salkowaski test); In the plant sample standard methods described by Harbone 1989; Sofowara 1993; Trease and Evans 1989 was used. These methods can give false-positive reaction in some cases so confirmatory tests should, always be carried out. The methods outlined are not unique in any way and are provided only as a guide. Portion of the extract was used to test for the following plant constituents: alkaloids, saponins, tannins, phlobatannnins, anthraquiunones, terpenes and cardiac glycosides carbohydrate and flavonoids. The screening procedures were used to test for the presences of these phytochemicals. The phytochemical analysis of *Haematostaphis barteri* stem bark crude water extract and the most active fraction of the fractionated component for the above chemical constituents were performed as described in appendix 1

Determination of the percentage packed cell volume (PCV)

This was done using the micro haematocrit method described by Cole, 1974.

Blood was directly taken from the tail of rats into a heparinised capillary tube to about ¾ of their length. One head of the tube was completely sealed using plastacine wax. The sealed capillary tubes were transferred to haematocrit centrifuge placed in such a manner that the sealed end points outward. The cover of the centrifuge was tightened to prevent blood spillage and was centrifuge for 5 minutes. There after, the capillary tubes were transferred to the haematocrit reader and the percentage packed cell volume is read directly (visually).

In vitro screening

Exactly 100 mg of the extracts were weighed and dissolved in 2ml of 5% DMSO (dimethyl sulphoxide). Serial dilution of this stock solution was done using 5% DMSO to obtain concentrations ranging from the stock (50mg/ml) to 6.25mg/ml. Assessment of the *in vitro* antitrypanosomal activity was performed in double or triplicates in 96 well microtitre plates (Flow laboratories inc., Mclean, Virginia, USA). In wells of the microtitre plates, 20µl of each of the extract was incubated at 37°C, with 40 µl of the infected blood containing about 30-50 parasites per field (obtained from a donor rat with about 10_6 *T. brucei brucei* and *T. Congolese* per ml of blood), as described under determination of Parasitemia was mixed with 5 µl of extract solution of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml as effective test concentrations in the reaction mixtures. To ensure that the effect monitored was that of the extract alone, a set of control was included which contained the parasites suspended in 5% DMSO (dimethyl sulphoxide) only. For reference, tests were also performed with the same concentrations of Nozomil® (a standard drug for African Trypanosomiasis that contains 10.5mg

diminazene diaceturate + 555 mg phenazone/g.) KEPRO B.V. Maagdenburgstraat 7421 Deventer Holland a commercial trypanocidal drug.

After 5 minutes incubation maintained at 37°C about 2µl of the test mixtures were placed on separate microscopic slides and covered with cover slips and the parasites observed every 5 min for total duration of sixty minutes. It should be note that under this invitro system adopted, parasites survived for about 4 hours when no extract was present. Cessation or drop in motility of the parasites in the extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of trypanocidal activity.

In vivo activity of the stem bark water extract

Thirty rats for each parasite (*T.b. brucei* and *T.congolense*) were divided into six groups of five rats each. The rats in five groups were intraperitoneally infected with *T. congolense* and *T. brucei brucei* respectively at inoculums concentration of 2 parasites per microscopic field approximately inoculating $1x10_6$ parasites intraperitonially per animal by intramuscularly inject into each rats 0.25 ml blood/PBS solution i.e. basically on Herbert and Lumsden comparison table. For several passages, approximately 80% blood solution was obtained by cardiac puncture into 1ml syringe containing 0.2ml EDTA. About 0.1-0.2 ml of blood collected as described above or blood (diluted with dextrose saline as the excipecient to contain approximately 2 parasites per microscopic field was injected into clean rats acclimatized under laboratory condition for two weeks. Parasitemia was monitored in the blood obtained from tail, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at ×40 magnification using the Rapid Matching method of Herbert and Lumsden (1976). Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with dextrose saline (PH 7.2). Logarithm values of these counts obtained by matching with the table of Herbert and Lumsden (1976) is converted to antilog to provide absolute number of trypanosomes per ml of blood by comparing to that of the table. The levels of the parasitemia monitored daily for the period 14 days of the experiment, where exactly, 20, 40 and 80mg/kg of the extract were the treatment dosage for groups 1, 2 and 3 injected intramuscularly, to infected treated with the extract and 3.5g/kg per body weight of Nozomil® (a standard drug for African Trypanosomiasis that contains 10.5mg diminazene diaceturate + 555 mg phenazone/g.) KEPRO B.V. Maagdenburgstraat 7421 Deventer Holland was given intraperitoneally to group 4 (infected treated with standard drug) for both parasites infected to rats, 2-3 days after the parasites were first detected in the bloodstream (6 days post infection); the remaining infected group (group 5) was left untreated (infected control). Group 6 was maintained uninfected untreated (normal) control. The pre-infection and terminal packed cell volumes of all the infected rats were determined by the microhaematocrit method.

CHAPTER THREE

LITERATURE REVIEW

African animal trypanosomiasis.

African animal trypanosomiasis (AAT) is a parasitic disease that causes serious economic losses in livestock from anemia, loss of condition and emaciation. Protecting animals from trypanosomiasis is difficult in endemic areas, as bites from tsetse flies and variety of other insects must to be prevented. Tsetse fly eradication program may help in controlling this disease, as well as other forms of trypanosomiasis that affect humans.

Etiology.

Most trypanosomes are transmitted by tsetse flies. Two tsetse-transmitted parasites, *T. brucei gambiense* and *T. brucei rhondiense*, cause human African trypanosomiasis (HAT)/sleeping sickness, which affects both humans and animals.

The remaining tsetse-transmitted trypanosomes primarily affect animals and cause Animal African trypanosomiasis (AAT). The most important species in this disease are *trypanosoma congolense, T. vivax* and *T. brucei* sub species *brucei*. *Trypanosoma congolense* can be classified into three types, which are called the savannah, forest and kilifi types. Other species such as *T. simiae* and *T. godfreyi* can also cause AAT. Some trypanosome infections in Africa cannot be identified as any currently recognized species. Concurrent infectious can occur with more than one species of trypanosome.

Trypanosomes

Trypanosome been a protozoan parasite that resides in the blood and tissue and is cyclically transmitted by tsetse flies of the genous *Glossina*, its main hosts are man, domestic and wild animals. Trypanosomes moves by twisting its body into **S** shapes. The movement comes from the flagellum and the direction is towards the free end of the flagellum.

Classification

Trypanosomes fall into two main division depending on the location of metacyclic form of trypanosomes in the insect.

Salivaria: Metacyclic forms are in the salivary glands and the proboscis where infection is inoculative. Species under this form are: **Dutonella** e.g. *T. Vivax*, **Nannomonas** e.g. *T. Congolense*, **Trypanozoo** e.g. *T. brucei*

Stercoraria: Metacyclic occupy a posterior position in the gut and are passed out in insect faeces. Infection is by contamination.

Distinguishing characters

Some of the distinguishing character of trypanosomes are:--Size and shape of the body, position of the nucleus and kinetoplast, presence or absence of free flagellum, the shape of the posterior end which can either be pointed sharply, oval or blunt e.g. shape of posterior end of *T. brucei* and its sub-group are blunt and *T.congolense*- round, position of the kinetoplast of *T. brucei* is sub-terminal and *T. congolense* is marginal and size of the kinetoplast of *T. brucei* –smaller while *T.congolense*-medium.

A summary of the above morphological characters

Species	**Morphology**	**Free Flagellum**	**Undulating membrane**	**Kinetoplast**	**Other characteristics**
T.congolense	Pleomorphic	Absent	Slightly developed	Marginal or Central sub-terminal	Posterior end Round or flat
T.brucei	Pleomorphic	Present in long and intermediate forms absent in short forms	Well developed	Small sub-terminal	Posterior end of Long forms is **pointed** Short forms is **Rounded** Intermediate forms is **blunt**

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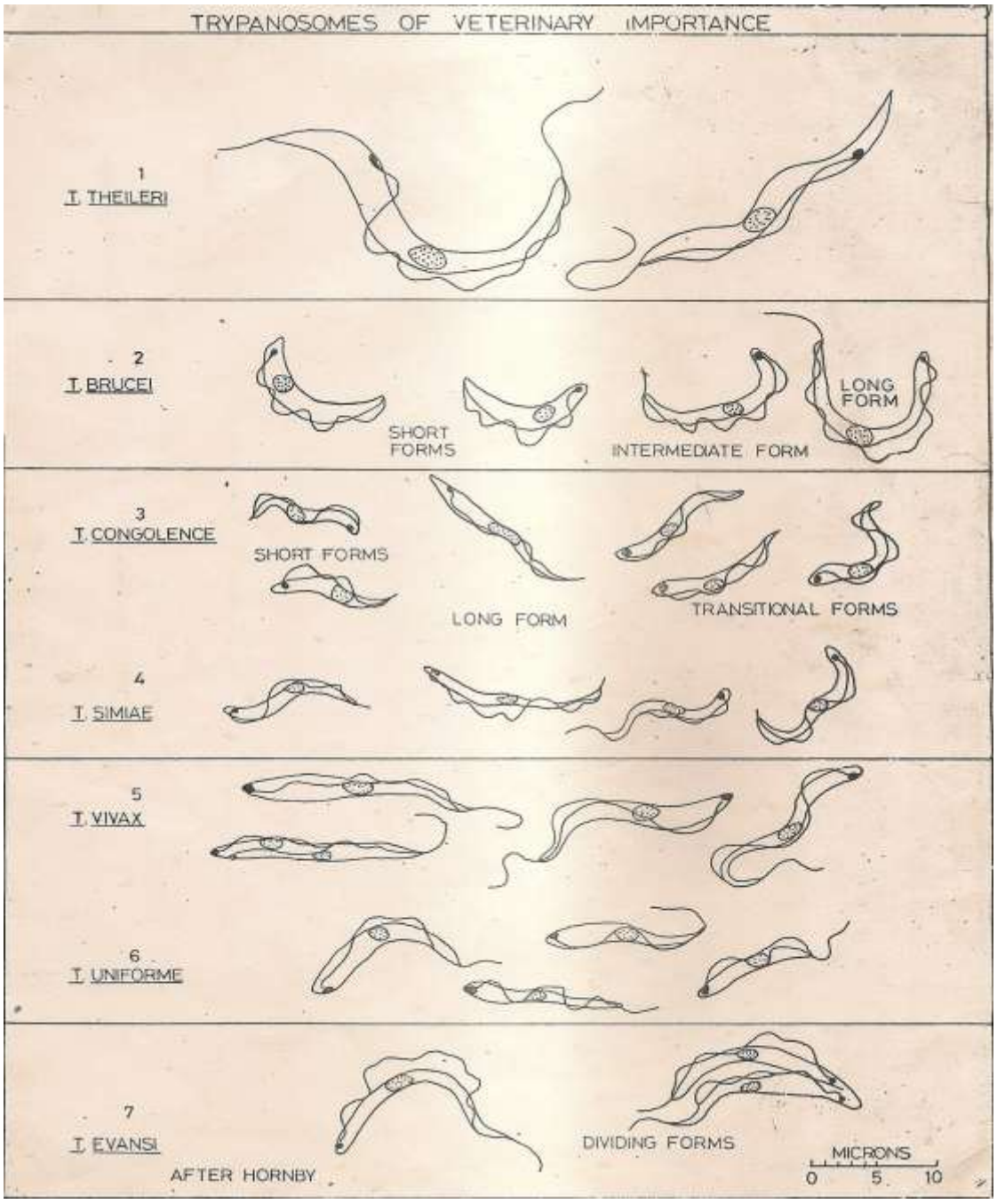


Figure 1: Showing the different sub-species of veterinary important trypanosomes.

Forms of trypanosomes

A variety of different forms appears in the lifecycle of trypanosomes, distinguished mainly by the position of the flagellum. For example: Amastigote has reduced or absent flagellum, Promastigote has flagellum anterior of nucleus free from cell body. Epimastigotes has flagellum anterior of nucleus connected by a short undulating membrane, Opisthomastigote has flagellum posterior of nucleus, passing through a groove in the cell and Trypomastigote has flagellum posterior of nucleus, connected by a long undulating membrane. These are stages characteristics of the trypanosome species in the bloodstream as well as infective metacyclic stages in the tsetse fly vector. Most trypanosomes have at least amastigote and promastigote stages. *Trypanosoma* appears in all five forms; with the trypanosomal stage occurring in the vertebrate host. *Trypanosoma brucei* sub-species have two forms in the bloodstream of a vertebrate host, the rapidly dividing long-slender form and the non-dividing short stumpy form. The stumpy parasites are adapted for uptake into the tsetse fly vector, and are non-proliferative in comparison with the slender forms.

The stages that occur only in insect are the promastigote and epimastigotes. All species of trypanosomatidae have a single nucleus and are either elongated with a single flagellum or rounded with a very short nonprotruding flagellum. Many members of the family are hetroxenous; they live in more than one host species over their life cycle (in the blood and/or fixed tissues in vertebrate and intestine of bloodsucking invertebrates). As the trypanosomes usually live in blood they are called hemflagellates.

Its response to stimuli.

Unique to African trypanosomes e.g. *trypanosoma brucei* is the expression of a variable surface glycoprotein (VSG) coat on the cell surface, which undergoes constant variation i.e. in order to evade the humoral immune system (antibody response). It is thought that recombination via double stranded DNA breaks from a repertoire of about 100 complete VSG genes, and a large numbers of VSG-related sequences, which is responsible for the vast diversity of the parasites (Taylor and Rudenko 2006). This recombination would retain effectiveness in immune evasion by maintaining diversity.

Trypanosoma parasites experience differentiation in their life cycle in response to changes in temperature, availability of nutrients, and immune system defense. These changes occur so that parasites can readily adapt to different host environments, which is by cycling between mammalian host and the insect vector. Specific surface coat protein including *Variable Surface glycoprotein* (VSG) and *Procyclic acidic repetitive proteins* (PARP) allows them to evade the immune systems of both species (Dean *et al* 2009). Stumpy form of trypanosomes can also be introduced to differentiate by *cis-aconitate* or *citrate* (CCA) intermediate in the Krebs cycle into procyclic forms (shedding the VSG coat for a *Procyclic acidic repetitive differentiation*(PARD) coat) (Czichos *et al* 1986) and that temperature reduction from 37ºC to 20ºC induced hypersensitivity of stumpy forms to CCA.

The primary mechanism by which trypanosome e.g. *T.brucei* can change forms in response to the CCA signal and to temperature drops is a group work of

surface proteins known as *protein associated differentiation* (PAD). These are transmembrane proteins convey the extracellular CCA signal to differentiate into procyclic form (Dean *et al* 2009). *T. brucei* slender forms do not have PAD proteins (except those which are in transition to the stumpy form), so, the CCA signal is relatively ineffective on them while the stumpy forms show the PAD 1 protein. A second protein, also the PAD 2 protein, is also found in stumpy forms, but is not diagnostic of stumpy forms (i.e. not all of them that have the PAD 2), while also allowing the transmission of CCA signal, is thermo regulated so that it up regulates dramatically at the lower 20ºC temperature than 37ºC temperature (Dean *et al* 2009).

This makes sense since the job of the PAD protein is to allow the trypanosome to switch to the procyclic form upon entering the fly and the 20ºC temperature would be indicative of the shift from the larger human body to the smaller fly body (Engstler and Michael 2004; Fean *et al* 2007)

Trypanosoma brucei complexity

Trypanosoma brucei brucei (infective to animals but not to humans) and the human pathogens *trypanosoma brucei gambiense* and *trypanosoma brucei rhodesiense* are morphologically indistinguishable. Each trypanosomiasis endemic area has associated with it several strains of biochemically and antigenically distinct trypanosomes. *T.b.gambiense* appears to be a homologenous group whereas *T. b. rhodesiense* appears to be made up of many different strains. Enzyme studies support the view that *T. b. rhodesiense* is a set of variants of *T. brucei* rather than a subspecies of *T. brucei*.

Transmission and life cycle of trypanosomes

African trypanosomiasis is transmitted by a small number of species of tsetse flies belonging to the genus *Glossina*. Both male and female tsetse flies suck blood and can therefore transmit the disease. Once infected a tsetse fly it remains a vector of trypanosomiasis throughout its life. The life span of tsetse fly is about 3 month.

The life cycle of *T.brucei* starts with the metacyclic trypomastigotes which can be inoculated through the skin when an infected tsetse fly takes blood meals. The parasites develop into long slender trypomastigotes which multiply at the site of inoculation and later in the blood, lymphatic system and tissue fluid. They carried to the heart and various organs of the body and in the later stages of infection they invade the central nervous system (CNS).

Trypomastigotes are injected by a tsetse fly when it sucks the blood. In the midgut of the fly, the parasites develop and multiply. After two to three weeks, the trypomastigotes migrate to the salivary glands of the tsetse fly where they become epimastigotes, multiply, and develop into infective metacyclic trypomastigotes. In summary, trypomastigote injected through skin when tsetse fly takes the host blood**,** then the trypomastigote multiply in the blood, lymph and in later stages, in CNS and trypomastigotes again is ingested by the tsetse fly. Parasites multiply in midgut of the tse tse fly migrate to the salivary glands and it becomes epimastigotes and multiplies. Develop into infective metacyclic trypomastigotes again.

Symptoms at stages of infection

Early stage of infection:

There is a high irregular fever with shivering, sweating, and an increased pulse rate. There are persistent headache, and usually pains in the neck, shoulders, and calves, and occasionally a delayed intense pain to knocks and pressure known as Kerandel´s sign. The lymph glands become swollen especially the lymph glands at the back of the neck are frequently involved (Winterbottom´s sign), while in some cases it is usually the glands under the jaw, in the arm-pit, at the base of the elbow, or in the groin which are involved. As the diseases progresses, the spleen becomes enlarged, and there is edema of the eyelids and face, sleeplessness, aimless scratching of the skin, and often a transient rash. In men, impotence may occur and in women, abortion or amenorrhoea. There is a rapid fall in haemoglobin, reduction in platelet numbers, and a significant rapid rise in the erythrocyte sedimentation rate (ESR). Myocardial symptoms may also develop.

Late stage of the diseases:

The trypanosomes invade the central nervous system,(CNS) giving symptoms of meningoencephalitis, including trembling, inability to speak properly, progressive mental dullness, apathy, excessive sleeping, and incontinence. There is usually rapid weight loss, continuing irregular fever, edema of the limbs and inability to walk without help. If untreated, coma develops and finally death.

Immune responses

The main immune response in African trypanosomiasis is a humoral one with stimulated B lymphocytes producing large amount of immunoglobulin M (Ig M) follow in the later stages of infection by immunoglobulin G (Ig G).

The antibodies are effective in destroying the trypanosomes until the organisms vary their surface antigenic structure, i.e. variant surface glycoprotein (VSG´s). Then the existing antibody becomes ineffective and the parasites multiply once more until the host produces another set of antibodies which are effective against the new VSG antigens. This process can be repeated many times and is thought to explain the temporary disappearance of the trypanosomes from the blood, necessitating repeated blood examinations to detect the parasites.

The level of Ig M rises rapidly and reaches a very high concentration in the blood. As the disease progresses Ig M can also be found in the cerebrospinal fluid. Protection against other diseases, especially bacterial infections, is reduced due to immune depression and the body's humoral responses being used against the trypanosomes.

Therapeutic Development by targeting Enzymes of glycolysis and their Sub cellular Compartments.

Considering subspecies of the African trypanosomes; *Trypanosoma brucei*, which cause human African trypanosomiasis, are transmitted by the tsetse fly, with transmission-essential life-cycle stages occurring in both the insect vector and human host. During infection of the human host, the parasite is limited to using glycolysis of the host sugar for ATP production. This dependence on glucose breakdown, presents a series of targets for potential therapeutic

development, many of which have been explored and validated as therapeutic targets experimentally (Verlinde *et al* 2001). These include enzymes directly involved in glucose metabolism (e.g., the *trypanosoma brucei* hexokinase), as well as cellular components required for development and maintenance of the essential sub cellular compartments that house the part of the pathways, i.e. the glycosomes.

In the procyclic form, parasites utilize the abundant amino acids in their surrounding to generate ATP through mitochondrial-based pathway. While glycolysis is important to the procyclic form (PF) parasites, these parasites can thrive in the absence of glucose if adapted to low glucose conditions, indicating that other metabolic pathways can compensate for the loss of glycolysis (Ter Kuile,2008; Drew *et al*.,2003).

In blood stream form (BSF) parasites, glycolysis of the host glucose provides the sole source of carbon for ATP production. This dependence on glycolysis for ATP coincides with reduced mitochondrial function, limiting the metabolic options available to the parasite and presenting a series of targets for potential therapeutic development.

These include enzymes that participate directly in glycolysis, proteins responsible for the enzyme import into glycosomes, and cellular components involved in the regulation of glycosome number and differentiation. More often, the target was the enzymes of glycolysis, with a particular focus on the first enzyme in the pathway, *Trypanosoma* hexokinase (THK). Additionally, compartmentalization of the pathway is critical to the success of the parasite, so, considering strategies aimed at disruption of mechanisms the parasites uses during the maturation and development of glycosomes. (Bakker *et. al*., 2000)

Glycolysis in the Bloodstream forms (BSF) of African trypanosomes

Metabolism of the host glucose through glycolysis is essential to the success of a BSF parasite i.e. the pathway is the sole source of ATP production in the mammalian infected lifecycle stage. The pathway is organized into subcellular compartments related to peroxisomes named glycosomes. The single membrane compartment houses the first seven enzymes of glycolysis (Wilson *et. al*., 2002). Under aerobic condition, these enzymes convert glucose to 3-phosphaglycerate, which is then further metabolized to pyruvate with the concomitant production of ATP by pyruvate kinase in the cytosol. The pyruvate is then secreted from the cell.

One key to the presence of compartmentalized glycolysis is related to regulation of energy metabolism. ATP and reducing equivalent depletion and production within the glycosome are balanced. ATP is consumed by the activity of the THKs and phosphofructokinase (PFK), while it is regenerated by the activity of the glycosomal phosphoglycerate kinase (gPGK). Additionally, NADH produced by glyceraldehydes-3-phospate dehydrogenase is balanced by NADH oxidation when glycerol 3-phosphate dehydrogenase (GPDH) metabolizes dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate (Gly-3-p). The resulting Gly-3-p is shuttled from glycosome to the mitochondria where electrons are ultimately transferred to water through the activity of a glycerol 3-phosphate oxidase complex (consisting of a mitochondrial glycerol 3-phosphate

dehydrogenase, ubiquinone, and trypanosomal alternative oxidase). The shuttle returns DHAP to the glycosome, allowing maintenance of the glycosomal redox balance.

The compartmentalization of a majority of the glycolytic pathway segregates important steps in the path to ATP synthesis and creates what could be considered additional obstacles to efficient energy metabolism. Bakker and colleagues try to explain this through a combination of computational and wet-bench experiments have found that compartmentalization of glycolytic enzymes that lack allosteric regulation prevents the unchecked consumption of ATP in the "turbo-explosion" of glycolysis (Bakker *et. al*. 2000). Because feedback inhibition does not limit THK and PFK activity, if these `downstream enzymes is unchecked by compartmentalization.

Metabolism processes in trypanosomes.

Organisms acquire organic material from their environments and convert this material into energy or their own substance (i.e., biomolecules). Cells are made of distinct classes of biomolecules with specific functions as an examples, with DNA, its building blocks (nucleotides) with major function as genetic material and RNA as template for protein synthesis, proteins building block are amino acids and function as cell structure and function, while lipids building blocks are fatty acids major function is membrane component and lastly carbohydrates with building blocks are sugars and major function as energy production.

The parasite exhibits a rapid growth and multiplication rate during many stages of its life cycle. This necessitates that the parasite, like all other organisms, acquires nutrients and metabolize these various biological molecules in order to survive and reproduce. Obviously, the parasite's metabolism will be intertwined with that of the host's because of the intimate relationship between the host and the parasite. These host-parasite interactions are further complicated by the complex life cycle of the parasite involving vertebrate and invertebrate hosts as well as different locations within each of these hosts. A better understanding of the parasite's metabolism may lead to the development of novel therapeutic strategies which exploit the uniqueness of the parasite. Below are brief discussions of selected aspects of parasite metabolism-with emphasis on the unique features of the parasite according to the classes and function of biomolecules.

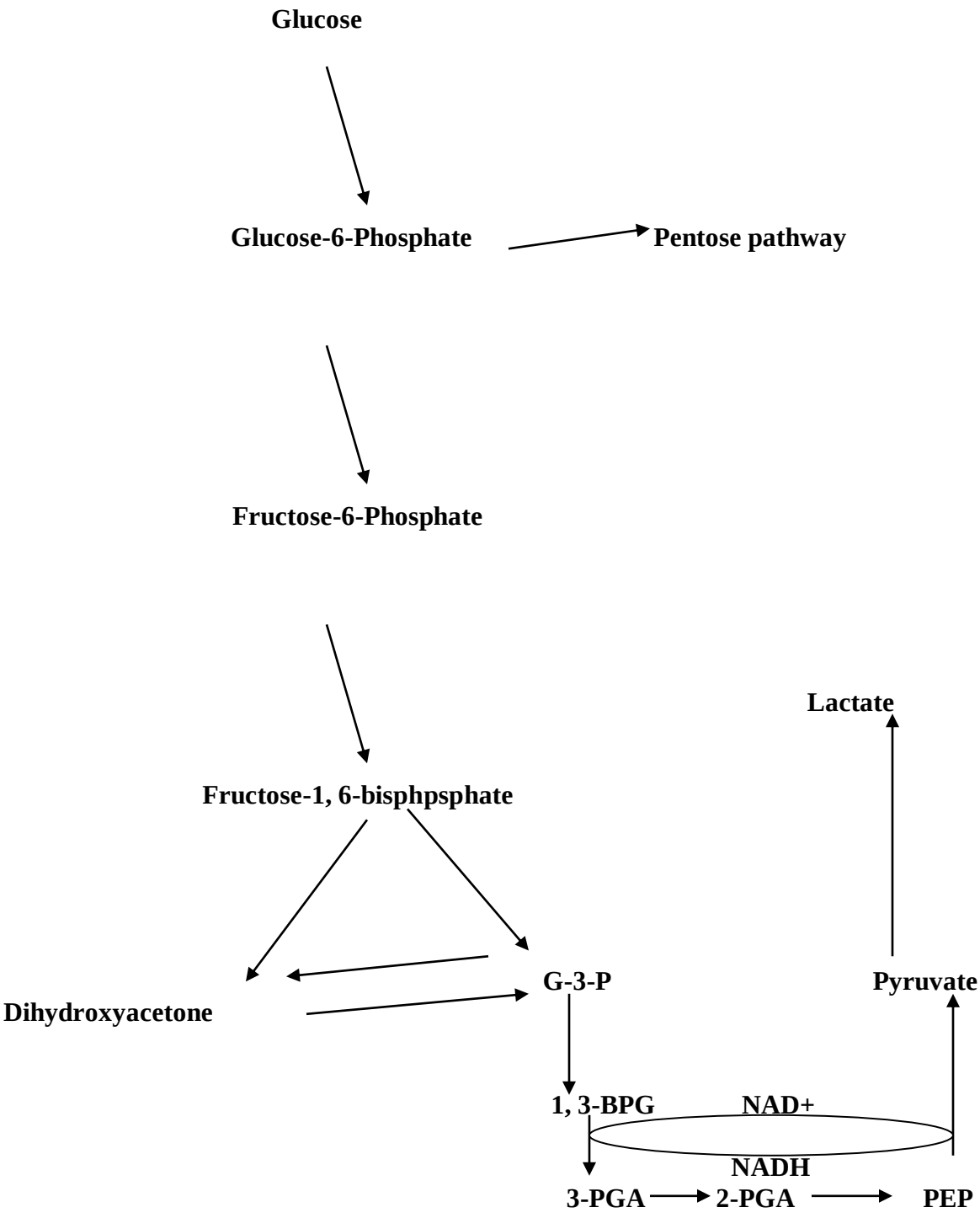
Carbohydrates as energy source in trypanosomes.

The blood-stage parasite actively ferments glucose as a primary source of energy. The metabolic steps involved in this conversion of glucose to lactate (referred to as glycolysis) are essentially the same as that found in other organisms. All of the enzyme activities have been identified in parasite and some of the genes cloned. The parasite exhibits a high rate of glycolysis and utilizes up to 75 times more glucose than that of uninfected (normal) erythrocytes. Most of the glucose is converted to lactate and the high lactate dehydrogenase (LDH) activity is believed to function in the regeneration of NAD^+ from NADH which is produced earlier in the glycolytic pathway by glyceraldehydes-3-phosphate dehydrogenase. The net result of glycolysis is to produce ATP which is the energy

currency of the cell. In other word, ATP is needed for anabolic and homestatic processes.

Aerobic metabolism involves the further catabolism of pyruvate (glycolysis intermediate preceding lactate) to carbondioxide and hydrogen atoms via the tricarboxylic acid (TCA) cycle. The hydrogen atoms are captured by the reduction of NAD+ to NADH. The electrons from the captured hydrogen are then fed into a chain of electron carriers and ultimately transferred to molecular oxygen to form water. ATP is generated by capturing energy during electron transport by the process known as oxidative phosphorylation. The TCA cycle and oxidative phosphorylation can generate up to 38 molecules of ATP per glucose molecule, whereas glycolysis only produces two molecules of ATP per glucose molecule. Nonetheless, the blood-stage of mammalian parasites does not exhibit a complete TCA cycle .An explanation for the inefficiency is the abundance of glucose in the mammalian blood stream. In contrast, the parasite does appear to exhibit a complete TCA cycle in the glucose-poor environment of the insect host. The TCA cycle and oxidative phosphorylation are generally carried out in the mitochondria of eukaryotes. These processes are generally assumed to be non-functional in the blood-stage parasite as evidenced by the acritae mitochondria.

Most (approximately 85%) of the glucose utilized by the parasite is converted to lactate. However, some of the glycolytic intermediates may be diverted for synthetic purposes. For example, enzymes of the pentose phosphate pathway have been identified. This pathway probably provides some of the ribose sugars needed for nucleotide metabolism and provides for regeneration of reduced NADPH to be used in biosynthesis or defense against reactive oxygen intermediates. Similarly, the further metabolism of pyruvate may provide intermediates in several biosynthetic pathways.


Glucose
Glucose-6-Phosphate
Pentose pathway
Fructose-6-Phosphate
Fructose-1, 6-bisphpsphate
Lactate
Dihydroxyacetone
G-3-P
Pyruvate
1, 3-BPG
NAD+
NADH
3-PGA
2-PGA
PEP

Parasite's Lipids utilization

Lipids are a major component of membrane. The rapidly growing parasite requires large amounts of lipids for this increase in parasite surface area and volume of internal membranes. This huge demand for lipids makes lipid metabolism an attractive target for anti-drugs and several potential drugs targeting lipid metabolism.

Membrane lipids are composed of a glycerol (3-carbon units) backbone which has a polar head group and two long chain fatty acid. Historically, the parasites has been considered to be incapable of synthesizing fatty acid de novo and restricted to obtaining preformed fatty acids and lipids from the host (salvage). However, several enzymes associated with the type II fatty acid synthesis pathway have been identified in parasite. The type II pathway is found in plants and prokaryotes, whereas the type I fatty acid synthetase is found in yeast and metazoan (Mitamura and Palacpac, 2003).

Several parasite enzymes involved in lipid synthesis from glycerides and fatty acids, as well as enzymes involved in the remodeling of lipid polar head groups have been identified. An enzyme capable of activating fatty acids (necessary for incorporation into lipids) has been localized to membranous structures found within the cytoplasm of the infected erythrocytes (Matesanz *et. al*, 1999).

Parasite's Proteins utilization

Proteins are composed of linear chains of amino acids which fold into 3-dimensional structures. Through their roles as enzymes or structural proteins, which are responsible for cellular structure and function. The blood-stage parasite obtains amino acids for protein synthesized from three sources: (1) degradation of ingested hemoglobin, (2) uptake of free amino acids from the host plasma (or cells), and (3) de novo synthesis. The most abundant source of amino acids is the ordered degradation of hemoglobin. The parasite digests up to 65% of the total host hemoglobin into amino acids. However, most of these amino acids are effluxes from the infected erythrocyte and only 16% of the digested hemoglobin is incorporated into parasite proteins (Krugliak, 2002).

Several amino acids are taken up by infected erythrocytes at accelerated rates. However, the amino acids formed via carbondioxide fixation and some of the exogenously added amino acids are not readily incorporated into proteins. Many of these amino acids (through transamination reactions) can interact with pathways involved in energy production and possibly serve as fuel sources. In addition, some amino acids serve as precursors or components of biosynthetic or other metabolic pathways. Of particular note is the proposal that glutamate dehydrogenase provides the reduced NADPH needed for glutamate reductase which presumably functions in redox metabolism.

Ribosomes are supramolecular complexes composed of ribosomal RNA and proteins. Their function is not to translate mRNA into protein. The mechanism of protein synthesis is presumably typical of other eukaryotes. Interestingly, different rRNA molecules are ex pressed during the vertebrate and invertebrate stages of the parasite's life cycle (McCutchan *et. al*, 1995). The functional significance of stage specific ribosome is not yet known.

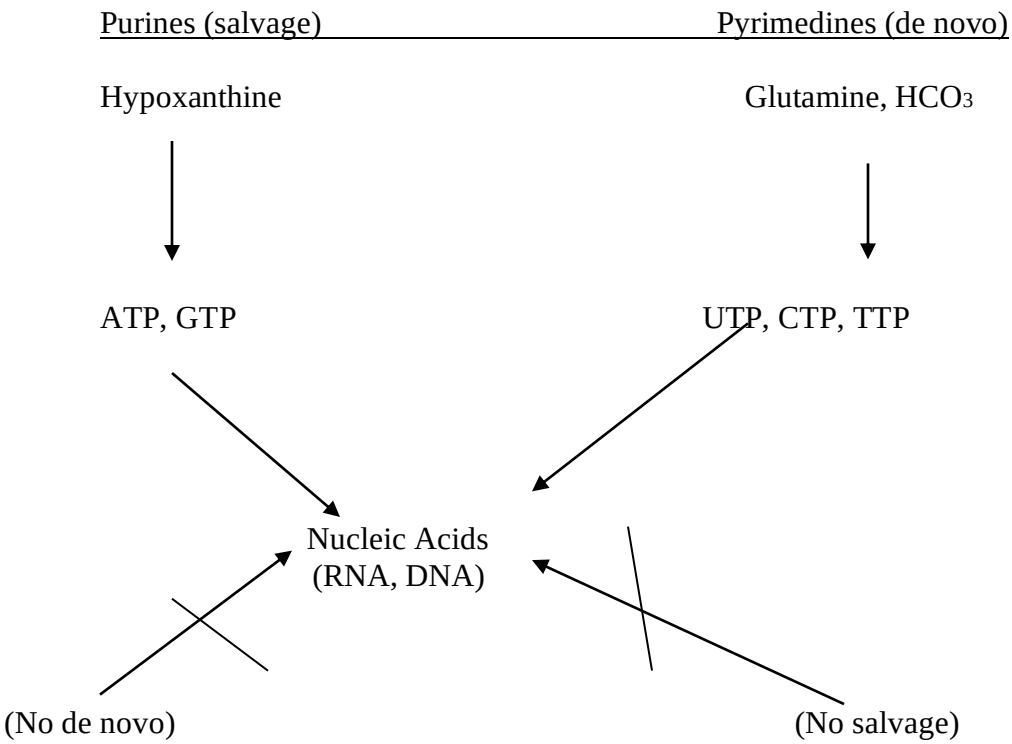
Parasite's Nucleotides utilization

DNA and RNA are polymers of nucleotides. Nucleotides consist of a ribose sugar group linked to either a purine (adenine and guanine) or a pyrimedine (cytosine, uracil and thymine) base. These bases can either be obtained via de novo synthesis or from the environment by the 'salvage' pathway. The parasite obtains preformed purines by the salvage pathway and synthesizes pyrimidines de novo. Since the host can obtain both types of bases by either pathway, it may be possible to exploit the parasite's limited capability in nucleotide metabolism.

The primary purine salvaged by the parasite is hypoxanthine which can be obtained from the host plasma. In addition, adenosine in the host plasma can be converted to hypoxanthine following dephosphorylation. Through a series of reactions the hypoxanthine is converted into ATP and GTP (or deoxy-ATP and deoxy-GTP) and incorporates in RNA (or DNA).

The parasite can not utilize preformed pyrimedines and must synthesize them from bicarbonate and glutamine. One step of pyrimedine synthesis involves an electron transport in which dihydroorotate dehydrogenase transfers electrons to an electron transport chain involving ubiquinone, cytochrome and molecular oxygen. This activity is probably located in the mitochondria and accounts for the microaerophillic requirements of the parasite. Pyrimedine synthesis also requires folates as co-factors.

Nucleotide metabolism in parasite



Parasite utilization of Vitamins and co-factors

Many biochemical processes require co-factor which do not directly participate in growth processes as do the bulk nutrients. Instead, vitamins are usually required in smaller amounts and are usually recycled. Pantothenate appears to be the only vitamins not supplied by the erythrocyte (Divo *et. al*, 1985) and is probably needed for the formation of acyl-Coenzyme A which is needed in lipid biosynthesis.

Folate and its derivatives are important co-factors in the synthesis of nucleotides and amino acids and especially for the transfer of methyl (one carbon) groups. The role of the dihydrofolate cycle in de novo pyrimethine synthesis is especially important. Dihydrofolate is reduced to tetrahydrofolate by dihydrofolate reductase (DHFR). Several anti- parasite drugs preferentially inhibit parasite DHFR. The tetrahydrofolate is methylated by serine hydroxymethyl transferase and the resulting methylene tetrahydrofolate functions as a methyl donor. For example, thymidylate synthase catalyzes the formation of dTMP from dUMP by transferring the methyl group from methylene tetrahydrofolate. During

this reaction the methylene tetrahydrofolate is converted to dihydrofolate, which then recycled.

Increased folates are needed to accommodate the demand for pyrimedines which are associated with DNA replication. The parasite cannot utilized preformed folate and must synthesize dihydrofolate from GTP, para-aminobenzoic acid and glutamate. Other sulfa containing drugs inhibit the de novo synthesis of dihydrofolate and combination of sulfa-drugs with pyrimethamine inhibits folate metabolism at two distinct places in the pathway.

Heme is an important component of many enzymes. The parasite cannot utilize the heme that is released as a result of hemoglobin degradation and must synthesize heme de novo. All of the enzymes necessary for the synthesis of heme are found within the parasite's genome. There is some uncertainty about whether heme is synthesized in the mitochondria or any other place.

Targets for therapeutic development.

In the African trypanosome, T. brucei hexokinase (THK), an activity composed of an unknown ratio of two proteins (THK1 and THK2) mediates the first step of glycolysis. Because the enzymes have the hallmarks of good targets for therapeutic development, considerable effort has been directed toward the development of THK inhibitors as potential antiparasitic compounds. First, both THK1 and THK2 are essential to the BSF parasite, as demonstrated by targeted gene silencing using RNA interference (RNAi) constructs specific to the unique 3'UTRs of the gene (Albert et al.2005; Chambers *et al*. 2008).In both cases, cell toxicity was observed after 3-5 days of RNAi exposure. Second, chemical inhibitors of THK1 are toxic to the parasite (Chambers *et al*. 2005; Wilson *et al*. 2002). Third, THK1 is likely different enough from host enzymes, sharing only 30-33% sequence identity with mammalian hexokinases (HKs), to suggest that it can be specifically targeted. Last, TbHK1 has unusual properties, including oligomerization into hexamers and is inhibited by compounds distinct from those which inhibit the mammalian enzymes, including fatty acids to suggest that specific inhibition is possible (Chambers *et al* 2008).

Trypanosomes Inhibitors

Structural-based inhibitor was developed by Wilson and colleagues to Trypanosoma hexokinase (THK) that were antitrypanosomal through modeling of THK to known Hexokinase (HK) structure. These glucosamine derivatives were tested and found to be competitive with respect to glucose, with *Ki* value similar to the *Km* value for glucose (Chambers *et al*. 2008). However, the compounds were not particularly toxic to blood stream forms (BSF) parasites, possibly because the compounds entered the cell by passive diffusion instead of impost against a concentration gradient. Alternatively, the compounds may have been imported by facilitate transporter, again failing to accumulate to sufficient concentrations for toxicity.

Trypanosome hexokinase inhibitors have also been identified in surveys of chemicals that inhibit HKs from other system (Dodson *et. al* 2011). The activity of such molecules identified by this approach is likely the result of conserved structural features of mammalian and trypanosome HKs (Newell *et al* 1991).

Lack of sensitivity of the trypanosome enzymes to other known HK inhibitors, including glucose-6-phosphate, 5-thio-D-glucose, and 3-methoxyglucose, suggest that the THKs are sufficiently unique for therapy development (Chambers *et al*. 2008). A group of bisphosphonates that are potent inhibitors of *T. cruzi* HK did not inhibit recombinant *Trypanosoma brucei* hexokinase (rTbHK), emphasizing the unique nature of the THKs (Hudock *et al*. 2007; Sanz-Rodriguez *et al*. 2006). Notably, rTbHK2, when oligomerized *in vitro* a catalytically inactive rTbHK1 variant, is active, and the activity is sensitive to ppi inhibition and, to a lesser extent, the bisphosphonate risedronate (Chambers *et al*. 2008).

Medicinal plants.

The plant kingdom represents an enormous reservoir of biologically active molecules and so far only a small fraction of plants with medicinal activity have been assayed. Nearly 50% of drugs used in medicine are of plants origin. This is the reason therefore much current research devoted to the phytochemical investigation of higher plants which have ethno botanical information associated with them.

The phytochemical isolated are then screened for different types of biological activity. Alternatively, crude extracts can be first assayed for particular activities and the active fractions then analyzed phytochemically. A variety of bioassay are now available for the phytochemist to use in such work (Bajaj, 1996).

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new source of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plant would further be valuable in discovering the actual value of folkloric remedies (Famsworth, 1996)

Biological buffers.

Many chemical reactions are affected by the acidity or basicity of the solution in which they occur. In order for a particular reaction to occur or to occur at an appropriate rate, the PH of the reaction medium must be controlled. Most biological molecules contains groups of atoms that maybe charged or neutral depending on PH, and whether these groups are charged or neutral has a significant effect on the biological activity of the molecules.

In multicellular organisms, the fluid within the cell and the fluids surrounding the cells have a characteristic and nearly constant PH.This PH is maintained in a number of ways and some of the most important biological buffer systems are the dihydrogen phosphate system and the carbonic acid system.

The phosphate buffer system operates in the internal fluid of the cells. This buffer system consist of dihydrogen phosphate ions(H_2PO_4) as hydrogen ion donor acid and hydrogen phosphate ion (HPO_4) as hydrogen ion acceptor (base).These two ion are in equilibrium with each other.

In mammals, cellular fluid has a PH in the range of 6.9 to 7.4 and the phosphate buffer is effective in maintaining this PH range. Another biological fluid in which a buffer plays an important role in maintaining its PH is the blood

plasma. The phosphate buffer saline solution is necessary to check the stability of materials that will be used for biological purposes, to maintain P_H of the solution and for safety practical.

Chromatography

Chromatography is a collective term referring to a group of separation processes whereby a mixture of solutes, dissolved in a common solvent, are separated from one another by a differential distribution of the solutes between two phases. One phase, the solvent is mobile and carries the mixture of solutes through the other phase, the fixed or stationary phase.

Chromatographic methods encompass a number of variations in technique in which the mobile phase ranges from liquids to gases and the stationary phase ranges from sheet of cellulose paper to the capillary glass tubes. These tubes are internally coated with covalently bonded complex or organic polymers.

Chromatographic methods are generally classified according to the physical state of the solute carrier phase that is the mobile phase. These branches are represented as liquid and gas-liquid chromatography, referring to the respective liquid and gaseous states of the mobile phase. These branches are further classified according to how the stationary phase matrix is contained for a particular chromatographic method. For example, liquid chromatography is divided into flat and column methods depending on whether the stationary phase is a thin layer mechanically supported on a sheet or is packed into a column. The column methods are classically referred to as liquid chromatography. They may be subdivided wherein the stationary phase is packed into glass or metal tube.

The chromatographic divisions based on the mobile phase may also be divided according to the mechanism of solute interaction with the stationary phase. The two mechanisms, adsorption and partition are the most commonly encountered for both solution and gas mobile phase separations. Adsorption chromatography (liquid-solid; [L/S] or gas-solid; [G/S] is process whereby solutes of a sample are separated by their differences in their attraction to the stationary versus the mobile phase. Partition chromatography, (liquid-liquid) [L/L]; or gas-liquid [G/L] is a process where by the solutes of the sample are separated by the differences in their distribution between two liquid phases [L/L] or between a gas and a liquid phase, [G/L]. In both cases the stationary phase is liquid, and the mobile phase is either a liquid or a gas.

The general principle of chromatography is the physico-chemical basis of its separation techniques which is principally distribution equilibrium. Distribution equilibrium refers to the differences in solubility and adsorption of a component in two immiscible phases

Separation equilibrium can be visualized as the distribution of a solute, [S], between two immiscible phases: upper phase [U] and lower phase, [L] at constant temperature and pressure. The ratio of the solute concentrations in the two phases determines the separation, which can be defined by an equilibrium concentration distribution coefficient, K_D:

$$K_D = C_U/C_L$$

C_U and C_L represent concentration of solute in the upper and lower phases respectively.

The distribution coefficient is sometimes referred to as **partition coefficient**.

Haematostaphis barteri.

Haematostaphis barteri is a tree or shrub 2-8m high, with a thick trunk up to 35 cm in diameter, a spreading, open crown, and leaves clustered at the far ends of the branches. The bark is scaly, grey-brown, with brownish slash with presence of colourless gum. The stems is glabrous crimson and the leaves has alternate, imparipinnate, globrous, 20-30 cm long, with 17-25 alternate or subpposite leaflets, narrowly elliptic or oblong, 5-8cm long and 1.5-3cm across, with notched apex and rounded or angular base. The flower has a cream-coloured, 3cm in diameter, with three imbricate petals. Its lax panicles composed of glabrous ellipsoid drupes, 2cm long, crimson when ripe. The flowering and fruiting is at the end of the dry season, usually before the first leaves appear. The usual habitats are Sudanese and Guinean savannahs on rocky soil. They are uncommon and scattered found in tropical Africa from Ghana to Sudan. The fruit is edible pulp tastes of resin and their seed provides edible oil (www.sciencedirect.com/science/articl--- ;www.medicinalplantsinnigeria.com/ga-----).

Uses and nutritive values of the plant

The fruit of *H. barteri* contains 26.7mg of ascorbic acid per 100g of the ice fruit, the leaves are used as eatable vegetables (bean soup) in the North Eastern part Nigeria as well as animals feeds (Eromosole 1994). The succulent sweet and sour pulp that fill the seed pod is used in large numbers of ways, for example the juice is used in wine production locally and also used in the place tamarin in preparing porridge (Iyedo1984). *Haematostaphis barteri* fruit also is an excellent source of vital nutrient (Amoo & Lagide 1999) based on the proximate composition of the dry fruit showed; that the dry fruit has a moisture content (5.6%); crude protein (1.5%); ash content (2.3%); fat is negligible; total titrable acidity (5.3%); reducing sugar were (glucose 66.9mg/g ; fructose 71.8mg/g; mannose 76.6mg/g and galactose 66.9mg/g), vitamin A (12.4mg/2g) and vitamin C (46mg/2g).

The seed of *H. barteri* contains six fatty acids with oleic (69.35%) and stearic (15.4%) the most abundant unsaturated fatty acids. The other unsaturated higher fatty acids, are eicosadienoic (6.92%) and oleic (2.74%) were detected and the total unsaturation for the oils was (79.01%) (Eromosole 2002).

A project work revealed similar on proximate and elementary analysis of the fruit of the plant as the moisture content was (8.1%); crude protein (10.3%); crude fibre (2.2%); fats negligible; ash content (4.5%); carbohydrate (1.5%); sodium 25mg/100g; potassium 108mg/100g; phosphorus 357.5mg/100g and vitamin C 152.8mg/100g respectively.

The leaves of the plant have the crude fibre (33.04%); crude protein (13.78%); carbohydrate, lipids, ash and moisture contents are within the range values expected for dry leaf vegetables. The leaves have all the essential amino acids in good quality. The anti- nutritional factors in the vegetables namely tannin, oxalates, and phytates are lower than the range of values reported from

most vegetables (Kubmarawa and Magomya, 2009). The boiled leaves with *Pseudoadrela kotschyi* and *Ficins ghaphalocarpa* are used for the treatment of malaria by "*Wa*" community in Ghana (Asase 2005). Other medicinal uses of the plants also is the decoction preparation of the stem bark which is taken as blood tonic especially in children and pregnant women and the flower is prepared for skin diseases medicines (Eromosele 1994; Ijomah et al 1997).

Trypanocidal activities of Natural product's secondary metabolite

The effect of 34 alkaloids of the piperidine, pyridine, tropane, isoquinoline, indole, quinolizidine, quinoline, purine, and steroidal types on the growth off *T.brucei brucei* and *T.congolense* was investigated in vitro. Berbamine,berberine,cinchonidine,cinchonine,emetine,ergotamine,quinidine,quinine andsanguinarine showed trypanocidal activities with ED_{50} values below 10µM. Berberine,emetine, and quinidine were the most active compounds, with ED_{50} values comparable to those of the antitrypanosomal drugs suramin and diminazene aceturate (Merschjohann *et al*.,2001). However, most of these compounds were also cytotoxic. In case of emetine, the ratio of cytotoxic/trypanocidal activity was only 3 while for quinidine it was 300, indicating that this latter compound could be a candidate for further drugs development. DNA intercalation in combination with protein biosynthesis inhibition, which is the major anticancer mode of these types of alkaloids, could be responsible for the observed trypanocidal and cytotoxic effect. (Merschjohann *et. al*., 2001). Bisbenzylisoquinoline alkaloids were studied for their *in vitro* trypanocidal activity against trypomastigote forms of the *T.cruzi*. The inhibitory activity of these compounds against trypanothione reductase, a target enzyme for chemotherapy against chagas disease, was also studied. Six (6) bisbenzylisoquinoline alkaloids (antioquinine, cepharanthine, daphnoline, limacine, cycleanine, and curine displayed an IC_{50}< 100 µM against *T.cruzi* (Fournet *et. al*., 1998). The trypanocidal activity of 13 isoquinoline alkaloids from various plants of the families *Fumariaceae* and *Memispermaceae* was studied in mice infected with *T. brucei*. Only capnoidine and corpaine showed slight activity when given in a single dose of 200mg/kg subcutaneously in DMSO excipient, and none of the compounds were active when given in 2% carboxymethycellulose-twen 80 excipient. Claviculine, limacine, and stylopine were fairly toxic (Dreyfuss *et. al*., 1987). Dasymachaline-N-oxide, an aporphine alkaloids from *Desmos dasymachalus* (Asruddin *et. al.* 2001), heyneanine and 12-methoxy-4-methylvoachaloline,2-indole alkaloids isolated from the root bark of *Tabernaemontana catharinensis* (Pereira, *et.al.*,1999),also displayed strong trypanocidal activity.

Several naturally occurring diterpenes, sesquiterpenes are reported for their trypanocidal activity.Pimaric acid, isopimaric acid and dehydroabietic acid 3-diterpenoids from *Pinus oocarpa* (Rubio *et. al*., 2005). Jatrogrossidione, the main diterpene of *Jatropha grossidentata,* jatrophone from *Jatropha isabellii* (Schmeda-Hirschmann, *et. al.*, 1996; Scio *et. al*., 2003), were tested against *T.cruzi in vitro*.Jastrogrossidione was able to clear the parasites at concentration of 1.5-5.0µg/ml, another diterpene from *Casearia sylvestris*,showed a pronounced activity on *T.cruzi* with a MIC of 0.59 µg/ml (Espindola *et. al*.,2004) .Kolavenol,

a diterpine from *Entada abyssinica*, showed a moderate trypanocidal activity against *T.b. rhodesiense*, with IC_{50} value of 2.5µg/ml (Freiburghaus *et. al*.,1998). *Goya zensolide*, from *Lychnophora* showed the highest trypanocidal activity against trypomastigote forms (IC_{50}=15.79 µg/ml) compare with other components. This compound also showed strong haemolytic activity, probably due to a non specific mechanism of action. In fact, the exocyclic methylene groups conjugated with a γ-lactone carbonyl group present in these molecules have a strong affinity for thiol groups of sulfhydryl-containing macromolecules, and may react with them by nucleophilic Michael-type addition, leading to deactivation of vital cellular functions (Grael *et. al* .,2005). With respect to triterpenes, urolic acid and oleanolic acid from *Arrabidaea triplinervia*, and their synthetic derivatives, showed *in vitro* activity against trypomastigote of *trypanosoma*. Ursolic acid was four times more active than oleanolic acid (Leite *et. al*., 2006).The presence of hydroxyl and/or carboxy group appeared to be necessary for the trypanocidal activity as could be deduced from the effect of the ester, and aldehyde derivatives. In addition to ursolic acid and oleanolic acid, gypsogenic acid; urjinolic acid, 2∞hydroxyursonic acid and maslic isolated from *miconia* species, displayed trypanocidal activity against blood stream trypamastigote forms of *trypanosoma* (Cunha *et. al*., 2003, 2006).The *in vitro* assays showed that ursolic acid, oleanolic acid and ursolic acid potassium salt were the most active (IC_{50}=17.1, 12.8, and 8.9 µM, respectively (Cunha *et.al*., 2006). In contrast, the mixture of 2∞hydroxyursonic acid and maslic acid, that posses a hydroxyl group at C-2 and C-3 positions, were much less potent than a mixture of ursolic acid and oleanolic acid (IC_{50}=48.5 and11.8 µM respectively). On the same line, urjinolic acid that differs from oleanolic acid by two additional hydroxyl groups (at C-2 and C-23) displayed weak trypanocidal activity (IC_{50}=76.3 µM) when compared with the other triterpenes (Cunha *et. al*., 2006).These results suggest that the free hydroxyl at C-3 and the polarity of the carbon C-28 are the most influential structural features for the *in vitro* trypanocidal of these triterpenes. In the *in vivo* assays, ursolic acid, its potassium salt and the mixture of ursolic acid and oleanolic acid were the most potent in reducing the parasitemia in mice, with ursolic acid and its potassium salt displaying up to 75.7% and 70.4% of parasitemia reduction, respectively.

Baibutoside, a cycloartane-type triterpene glycoside, isolated from the root of *Astragalus baibutensis*, along with actylastragaloside and astragaloside, were tested for *in vitro* antiprotozoal activity. They display notable activity against *T.b.rhodesiense*, with actylastragaloside being the most potent (IC_{50}=9.5µg/ml).Acetylastragaloside was also lethal to *T.cruzi* (IC_{50}=5.0µg/ml, however, it exhibits some cytotoxicity on mammalian cells (Calis *et. al*., 2006). Vernoguinosterol, vernoguinoside, 2-stimastane derivatives from *Vernomia guineensis*, exhibited moderate inhibitory activity against four (4) strains of bloodstream trypomastigote of *T. b.rhodesiense* with IC_{50} values in the range of 3 to 5µg/ml (Tchinda *et. al*., 2002).

A series of flavonoids aglycons and glycosides were evaluated for their *in vitro* activities against *T.b. rhodesiense* and *T. cruzi*. Their cytotoxicity against mammalian L6 cells were also assessed and compared to their antiparasitic

activities. The overall best in vitro trypanocidal activity for *T.b. rhodesiense* was exerted by 7, 8-dihydroxyflavone (IC_{50} = 68ng/ml) and catechol (IC_{50} = 0.8µg/ml. The tested compounds lacked cytotoxity in vitro and in vivo (Tasdemir *et. al*., 2006). Nine flavonoids from *Sophora flavescens,* also displayed trypanocidal activity against epimastigotes of T.cruzi, with IC_{50} values between 3.6-18.0µM (Matsuo et. al.,2003) of calycapterin, xanthomicrol, isokaempferide , luteolin, apigenin, among others 9 isolated flavonoids from *Dracocephalum kotschyi*, only isokaempferide and apigenin showed lethality against epimastigotes of *T. cruzi* (Gohari *et. al*., 2003). Flavonoids from *Lychnophora staviodes* were evaluated for their trypanocidal activity. Quercetin-3-methyl ether which was the most potent compound showed no lysis activity, and thus represents a promising compound for use against *T.cruzi* in blood banks (Takeara *et. al*., 2003). The investigation of the trypanocidal constituents of *Trixis vauthieri* and *Millettia griffoniana* exhibited moderate trypanocidal activity (Namga *et. al*., 2005)

A series of synthetic quinines were tested for their trypanocidal activity *in vitro* against trypanosoma epimastigotes. Among the tested compounds, Furanoquinolinediones showed potent trypanocidal activities but, only the 1, 5-regioisomer was found active as a redox cycling agent (Tapia *et. al*., 2004). Naphtho [2, 3-b] thiophen-4, 9-quinone and derivatives were prepared and evaluated for their trypanocidal activity by their effect on:-

Growth of epimastigotes forms of *trypanosoma in vitro*, Lysis of trypomastigote forms in murine blood and inhibition of the recombinant enzyme trypanothione reductase.

Most of the compounds were active against epimastigotes in culture but exhibited weak activity against trypomastigote in murine blood as well against the enzyme trypanothione reductase. The antiprotozoal activity of 1, 4-naphthoquinones are considered as subversive substrates of trypanothione reductase, one of the most promising targets for antitrypanosomal drug development (Blumenstiel *et. al*., 1999, Salmon-chemin *et. al*., 2000). This enzyme is believed to play a crucial role in the maintenance of the redox equilibrium in *trypanosoma* parasites. It is believed that the mechanism of action of naphthoquinones derivatives involves their absorption by the parasites and subsequent reduction to semiquinones and quinols. These compounds are capable of reducing molecular oxygen in hydrogen peroxide and super-oxide anions thus forming hydroxyl radicals which cause damage to the parasite plasmatic membrane and also inhibit some biosynthetic pathways. Their trypanocide activity is believed to involved the generations of oxygenated intracellular species .So, it must be related to the compound lipophilicity (related to the size and polarity) and it reduction potential (related to the electron-withdrawing ability) (Goulart *et. al*., 1997)

The trypanocidal effect of lignan lactones, was evaluated *in vitro* against intracellular amastigote forms of *trypanosoma* and confirmed *in vivo*, showed that (-) Hinoknin was the most active compound *in vitro* with IC_{50} of 0.7 µM. *In vivo* assays showed significant reduction of Parasitaemia after administration of (-) Hinoknin and (-) 6, 6'-dinitrohinokinin (Saraiva, *et. al*., 2007; de Souza *et. al*., 2005).The antimutagenic effect of (-) hinokinin on the chromosome damage

induced by the chemotherapeutic agent doxorubicin was investigated. Animals related with different doses of (-) hinokinin (10, 20 and 40mg/kg exhibited micro nucleated cell frequencies similar to that of the negative control (Medola *et. al*., 2007). Thus, (-) hinokinin not only has no genotoxic effect, but is also effective in reducing the chromosome damage induced by doxorubicin. (-)Hinokinin also exerted a significant antioxidant effect on parasite mitochondria, which might be one possible mechanism by which this compound may exert a protective effect on the chromosome damage induced by the free radicals generated by doxorubicin (Medola *et. al*., 2007). Some neolignans from *Piper regnellii* showed moderate activity against epimastigotes forms of trypanosoma with IC_{50} values of 7.0-8.0µg/ml (Luize *et. al*., 2006). After methylation, this compound showed a lessened inhibitory activity on the growth of the protozoan, suggesting that the loss of the hydroxyl group reduces the activity. Eupomatenoids-5, one of the compounds was significantly more active than benznidazol, and showed low cytotoxic effects against Vero cells, but caused no lysis in sheep blood at concentrations which inhibit the growth of epimastigotes forms (Luize *et. al*., 2006

Other phenolic types of compounds are described for their trypanocidal activity. Isolated compounds from *Garcinia intermedia* and *Calyphyllum brasiliense*, were evaluated against epimastigotes and trypomastigote *trypanosoma*. Most of compounds displayed moderate activity (Abe *et. al*., 2004).

Compounds from *Garcinia subelliptica* (Guttiferae),were evaluated for their trypanocidal activity, with garciniaxanthone B and subelliptenone B been the most active with IC_{50} values 66 and 51 µM against epimastigotes and 8 and 2 µM against trypomastigote respectively, compared to 24 µM (epimastigotes) and 2 µM (trypomastigote) for gentian violet (Abe *et. al*., 2003). The inhibitory activity of 13 coumarins was assayed against *T. cruzi*-GAPDH. Chalepin was the most active compound, with IC_{50}=64 µM (Vieira *et. al*., 2001). Series of simple phenolics also displayed moderate trypanocidal activity against *T. cruzi* and *T.b. rhodiense* (Tasdemir *et. al*., 2006).

Caffeic acid and 4, 5-di-O-E-cafeoyl quinic acid from *Lychnophora pohlii*, displayed some trypanocidal activity against trypanosoma trypomastigote forms, with % lysis of 55.07 at 500µg/mL (Grael *et. al*., 2005).

Since the available trypanocidal drugs are unsatisfactory, several synthetic and naturally occurring compounds have been evaluated for their antitrypanosomal activity .(WHO2000;*http*://*www*.*who*.*int*/*ctd*/*chagas*/*burdens*.*htm*;Kirchoff 1993)

However, previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azaanthraquinone) highly aromatic planar quaternary alkaloids, barbarine and harmarine (Hopp *et. al*., 1976; Nok, 2001). A literature survey indicated that several flavonoids have antitrypanosomal activity, some showing an interesting selectivity such as quercetagetin (Raz, 1998, Camacho *et. al*., 2000b; Tarus *et. al*., 2002). *C. sieberiana* was also shown to contain anthracenic derivatives (Duquenois and Anton, 1968) which could also explain a part of the observed activity as a recent study has shown that azaanthraquinone has interesting effect on *Trypanosoma congolense* (Nok, 2002)

and Tri-n-Butyltin Oxide to have activity against *T. brucei brucei* (Nok *et. al*., 1992) .As a recent paper analyzed the antitrypanosomal activity of alkaloids from different types, some of which being active (Merschjohann *et. al*., 2001).

However, the antimicrobial activity of the stem bark extracts of *Haematostaphis barteri* was tested against some microbial agent using TLC direct-autobiographic methods and was found to be effective, though preliminary chemical analysis revealed the presence of flavonoids, tannins among others (Asase *et. al*., 2008 ;Kubmarawa *et. al*. 2007).

Some plant extracts have been demonstrated to contain potent trypanocidal constituents (Igweh and Onabanjo 1989; Owolabi *et. al*., 1990; Atawodi *et. al*., 2005; Abu *et. al*., 2009) just to mention few among them. So far, different solvent extracts of the different part of *Khaya Senegalensis* have been shown to possess both *in vivo* and *in vitro* activity against *T.brucei* and *T. evansi*. (Wurocheke and Nok, 2004; Atawodi 2005; Adeiza *et. al*., 2010; Umar *et. al*., 2010). From those report, reveals that different parts of water, ethanolic, and methanolic extractable phytochemicals that possess both *in vitro* and *in vivo* activities against *T. brucei* and *T. evansi*. Likewise an *in vitro* activity of *P. Laxiflora* and *C. sieberiana* on trypanosomes and *in vivo* study with mice infected with *T. b. gambiense*, root water decoction and maceration of *C. sieberiana* were found to be inactive (Youan *et. al*.,1997), while Hoet *et. al*., (2004) found an activity in vitro for the leaf extract, which according to Neuwinger (2000) is used in many traditional medicines.

Inhibitory mode of actions

It has been suggested by Sepulveda-Boza and Cassels (1996) that natural products exhibits their trypanocidal activity through interference with redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. Phytochemicals in contrast to synthetic pharmaceuticals based upon single chemicals may exert their effects through the additive or synergistic action of several chemical compounds acting at a single or multiple target sites associated with a physiological process (Tyler, 1999). It is also known that some agents act by binding with the kinetoplast DNA of the parasite (Atawodi *et. al*., 2003). Many of the active compounds in inhibiting culture growth also inhibited cell respiration, suggesting that these drugs act by blocking mitochondrial electron transport. Some of the mode of action was found to exhibit trypanostatic activity, similar to the early reports of the trypanostatic effect of ethanolic extract of *F. abida*. Trypanostatic effect is said to suppress the activity of the parasite there by sustaining the life of the infected rats (Tijani *et al* 2009)

Some trypanocidal effects of alkaloids appear to be correlated with their antioxidative activities (Morello *et. al*., 1994).For example, (-) Hinokinin also exerted a significant antioxidant effect on parasite mitochondria, which might be one possible mechanism by which this compound may exert a protective effect on the chromosome damage induced by the free radicals generated by doxorubicin (Medola *et. al*., 2007).

CHAPTER FOUR

4.0.0 RESULTS & DISCUSSION

4.1.0 Results:

The aqueous crude extract of the *H. barteri* stem bark showed *in vitro* antitrypanosomal activity in a dose-dependant fashion (Figures 2-5) for both *T. congolense* and T. *brucei brucei.* The activity seemingly to be higher in *T. congolense* to *T.brucei brucei.* At 50 and 25mg/ml, water crude extract of *H. barteri* stem bark exhibited strong trypanocidal activity within 30-35 minutes against both parasites species. The survival time for both parasites in various concentrations (50; 25; 12.5 and 6.25mg/ml) of the crude extract of *H. barteri* inhibit the parasites motility. Parasites survival time was concentrations dependent with the trypanosomes surviving for longer periods in lower concentrations (6.25 and 12.5mg/ml) and for shorter periods in higher concentrations (25 and 50mg/ml) as shown in table AP: 1. At these concentrations, morphology of red blood cells was unaffected. The standard trypanocide (Diminazene aceturate) used as standard control completely killed the parasites at concentration of 100µg/ml within 5 minutes of incubation. The negative control consisting of parasites incubated with PBS or 10%DMSO showed active parasite even for more than 4 hours. In the in vitro activity of the fractionated crude extract of *H. barteri* stem bark with methanol, ethyl acetate, acetic acid and water as solvents, the methanolic fraction showed the highest activity as shown in figure 10; 11 and 13.

In the *invivo* antitrypanosomal activity, all rats infected with *T.brucei brucei* and *T.congolense* manifested varying degree of clinical trypanosomiasis that include weakness, and rough hair coat, later there was parlous of the mucous membranes of the eye and the foot pads. These signs were progressive and more severe in the infected untreated group. The survival time of table 2 & 3 shows that the infected untreated group (Group5) has a less survival days for both the parasites as compare with the infected treated groups with the crude extract (Groups 1, 2 and 3). The groups treated with the standard drug and uninfected control had a 100% survival.

The parasiteamia of both controls and the infected treated groups are presented in Figure 8 & 9 for *T. brucei brucei* and *T. congolense* respectively. While a progressive increase in parastemia was observed in the infected controls; treatments with the crude extract doesn't significantly lowered the level of parasitaemia ($P<0.05$) when compared to the infected untreated rats, though there was an extension of the survival duration with a day or two in comparism to the infected untreated rats infected with both parasites. While the parasite totally disappeared from the bloodstream of Nozomil® treated infected group on days 8 i.e. 3 days post treatment in the case *T. brucei brucei* and on 14 days post infection for the *T. congolense* parasites. However, we have also observed that the methanolic fractionated extract of *H. barteri* stem bark was found to exhibit trypanostatic activity *in vivo* in the *T. brucei brucei*. Trypanostatic effect is said to suppress the activity of the parasites there by sustaining the life of the rats when compared to the control groups as showed in figure 14. The uninfected control

showed no signs of trypanosomiasis or other infections throughout the period of experimentation

Both parasites infection in this work caused significance fall in PCV of infected rats; figure 6 & 7 indicative of anaemia but no significant effect on the change in body weight when compare with the normal control especially in *T. brucei brucei* as shown in figure 8 & 9. However, treatment with Nozomil® to the infected animals significantly reduced the magnitude of decline in PCV; administration of the extract at the various doses seems not to have effect on the disease-induced anemia. The PCVs of the two groups of uninfected animals remained relatively constant as still shown in table AP: 5 & 6; or in the same figure 6 & 7. From the phytochemical studies the types of compounds present in crude and the fractionated fraction are showed in table 1.

Table 1: Qualitative phytochemical analysis result of water crude and the fractionated fractions extract of *H. barteri*

PHYTOCHEMICALS	CRUDE EXTRACT	**FRACTIONATED EXTRACTS**			
		WATER	ACETIC. ACIDS	ETHYL ACETATE	METHANOL
PHLOBATANIN	--	--	--	--	--
SAPONINS	+	+	+	+	+
FLAVONOIDS	+	+	+	+	+
STEROIDS	--	--	--	--	--
TEPENOIDS	+	+	+	+	+
CARDIAC GLYCOSIDE	+	+	--	--	+
ALKALOIDS	+	+	--	--	+
CARBOHYDRATES	+	+	--	--	+
TANINS	+	--	+	+	+
ANTHRAQUINONE	+	+	--	--	--

TABLE 2: Survival days of rats infected with T.congolense and treated with either Diminazene aceturate (Nozomil®) or the *H.barteri* stem bark crude water extract

GROUP TREATMENT	NUMBER IN A GROUP	NUMBER OR % DEAD	RANGE IN DAYS OF DEATH
Infected treated with the extract at 20mg/ kg body weight	5	5 (100%)	16-18
Infected treated with the extract at 40mg/ kg body weight	5	5 (100%)	16-19
Infected treated with the extract at 80mg/ kg body weight	5	5 (100%)	17-20
Infected treated with standard drug at 3.5g/kg	5	Nil (0%)	No death
Infected control (Infected untreated)	5	5 (100%)	15-18
Normal control (Uninfected untreated)	5	Nil (0%)	No death

TABLE 3: Survival time of rats infected with *T. brucei brucei* and treated with either Diminazene aceturate (Nozomil®) or the *H. barteri* stem bark crude water extract and their controls.

GROUPS TREATMENT	NUMBERS IN A GROUP	NUMBER OR % OF DEATH	RANGE IN DAYS OF DEATH
Infected treated with extract at 20mg/ml kg (Dose I)	5 rats	5 (100%)	7-10
Infected treated with extract at 40mg/ml kg (Dose II)	5 rats	5 (100%)	7-10
Infected treated with extract at 80mg/ml kg (Dose III)	5 rats	5 (100%)	9-11
Infected treated with standard drug	5 rats	0 (0%)	No death
Infected control (infected untreated)	5 rats	5 (100%)	7-9
Normal control (uninfected untreated)	5 rats	0 (0%)	No death

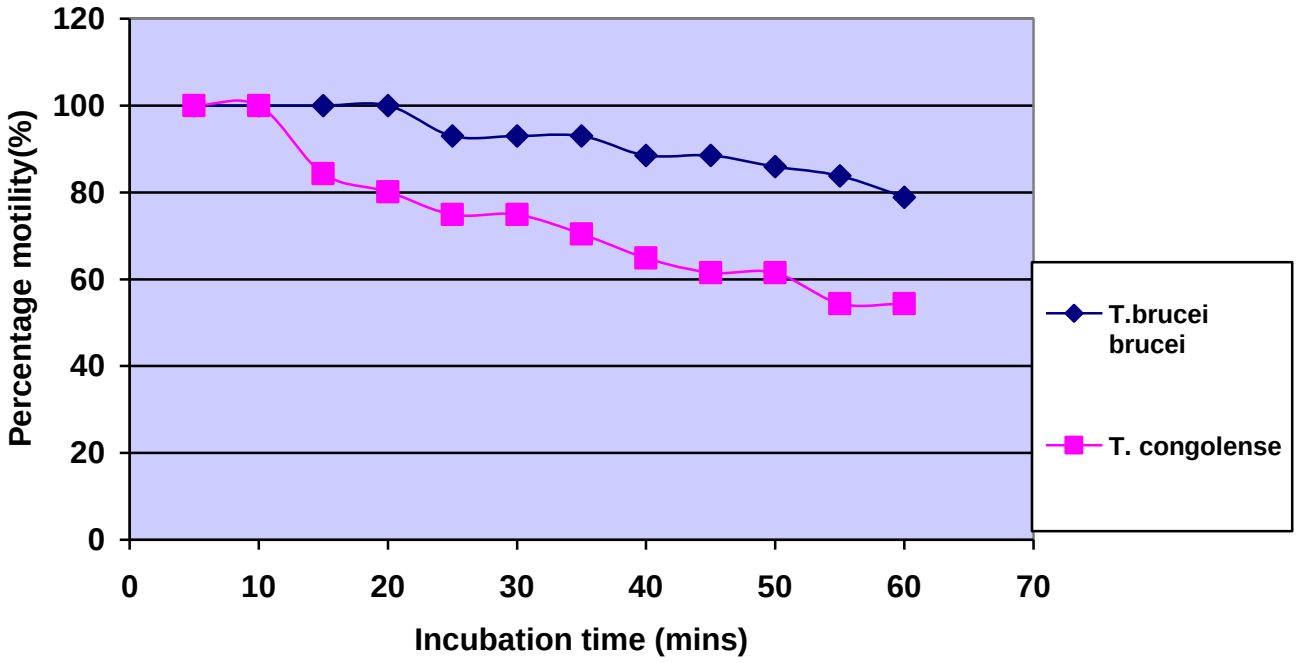


Figure 2: Profile of incubation time against motility of *T.brucei brucei* and *T.congolense* for *H. barteri* stem bark crude water extract at concentration 6.25mg/ml to infected bloods.

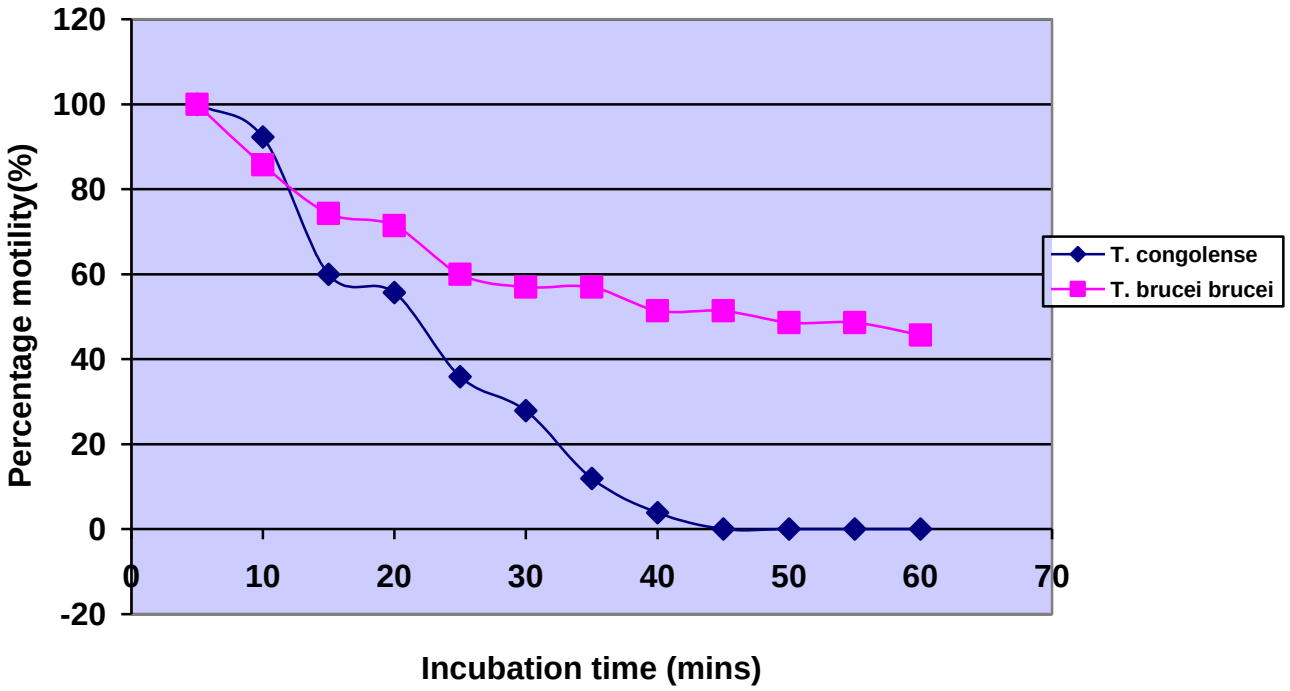


Figure 3: Profile of incubation time against motility of *T. brucei brucei* and *T. congolense* for *H. barteri* stem bark crude water extract at concentration of 12.5mg/ml to infected bloods.

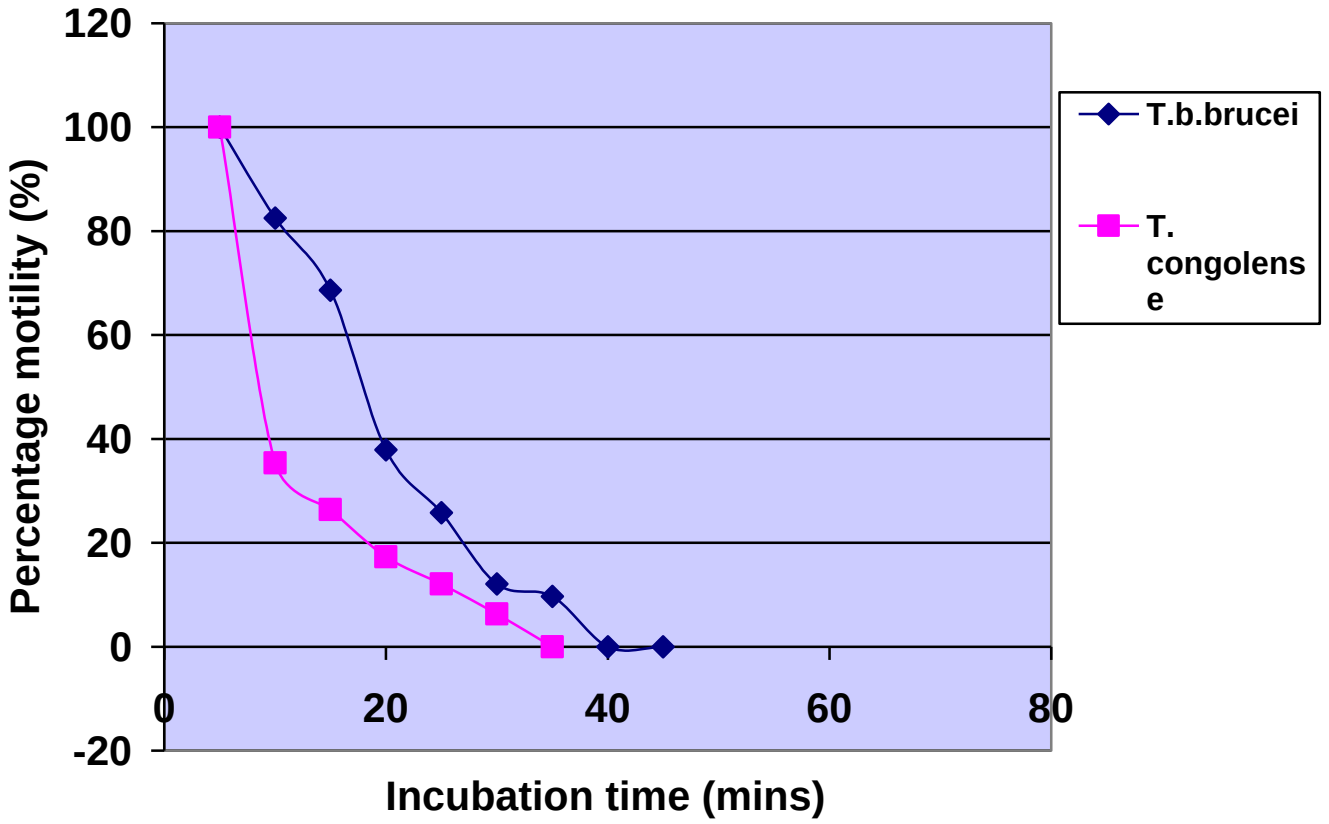


Figure 4: Profile of incubation time against motility of *T. brucei brucei* and *T. congolense* for *H. barteri* stem bark water crude extract at concentration 25mg/ml to infected bloods

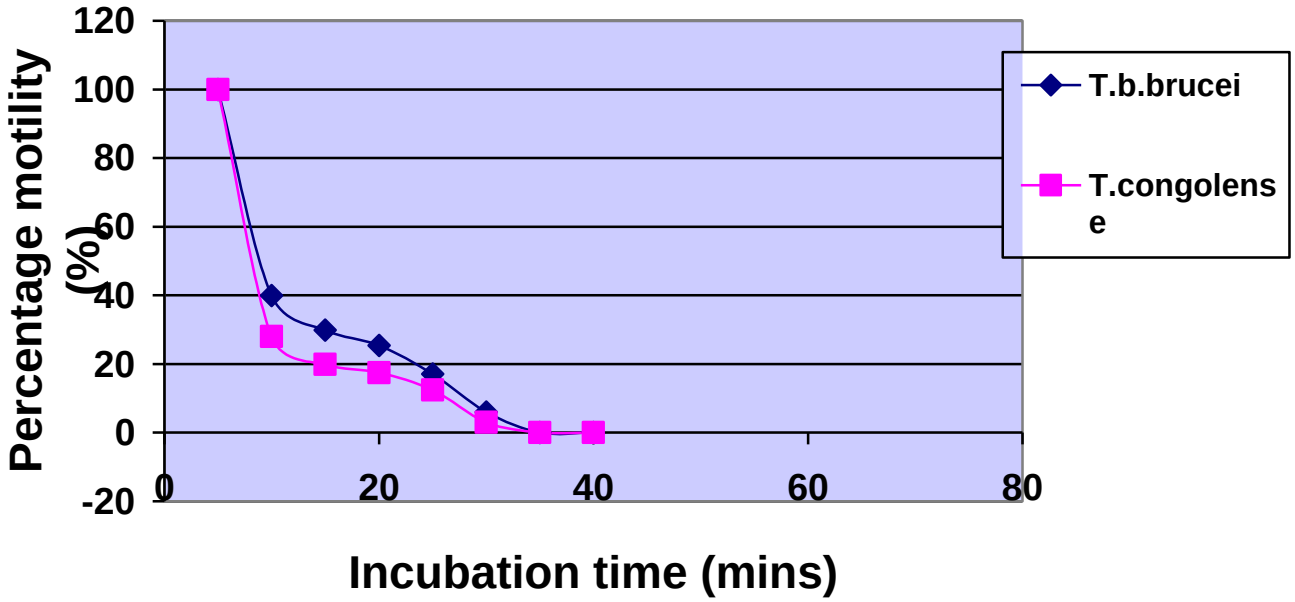


Figure 5: Profile of incubation time against motility of T. brucei brucei and T. congolense for *H. barteri* stem bark crude water extract at concentration 50mg/ml to infected bloods

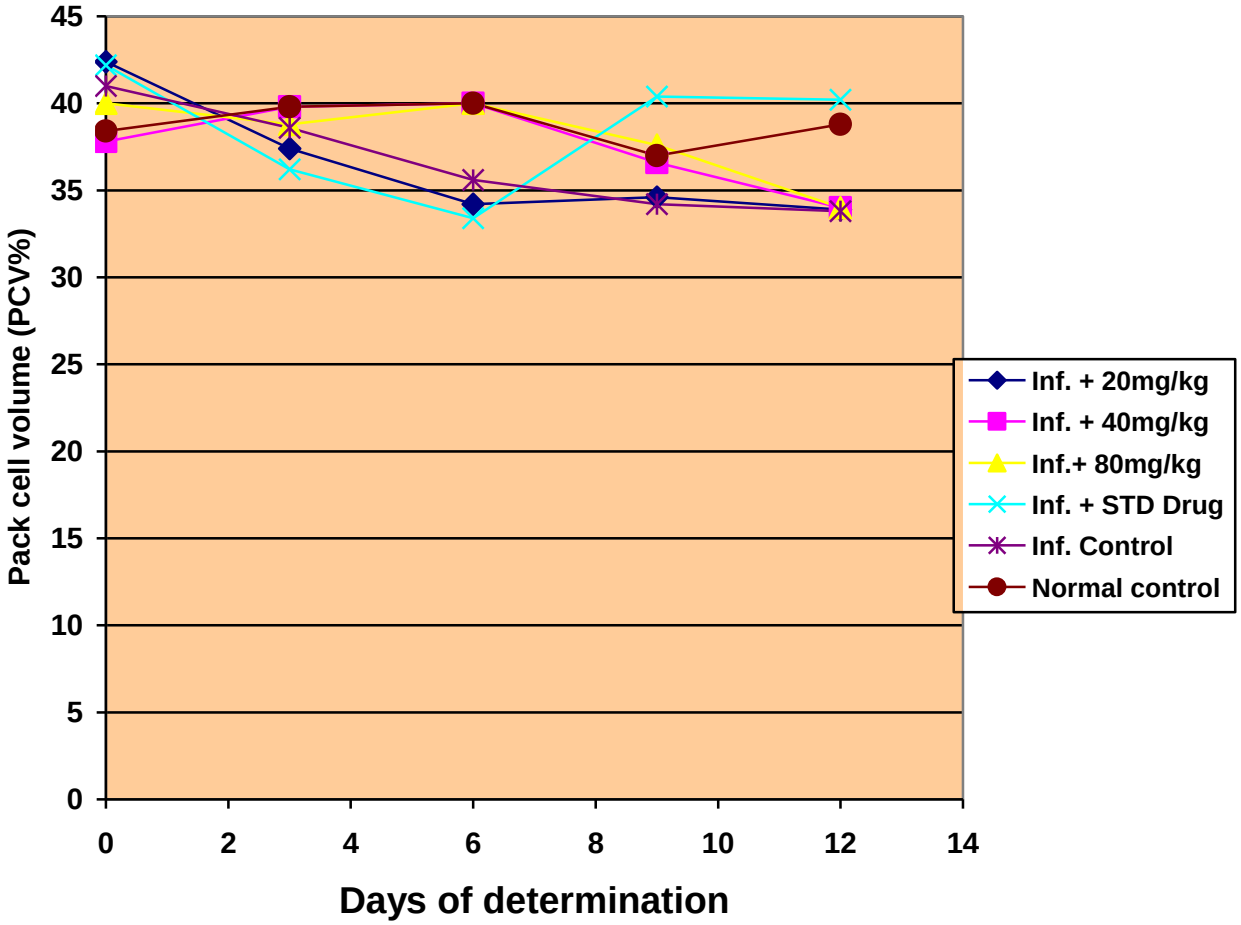


Figure 6: Effect of H. barteri stem bark water crude extract and diminazene treatment and thier controls on the PCV of rats infected with T.congolense.

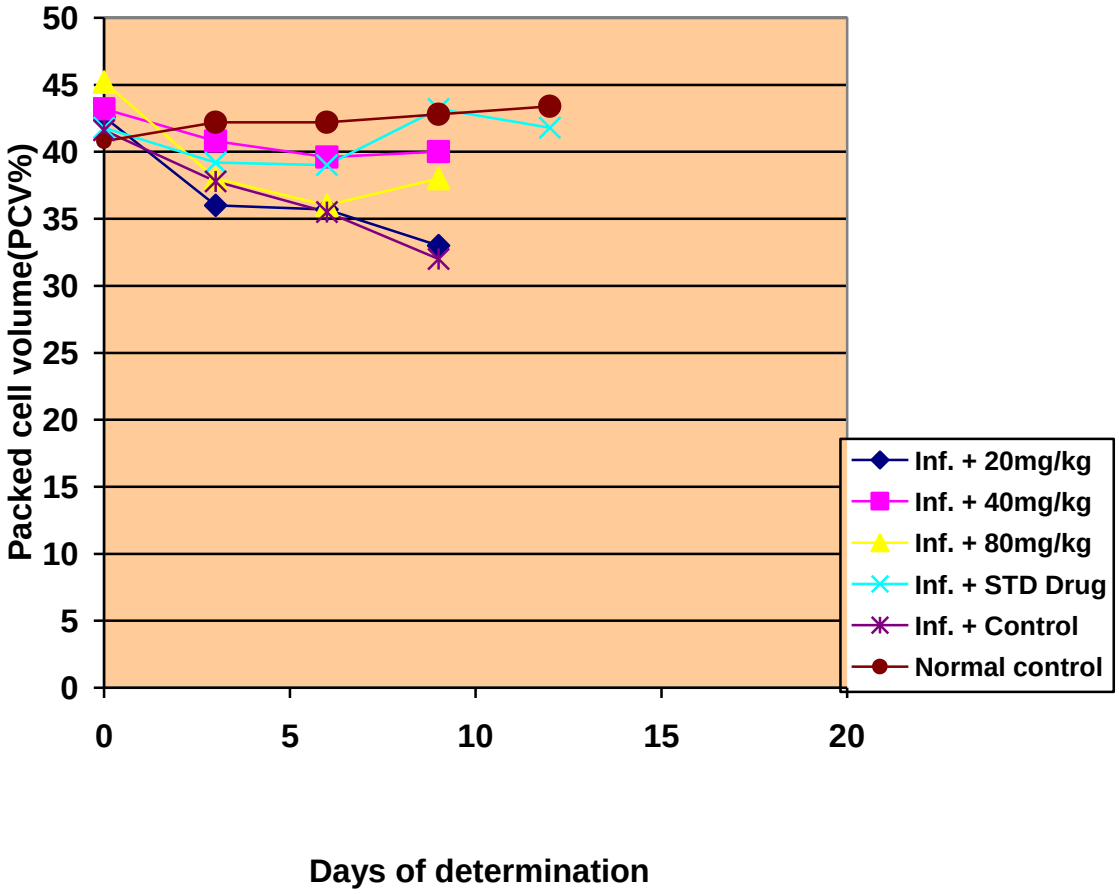


Figure 7: Effect of water crude extract of H. barteri stem bark, deminizene and their controls on the PCV level of *T.brucei brucei* infected rats

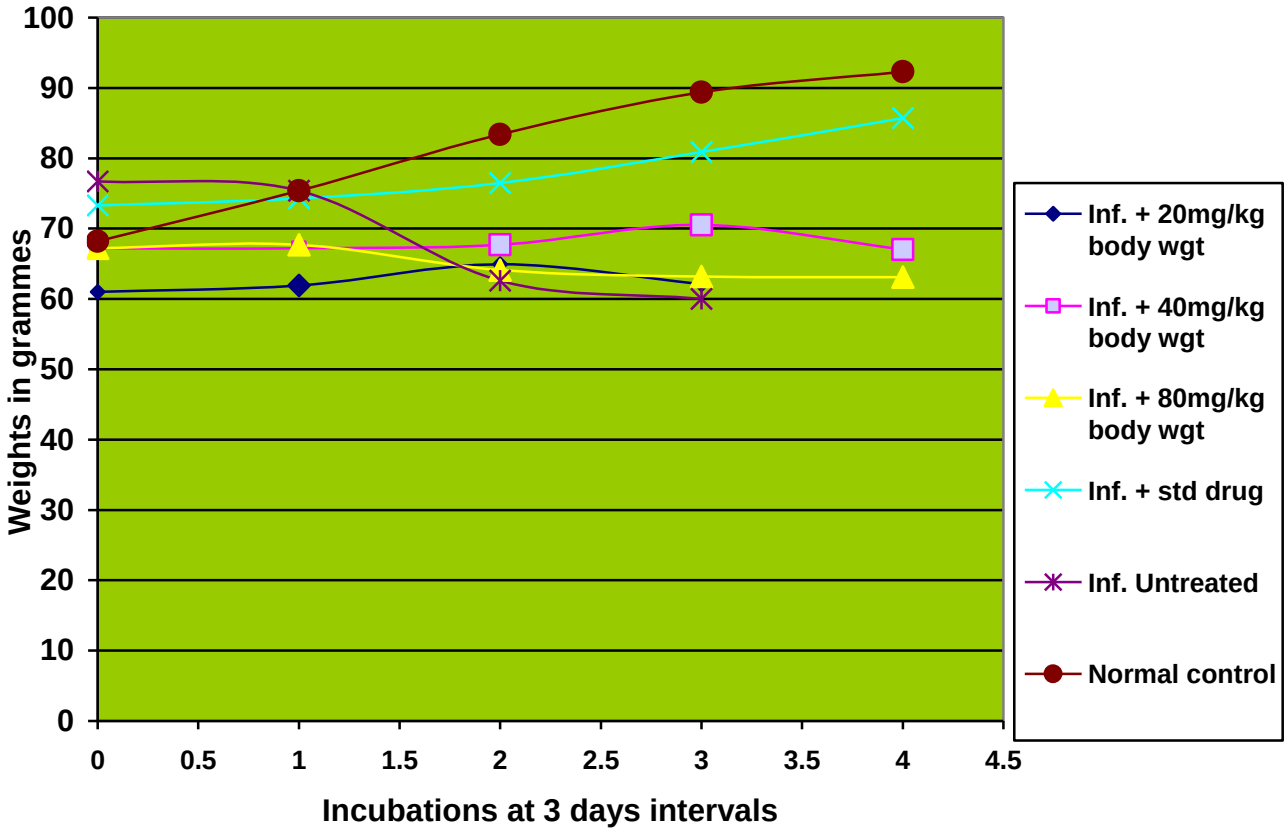


Figure 8:Change in weights in grams (g) of treated rats infected with T. brucei brucei at three days intervals from day zero of infection.

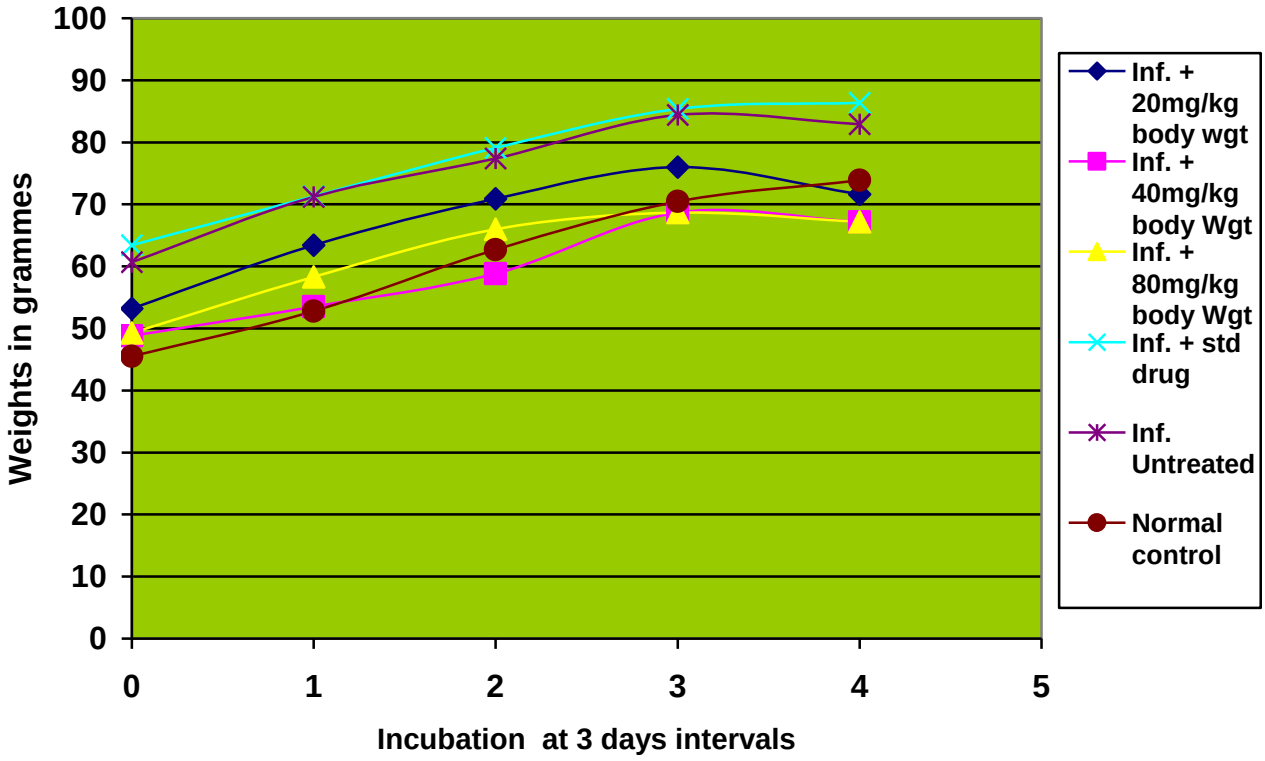


Figure 9:Change in weights in grams (g) of the treated rats infected with T.congolense at three days intervals from day zero of infection

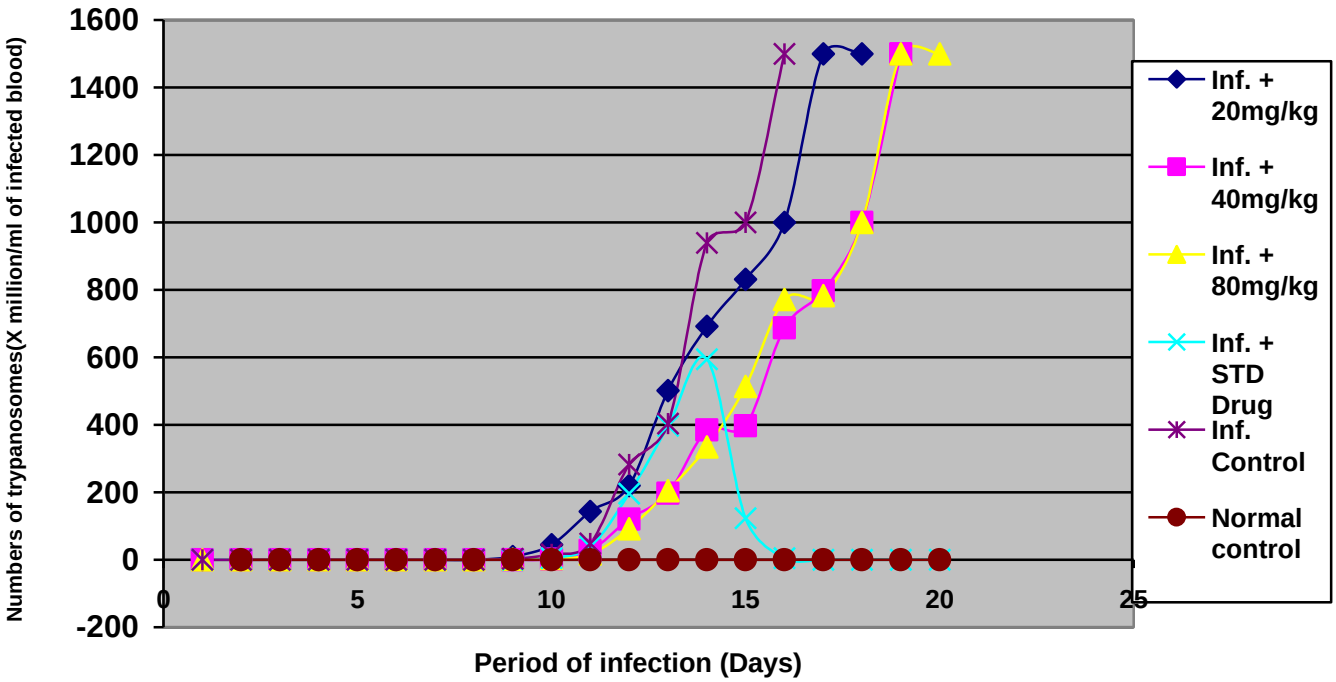


Figure 10:Effect of *H. barteri* stem bark, water crude extract, diminazene treatment and their controls on *T. congolense* infected rats

Table4: Average number of parasites per microscopic field per rats infected with *T.congolense* treated with the crude water extract for 7days and the standard drug group.

Days of Incubation from zero	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th
GRP1 20mg/kg BW	0	.305	2.40*	11.4 *	36.4 *	56.0 *	128 *	174 *	212 *	256	>256	>256	--	--
GRP2 40mg/kg BW	0	.308	.406 *	.490 *	7.00 *	30.6 *	50.2 *	98.2 *	101 *	176	204	256	>256	--
GRP3 80mg/kg BW	0	.115	.425 *	1.01*	4.40 *	23.4 *	52.6 *	85.2*	131 *	197	200	256	>256	>256
Std Ctrl	0	.030	.058	1.22	9.75	50.0	101	152*	31.3	1.25	0	0	0	0
Inf. Ctrl	0	.030	.630	4.01	12.2	72.0	103	240	256	>256	--	--	--	--

* = Signify treatment

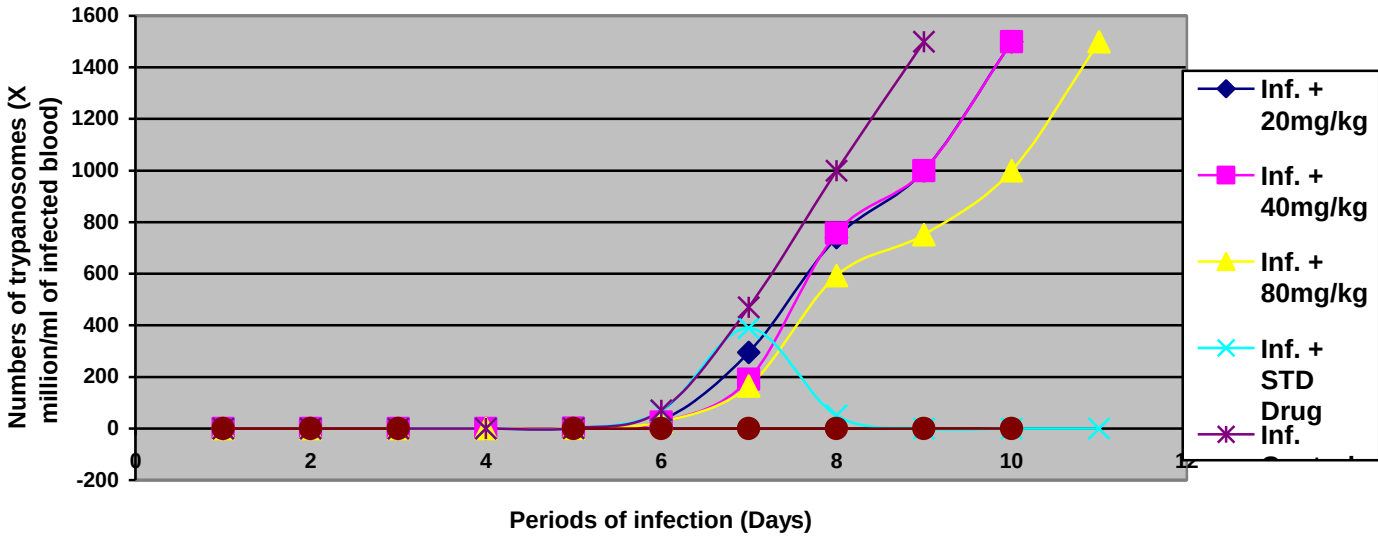


Figure 11:Effect of H. barteri stem bark water crude extract, diminazene treatment and their controls on *T.brucei brucei* infected rats

Table 5: Average numbers of parasites per microscopic field per rats infected with *T. brucei brucei* treated with the crude water extract for 5 days and the standard drug group

Incubation & treatment days	5th	6th	7th	8th	9th	10th	11th
20mg/kg body weight with extract	0.660*	8.0*	75.0*	184.0*	256*	>256	
40mg/kg body weight with extract	0.125*	6.2*	48.4*	151.0*	256*	>256	
80mg/kg body weight with extract	0.100*	6.4*	42.0*	151.2*	192*	256	>256
3.5g/kg body weight with std drug	0.100	17.2	99.2*	12.8	0	0	0
Infected not treated	0.106	18.0	120	256	>256		

* - Signify treatment

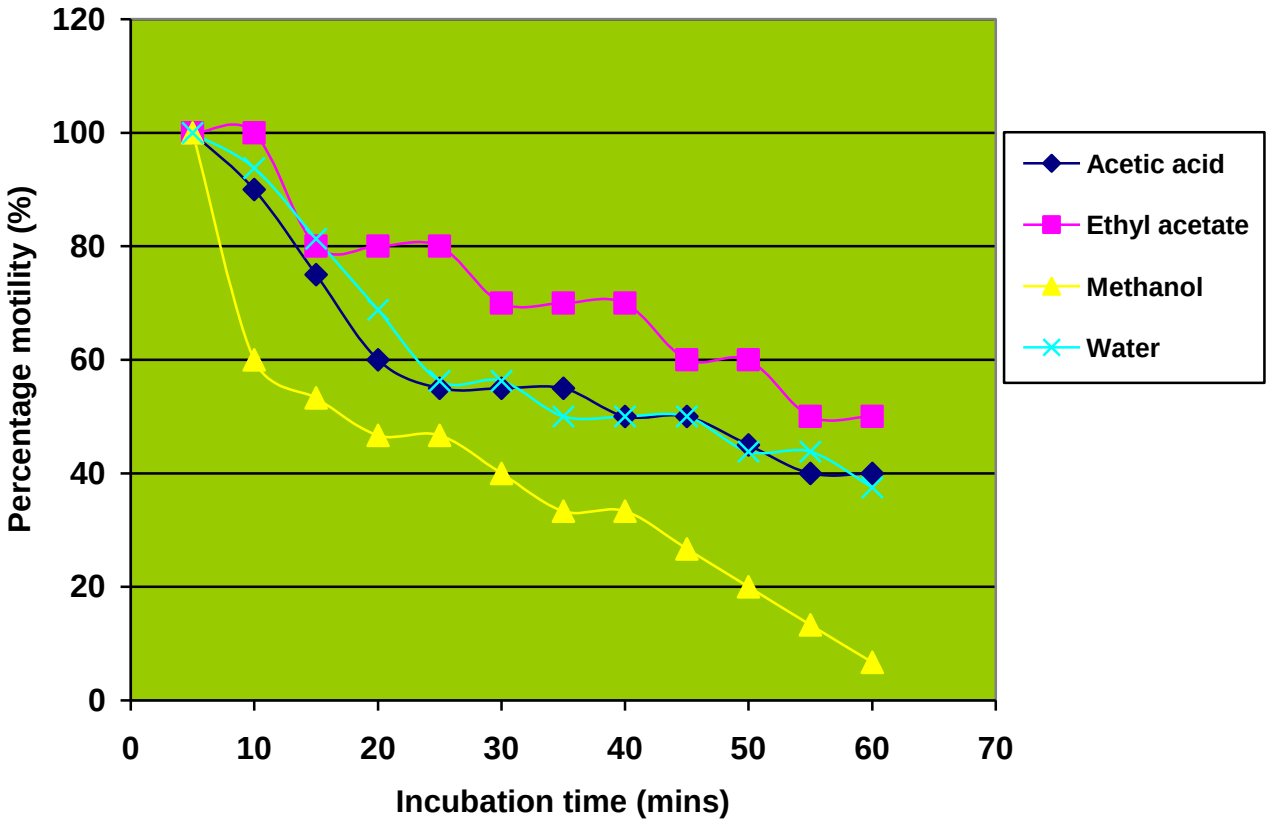


Figure 13: Profile of incubation time against motility of *T. brucei brucei* for fractionated H. barteri stem bark extract at concentration 12.5mg/ml to infectected blood.

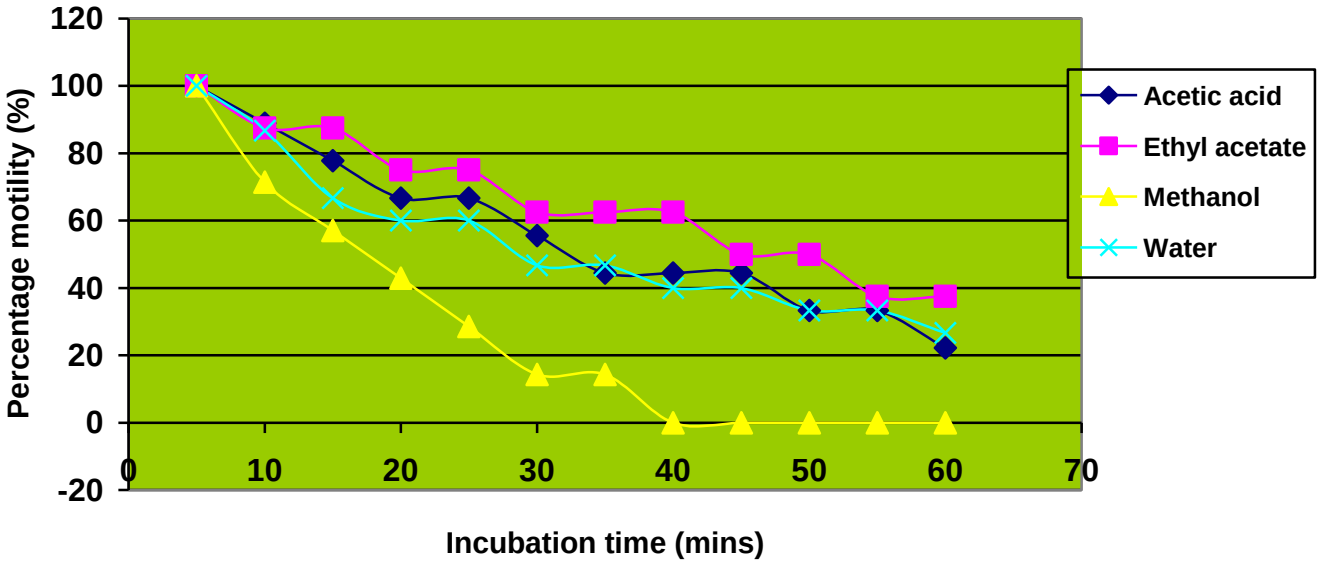


Figure 14: Profile of incubation time against motility of *T. brucei brucei* for fractionated H. barteri stem bark extract at concentration 25mg/ml to infected blood.

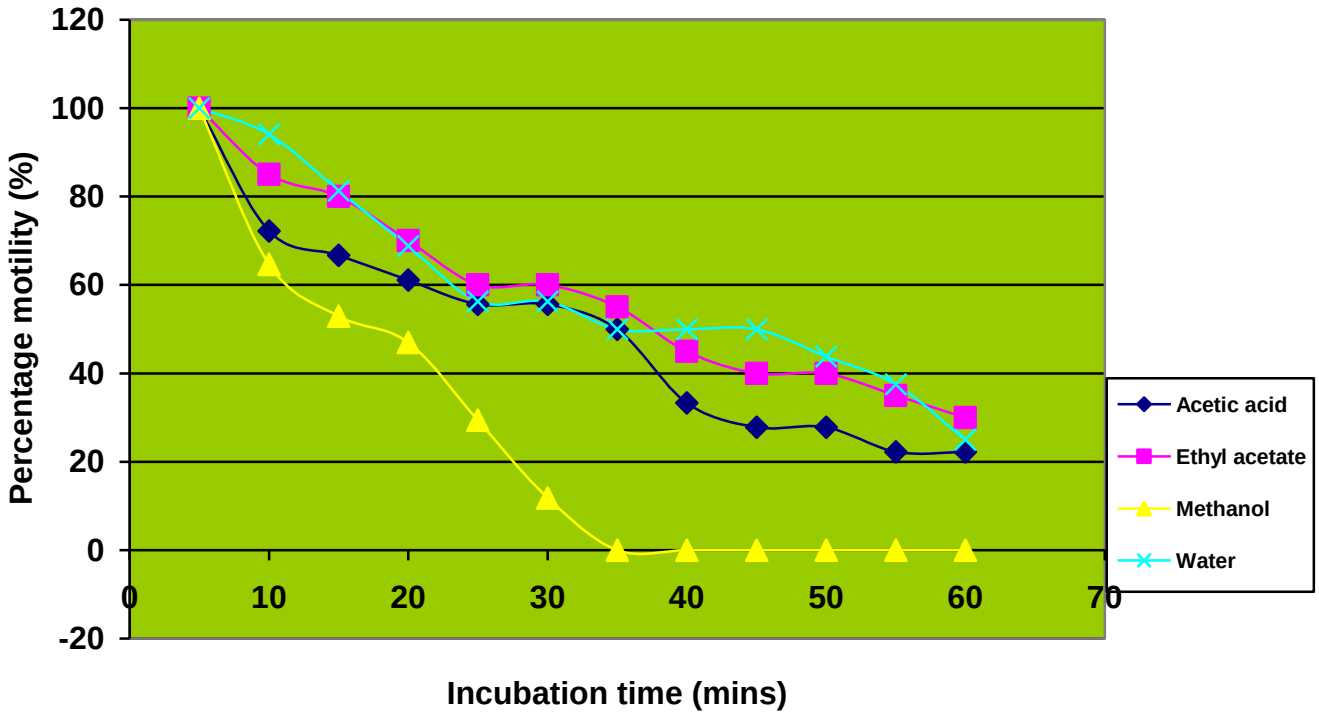


Figure 15: Profile of incubation time against motility of *T. brucei brucei* for fractionated H. barteri stem bark extract at concentration 50mg/ml to infected blood.

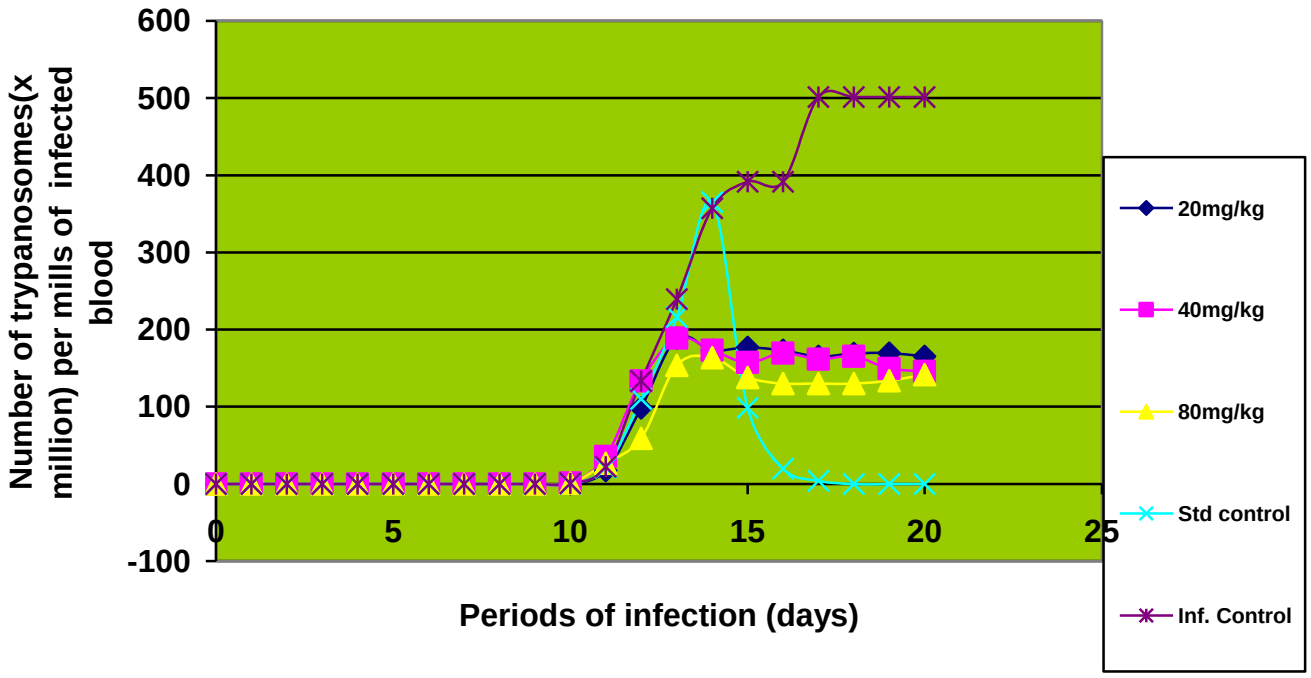


Figure 16:Profile effect of fractionated methanolic fraction of *H. barteri* stem bark extract on *T. brucei brucei* infected rats.

Table 6: Average numbers of parasites per microscopic field per rats infected with *T. brucei brucei* treated with the methanolic fraction of the extract for 7 days and the standard drug group

Incubation and treatments days	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th
20mg/kg body weight of extract	0.1	4.4±3.9b*	25±18b*	48±12b*	44±9.2b*	45±8.1b*	44±6.4b*	42±8.5b*	43±3b	42±2b
40mg/kg body weight of extract	0.3	9.0±1.7b*	33.3±9.6b*	48±3b*	44±5.1b*	40±5.5b*	43±3.6b*	41±12b*	38±3b	37±2.3b
80mg/kg body weight of extract	0.3	6.8±2.5b*	15±4.4b*	39±4.3b*	39±5.8b*	35±4.1b*	33±6.1b*	33±3b*	34±3b	36±2b
3.5g/kg body weight of Std drug	0.1	5.3±2.3a	28±12.5a	55±12a	93±11a*	25±5b	5±2b	1b	0	0
Infected untreated	0.2	5.6±2.4a	33.3±6.9a	61±8.8a	91±12a	100±0a	100±0a	128±0a	128±0a	128±0a

4.20 Discussion

In addition to those plants demonstrated to have activities against trypanosomes, *Haematostaphis barteri* stem bark also seem to contain some water extractable phytochemicals that possess *in vitro* activities against *T.b. brucei* and *T. congolense*. By considering parasites motility which constitutes a relatively reliable indicator of viability of most zoo flagellate parasites (Kaminsky *et.al*., 1996), cessation or drop in motility of trypanosomes therefore serves as a measure of antitrypanosomal potential of the crude water extract when compare to control in this study. The quantitive difference in antitrypanosomal activities by the plants could be attributed to the variation(s) in the concentrations. In natural products with trypanocidal activity can belong to a variety of phytochemicals classes as been identified (Hopp *et al* 1976; Oliver-Bewer 1986). Although the investigation did not involve structure elucidation, literature search reveals that extracts showing potent trypanocidal activity have also reported to contain flavonoids, alkaloid, terpenes and phenolic compounds. Based on the phytochemical screening performed on the crude water extract and fractionated extract in table 1 indicate some of their presence as earlier reported by Kubmarawa *et al* 2007.From the phytochemical studies the type of compounds which could be responsible for the antitrypanosomal activities are associated with the metabolites and the result obtained showed that the fractionated methanolic extract of *H. barteri* contain some metabolites that suppresses the activity of trypanosome rather than killing the parasite (*T. brucei brucei*) completely in vivo.

H. barteri just demonstrated strongly reduce trypanosomes motility in vitro within few minutes to very low number and completely ceased the motility as shown at 25 and 50mg/ml within 35 and 40 minutes i.e. before 1hour in this present study. The report of therapeutic non-activity of stem bark water crude extract of *H. barteri* against infected rats in vivo was observed as shown in figure 11 and 12 in this study. That the plants tested showed in vitro trypanocidal effect only is not unusual since earlier reports by Freiburghaus *et al* 1996, 1997, 1998; Asuzu and Chineme 1990, Nok *et al* 1993, Owalabi *et al* 1990, Wurochekke and Nok (2004) have clearly indicated that plants could possess potent trypanocidal in relatives.

The observed *in vitro* anti-*T. brucei brucei* and *T. congolense* activity on the stem bark aqueous crude and methanolic extract fraction of this plant support earlier reports that some plant extracts possess in vitro activities against trypanosomes (Asuzu and Chineme, 1990; Nok *et. al*., 1993; Ibrahim *et. al*., 2008; Umar *et. al.*, 2010).

In this parasitological finding, it shows that when the infected rats was treated with the aqueous crude extract of *H. barteri*, the course of infection was modified, that is, the extract did not significantly reduce the level of parasitaemia, but however, it prolonged the survival time of the infected rats with a day or two

and reduce the extend of anaemia as compared with that of infected control group (group 5) for both parasites in figure 9 and 10; table 5 and 6.

Treatment with the standard drug (diminazene diaceturate) resulted in the sharp reduction in the parasites count from day 5 post infection (post treatment) with the parasites completely cleared from the blood on day 8 post infection (3 days post treatment) and there was no relapse recorded in this group (group 4) during the study and there after 60 days. The uninfected control group (group 6) showed no signs of trypanosomiasis or other infections throughout the period of experimentation.

The failure of *H barteri* aqueous extract to achieve an effective therapy in vivo might be a reflection of no viable active components with anti-trypanosomal activity in the aqueous crude extract or there maybe inactivation of the active components in vivo. Moreover, a plant with high in vitro trypanocidal activity may have no in vivo activity and vice versa, which can also be as a result of peculiarities in the metabolic disposition of the plants chemical constituents (Atawodi *et al* 2003). So, *H. barteri* found to be effective in vitro but not active in vivo is not surprising. It is also possible for a strongly trypanocidal agent activity on *T. brucei brucei* can be weak to *T. congolense* or in the other way round though the trypanocidal agent may be the same. This suggests species-dependent factors may also play a role in susceptibility. For instance, unlike some other species mammalian *T.b. brucei* has no functional kinetoplast DNA (Fairlamb1982) while *T. congolense* possess no functional flagellum (Hoare, 1972) which can be a factor.

Also the result by Freiburghaus (1996) has also clearly indicated that different solvent extracts of the same plant may exhibit different trypanocidal activity just as extracts of different parts of same plants. Therefore, the statement that a plant is trypanocidal or not should be taken within the context of the solvent used and the parts investigated and the sub-species of trypanosomes tested upon.

However, previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azaanthraquinone), aromatic planar quaternary alkaloids, barbarine and harmarine (Hopp *et. al*., 1976; Nok, 2001) which some are found to be present in this plant's crude water extract and also in some of the fractionated extracts. The mechanism of action maybe relayed on the suggestion by Sepulveda-Boza and Cassels (1996), that natural products exhibits their trypanocidal activity through interference with redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This can be possible because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also reported that some agents act by binding with the kinetoplast DNA of the parasite (Atawodi *et. al*., 2003); such agent cannot have any activity against *T.b. brucei* because it has no functional kinetoplast DNA. Since the stem bark water extract of *H. barteri* has also been shown to be effective in vitro to both the sub-species parasite, it is thus possible that this extract act through other one or more of these mechanisms. The exert mechanism for the in vitro action of this extract is unknown since the active ingredient(s) were not isolated. However, it appears

reasonable to speculate that this extract may belong to the group that acts by static action affecting growth and multiplication of trypanosomes before cessation of motility of them gradually and out rightly in vitro.

Anaemia is a constant feature of trypanosomes infections whose severity is linked to the level of parasitaemia (Umar, *et. al*., 2000). The extract did not affect the severity of anaemia infected animals because the etiological factors involved in the heamolysis have been established before the extract treatment, so the falls in the packed cell volume (PCV) is as a result of the parasites infection. The Nozomil® treated infected rats showed significant improvement in PCV from infection levels perhaps because the drug was able to eliminate parasites from the blood to the levels undetectable by microscopic examination without relapsing back. However, the antitrypanosomal activity of this plant´s crude extract reported herein appears to be relatively less infective in *T.b. brucei* compare to *T. congolense* which is noticed when their respective in vitro profiles are compared though the difference is not all that significant. The present study demonstrated that the water crude extract of the *H. barteri* at the effective concentrations significantly inhibited both *T. brucei brucei* and *T. congolense* organisms in vitro as similar result were recorded by Mikail *et al* (2002) using garlic on *T. brucei brucei.* The observation after parasites inoculated intraperitoneally to rats produces parasitaemia within 3-4 days associated with gradual loss of conditions, as facial edema may result from release of vaso active substances is not an indication of the extract usefulness. The observed symptoms resemble an earlier finding recorded with *T.b. brucei* in mice and rats (Anika *et al* 1987).

Study using *H. barteri* stem bark water extract in vivo shown that definite statement can be made on the anti-*T. brucei brucei* and *T. congolense* activity of the plant which is found to be very weak in *in vivo* activity or slightly effective. Though speculation were made why extracts of some plants such as that of *H. barteri* failure of the stem bark crude water extract to show any trypanocidal action depicts that the anti-trypanosomes are lacking in the stem bark as opposed to the claims by the healers which according to the existing literature is used in ethanomedicine in the management of trypanosomiasis.

However, it was observed that the fractionated methanolic extract of *H. barteri* stem bark water extract on *T. brucei brucei* was found to exhibit trypanostatic activity in vivo, similar to the early reports of the trypanostatic effect of ethanolic extract of *F. abida* and also known to be effective in the management of anaemia induced by *T. b. brucei* in rats as reported same with the above plant (Tijani *et al* 2009).

Trypanostatic effect is said to suppress the activity of the parasite there by sustaining the life of the rats when compared to the infected control group. It is worthy to mention that some plants have already been investigated for their antitrypanosomal activity in other studies. For instance, water, methanol and dichloromethane extracts of the leaves of *T. avicennioides* tested, but only methanolic extracts was active on *T.b. brucei* (Bizimana *et al* 2006). Stem bark extracts of the same plant species has shown in vitro effect against *T.b. brucei* (Shuaibu *et al* 2008).

In the present study the *H. barteri* stem bark water crude extracts tested for its *in vivo* anti-trypanosomal activity gave a result that are efficaciously comparable to those of previous investigators on some plants who their extracts were active *in vitro* on *T.b. brucei* and *T. congolense* but not significantly active in vivo. Partially, fractionating of the extract using column with coarse silica gel with four different solvent reveals significant in vivo anti-trypanosomal function of the methanolic fraction against *T. brucei brucei* as showed in figure 16

This study has provided evidence that *H.barteri* fractionated methanolic extract exhibits trypanostatic effect which is often associated with reduction in anaemia and promotes weight gain just similar to that of T. congolense in crude water extract investigated earliar in experimental rats with Animal African trypanosomiasis (AAT) similar to other previous study (Ogbadoyi *et al* 1999)

Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis (Biizimana *et al* 2006) and also the primary cause of death (Mamo and Holmes 1975), the stem bark of *H. barteri* is used in treating anaemia analgeric Trypanostatic effect of the fractionated plants methanolic extract was explained with corresponding increase in PCV which was speculated to prolong the life span of the treated animals by either reducing the parasite load, improving the PCV or neutralizing the toxic metabolites produced by trypanosomes as stated by (Abubakar et al 2005).

Anaemia is a constant feature of trypanosomes infections whose severity is linked to the level of Parasitemia (Umar, *et. al*., 2000). Based on these results and the fact that plant part extracts are traditionally used in the treatment of African trypanosomiasis, we analyzed in greater detail the antitrypanosomal activities of the stem bark water extracts and fractionated methanolic extract of the *H. barteri* plants. Nevertheless, the activity value for the active extracts in vitro were still significant compared to the values obtained for commonly used trypanocidal drugs diminazene aceturate. However, since the crude extracts have very complex composition, purification might lead to partially pure compounds with high increased activity as showed in figure 16 in comparing with figure 10 and 11 for crude extract. Such difference between the results and those of previous may be due to the known variation in the purity of chemical composition though the plants variations may also be due to the geographical area and the time or season of collection (Shuaibu *et al* 2008) though these later factors has been taken care of.

In the in vivo studies with rats infected with *T. brucei brucei* and *T. congolense*, this seems to be the first report of in vivo and in vitro activity of this plant. The results of the present study confirmed that the use of medicinal plants in folk's medicine contribute significantly to primary health care, and that natural products are potential source of new drugs for the treatment of tropical diseases caused by trypanosomes or other parasites. The high activity values obtained in this plant in vitro render it candidate for the isolation of anti- trypanocidal compounds which could be developed into new lead structure for long development. Therefore, the trypanocidal effects of the extracts will require further experimentation even after the fractionation using column chromatographic techniques.

From the phytochemical studies the type of compounds which could be responsible for the anti-trypanosomal activities are associated with the metabolites and the result obtained showed that the fractionated extracts of *H. barteri* contain some metabolites that suppresses the activity of the trypanosomes a little rather than killing the parasite completely *in vivo*. So, there is a need to study the activity using some different solvents for extraction and also to try other sub-species of trypanosomes before any generalizations on therapeutic potentials. But further work on the toxicology, isolation and identification of bioactive components would certainly reveal whether this plant could be exploited for the development of new generation of trypanocides. Further more information reported in this study could be useful in assessing the overall anti-trypanosomal activity of this plant. But also note that, Inhibition of parasites motility here is not enough as indication of possible usefulness of the stem bark extracts as an anti-trypanosomal against *T.b. brucei* and *T. congolense*.

Since the *trypanosoma* parasite is exclusively extra-cellular. It lives and multiplies in blood and tissue fluids of their mammalian host. The major problem in the treatment of trypanosomiasis and chagas diseases is at their second stage (invasion of the central nervous system) probably because parasitaemia, albeit low grade, was persistent in the animals for trypanosomiasis, and cell distribution of intracellular *Trypanosoma* amastigote during both acute and chronic phases for chagas diseases though some plants has been demonstrated to be effective against trypanosomes which can be as a result of peculiarities in the metabolic product of the plants chemical constituents which are still very effective. The present study is a step towards validation of folkloric use of these medicinal plants as antitrypanosomal agents. In conclusion, we should not forget the earlier statement"plant also may have high in vitro anti-trypanosomal activity but may have no in vivo activity and vice versa, because of peculiarities in the metabolic disposition of the plant's chemical constituents".

We therefore concluded finally that the crude water extract of *H. barteri* stem bark possessed only *in vitro* anti-*T. brucei brucei* and *T.congolense* activity but for fractionated methanolic fraction it possessed *in vitro* and slightly *in vivo* against *T. brucei brucei.*

REFFERENCES

Abu, K.M., E.A., James, B.D., Njoku, G.C. and Sallau, A.B. (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna State of Nigeria. *J. Ethanopharmacol*. **7a**; 279-282.

Abubakar, A., Iliyasu, B., Yusufu A. B., Onyekwelu, N.A., Igweh, A. C., Shamaki, B.U., Afolayan, D.O. and Ogbadoyi, F.O;(2005) Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in wistar rats. *Biokemistri.* 17: 95-99.

Adeiza, A. A., Makeri, H. K. and Mohammed, M. (2000). Comparative *in vitro* trypanocidal activities of water and methanol extracts of three part of *Khaya Senegalensis* on *trypanosoma evansi. J. Medicinal plants Research* **4**(14):1365-1368.

Anika, S. M., Shelty, S. N., Asuzu, I. U. and Chime, A.B. C. (1987). Effects of some trypanocides and inflammatory agents in experimental trypanosoma brucei infection in mice. *Expl. Parasitol* **2**: 9-15.

Asase, A., Tesuo, K., Renee, J., Grayer, G., Monique S.J., Alfred, A., Oteng, Y., George, T. (2008). Chemical constituents and antimicrobial activity of medicinal plants from Ghana: *Cassia sieberiana, Haematostaphis barteri, Mitragyne* and *Pseudocedrala kotschyi. Ethanobotanical study of some Ghanaian anti-malaria plants 275-276*

Asaruddin, M. R., Kiuchi, F., Honda, G. (2001). Trypanocidal constituents of Desmos-dasymachalus. *Nat. Med*. **55**, 149-151.

Atawodi S E (2005). Comparative in vitro trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. *Afri. J. Biotechnol.,* 4(2): 177-182.

Atawodi SE, Bulus T., Ibrahim S., Ameh D.A., Nok A.J., Mamman M, Galadima M, (2003). In vitro trypanocidal effects of methanolic extracts of some Nigeria savannah plants. *Afri. J. Biotechnol***., 2**(9):317-321.

Bajaj, Y.P.S. 1996.Medicinal and aromatic plants, *Volume* **9**, 341-348 Spinger, Berlin.

Bakker, B.M., Mensonides, F.I.C., Teusink, B., Van Hoek, P., Michels, P.A.M., and Westerhoff, H.V. (2000), "Compartmentation protects trypanosomes from the dangerous design of glycolysis," *Proceeding of the National Academy of Sciences of the United States of America*," **5** (97).2087-2092.

Bakker, B.M., Michels, P.A.M., Opperdoes, F. and H. V. Westerhoff, H.V. (1997), "Glycolysis in bloodstream form *Trypanosoma brucei* can be understood in terms of the kinetics of the glycolytic enzymes," *J. Bio. Chem.*, **6** (272) 3207-3215

Bizimana N., Tietjen, U., Zessin, K. H., Diallo, D., Djibril, C., Milzig, M.F. and Clausen, P.H :(2006) Evaluation of medicinal from Mali for their in vitro and in vivo trypanocidal activity.*Ethnopharmacol*. 103(3) 350-356.

Blumenstiel, K., Schoneck, R., Yardley, V., Croft, S. L., Krauth-Siegel, R. L. (1999). Nitrofuran drugs as common subversive substrates of Trypanosoma lipoamide dehydrogenase and trypanothione reductase. *Biochem. Pharm*. **58**, 1791-1799.

Calis, I., Koyunoglu, S., Yesilada, A., Brun, R., Ruedi, P., Tasdemir, D. (2006). Antitrypanosomal cycloartane glycosides from Astragalus baibutensis. *Chem. Biodivers*. **3**, 923-929.

Camacho, M.d.R., Mata, R., Castaneda, P., Kirby, G.C., Warhurst, D.C., Croft, S.L., Phillipson, J.D., 2000b. Bioactive compounds from *Calaenodendron mexicanum*. *Planta. Medica.* **66**, 463-468.

Chambers, J. W., Fowler, M. I., M. Morris, M.T. and Morris, J.C., (2008). "The anti-trypanosomal agent Ionidamine inhibits Trypanosoma brucei hexokinase *1," Mol. Biochem. Parasitol.* **2** (158), 202-207.

Chambers, J.W., Kearns, M. T., Morris, M. T., and Morris, J. C. (2008). "Assembly of heterohexameric trypanosome hexokinase reveals that hexokinase 2 is a regulable enzyme," *J. Biol. Chem.* **22** (283) 14963-14970.

Coutou, V., Guegari, F., Plazolles, N., Baltz, T. (2010*).* Complete in vitro life cycle of *Trypanosoma congolense.* Development of Genetic tools. Plos Negf. Trop. Dis**.** **4**(3):618.

Cunha, W. R., Crevelin, E. J., Arantes, G. M., Crotti, A. E. M., Andrade e Silva, M. L., Furtado, N. A. J. C., Alberquerque, S., Ferreira, D. S. (2006). A study of the trypanocidal activity of triterpene acids isolated from Monica species. *Phytother. Res*. **20** 474-478.

De Souza, V. A., da Silva, R., Pereira, A. C., Royo, V. A., Saraiya, J., Montaheiro, M., de Souza, G.H.B., da Silva F.A. A., Grando, M. D., Donate, P.M., Bastos, J. K., Albuquerque, S., e Silva, M. L. A.(2005). Trypanocidal activity of (-)-cubebin derivatives against free amastigotes forms of *trypanosoma cruzi. Bioorg. Med. Chem. Lett*. **15** 303-307

Divo, A.A., Sartilli,A.C. Palton, C.L. and Bia, F.J. (1988) "Activity of fluoroquinone antibiotics against plasmodium falciparium invitro,"*Antimicrob. Agents Chemotherapy,* **8** (32) 1182-1186.

Dodson H. C., Lyda, T. A., Chambers, Y. W., Morris, M. T., Christensen, K. A. and Morris J. C. (2011). Quetcetin, a fluorescent bioflovonoid, inhibits *Trypanosoma brucei* hexokinase 1.*Expl. Parasitol*. 127(2) 423-428.

Dreyfuss, G., Allais, D. P., Gruinaudeau, H., Bruneton, J. (1987). Trypanocidal activity of isoquinoline alkaloids in mice. *Ann. Pharm. Francaises* **45;** 243-248.

Duquenois, P., Anon, R., 1968. Chemical study of *Cassia sieberiana* leaves. *Planta Medica*. **16,** 184-190.

Espindola, L. S., Rossy Vasconcelos, J. Jr., de Mesquita, M. L., P., de Paula, J. E., Mambu, L., Santan, J. M. (2004). Trypanocidal activity of a new diterpene from Casearia sylvestris var. lingua. *Planta. Med*. **70**, 1093-1095.

Famsworth, N. R. Biological and phytochemical screening of plants. *J. Pharm. Sci*. **55**:225-276.

Freburghaus F., Kaminsky R., Nkuna MHN. Brun R.(1996). Evaluation of African Medicinal for their in vitro trypanocidal activity. *J. Ethnopharmarcol. 55:1-11*.

Founet, A., Inchauti A., Yaluff, G, Royas de Arias, A., Guinaudeau, H., Bruneton, J., Breidenbach, M. A., Karplus, P. A., Faerman, C. (1998). Trypanocidal bisbenzylisoquinoline alkaloids are inhibitors of trypanothione reductase. *J. Enz. Inhibit*. **13**, 1-9.

Ginsburg H. (1994). Transport pathways in the parasite infected erythrocyte. *Biochem. Pharmacol.* **48**, 1847-1856.

Gohari, A. R., Saeidnia, S., Mastuo, K., Uchiyama, N., Yaguura, T., Ito, M., Kiuchi, F., Honda, G. 92003). Flavonoid constituents of Dracocephalum kotschyi growing in Iran and their trypanocidal activity. *Nat. Med*. **57** 250-253.

Goulart, M. O. F., Zani, C. L., Tonholo, J., Freitas, L. R., De Abreu F.C., Oliveria, A. B., Rasian, D. S., Starling, S., Chari, E. (1997). *T*rypanocidal activity and redox potential of heterocyclic and 2-hydroxynaphthoquinones. *Bioorg.Med. Chem. Lett*. **7**, 2043-2048.

Grael, C. F. F., Albuquerque, S., Lopes, J. L. C., (2005). Chemical constituents of Lychnophora pohlii and trypanocidal activity of crude plant extracts and of isolated compounds. *Fitoterapia* 76, 73-82.

Harbone J.B., (1973). Phytochemical Methods. A guide to modern techniques of plant Analysis. London, Chapman and Hall LTD. 7-8.

Herbert, W.J., and Lumsden W.H. (1976).*Trypanosoma brucei*. A rapid "matching" method for estimating the host's parasitaemia. *Exptl. Parasitol*; 40:427-431.

Hoet, S., Opperdoes F., Brun, R., Adjakidje V., Quetin-Leclecq, J. (2004) In vitro antitrypanosomal activity of ethno pharmacologically selected Beninese plants *J.Ethnopharm*. 91: 37-42.

Hopp KH, Cunnigham IV, Bromel MC, Schermester LJ, Wahba KSK (1976). *In vitro* antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*, *Llyydia*, **39** (5): 375-377.

Igweh AG, Onabanjo AO (1989), chemotherapeutic effects *Annona senegalensis* in *T. brucei brucei* infection in mice**.** *J. Ethnopharmacol***., 30**: 307-313.

Kaminsky F, Nkuna MHN, Brun R (1996). Evaluation of African medicinal plants for their in vitro trypanocidal activity. *J. Ethnopharmacol*., 55: 1- 11.

Kessler, P.S., Parson, M. (2005), "Probing the role compartmentation of glycolysis in procyclic form *Trypanosoma brucei*; RNA interference studies of hexokinase and phosphofructosekinase," *Journal of Biological Chemistry*, **10**(280) 9030-9036.

Kirchoff L.V (1993). American Trypanosomiasis (chagas disease)-a tropical disease now in the United States. *N.Engl. J.Med*.**329**, 639-644.

Kirk K., Staines H.M., Martins R.E. and Saliba K.J. (1999).Transport properties of the host cell membranes. Foundation Symposium No **226**, 55-73.

Krauth-Siegel, R.L., Muller, J.G., Loltspeich, F. and Schirmer, R. H. (1996). Glutathione reductase and glutamate dehydrogenase of parasite. *Eur. J. Biochem*. **235**, 345-350.

Kubmarawa D, Ajoku GA, Enwerem N. M and Okorie DA (2007) Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *Afri. J. Biotechnol***. 6**(14) 1690-1696.

Lang-Unnash N.and Murphy A.D. (1998). Metabolic changes of the parasite during the transition from the human to insect host. *Annu. Rev. Microbiol***. 52**, 561-590.

Launer SA., Rathod PK., Ghori N. and Haldar K. (1997). A membrane network for nutrient import in red cells infected with parasite**.** *Science* **276**, 1122-1125.

Leite, J. P. V., Oliveira, A. B., Lombardi, J. A., Filho, J. D. S., Chari, E. (2006). Trypanocidal activity of triterpenes from Arrabidaea triplinervia and derivatives. *Biol. Pharm. Bull.* **29**, 2307-2309.

Luize, P.S., Ueda-Nakamura, T., Dias, Filho, B.P., Cortez, D. A. G., Nakamura, C. V. (2006). Activity of neolignans isolated from Piperregellii against *trypanosomal cruzi. Biol. Pharm. Bull*. **29**, 2126-2130.

Matesanz F., Duran-chica I., Alcina A. (1999). The cloning and expression of pfacs1 parasite fatty acyl coenzyme a synthetase-1 targeted to the host erythrocyte cytoplasm. *J. Mol. Biol*. **291**, 59-70.

Mamo E and Holmes P. (1975). The erthrokinetics of zebu cattle chronically infected with *T. Congolense*. Research in Veterinary science.18; 105-106

McCutchan, T.F., Li J. McConkey G.A., Rogers M.J. and Waters A.P. (1995). The cytoplasmic ribosomal RNAs of parasites. *Parasitol. Today* **11**, 134-138.

Medola, J.F., Cintra, V.P., Pesqueira e Silva,E. P., de Andrade Royo, V. da Silva, R., Saraiva, J., Albuquerque, S., Bastos, J. K., Andrede e Silva, M. I., Tavares, d. c. (2007). *(-)-Hinokinin* causes antigenotoxicity but not genotoxicity in peripheral blood of wistar rats. *Food Chem. Toxicol*. **45**,638-642.

Merschjohann, K., Sporer, F., Steverding,D., Wink,M., (2001). Invitro effect of alkaloids on bloodstream forms of trypanosoma T.brucei and T.congolense. *Planta Med***. 67,**623-627.

Morello, A., Liphenca, I., Cassel, B. K., Speisky, H., Aldunate, J., Repetto, Y. (1994). Trypanocidal effect of boldine and related alkaloids upon several strains T.cruzi. *Pharm. Toxicol. Endocrim.* **107c;** 367-371.

Murray M.P.K.Murray and Mc Inlyre (1977) .An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. Roy. Soc. Trop. Med. Hyg*. 71; 325-326.

Mutamura T. and Palacpac NMQ. (2003). Lipid metabolism in parasite infected erythrocyte: possible new target for chemotherapy. Microbes and infections **5**, 545-552.

Neuwinger, H. D.,2000. African Traditional Medicine: A Dictionary of Plants Use and Applications. *Medpharm*, Stuttgart.

Ngamga D., Yankep, E., Tane, P., Bezabih, M., Ngadjui, B. T., Fomum, Z. T., Abegaz, B. M., (2005). Antiparasitic prenylated isoflavonoids from seeds of Millettia graffoniana. *Bull. Chem. Ethiopia* **19**, 75-80. Soc

Nok, A. J.(2002) *Azanthraquianol inhibits respiration and invitro growth of long and slender blood stream forms of Trypanosoma Congolense cell. Biochem. Function,* **20**:205-212.

Nok A. J, Esievo KAN, Adaudi A, Isaac AI, Emmanuel GC, Solomon OM (1992). Trynocidal activity of an organotin compound (Tri-n-Butyltin Oxide) toward Trypanosoma brucei. *J. Clin. Biochem. Nutr*., **13**: 81-85.

Nok, A. J., Esiero, K.A.N., Hongdet, I., Arowosafe, S., Onyenkwe, P.C., Gimba, C.E., Kagbu, J.A. (1993) *Trypanocidal potentials of Azodichracta indica invivo activity of leaf extract against. T. brucei brucei. Clin. Biochem. Nutr*. **15**:113-118.

Owolabi OA, Makanga B, Thomas EW, Molyneux DH, Oliver RW, (1990). Trypanocidal potentials of African woody plants. *In vitro* trials of *Khaya gradifolia* seed extracts. *J. Ethnopharmacol*., 30: 227-231.

Pareira, S., Dias, D. A., Franca, S. C., Sampaio, S. V., Toldo, M. P. A., Albuquerques, S. (1999) Trypanocidal activity from Tabernaemontana calharinensis. *A. DC. Acta Horticultural* **501,** 165-170

Raz, B., Iten, M., Grether-Buhler,Y., Kaminsky, R., Brun, R., 1997.The Almar Blue® assay to determine drug sensitivity of African trypanosomes (*T.b. rhodesiense* and *T.b. gambiense)* in vitro. *Acta. Tropica **68**, 139-147.*

Rubio, J., Calderon, J. S., Flores, a., Castro, C., Cespedes, C. L. (2005). Trypanocidal activity of oleoresin and terpenoids extracted from Pinus oocarpa. *J. Biosci*. **60**, 711-716.

Salmon-Chemin, L., Lemaire, A., De Freitas, S., Deprez, B., Sergheraert, C., Davioud-Charvet, E. (2000*).* Parallel synthesis of a library of , 4-naphthoquinone and automated screening of potential inhibitors of trypanothione reductase from trypanosoma cruzi. *Bioorg. Med. Chem. Lett*. **10**, 631-635.

Saraiva J., Vega C., Rolon, M., de Silva, R. e Silva Marcio, L.a., Donate, P. M., Bastos, J. K., Gomez-Barrio, A., de Albuquerque, S.(2007*).* In vitro and invivo activity of lignan lactones derivatives against Trypasoma cruzi. *Parasitol. Res.* 100, 791-795.

Scio, E., Ribeiro, A., Alves, T. M. A., Romanha, A. J., Dias de Souza Filho, j., Cordell, G. A., Zani, C. L. (2003) Diterpenes from Alomia myriadenia with cytotoxic and trypanocidal activity. *Phytochemistry* **64**, 1125-1131.

Schmeda-Hirschmann, G., Razmilic, I., Sauvian, M., Morretti, C., Munoz, V., Ruiz, E., Balanza, E., Fournet, A. (1996). Antiprotozoan activity of jatrogrossidione from jatropha grossidentata and jatrophone from jatropha isabellii. *Phytother. Res.* **10,** 375-378.

Sepulveda-Boza S, Cassels BK (1996). Plants metabolites active against *Trypanosoma cruzi*. *Planta Medica,* 62: 98-105.

Shuaibu M., N., Wuyep P.T.A. Yana T., Hirayama K., Ichinose A., Tanaka T. and Konno I (2008): Trypanocidal activity of extracts and compound from the stem bark of *Anogeissus Leiocarpus* and *Terminalia aricennoioides*. *Parasitol. Res*. 102(4) 697-703.

Sofowora, A., (2008) Medicinal plants and Traditional Medicines in Africa. Spectrum Books LTD, Ibadan Nigeria 289.

Takeara, R., Albuquerque, S., Lopes, N. P., Lopes, J. L. C. (2003). Trypanocidal activity of Lychnophora staaviodes (Vernonieae Asteraceae) *Phytomedicine* **10**, 490-493.

Tapia, R. A., Salas, C., Morello, A., Maya, J. D., Toro-Labbe, A. (2004). Synthesis of dihydronaphthofurandiones and dihydrofuroquinolines with trypanocidal activity and analysis of their stereoelectronic properties. *Bioorg. Med. Chem.* **12,** 2451-2458.

Tarus PK, Machocho AK, Lanmgat-Thoruwa CC, Chabra SC (2002) Flavonoids from *Tephrosia aequilata*.*Phytochemistry*, **60**:375-379.

Tchinda, A. T., Tsopmo, A., Tane, P., Ayafor, J. F., Connolly, J. D., Sterner, O. (2002). Vernoguinosterol and vernoguinoside, trypanocidal stigmastane derivatives from vernonia guineensis. *Phytochemistry* **59,** 371-374.

Tijani A Y, Uguru M.D. Salawu O A., Abubakar A.Onyekwe N O.and Akingbasote J.A.(2009). Effect of *Faid Albida* on some biochemical parameter of infected with *trypanosoma brucei brucei. Afri. J. pharm. Pharmacol.* 3(1)

Trease G. E. and Evans, W. C. (1985). Introduction and General Methods. In *Pharmacognosy, 12th Edition*, published by Alden press, Oxford London 469-474.

Tyler VE (1999) Phytomedicines: back to the future. *J. Nat. Prod.*, **62**: 1589-1592.

Umar IA, Toh ZA, Igbalajobi FI, Igbokwe IO, Gidado A (2000). The effects of orally administered vitamin C and E on severity of anaemia in *Trypanosoma brucei*-infected rats. *Trop.Veterinarian***, 18**:71-77.

Umar, I.A., Ibrahim, M.A., Fari, N.A., Isah, S., Balogun, D.A. (2010) *In vitro* and *In vivo antitrypanosoma evansi* activities of extracts from different parts of *Khaya sengalensis***.** *J. Cell Ani. Biol.* **4**(6): 91-95.

Verlinde, C. L. M. J., Hannaert, V., Blonski, C. (2001). Glycolysis as a target for the designed of new anti-trypanosome drugs. *Drug Resistance Updates* **4** (1) 50-65.

Walker, P. J. (1972). Capillary concentration technique applicable to infections of T. congolense in cattle. *Trans. R. Soc. Trop*. 66(2) 348.

Wilson R.J.M. (2002) Progress with parasite plastids *J. Mol. Biol*. **319**, 257-274.

Wilson, M. Sanjouand, M., Perie, A. H., Hannert, J. V., and Opperadoes, F. (2002), "Sequencing, modeling, and selective inhibition of *Trypanosoma brucei* hexokinase," *Chem. and Bio*. **7** (9) 839-847.

Woo, P. T. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta. Trop*. 27(4) 384-386.

World Health Organization (2000) WHO report on global surveillance of epidemic prone infections disease. WHO/CDS/CRS/ISR/2000-1. http://www.who.int/ctd/chagas/burdens.htm.

Wurochkke, A.U. and Nok, A.J. (2004) In vitro antitrypanosomal activities of some medicinal plants used in the treatment of Trypanosomiasis in Northern Nigeria. *Afr. J. Biotechnol.* 3(a) 481-483.

Youan, B.B.C., Coulibaly, S., Miezan, T. B., Doua, F., Bamba, M., 1997. In vivo evaluation of 16 plants extracts on mice inoculated with *Trypanosoma brucei gambiense*. *Bulletin of the world Health Organization* **75,** 343-348.

APPENDIX I

Abbreviations

ATP	Adenine triphosphate
THK	Trypanosomes hexokinase
NAD^+	Nicotinamide adenine dinucleotide (reduced form)
NADH	Nicotinamide adenine dinucleotide (oxidized form)
PEP	Phosphoenol pyruvate
PFK	Phosphofructosekinase
LDH	Lactate dehydrogenase
DNA	Deoxyribonucleic acid
PCV	Packed cell volume
GPDH	glycerol-3-phosphate dehydrogenase
BSF	Blood stream form
dTMP	deoxy-thymidylmonophosphate
dUMP	deoxy-Uridylmonophosphate
DHFR	Dihydrofolate reductase
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
UTP	Uridine triphosphate
CTP	Cytidine triphosphate
TTP	Thymedine triphosphate
VSG	Variable surface glycoprotein
BPG	Biphosphoglycerate
PGA	Phosphoglyceraldehyde
2PG	2-Phosphoglycerate
3PG	3-Phosphoglycerate
PARD	Procyclic acidic repetitive proteins
HK	Hexokinase
TLC	Thin layer chromatography
gPGK	glycosomal Phosphoglycerate kinase
IC_{50}	Inhibitory concentrations for 50% of the total number
ED_{50}	Effective dose for 50% of the total number
KD	Distribution coefficient
RNA	Ribonucleic acid
AP	Appendix
GTP	Guanine triphosphate
DHAP	Dihydroxyacetone phosphate
CCA	Cis-aconitate or citrate
PAD	Protein associated differentiation
TCA	Tricarboxylic acid
ESR	Erythrocyte sedimentation rate
CNS	Central nervous system
PF	Procyclic form
Ki	Initial enzymatic constant
Km	Michaelis-Menten Constant

DSMO	Dimethyl sulphoxide
EDTA	Ethylene diamine tetraacetate
pH	Hydrogen ion concentration
BSF	Bloodstream form
PBS	Phosphate buffer saline
HCT	Haematocrit centrifugation techniques
NITR	Nigerian Institute for Trypanosomiasis Research
ECWA	Evangelical Church of West Africa
PFK	Phosphofructosekinase
Ctrl	Control
STD	Standard
Inf.	Infected
BW	Body weight

LIST OF EQUIPMENTS
- Centrifuge: - Centrifuge 72 Tuttlingen West Germany Helich Universal
- Hemocytometer: - Mettler instrument, Switzerland.
- Micro haematocrit centrifuge Tuttlingen West Germany
-Micro haematocrit scale:-Halstead corning Ltd, Essex England
-Weighing balance:- Mettler P.165 Grallen Hamp Mettler instruments Switzerland
-Water bath: - Geseuchaft fur labor technik, M.B.H. and Company, West Germany
- Light Microscope binocular (Olympus England)
-Heparinized capillary tube Superior Marienfield, Germany.
-Weighing balance Ohaus Corporation Prime Brook, New Jersey USA
Water bath--Gallen Kamp Thermo series 95 Hungary product

LIST OF CHEMICALS
Acetic acid- glacial Analar R' BDH Chemical Ltd Poole England
Methanol Sigma –Alorich Country of origin Germany
Ethyl acetate Analar R' BDH Chemical Ltd Poole England
Silica Gel –Self-indicating M&B Laboratory chemical May & Baker Ltd Dagen Ham England
Goya Extra virgin olive oil
Fehling solution A & B, Analar R' BDH Chemical Ltd Poole England
Draggendrofs´ reagent Mayer's reagent
Ethanol Analar R' BDH Chemical Ltd Poole England
Tetraoxosulphate VI—Analar R' BDH Chemical Ltd Poole England
Hydrochloric acids---Analar R' BDH Chemical Ltd Poole England
Chloroform—M&B Laboratory chemicals May & Baker Ltd Dagen Ham England
Dextrose saline—5% Glucose intravenous infusion
Ferric chloride—M&B Laboratory chemicals May & Baker Ltd Dagen Ham England

PHYTOCHEMICALS TEST.

Test for reducing sugar (Fehlings test)

To 0.5g of the extract in 5ml of water and was added to boiling Fehlings reagent (solution) **A** and **B** in a test tube, observe the solution for the colour change from light blue to yellowish-brownish

Test for anthraquinone

To 0.5g of the extract added 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test

tube and 1ml of dilute ammonia was added .The resulting solution was observed for colour change.

Test for terpenoids (Salkowski test)

To 0.5g of the extract with 2ml of chloroform and,3ml of concentrated sulphuric acid carefully added the side to form a layer A reddish brown colorations of the interface indicates the presence of terpenoids.

Test for steroids

Two ml of acetic anhydride was added to 0.5g of the powered extract with 2ml H_2SO_4. The colour changed from violet to blue or green indicating the presence of steroids.

Test for flavonoids

Any among the three methods can be used:
First, dilutes ammonia (5ml) was added to a portion of an aqueous filtrate of the extract then added concentrated sulphuric acid. A yellow colouration that disappears on standing indicates the presence of flavonoids.
Secondly, a few drops of 1% aluminium solution were added to a portion of the filtrate yellow coloration indicates the presence of flavonoids.
And lastly, a portion of the extract was heated with 10ml of ethyl acetate over a steam for three minutes, then mixture was filtered and 4ml of filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins

To 2g of the powdered extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then the formation of emulsion indicates the presence of saponin

Test for cardiac glycoside (Keller kwainite).

To 0.5g of the extract with 5ml of water ,2ml of glacial acetic acid with a drop of ferric chloride solution. This was under layer with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxy sugar characteristics of cardinolides. A violet ring may appear below the brown ring, while in acetic acid; a layer is greenish ring instead of brown above and gradually spread.

Test for tannins

To 0.5g of the powdered extract sample was boiled in 20ml of water in a test tube and boiled and then was filtered. To the filtrate few drops of 0.1% ferric chloride were added, brownish-green observation or blue-black colouration indicates the presence of tannins.

Test for alkaloids

To 0.5g of the extract was diluted to 10ml with acid alcohol and boiled, filtered. To 5ml of the filtrate was added 2ml of dilute ammonia and 5ml of chloroform was added and shake gently to extract the alkaloid base and the chloroform layer was divided to two portions. Mayer's reagent was added to one portion, Draggendroffs reagent to the other portion. The formation of a cream (with Mayer's reagent) while reddish brown precipitated (with Draggendroffs reagent) indicates the presence of alkaloid.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant extract was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Parasitemia level estimation procedures

Wet preparation:

1. **Whole blood-** A drop of fresh blood is put on a slide and covered with a coverslip. The preparation is examined microscopically with subdued light or by phase contrast. Trypanosome movement is examined for at least 20 microscopic fields.
2. **Concentration techniques-** These are used when the parasites are scanty and the presence of red cells obscure their presence. Method applied includes:

a) Anion exchange/centrifugations technique (Lumsden et.al, 1977, 1979)

This is used to separate the trypanosomes from the cellular elements in the blood. Heparanized blood is passed through cellulose column, which is equilibrated against a buffer of the specific animal blood to be separated.

Principle:

Red blood cells are more negatively charged than the trypanosomes. Therefore, they are held in the cellulose of the column as trypanosomes are eluted. The elute is then centrifuged to the concentrate the trypanosomes for microscopical examination.

Procedure

(i) Prepared column using barrels of 2-ml disposable syringe using DE52 cellulose Lunham and Godfrey (1970)

(ii) Prim the column by filling to the brim with PSG and allowing draining twice.

NB: Ionic strength of PSG should be 0.145 appropriate to bovine blood. This is prepared by mixing 4 volumes of PBS with 6 volumes of distilled water and adding glucose 1% w/v

iii) Discharge 100µl from capillary tube and drain into the DE52 cellulose bed.

(iv) Add a few drops of PSG on top of the column

(v) Collect elute in a prepared centrifuge tube, centrifuge and examine under the microscope.

b) Haematocrit centrifugation technique (HCT) (Woo, 1970).

Prepare a glass chamber as follows:

(i) Used two pieces of rectangular glass 25×10×1.2 mm-thick cut from a microscope slide

(ii) Fix them at 1.5 mm apart on a microscope slide

(iii) Fix a coverslip onto these two pieces of slide to form a chamber

(iv) Fill this chamber with water

(v) Centrifuge infected blood in a capillary tube for 5 minutes at 10,000 rpm

(vi) Insert the capillary with the sealed end into this flooded chamber making sure that the buffycoat-plasma junction is at the centre

(vii) Rotate the capillary tube gradually as you examine under the microscope at ×10 objective.

c) Capillary concentration technique (CCT) (Walker, 1972)

T. Congolense has a tendency of being retained amongst red blood cells. This technique was designed to create a large differential density between red blood cell (RBC) and the parasite. This is done by mixing blood with a strongly hypertonic non-toxic medium prepared as follows:

1) 9% glycerol

2) 9% magnesium sulphate

3) 0.1% Tris buffer, PH 8.2
4) Phenol red 1:100,000

Procedure

- **(i)** **M**ix 50µl of the infected blood with 50µl diluents in a micro titre titration plate
- **(ii)** **A**llow to stand for 15 minutes
- **(iii)** Fill capillary tubes ¾ full, seal on end with plastacine
- **(iv)** Spin using haematocrit centrifuge for 2 minutes
- **(v)** Place the spun capillary tubes on a slide, cover buffycoat with a few drops of diluents under coverslip
- **(vi)** Examine under the microscope as for the HCT

d) Dark ground/phase contrast buffycoat technique (DG) (Murray et, al., 1977).

i) Fill capillary tube with about 70µl infected blood

ii) Centrifuge for 5 minutes at 10,000rpm using micro haematocrit centrifuge

iii) Cut the capillary tube with diamond pencil 1mm below the buffy coat to incorporate the uppermost layer of RBC and 1cm above to include plasma

iv) Spread the contents gently on a clean slide, mix and cover with 22×22-mm cover slip

v) Examine unto 200 microscope fields for parasites under the microscope.

e) Herbert and Lumsden (1976)

Briefly the method involved microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS) P.H 7.2. Logarithm value of these counts obtained was matched with the table of Herbert and Lumsden and converted to antilog to provide absolute number of trypanosomes (parasite) per ml of blood.

Principle

The technique proposed depends upon matching the microscopic appearance of a wet film of eights microscopic fields. These pictures represent, respectively, concentrations of trypanosomes decreasing in number by having steps from 256 to 2 organisms per field.

The principle of having steps is extended, below the level of 2 organisms per field by examining more than 1 field. Clearly, at the level of 1 trypanosome per field, 20 fields should exhibit about 20 organisms, at the level of 0.5 per field, 10; and so on until, at a concentration of 0.0625 organisms per field. At the upper level of this range it is not necessary to examine the full 20 fields as a table can readily be constructed to show equivalences for levels of parasitaemia at halving intervals from 1 to 0.0625 organisms per field, when 5, 10; or 20 fields are examined.

Herbert and Lumsden *Trypanosoma brucei* a rapid "matching" method for estimating the Host's Parasitemia table

Table for wet films of blood from mice infected with trypanosoma brucei viewed at ×400 magnifications

Organisms' Per field		(Antilog) Equivalent log Number of organisms per milliliter of blood	No of organisms per ml of blood
>256		>9.0	>1000, 000,000
M	256	9.0	1000,000,000
A	128	8.7	501,200,000
T	64	8.4	251, 200,000
C	32	8.1	125,900,000
H	16	7.8	63,100,000
I	8	7.5	31,620,000
N	4	7.2	15,850,000
G	2	6.9	7,943,000

Organisms in:--

20 Fields	10 Fields	5 Fields				
			C			
		4-5	**O**	1	6.6	3,981,000
		2-3	**U**	0.5	6.3	1,975,000
	2—3		**N**	0.25	6.0	1,000,000
2-3			**T**	0.125	5.7	501,200
1			**I**	0.0625	5.4	251,200
0			**N**	<0.0625	<5.4	<251,200
			G			

For example to inoculate approximately 1million parasites to a rat using inoculums of 1 per field.

Number of organism/ml of blood =3,981,000----------1

1,000,000----------x

X=1,000,000/3,981,000

=0.25ml of the blood will be given.

STUDENTS T-TEST

Test of difference or comparison between two means.

The student's t-test is a parametric test of difference between two samples. It is applicable only to data measured at interval scale. Test at 5% if there are differences in the two means or samples.

Note of the following:-

Ho: There is no significant difference in between the means or the two samples.

H1: There is significant difference in between the means or the two samples.

Significant level taken is 5% or 0.05.

Formula for t-test is:-

$$\mathbf{t}\ \text{cal} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S^2_p\,(1/n_1 + 1/n_2)}}$$

n = sample size

$$S^2_p = \frac{(n_1 - 1)\,S^2 1 + (n_2 - 1)\,S^2 2}{n_1 + n_2 - 2}$$

S = Standard deviation

$\bar{\times}$ = Mean average

Rule of Thumb:

Accept Ho if calculated value of t calculated is less than the table value.

Reject H_1 if calculated value of t is greater than the table value.

For example, if calculated value is less than the table value, we accept Ho and reject H_1. We therefore conclude that there is no significant difference between the means or samples.

APPENDIX II

Table AP 1: In vitro activities at different concentrations of the water crude extract in 5 minutes interval for 1 hour considering % motility of parasites as the index.

Concentration/ % Motility	Time(minutes) time	5	10	15	20	25	30	35	40	45	50	55	60
6.25mg/ml:T.b	%	100	100	100	100	93	93	93	89	89	86	84	79
6.25mg/ml:T.c		100	100	84	80	75	75	70	65	62	62	54	54
12.5mg/ml:T.b		100	86	74	71	60	57	57	51	51	49	49	46

12.5mg/ml:T.c		100	92	60	56	36	28	12	4	0	0	0	0
25mg/ml:T.b		100	83	69	38	26	12.	10	0	0	0	0	0
25mg/ml:T.c		100	36	27	17	12	6	0	0	0	0	0	0
50mg/ml:T.b		100	35	30	27	17	6	0	0	0	0	0	0
50mg/ml:Tc		100	28	20	18	12	3	0	0	0	0	0	0

TABLE AP 2: The changing weights in grams (g) of the treated rats infected with *T. Congolense* at three days intervals from zero day of infection. Values are expressed as means ± SD, (n=5).

GROUPS	ZERO DAY	3^{RD} DAY	6^{TH} DAY	9^{TH} DAY	12^{TH} DAY
Inf. + 20mg/ml kg	53.2±8.9	63.4±7.4	70.9±9.1	76.1±10.1	71.6±8.6
Inf. + 40mg/ml kg	48.8±4.5	53.5±5.7	58.8±7.1	68.7±7.6	66.1±8.1
Inf. + 80mg/ml kg	49.3±7.8	58.3±11.1	66.0±11.2	68.7±11.8	67.2±13.9
Inf. + STD DRUG	63.4±8.8	71.3±12.8	79.1±10.9	85.4±9.0	86.4±8.2
Inf. Control	60.7±10.9	71.2±11.3	77.4±14.9	84.4±15.5	82.9±17.2
Normal control	45.5±6.8	52.8±5.3	62.7±9.7	70.5±10.0	73.9±10.5

Table AP 3: The changing of weights in grams (g) of the treated rats infected with the *T. brucei brucei* at three days intervals from zero day of infection. Values are expressed as mean ± SD, (n=5)

GROUPS	ZERO DAY	3^{RD} DAY	6^{TH} DAY	9^{TH} DAY	12^{TH} DAY
Inf. + 20mg/ml kg	61.5±5.12	61.9±8.05	64.9±8.74	62.1±0	
Inf. + 40mg/ml kg	67.2±8.23	67.7±7.96	70.5±9.14	67.3±0	
Inf. + 80mg/ml kg	65.1±5.97	67.7±6.34	64.4±5.56	63.1±0	
Inf. +STD DRUG	73.2±21.54	78.3±18.87	82.2±19.46	84.9±14.79	85.7±13.61
Inf. Control	76.7±5.87	75.4±6.00	62.6±14.10	60±0	
Normal control	68.2±8.07	75.4±10.3	83.4±10.96	89.4±11.96	92.3±12.90

Table AP 4: The packed cell volume (PCV %) of the rats infected with *T. brucei brucei* at three days intervals from zero day of infection.

GROUPS	ZERO DAY PCV%	3^{RD} DAY PCV%	6^{TH} DAY PCV%	9^{TH} DAY PCV%	12^{TH} DAY PCV%
Inf. + 20mg/ml kg	$42.6±3.9^{a}$	$36.0±5.9^{a}$	$35.7±7.2^{a}$	$33±2.4^{b}$	All died
Inf. + 40mg/ml kg	$43.2±4.3^{a}$	$40.8±3.6^{a}$	$39.6±3.0^{a}$	$37.0±3.8^{a}$	All died
Inf. +	$45.2±4.6^{a}$	$38.0±1.9^{a}$	$36.7±2.9^{b}$	$38.0±2.1^{a}$	All died

80mg/ml kg					
Inf. + STD DRUG	41.8±3.0[a]	39.2±0.8[a]	39.0±1.9[a]	43.2±1.8[a]	41.8±1.9[a]
Inf. Control	41.6±3.0[a]	37.8±2.4[a]	34.5±2.1[b]	32±1.5[b]	All died
Normal control	40.8±7.4[a]	42.2±4.0[a]	42.2±1.9[a]	42.8±6.1[a]	43.4±2.1[a]

Values are expressed as mean ± SD, (n=5) and values with different superscripts are statistically different (p<0.05) to normal control.

TABLE AP 5: Packed cell volume of rats infected with *T. congolense* determined at three days intervals from zero day of infection.

GROUPS	ZERO DAY PCV %	3RD DAY PCV%	6TH DAY PCV%	9TH DAY PCV%	12TH DAY PCV%
Inf. + 20mg/ml kg	42.4±5.4[a]	37.4±2.0[a]	34.2±1.3[b]	34.6±4.2[a]	33.9±2.7[b]
Inf.+ 40mg/ml kg	37.8±3.5[a]	39.8±2.6[a]	40.0±3.8 [a]	36.6±3.6[a]	34.0±3.7[b]
Inf. + 80mg/ml kg	40.0±5.5[a]	38.8±2.9[a]	40.0±3.6 [a]	37.6±2.5[a]	34.0±2.4[b]
Inf. + STD DRUG	42.2±2.9[a]	36.2±1.5[b]	33.4±3.8 [b]	40.4±3.6[a]	40.2±2.9[a]
Inf. Control	41.0±4.1[a]	38.6±1.7[a]	35.6±1.5[b]	37.2±4.1[a]	33.4±1.7[b]
Normal control	38.4±4.2[a]	39.8±1.5[a]	40.0±2.6[a]	37.0±2.7[a]	38.8±1.9[a]

Values are expressed as mean ±SD, (n=5) and values with different superscripts are statistically different (p<0.05) to normal control.

Table AP 6: Estimated numbers of parasites (×10^6) per mills of infected blood with *T. brucei brucei* treated with the crude water extract and standard drug group

Incubation and treatment days	5th	6th	7th	8th	9th	10th	11th
20mg/kg body weight with extract	2.6	31.6	294.4	740.8	1000	1500	
40mg/kg body weight with extract	0.5	24.5	190.4	758.0	1000	1500	
80mg/kg body weight with extract	0.4	25.3	165.2	592.0	751.8	1000	1500
3.5g/kg body weight with std drug	0.4	67.8	389.4	50.5	0	0	0
Infected not treated	0.42	70.98	469.8	1000	1500		

Table AP 7: ---Estimated numbers of parasites (x10^6) per mills of blood of infected rats with *T.congolense* treated with *H.barteri* and standard drugs

Days of incubation from 7th day	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th
GRP1 20mg/ml	.06	.125	9.50	45.0	143.2	219.8	501.2	691.5	831.2	1000	1500	>1500	---	---
GRP2 40mg/ml	.06	.128	1.25	1.95	27.7	120.4	197.0	385.2	397.0	687.6	796.8	1000	1500	---
GRP3 80mg/ml	.06	.46	1.7	3.9	17.4	92.0	204.5	334.4	513.7	771.4	783.1	1000	1500	>1500
Std. Ctrl	.06	.13	.25	4.9	38.5	190.3	397.6	594.2	122.8	4.91	0	0	0	0
Inf. Ctrl	.06	.13	2.5	15.9	48.2	282.6	404.1	939.8	1000	1500	---	----	---	

Table AP 8: In vitro activity at 12.5mg/ml for fractionated extract at 5 minutes intervals for 60 minutes, by considering % motility of parasite (*T. brucei brucei*) as an index

		TIME (Minutes)											
FRACTIONS		5	10	15	20	25	30	35	40	45	50	55	60
Acetic acid	Percentage (%) motility	100	90	75	60	55	55	55	50	50	45	40	40
Ethyl acetate		100	100	80	80	80	70	70	70	60	60	50	50
Methanol		100	60	53	47	47	40	33	33	26	20	13	7
Water		100	94	81	69	56	56	50	50	50	44	44	38

Table AP 9 : In vitro activity at 25mg/ml for fractionated extract at 5 minutes intervals for 60 minutes, by considering % motility of parasite (*T. brucei brucei*) as an index

		TIME (Minutes)											
FRACTIONS		5	10	15	20	25	30	35	40	45	50	55	60
Acetic acid	Percentage (%) motility	100	89	78	67	67	56	44	44	44	33	33	22
Ethyl acetate		100	88	88	75	75	63	63	63	50	50	38	38
Methanol		100	71	57	43	29	14	14	0	0	0	0	0
Water		100	87	67	60	60	47	47	40	40	33	33	27

Table AP 10 : In vitro activity at 50mg/ml for fractionated extract at 5 minutes intervals for 60 minutes, by considering % motility of parasite (*T. brucei brucei*) as an index

	TIME (Minutes)												
FRACTIONS		5	10	15	20	25	30	35	40	45	50	55	60
Acetic acid	Percentage (%) motility	100	72	67	61	56	56	50	33	28	28	22	22
Ethyl acetate		100	85	80	70	60	60	55	45	40	40	35	30
Methanol		100	65	53	47	29	12	0	0	0	0	0	0
Water		100	94	81	69	56	56	50	50	50	44	38	25

Table AP 11: -Estimated numbers of parasites ($x10^6$) per mill of blood of infected rats with *T. brucei brucei* treated with methanolic fraction *H.barteri* and standard drug

Incubation and treatment days	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	
20mg/kg body weight of extract	0.5	17.4	98.4	188.9	173.1	177.0	173.1	165.2	169.2	169.2	165.3	
40mg/kg body weight of extract	1.2	35.4	133.4	188.9	173.1	157.4	169.2	161.3	165.2	149.5	145.6	
80mg/kg body weight of extract	1.2	27.0	59.2	153.4	168.4	137.7	129.8	129.8	129.8	133.8	141.6	
3.5g/kg body weight of std drug	0.5	21.0	110.2	216.4	365.0	98.4	19.8	3.98	**0**	**0**	**0**	
Infected not treated	0.8	22.2	133.4	239.4	357.2	391.6	391.6	501.2	501.2	501.2	501.2	