IN VITRO TRYPANOLYTIC POTENTIAL OF AFRICAN HEDGEHOG (*ATELERIX ALBIVENTRIS L.*) SERUM AGAINST *TRYPANOSOMA CONGOLENSE*

BY

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A DESSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIAIN PARTIAL FILFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF AMASTER OF SCIENCE DEGREE IN BIOTECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY,
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FEBUARY, 2019

DECLARATION

I declare that the work in this dissertation entitled "***In vitro* trypanolytic potential of African hedgehog (*Atelerix albiventris*) serum against *Trypanosoma congolense*"** has been performed by me in the Department of Biochemistry The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report dissertation was previously presented for another degree or diploma at this or any other Institution.

Name of student	Signature	Date
P14SCBC8116		

CERTIFICATION

This project report dissertation entitled ***IN VITRO*TRYPANOLYTIC POTENTIAL OF AFRICAN HEDGEHOG (*ATELERIX ALBIVENTRIS*) SERUM AGAINST *TRYPANOSOMA CONGOLENSE*** by ABDULLAHI LEMA ABUBAKAR meets the regulations governing the award of the degree of Master of Science in Biotechnology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to the Almighty Allah and the trusted messenger, Muhammad (s).

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ABSTRACT

African Trypanosomosis remains a major disease of humans and animals in sub-Saharan Africa and is responsible for considerable mortality and socio-economic impacts in the sub-region. There is no available vaccine and the available chemotherapeutic options are considered unsatisfactory due to toxicity and drug resistance. This study was designed to evaluate the possible trypanolytic potential of African Hedgehog serum and plasma for contribution to the future development of a new management option for the disease. Five adult hedgehogs and 5 laboratory mice were infected intraperitonially with 10^6*Trypanosoma congolense* cells and monitored for parasitemia and survival. The hedgehogs were resistant to the infection as no parasite was detected and none of the hedgehogs died even after 21 days post infection while the mice all died within 12 days post-infection. Further, serum and plasma were prepared from twenty adult hedgehogs and diluted serially in phosphate-buffered saline (PBS) to obtain 1×, 10×, 100× and 1000× dilution. Twenty micro liters of each dilution were added to 5 µl of *T. congolense* parasitized blood and incubated over a period of 1h at room temperature. Positive and normal controls contained a standard drug Diminazene Aceturate (DMA) and PBSG, respectively. The parasites were observed under microscope for motility and viability after 5, 15, 30, 45 and 60 minutes. The 10× diluted serum had higher significant ($p<0.05$) trypanolytic activity than the standard drug and PBS, while all the plasma dilutions had lower trypanolytic activity than the standard drug. A Bioassay-guided fractionation with ion exchange and gel filtration chromatography followed by characterization with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) revealed a proteinaceous trypanolytic factor with a molecular mass of 110 kDa. For the first time, it has been demonstrated that hedgehog serum has significant trypanolytic activity against *T. congolense* suggesting that the trypanolytic factor in hedgehog serum can be exploited for the development of new therapy for Trypanosomosis.

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LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
AAT	Animal African Trypanosomiasis
APOA-1	Apolipoprotein A-1
APOL-1	Apolipoprotein L-1
APS	AmmoniumPersulfate
BCT	Buffy Coat Technique
BIIT	Blood Incubation Infectivity Test
CSF	Cerebro Spinal Fluid
DTT	Dithiotretol
DEAE Cellulose	Diethyl Amino Ethyl Cellulose
DMA	Diminazene Aceturate
DMSO	Dimethyl Sulfoxide
EDTA	Ethylene Diamine Tetra acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
HAT	Human African Trypanosomiasis
HCT	Hematocrit Concentration Technique
HDL	High Density Lipoprotein
HPR	Haptoglobin Related Protein
IEC	Ion Exchange Chromatography
ITS- PCR	Internal Transcribed Spacer Polymerase Chain Reaction
LAMP	Loop Mediated Isothermal Amplification method
NASBA	Nucleic Acid Sequence Based Amplification Assay.
NITR	Nigerian Institute of Trypanosomiasis Research

PARP	Procyclic Acidic Repetitive Protein
PBS	Phosphate Buffer Saline
PBSG	Phosphate Buffer Saline Glucose
PCR	Polymerase Chain Reaction
SD	Standard Deviation
SDS PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SPSS	Statistical Package for the Social Sciences
SRA	Serum Resistance Associate
T. brucei	*Trypanosoma brucei*
T. vivax	*Trypanosoma vivax*
TEMED	Tetramethylethylenediamine
TgsGP	*Trypanosoma gambiense* Specific Glycoprotein
TLF1	Trypanolytic Factor 1
TLF2	Trypanolytic Factor 2
VSG	Variant Surface Glycoprotein
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

African trypanosomes are protozoan parasites that affect both humans and animals in sub-Saharan Africa, and are a major constraint to health and agricultural development (Cooper *et al.*, 2016). African Animal Trypanosomosis (AAT) is usually caused by *Trypanosoma congolense*, *T. brucei brucei* and *T. vivax* which affect health of cattle and other livestock (Atawodi, 2005), while Human African Trypanosomosis (HAT) is caused by two sub-species of *T. brucei, T. b. rhodosiense* in Eastern Africa*, and T. b. gambiense* in West and Central Africa (Atawodi, 2005). Thus, the significance of Trypanosomosis to human health, nutrition and economy is significance. Most trypanosomes are transmitted through the bite of tsetse flies (*Glossina spp*) during their blood meal on infected host (Vanhamme and Pays 2004).

African Trypanosomosis remains an impediment to human and animal health and is responsible for considerable mortality and a major obstacle to socio-economic development of the rural areas affected. Available chemotherapies for the disease are unsatisfactory due to their toxicity and resistance. Human African Trypanosomosis (HAT) threatens the lives of over 70 million people (Director, 2005; Atawodi, 2005). On the other hand, AAT is one of the major limitations to livestock production in rural areas where livestock farmers to subsist on less than 1.24 USD per day (Perry and Sones, 2007). Therefore, control of AAT is imperative due to its massive impact on the livelihood of rural communities.

Trypanosomes have been known to infect a wide variety of mammals; however some mammals are not susceptible to trypanosome infection, similarly it has been known for over a century that the serum of humans and other primates are toxic to most African trypanosome species (Cooper *et al.,* 2016). Two structurally distinct Trypanolytic Factor (TLF; TLF1 and TLF2) complexes have been identified to be components of high density lipoprotein (HDL) in humans and other primates (Lugli *et al.,* 2004). The two separate classes of High Density Lipoprotein (HDL) particles with lytic activity, share several protein components, including apolipoprotein A1 (ApoA1), haptoglobin related protein (Hpr) and apolipoprotein L1 (apoL1). (Tomlinson *et al.*, 1997; Raper *et al.,* 1999; Bullard *et al.,* 2012)

Both TLF1 and TLF2 utilize the same lytic component in the form of the ionic channel-forming protein, ApoL1 (Rifkin, 1991; Bishop, 2001 and Lugli *et al.,* 2004). Following internalization, ApoL1 undergoes a pH-dependent conformational change in the endolysosome pathway which releases it from the TLF complex (Hager *et al.,* 1997; Muranjan *et al.,* 1998) and promotes insertion into parasite membranes (Molina *et al.,* 2000; Richard and Wheeler, 2010), leading to the lytic effect.

Serum from Cape buffalo kills sub species of *Trypanosoma brucei* (*T. b. rhodosiense*, *T. b. gambiense*), *T. vivax, T. congolense,* and *T. evansi in vitro* (Reduth *et al.*, 1994) or prevents their replication. This activity is absent in the serum of a wide variety of domestic animals (Reduth *et al.*, 1994).

The African hedgehog *Atelerixalbiventris*is a species of interest in lipoprotein research (Sparrow *et al.,*1995). Hedgehog is among the few species whose serum contains lipoprotein; the spectrum of HDL-like lipoproteins displayed a protein with apoA1-like properties as their prominent Apo lipoprotein component (Sparrow *et al.,* 1995), however apoA1 is confirmed to be a component of the TLF (Tomlinson *et al.,* 1997).

1.2 Statement of the Research Problem

Trypanosomosis remains a major threat to both human and animals in sub-Saharan Africa, and is responsible for 3 million livestock deaths (WHO,2012).African Trypanosomosis is of great public health importance and still remains a disease with unsatisfactory medical control (Edoga*et al.*, 2013).*Trypanosomacongolense* is the most prevalent and wide spread pathogenic trypanosome in Tropical Africa (Peacock *et al.*, 2012). Therefore the need for alternative treatment options that are safe, effective and affordable is urgent.

1.3 Justification

African trypanosomes have been known to infect a wide variety of mammals, but some mammalian sera are capable of trypanolysis. Hedgehogs, humans and other primates apolipoproteins share high sequence similarities and apoA1 of human and other primates are also confirmed to be part of the trypanolytic factors. Recently there are no reports on trypanolytic potentials of the hedgehog serum and plasma. Therefore, this work was conceived to investigate the presence of trypanolytic factors in the blood of the African hedgehog as an effort towards the discovery of new and safe therapeutic agents for the treatment of AAT and HAT.

1.4 Null Hypothesis

African hedgehog serum and plasma does not have trypanolytic activity.

1.5 Research Aim

This study was aimed at studying the trypanolytic potential of African hedgehog serum and plasma.

1.6 Specific Objectives

i. To investigate the trypanolytic activity of the African hedgehog crude serum and plasma *in vitro.*

ii. To partially purified the trypanolytic factor in hedgehog serum using bioassay guided fractionation (Ion Exchange and Gel Filtration Chromatography)

iii. To characterize the components in the fraction with the highest trypanolytic activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Trypanosomosis

Animal African Trypanosomosis (AAT) is also known as "*Nagana", "dura",* tsetse fly disease or the wasting disease depending on the host or the region (Lopes *et al.,* 2010) it affects both wild and domestic animals such as cattle, sheep, dogs, pigs, horses, camels and others. The infection can become fatal if untreated. Trypanosomosis in cattle is caused by *T. congolense*, *T. vivax*and to a lesser extent, *T. b. brucei*, (O'Gorman *et al.*, 2006; O'Gorman *et al.*, 2009). There are several clinical signs of the disease which include; fever, listlessness, emaciation, hair loss, discharge from the eyes, oedema, anaemia and paralysis hence weakening the animal (Steverding, 2008).

2.2 Distribution of African Trypanosome

The distribution of trypanosomes of medical importance varies with locality and depends on the interaction between the tsetse flies (fig 2.1), domestic and wild animals (Simukoko *et al.,* 2007). Between 2008 and 2009 Nigeria was among the African countries, respectively, reporting fewer than 100 new cases of HAT per year, while Democratic Republic of Congo and Central African Republic reported > 1,000 new cases per year. The prevalence of Trypanosomosis varies from one country to another (WHO, 2012). In Nigeria, Trypanosomosis seems to be re-emerging as an important livestock disease, assuming major clinical importance in domestic animals (WHO, 2012).

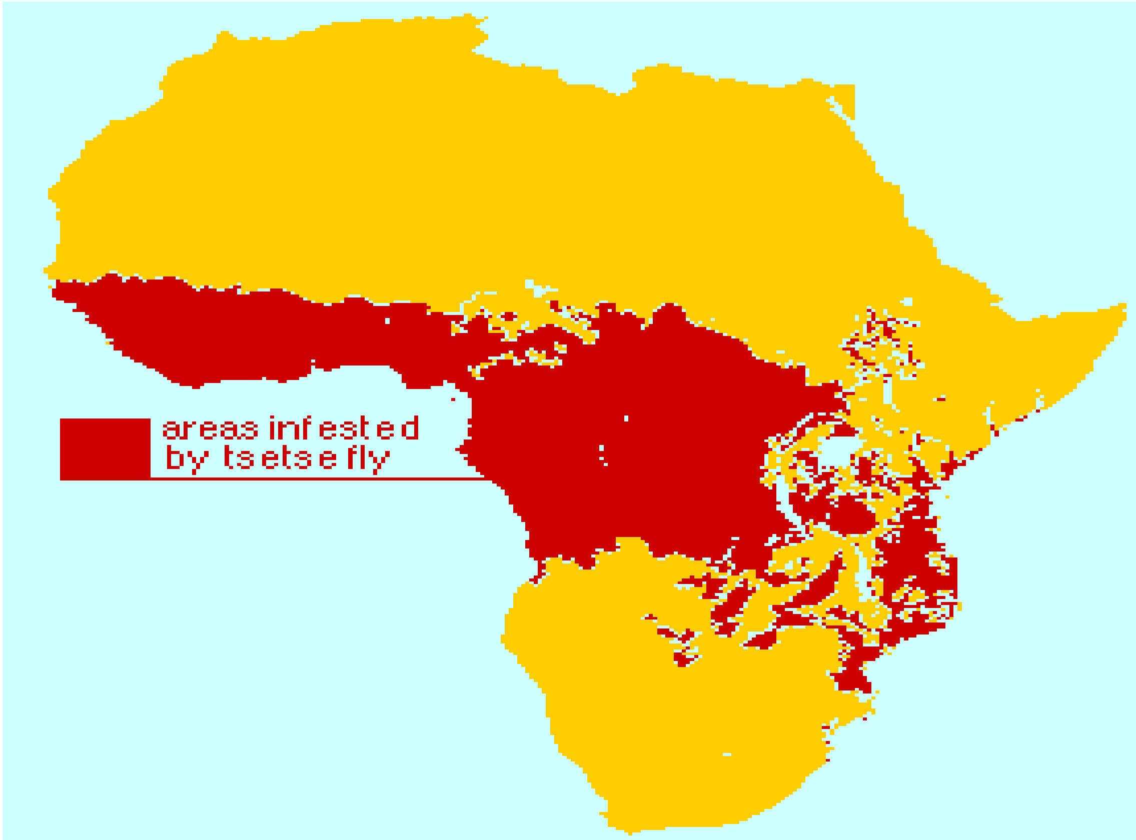


Figure: 2.1: Map of Africa showing the distribution of tsetse flies. Adapted from FAO (1998).

2.3 African Trypanosomes

African Trypanosomosis is one of the many constraints of Africa's struggle against poverty through livestock farming. It is an important infection that affects humans and livestock in poor African countries PAAT, (2007).

Trypanosomes are transmitted mainly by the tsetse flies of the genus *Glossina*. Some trypanosomes can also be transmitted mechanically by blood sucking flies such as tabanids, horse flies and stomoxys (Baral, 2010). The species transmitted by tsetse flies include; *T. brucei* and the sub species, *T. congolense* and *T. vivax* which undergo development in the tsetse fly vector (Welburn and Maudlin, 1999). *Trypanosoma evansi* is transmitted mechanically and thus the infection can be found outside the tsetse belt (Baral, 2010). Trypanosomes that are transmitted by tsetse flies can be grouped as haematic (*T. vivax, T. congolense, T. simiae)* which cause anemia, or humoral T. *brucei* sub species (Baral, 2010).

2.3.1 *Trypanosoma congolense*

Trypanosoma congolense is an extracellular protozoan parasite that causes devastating livestock infections and is the most prevalent and wide spread pathogenic trypanosome in tropical Africa being found in ruminants, pigs, dogs and other domestic animals (Peacock *et al.,* 2012).It is a strictly intravascular parasite whereas *T. brucei* can leave blood vessels and invade tissues implying differences in virulence mechanisms, host/pathogens relationships and their pathogenic effects(Simarro *et al.,* 2012).In the mammalian host *T. congolense* is a small trypanosome, shorter in length than *T. brucei*, and without conspicuous undulating membrane (Peacock *et al.*, 2012). Like other African trypanosomes, *T. congolense* developed several immune evasion strategies, including antigenic variation and the main symptoms of *T. congolense* are; low body weight, pancytopenia *i.e* anemia, neutropenia, lymphopenia, thrombocytopenia as well as increasing host susceptibility to secondary infections (Simarro *et al.*, 2012). *T. congolense* is the major cause of AAT in livestock (Simarro *et al.*, 2012).

2.3.2 *Trypanosoma vivax*

Trypanosoma vivax is a heterogeneous parasite that can be transmitted cyclically by tsetse flies (*Glossina spp*) and also mechanically by hematophagous flies (Gardiner, 1989). Its hosts include; a domestic animal such as bovines and ovines where it is pathogenic and at times causes a hemorrhagic disease (Thumbi *et al.*, 2010). The severity of *T. vivax* infection varies with host resistance and the parasite strains. The clinical signs of the infection are consistent with those of other trypanosome infections which include; anemia, oedema, abortion, nose discharge and progressive weight loss (CFSPH, 2009). Cattle infected with *T. vivax* have a higher parasitemia and a more acute disease than those infected with *T. congolense* (Taylor and Bea, 1999).

2.3.3 *Trypanosoma brucei brucei*

This is a *T. brucei* sub species and is distributed within the tsetse-belt region due to the distribution of the *Glossina* vector (Barrett *et al.*, 2003). The parasite is restricted within the sub-Saharan Africa and causes a mild infection in cattle. Unlike the other subspecies of *T. brucei* is not human infective because it is susceptible to trypanosome lytic factors associated with high density lipoproteins like the human Apo lipoprotein L1 found in human blood (Vanhamme *et al.*, 2003).

2.3.4*Trypanosoma simiae*

Trypanosoma simiae is tsetse-transmitted and belongs to the subgenus Nannomonas. It is not known to cause any disease in cattle but is severely pathogenic in pigs, goats, camels and horses (Kaufmann, 1996).

2.3.5 *Trypanosoma evansi*

Trypanosoma evansi belongs to the trypanozoon group and is morphologically indistinguishable from *T. brucei* sub species. It is the causative agent of "surra" which affects camels, horses, cattle, pigs, buffaloes and dogs (Baral, 2010). It is transmitted mechanically by blood sucking flies like the tabanids; stomoxys as such it can be found outside the tsetse belt. The parasite cannot undergo cyclical development in the vector. Symptoms of "surra" include; abortion, central nervous system disorders and diminished productivity in animals (Baral, 2010). *T. evansi* can also cause disease in man (WHO, 2005; Joshi *et al.*, 2005).

2.4 Life Cycle of African Trypanosomes

The life cycle of trypanosomes involves differentiation into several development stages in both the tsetse vector and the mammalian host (Figure: 2.2). Following a blood meal, the tsetse fly infects the mammalian host with metacyclic trypomastigotes into the skin tissue which multiply at the site of infection before invading blood and lymph node tissues (Peacock *et al.*, 2012). On invading the host's blood tissues the metacyclic trypomastigotes transform into long slender bloodstream forms that are covered with the Variant Surface Glycoprotein (VSG) coat (Baral, 2010). A non-infected tsetse fly gets infected with trypomastigotes during a blood meal on an infected host. Once inside the tsetse fly, initial infection is established in the mid gut. The bloodstream forms undergo a series of morphological and biochemical changes where the VSG coat is shed off and replaced with a coat of procyclic acidic repetitive protein (PARP) or procyclin, but the major surface molecules of *T. congolense* are carbohydrates rather than glycoprotein as in *T. brucei*(Baral, 2010; *Peacock et al.,* 2012). Before transformation into procyclic trypomastigotes the procyclic trypomastigotes which are long and slender multiply by binary fission before leaving the midgut to the anterior. The procyclic trypomastigotes once inside the anterior gut transform into elongated and asymmetrically dividing epimastigotes(Peacock *et al.*,2012). These multiply actively in the proboscis and then move to the salivary glands where the final transformation takes place. Inside the salivary glands, the epimastigotes continue to multiply thus generating short epimastigotes that attach to the salivary gland epithelium. The attached epimastigotes differentiate into VSG-coated metacyclic trypomastigotes that are suited for mammalian bloodstream invasion. The cycle takes about three weeks and the fly remains infective for life (Chappuis *et al.,* 2005).

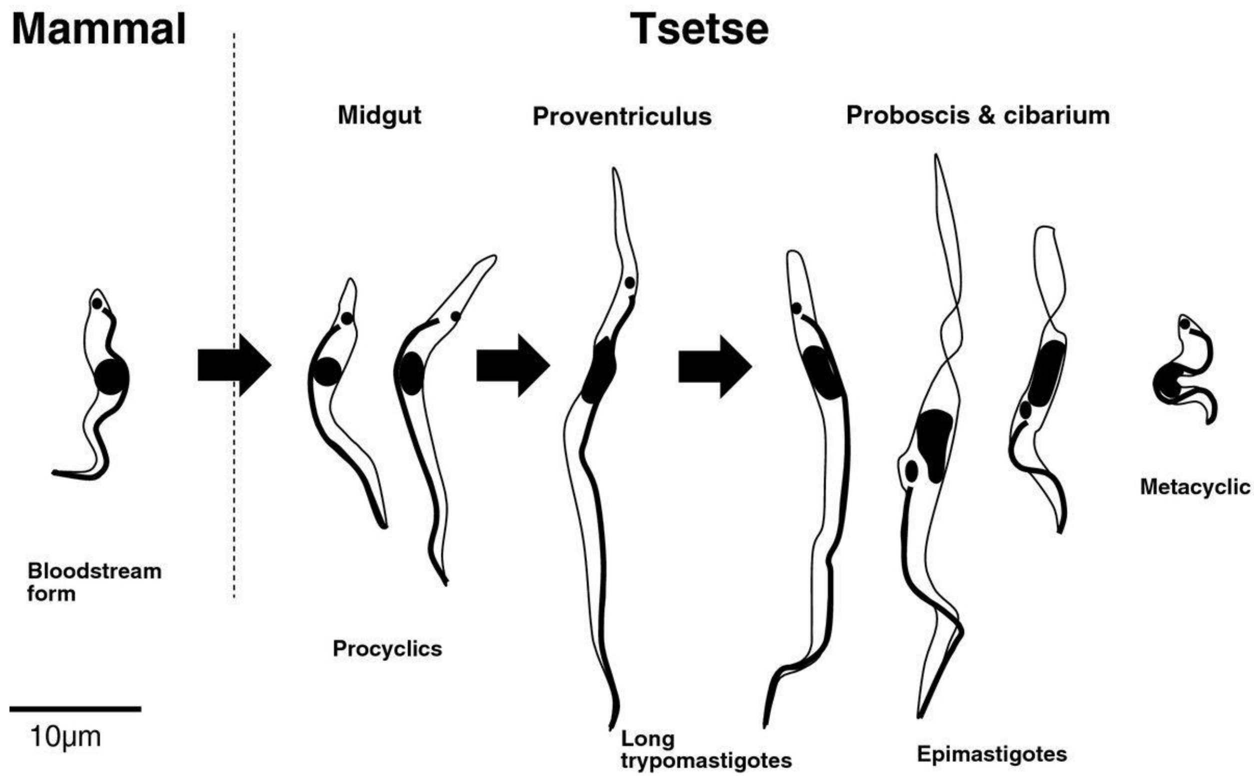


Figure: 2.2 the life cycle of *Trypanosoma congolense* (Peacock et *al*., 2012**).**

2.5 Diagnosis of Trypanosomosis.

The diagnosis of Trypanosomosis requires a high degree of training and expertise where the parasite must be detected in humans before treatment can be given. This relies on conventional techniques such as lymph node puncture, blood film examination or more elaborate techniques to concentrate parasites in the blood like Hematocrit concentration technique (HCT), (WHO, 2012). The concentration of trypanosomes in the blood rise and fall often decreasing below detection levels especially in *T. b. gambiense* infection thus examinations are usually repeated so as to demonstrate the parasite in body fluids. The different techniques used for diagnosis of trypanosome infection include; use of clinical signs, parasitological diagnosis, serological and molecular techniques (WHO, 2012).

2.5.1 Clinical diagnosis

Diagnosis of both HAT and AAT based on clinical signs alone is complicated due to lack of specific clinical signs of the disease (Nantulya *et al.,* 1987) the clinical symptoms (persistent headache, high fevers, general body weakness and weight loss) can mimic those of other diseases like malaria, filariasis which are also endemic in tropical regions (WHO, 2012).

2.5.2 Serological diagnosis

Serological techniques play a major role in the diagnosis and epidemiological assessment of Trypanosomosis especially in HAT; however, most of these methods cannot detect active infection since they rely on the presence of circulating antibodies which may be due to a past infection since antibodies can persist for up to three years after cure (Paquet *et al.,* 1992).

2.5.3 Parasitological diagnosis

Traditional parasitological methods are routinely used to demonstrate presence of trypanosomes in body fluids such as blood, Cerebrospinal fluid (CSF). Microscopy has low sensitivity; it leaves a big portion of trypanosome infections undetected (Majiwa *et al.*, 1994) due to the fluctuating nature of parasitemia and also it cannot detect parasites sequestered in other organ tissues (WHO, 2012). Parasitological tools are continually used in the field as they can be carried out directly and give results as well as being practical in resource poor settings (Cox *et al.*, 2010). Parasitological methods involve demonstration of trypanosomes in wet, thin and thick blood films in addition to cerebrospinal fluid (CSF), chancre and lymph node aspirates. Detection of trypanosomes in cerebrospinal fluid confirms the involvement of the central nervous system and this differentiates between the haemolymphatic (early) stage and meningo-encephalitic (late) stage which is essential for the correct choice of treatment for HAT.

2.5.4 Molecular diagnosis

Molecular diagnostic tools have been developed with high specificity and sensitivity (Cox *et al.*, 2005) and can detect trypanosomes even in cases of low parasitemia. Up till now molecular techniques were not easily applicable to field conditions owing to the amount of time required to process samples by conventional methods like the phenol-chloroform extraction method prior to analysis and the equipment needed. This has been overcome by improved extraction kits and preserving of DNA especially using dried blood spot (DBS) which makes sample collection in the field with a view of diagnosis by molecular methods simpler(Picozzi*et al.*, 2002). The molecular tools include; Polymerase Chain Reaction (PCR), PCR ELISA, PCR hybridization, Loop Mediated Isothermal Amplification method (LAMP) and Nucleic Acid sequence based amplification assay (NASBA), Serum Resistance Associate (SRA PCR), *Trypanosoma gambiense* Specific Glycoprotein (TgSGp PCR) (Picozzi *et al.*,2002).

2.5.4.1 Internally transcribed spacer polymerase chain reaction (ITS- PCR)

The ITS- PCR amplifies the internal transcribed spacers (ITS) regions that are located in the ribosomal genes. The ITS regions have inter-species length variations and a high copy number which are desirable qualities for molecular markers in identification and differentiation of trypanosome species (Cox *et al.,* 2005). The nested ITS- PCR amplifies both ITS regions of the ribosomal RNA gene. Using two sets of primers (Cox *et al.*, 2005) the PCR can detect Trypanozoon group*, T. congolense, T. vivax, T. simiae* and *T. theileri* and also mixed infections in a sample.

2.5.4.2 *Trypanosoma brucei gambiense*- surface glycoprotein PCR

Trypanosoma brucei gambiense- surface Glycoprotein PCR amplifies the TgsGP gene which codes the *T. b. gambiense* specific flagella pocket glycoprotein which is specific to *T. b. gambiense* (Radwanska *et al.,* 2002). This PCR is used to differentiate *T. b. gambiense* from *T. brucei* subspecies since TgsGP gene is specific to this sub species. It is a nested PCR using two sets of primers (inner and outer) (Radwansk *et al.,* 2002; Maina *et al.,* 2007).

2.5.4.3 Serum resistance associated PCR

Serum Resistance Associated- PCR is based on the serum resistance associated gene which confers resistance to *T. b. rhodosiense* strains from lysis by the trypanolytic factors present in human serum (Radwanska *et al.,* 2002). The SRA gene is specific to *T. b. rhodosiense* which distinguishes it from the other *T. brucei* sub species. This is achieved by nested PCR using two sets of primers (Radwanska, *et al.,* 2002; Maina *et al.,* 2007).

2.6 Trypanotolerance

It has been shown that different mammalian hosts respond differently to trypanosome infection. Some hosts are genetically resistant to trypanosome infection both in natural and experimental conditions. The BALB/c and C57B1/6 mice have varying susceptibilities to trypanosome infection. The C57B1/6 mice are resistant to Trypanosomosis compared to the BALB/c mice which are susceptible (Kaushik *et al.,* 2000; Yoshihara *et al.,* 2007). This occurrence has also been observed in some cattle breeds like the *Bostaurusm* (N'Dama breed) found in West Africa which is relatively resistant compared to the *Bosindicus* (Zebu breed). N'Dama remains healthy and productive during infection and perhaps acquired resistance to Trypanosomosis through their long coexistence with the trypanosomes. They have evolved a superior mechanism that controls trypanosome proliferation and anemia (Yoshihara *et al.,* 2007).

The mechanism by which these hosts are able to tolerate African Trypanosomosis is complex thus not well understood (Yoshihara *et al.,* 2007). It is thought to involve a strong immune response towards the parasite antigens as well as cytokine synthesis with lethal effects on trypanosome proliferation (Lutje *et al.,* 1996). Several studies report that there is a difference in cytokine production between resistant and susceptible hosts (Lutje*et al.,* 1996; Namangala*et al.,* 2009). Studies by (Taylor *et al.,* 1996; Magez *et al.,* 2006 and Baral *et al.,* 2007) showed that trypanotolerant hosts elicit a stronger antibody production than the susceptible ones. In trypanotolerant mice there is an increased production of interleukin (IL) 10 and IL-14 coupled with a stronger antibody response to trypanosome VSGs during infection (Namangala *et al.,* 2009).

2.7 Trypanosome Lysis by Human Serum

African trypanosomes have a large number of mammalian hosts. *T. brucei* and *T. congolense,* for instance, are able to infect most game as well as domestic animals. However, humans are naturally resistant to infection by these parasites. In 1912 Laveran and Mesnil had already noted that normal human serum (NHS) was able to lyse *T. brucei,* and reported that this was not associated with immunoglobulin or complement activation. Laveran also uncovered trypanolytic activity in the serum of some baboons (*Papio*) andmandrill *(Mandrillus*) species, although considerable differences in activity were noticed, the *Papiopapio* serum being much more efficient than human serum, and the activity of mandrill serum being low and inconsistent. Subsequent studies extended the trypanolytic property to gorilla serum, and confirmed the low and irreproducible nature of the activity of mandrill serum, which was also observed for the serum of *Papiohamadryas* (Seed *et al.,* 1990; Kageruka *et al.,* 1991; Poelvoorde *et al.,* 2004). Worth mentioning are differences between humans and gorillas on hand and baboons on the other hand, as lytic factors from these two groups showed distinct antigenic and functional properties (Seed *et al.,* 1990; Poelvoorde *et al.,* 2004). Therefore, the ability to lyse trypanosomes was probably acquired by at least two primate species independently

2.9 The Trypanolytic Factor

The mechanism of action and active component of trypanolytic factor in humans and other primates is a class of (HDL) particles (Rifkin *et al.,* 1976; pays *et al.,* 2006). There are two subclasses of trypanolytic HDL, TLF1 in the HDL3 fraction (the densest HDL sub-fraction) and TLF2, which is arguably too large and dense to be considered a normal HDL particle (Hadjuk *et al.,* 1989; Gillet and Owen 1991; Raffer *et al.,* 1996).

Endocytosis and trafficking of TLF to the low pH of the lysosome is required for lytic activity; inhibition of uptake prevents lysis (Lorenz *et al.*, 1994; Shimmaru *et al.*, 2001; Vanhamme and Pays, 2004).

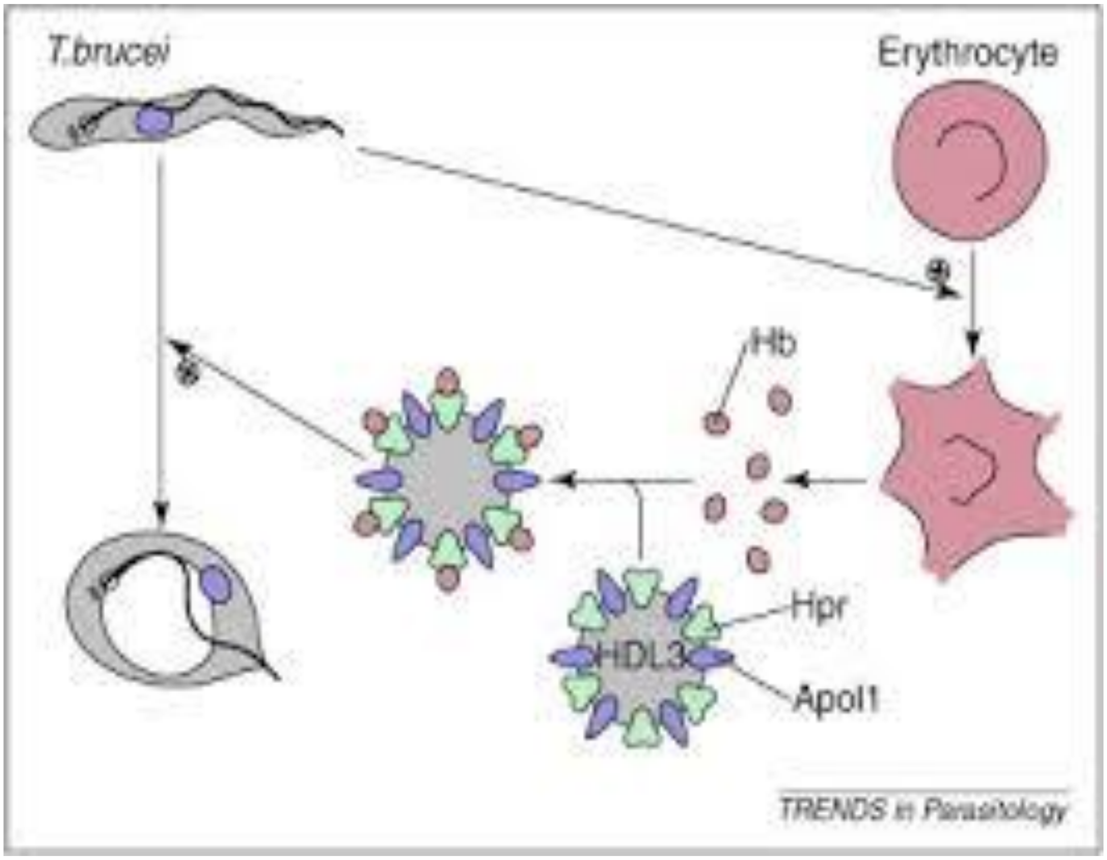


Figure: 2.3 Mechanism of Trypanolytic Activity in Human (Richard, 2010), Adapted from Trend in Parasitology

2.9.1Haptoglobin related protein (Hpr)

It is reported by different investigators that trypanolytic activity results from presence of apoL1 and Hpr on the same subset of HDL particles (Pe´rez-Morga *et al.,* 2005; Vanhollebeke *et al.,* 2007). TheapoL1 alone, either recombinant or native, are sufficient to cause complete trypanolysis, although less rapidly than when Hpr is present (Vanhamme *et al.,* 2003; Shiflett *et al.,* 2005; Vanhollebeke *et al.,* 2007). In particular, natural human mutants lacking Hpr but possessing apoL1 are not susceptible to trypanosome infection, but the rate of trypanosome killing by the serum from these individuals were found to be lower than normal (Vanhollebeke *et al.,* 2007).

2.10 African Hedgehog (*Atelerixa albiventris*)

African Hedgehog *Atelerixo albiventris* are wide spread in rural sub-urban and urban environments. These animals belong to the kingdom, Animalia, phylum chordate, class Mammalia order *Erinaceomorpha,* family *Erinaceidae* (Rilley and Chomel, 2005).

The African hedgehog is about 6-8 inches long and weighs about 18-25g/kg, the females are typically larger than the males**.** They have round, dark eyes, oval brownish ears, and short, white hairs on their brows, cheeks, and bellies. The hairs on their backs and sides are modified into ½-1 inch long, un barbed spines, Their back feet have only four toes; their front feet have five toes, their lifespan in the wild is about 2 to 3 years while in captivity is about 8 to10 years (Wrobel and Brown,1997).

They also have a high tolerance for toxins and have been seen consuming toxic plants, poisonous amphibians, and even scorpions, if they encounter a predator, hedgehogs roll tightly into a ball, protecting their head, belly, and legs. Hedgehogs prefer moderate temperatures and will often burrow and enter usually a state of aestivation in the summer or hibernation in the winter (Wrobel and Brown, 1997).

Figure: 2.4 African Hedgehog (*Atelerixalbiventris)*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Protein standard (Precision Protein Plus), Gel Loading Buffer II (Applied BiosystemAmbion USA), 4 to 20% mini Precast Gel (Bio Rad USA), DEAE Cellulose, (Santa Cruz Biotechnology, Inc), Sephadex G50 (Sigma), Coomasie Brilliant Blue R-250(Thermo Fisher Scientific USA), Agarose (Hydra Gene), Quick-DNA^{TM} Miniprep plus kit, DNA extraction kit, primers, Micro titter plate (Flow laboratories Inc., McLean, Virginia 22101, USA), DiminizineAceturate(445mgdiminazenediaceurate + 555 mg phenazone/g). All other reagents used in this study were of molecular biology and analytical grade and were purchased from reputable supplies companies.

3.1.1 Experimental animals

About 20 adult male hedgehogs were captured by hunters behind Umaru Musa Yaradua International Airport, Katsina State Nigeria. They were transported to Animal house, Biochemistry Department Ahmadu Bello University, Zaria, in a well-ventilated box; they were fed with groundnut and water *adlibitum*, and allowed to acclimatize for 7 days before blood samples were collected. Twenty mice was purchased from department of pharmaceutical science ABU, Zaria

3.1.2 Identification of the hedgehogs

The hedgehogs were identified by the Museum of Natural History Department of Biological Science Ahmadu Bello University, Zaria. The voucher number is No. 9a

3.1.3 Test Parasite

Trypanosomacongolense were obtained from stabilates maintained at Nigerian Institute of Trypanosomiasis and Onchocerciasis Research (NITOR) Kaduna, Kaduna State. The parasites were maintained in the laboratory by continuous passaging in rats until required. Passaging was considered necessary when parasitemia was in the range of 25 – 32 parasites per field (usually 8 to 10 days post infection). In passaging, 1 x 10^3 of the parasites was introduced intraperitonially into rats in 0.1 to 0.2 ml blood/PBS solution. For several passages, approximately 80% blood solution (v/v) was obtained by cardiac puncture into 1ml syringe containing 0.2 ml EDTA (1% w/v). About 0.1 to 0.2 ml of the blood collected as described above or blood (diluted with PBS which contain approximately 1x 10^3parasite/ml) were injected into clean rats and allowed to acclimatize under laboratory condition for at least one week.

3.2 Methods

3.2.1 Experimental Infection

The five hedgehogs were anaesthetized with ketamin injection and passage with 0.2ml of 10^6 cells of *Trypanosoma congolense* intraperitonially. Similarly the five mice was also passage with 0.2ml of 10^6 cells of *Trypanosoma congolense* intraperitonially and the parasitemia was monitored for 14 days post infection under microscope ×40 objective lengths. The survival analysis was also observed.

3.2.2 DNA isolation

The genomic DNA of the parasite was isolated using Quick-DNA™ Miniprep plus kit. Blood samples (200 µl) were added into micro centrifuge tubes containing 200 µl of biofluid and cell buffer. Twenty microlitre (20 µl) of protease were added and mixed thoroughly and the tube was incubated at 55 °C for 10minutes, binding buffer (420 µl) was added to the sample and the mixture transferred into a zymo-spin column in a collection tube and centrifuged at 12000 ×g for 1minutes and the collection tubes was discarded with the flow through. DNA prewash buffer was then added to the column in a new collection tube and centrifuged for 1min and then the collection tube were emptied and 700 µl of g-DNA wash buffer was added and centrifuge for 1 minutes, the collection tube was emptied and transferred into new micro-centrifuge tube, DNA elution buffer (50 µl) was added and incubated for 5minutes at room temperature and the centrifuged for 1minute and the extracted DNA was stored at -20°C

3.2.3 Parasite Identification by Nested PCR

The parasites obtained were identified by Polymerase Chain Reaction (PCR) using ITS-1 genus specific primers according to the method of Adams *et al.*, 2006. Both forward and reverse primers used are listed in Table 3.1.

Table 3. 1: Primer sequence used

Primer	Sequence 5′-3′
Tryp1	5′-AAG CCA AGT CAT CCA TCG-3′
Tryp2	5′-TAG AGG AAG CAA AAG-3′
Tryp3	5′-TGC AAT TAT TGG TCG CGC-3′
Tryp4	5′- CTT TGC TGC GTT CTT-3′

Adams *et al*., 2006

PCR was performed in 25 µl reaction volume. Cycling conditions were as follows: 1 cycle at 95 °C for 60s followed by 35 cycles at 94 °C for 60s, 54 °C for 60s, 72 °C for 30s, and final extension at 72 °C for 300s. For nested PCR two consecutive PCR reactions were carried out; a set of outer primers TRYP 3 and 4 were used in the first round of the reaction, followed by inner primers TRYP 1 and 2 in the second round. The PCR product (0.5 µl) from the first round of the reaction was added to 25 µl of the second round reaction in a fresh PCR tube. Cycling conditions for nested PCR were the same as for the standard PCR reaction (Adams *et al.*, 2006).

The component of the premix includes nuclease free water, magnesium chloride, taq DNA polymerase, DNTPs, ITS-1 Primers both inner and outer.

3.2.4 Determination of parasitemia

Parasitemia was monitored in blood obtained from the donor animal whose 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 (Herbert and Lumsden, 1976). The method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline PBS at pH 7.2. Logarithm values of these counts obtained by matching with the Table of Herbert and Lumsden (1976) which is converted to antilog to provide absolute number of trypanosomes per ml of blood.

3.2.5 Blood sampling

All the experiments reported in this study were performed on blood samples from apparently healthy hedgehogs. In each case, blood was taken from a hedgehog that has been fasted overnight for approximately 18 hours. The animals were slightly anaesthetized with ketamin injection, and the blood withdrawn by heart puncture. The blood was then collected on EDTA coated tube. The plasma was separated by centrifugation for 15 minutes at 2,000 ×g. The serum samples were collected in on plain containers without EDTA.

3.2.6 *In vitro* trypanolytic assay

The serum and plasma were serially diluted in phosphate buffer saline (PBS). One milliliter of the serum was used as the stock, 100 µl of the serum was taken from the stock to the next tube + 900 µl (PBSG) up to the last tube to prepared1×, 10, 100× and1000× Dilutions.

Assessment of *in vitro* trypanolytic activity was performed in triplicates in 96 well microtiter plates. Twenty micro liters (20 µl) of blood containing about 20 to 25 parasites per field was mixed each with 5 µl of serum and plasma dilutions. To ensure that the effect monitored was that of the serum and plasma, a set of control was included which contained the parasite suspended in phosphate buffered saline glucose (PBSG) only. Concentrations of diminazene aceturate (DMA) 200 mg/ml antitrypanosomal drug were used as the standard control. After 5 minutes of incubation Eppendorf tubes maintained at room temperature temperature, the test mixtures were placed on separate microscope slides and covered with cover slips and the parasites were observed for viability and motility under microscope ×40 at 5, 15, 30, 45 and 60 minutes. under this *in vitro* system adopted, parasites can survived for about 4 hours in the absence of serum or plasma dilutions. The number of viable cell or unlysed cells in the serum and plasma dilutions compared to parasite loaded control was taken as measure of trypanolytic activity (Atawodi, 2005).

3.2.7Bioassay guided fractionation by Ion exchange chromatography (IEC)

The serum was fractionated by ion exchange chromatography on DEAE Cellulose (Wilken and Nikolov, 2001; Jiang *et al.,* 2002). The column was equilibrated with potassium phosphate buffer (0.25 M; pH 7.4). The final height of the column was between 7 to 8 cm. about 15 ml solution of elution buffer was prepared from the stock (0.25M) solutions. The concentrations0.05M, 0.1M, 0.15M, 0.2M and 0.25 M, with an increment of 0.05M of potassium phosphate buffer. Crude serum (1 ml) was loaded into the column. The column (15×15cm) was rinsed with potassium phosphate buffer (0.25 M; pH 7.4). Test tubes were used to collect 15 fractions (3 ml of eluate in each tube) and the absorbance was determined at 280 nm for protein estimation. The biological activities of each fraction were assayed against *T. congolense.*

3.2.8Protein content determination

Twenty micro litter of the fractions were loaded into a micro plate well containing Bradford reagent (200 µl) which was prepared in a 10-fold dilution from the commercialized stock solution. The mixture was incubated for 10 min before being measured spectrophotometrically at 595 nm. For blank, the sample was replaced with20 µl demonized water. All experiments were all carried out in triplicates.

3.2.9 Bioassay guided fractionation by gel filtration chromatography

Further purification of the trypanolytic factor was carried out using gel filtration involving, the column were packed with Sephadex G50 and the Flow rate was set at 1.0 ml/min. The column was equilibrated with potassium phosphate buffer (0.25 M; pH 7.4) and then the combined fractions (100 µl) were loaded into the column. Three milliliter fractions of eluatewere collected and the protein content was analyzed using the Bradford protein assay.

3.2.10 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins in the samples were resolved on 4-20% Polyacrylamide gel and the gel was stained with Coommasie brilliant blue R-250 according to the method of Laemmli, (1970). The most active fractions from the crude serum was mixed with sample buffer in the ratio of 1:1 (25ul each) in micro centrifuge tubes, the mixtures were short spin and heated in water bath for 10 minutes at 90^{o}C. The samples were allowed to cool to room temperature and then loaded onto the polyacrylamide gradient gel. Fourty micro liter (40 µl) of the sample was loaded carefully into the well. After the run, the gels were stained using Coommasie brilliant blue R-250, gel were destained on a shaker by changing water from time to time until the protein band was seen clearly.

3.3 Statistical Analysis

The data collected were analyzed using one way ANOVA (Analysis of variance) turkey post hock and $p \leq 0.05$ was considered statistically significant. Other data collected are presented using charts plates and figures.

CHAPTER FOUR

4.0 RESULTS

4.1 Parasite Identification

The PCR agarose gel electrophoregram results of the parasite identification are presented in (Plate 4.1 and Table 4.1) the parasite was confirmed to be *T. congolense* with 560bp based on the expected band size reported by Adams *et al.*, 2006.

4.2 The Parasitemia and Survival Analysis of Hedgehog and Mice

The result of parasitemia and survival analyses between hedgehog and mice were presented in figure 4.1 and 4.2. It was observed that no parasite detected in hedgehog 14 days post infection therefore hedgehogs are resistant to infection with *T. congolense* while in mice it was observed that all the mice were died at 12 days post infection.

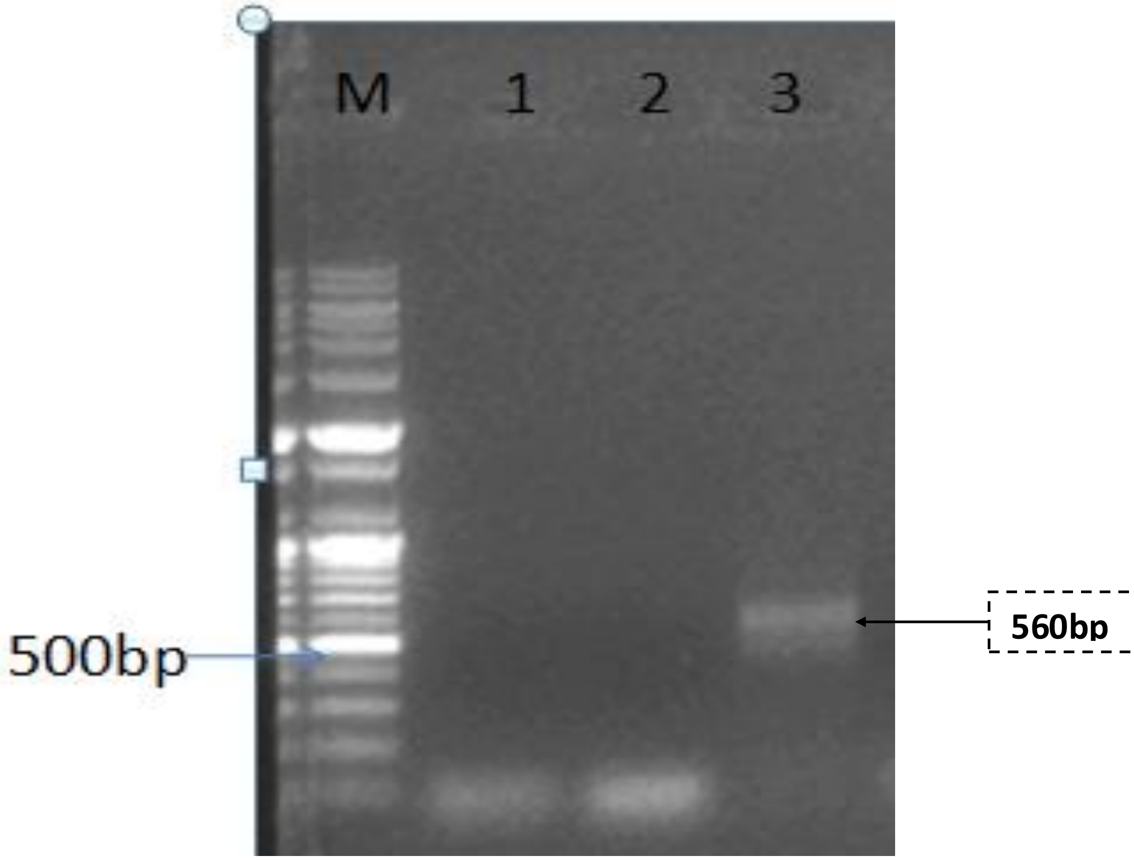


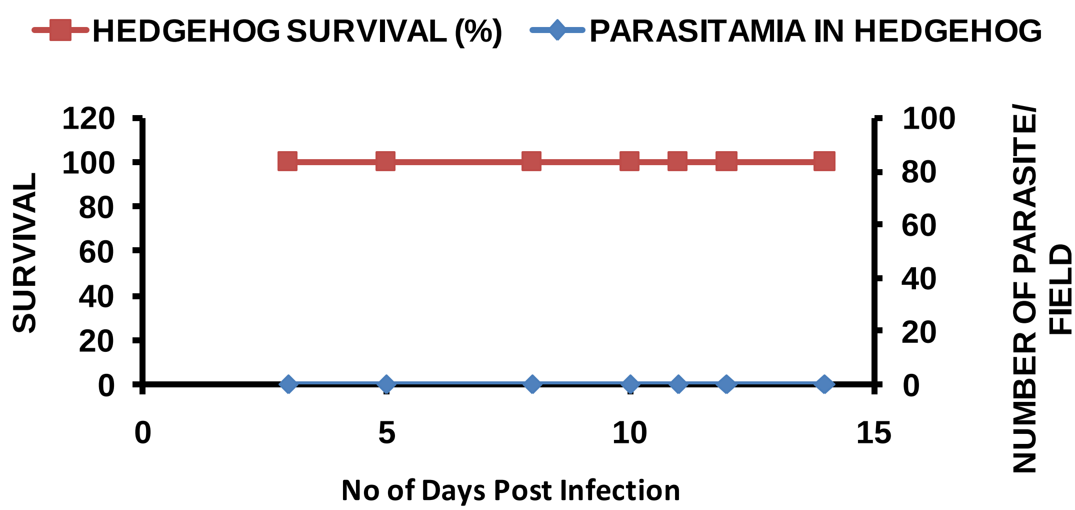
Plate: 4.1 Agarose Gel Electrophoregram of PCR showing the result for parasite identification.

Lane M is the DNA marker, 1 and 2 is negative control while lane 3 is positive for *T. congolense* with 560bp

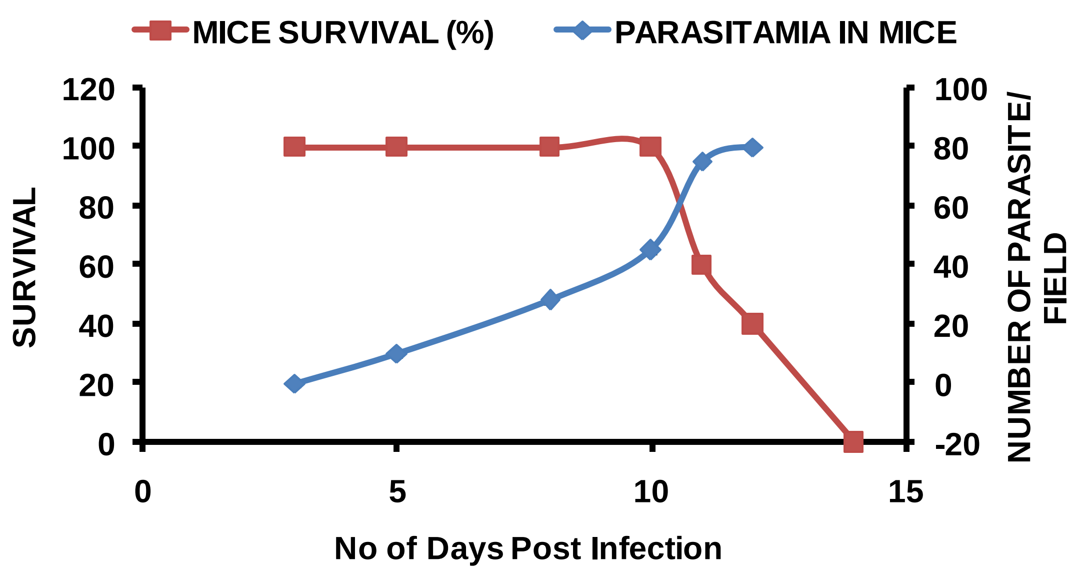
Table 4.1: Expected band size based on ITS-Primer

ITS 1 Band Size	Expected species based on ITS-1
640bp	*T. congolense savanna*
	T. congolense forest
560bp	*T. congolense*
430bp	*T. brucei*
380bp	*T. vivax*

(A)



(B)



Parasitemia and survival analysis of hedgehog days post infection with *T. congolense* (Fig: 4.1a) while (Fig.4.1b) shows the parasitemia and survival analysis of mice days post infection with *T. congolense*.

4.3 Assessment of *in vitro* trypanolytic activity of hedgehog serum and plasma

As shown in (Figure 4.2 and 4. 3). There was significant ($p<0.05$) increase trypanolytic activity by crude serum 10× when compared with the standard (DMA) and PBS after one hour of incubation, however in crude plasma dilutions, DMA was shown to have significant ($p<0.05$) increase in trypanolytic activity when compared with the plasma dilutions and PBS. Similarly when both 1000× and 1× concentrations of serum and plasma dilutions were compared in (Figure 4.4 and 4.5), it was observed that serum dilutions were found to have significant ($p<0.05$) increase in trypanolytic activity when compared with the plasma dilutions after one hour of incubation.

4.4 Bioassay Guided Fractionation

The result indicated that three fractions from ion exchange chromatography (fractions 2, 5 and 6) were the most active as shown in figure 4.5 because they have high trypanolytic activity against *T. congolense* at lower protein concentrations. Similar results were observed from gel filtration chromatography figure 4.6 were 4 fractions (fraction 4-7) were observed to be the most active fractions, because they also show high trypanolytic activity at lower protein concentrations.

4.5 Characterizations of Trypanolytic Factor

The results found in plate 4.2 indicates that the trypanolytic factor is a protein with a molecular mass of 110KDa as was observed to be present in all the active fractions of both ion exchange and gel filtration chromatography.

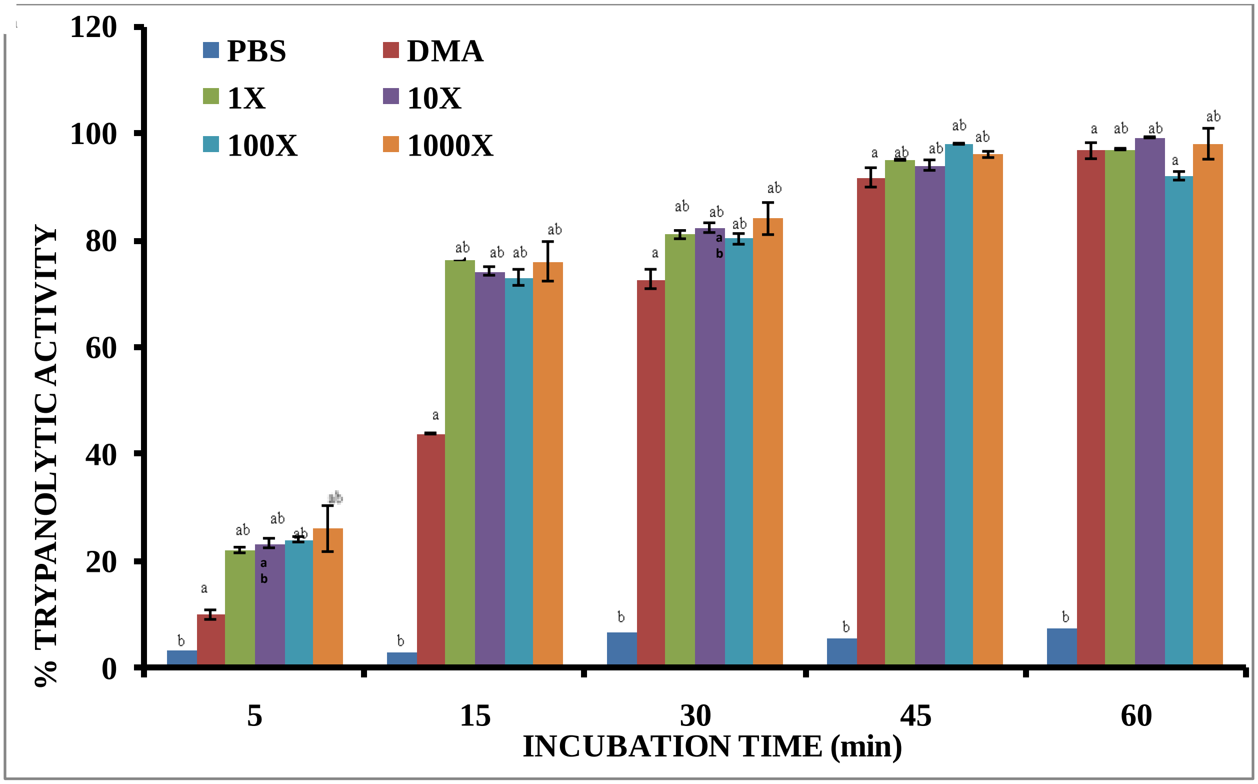


Figure 4.2: *In vitro* trypanolytic activity of hedgehog crude serum against *Trypanosoma congolense* after one hour of incubation**.**

KEY: PBS = Phosphate Buffered Saline DMA = Diminazene Aceturate

Note that a indicates significant difference (P<0.05) with DMA while b indicate significant difference with PBS (P<0.05), ab indicate significant difference to both a and b.

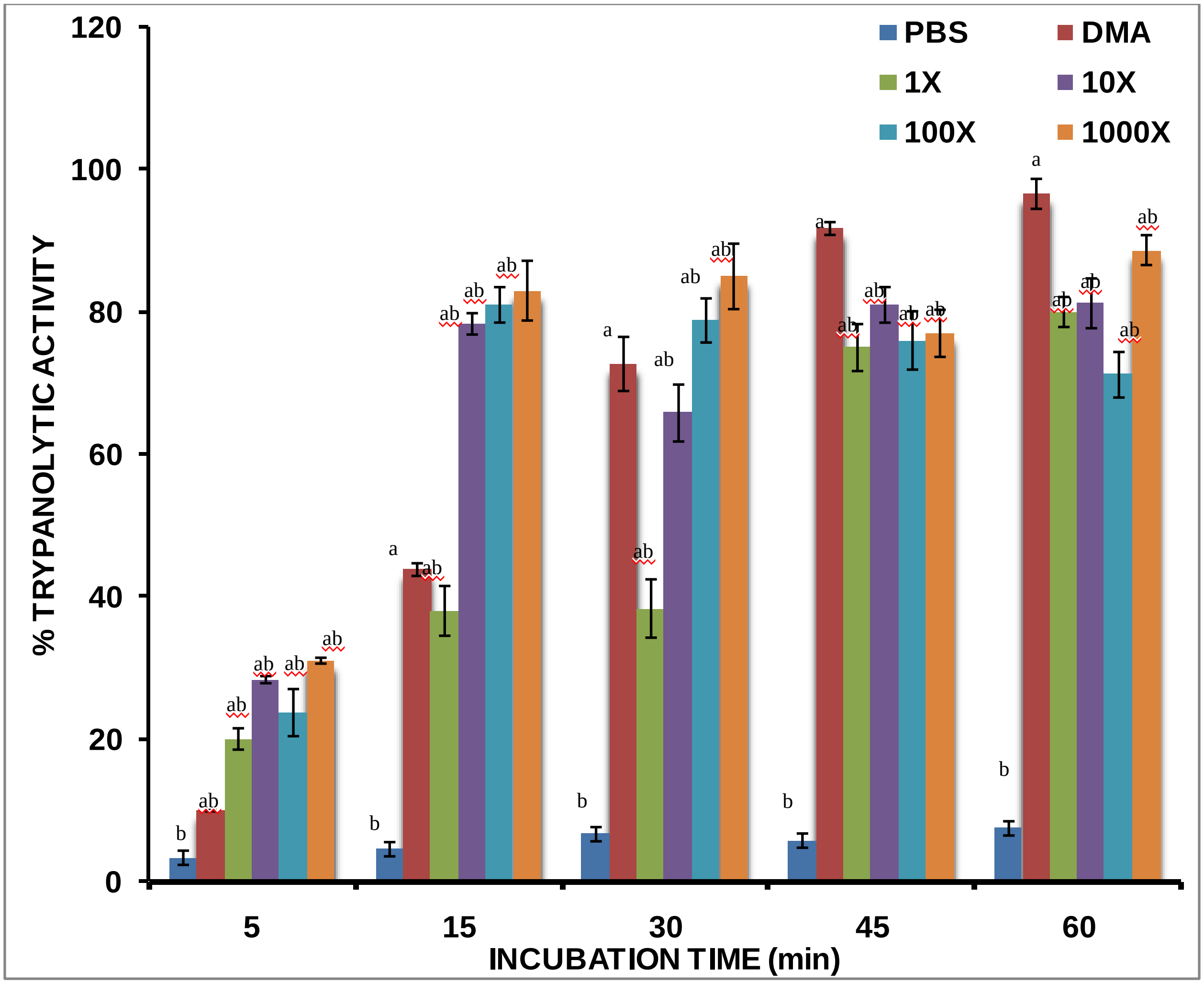


Figure: 4.3 *In vitro* trypanolytic activity of hedgehog crude Plasma dilutions against *Trypanosoma* congolense after one hour of incubation

KEY: PBS = Phosphate Buffered Saline DMA = Diminazene Aceturate

Note that a indicates significant difference (P<0.05) with standard while b indicate significant difference with PBS (P<0.05), ab indicate significant difference to both a and b.

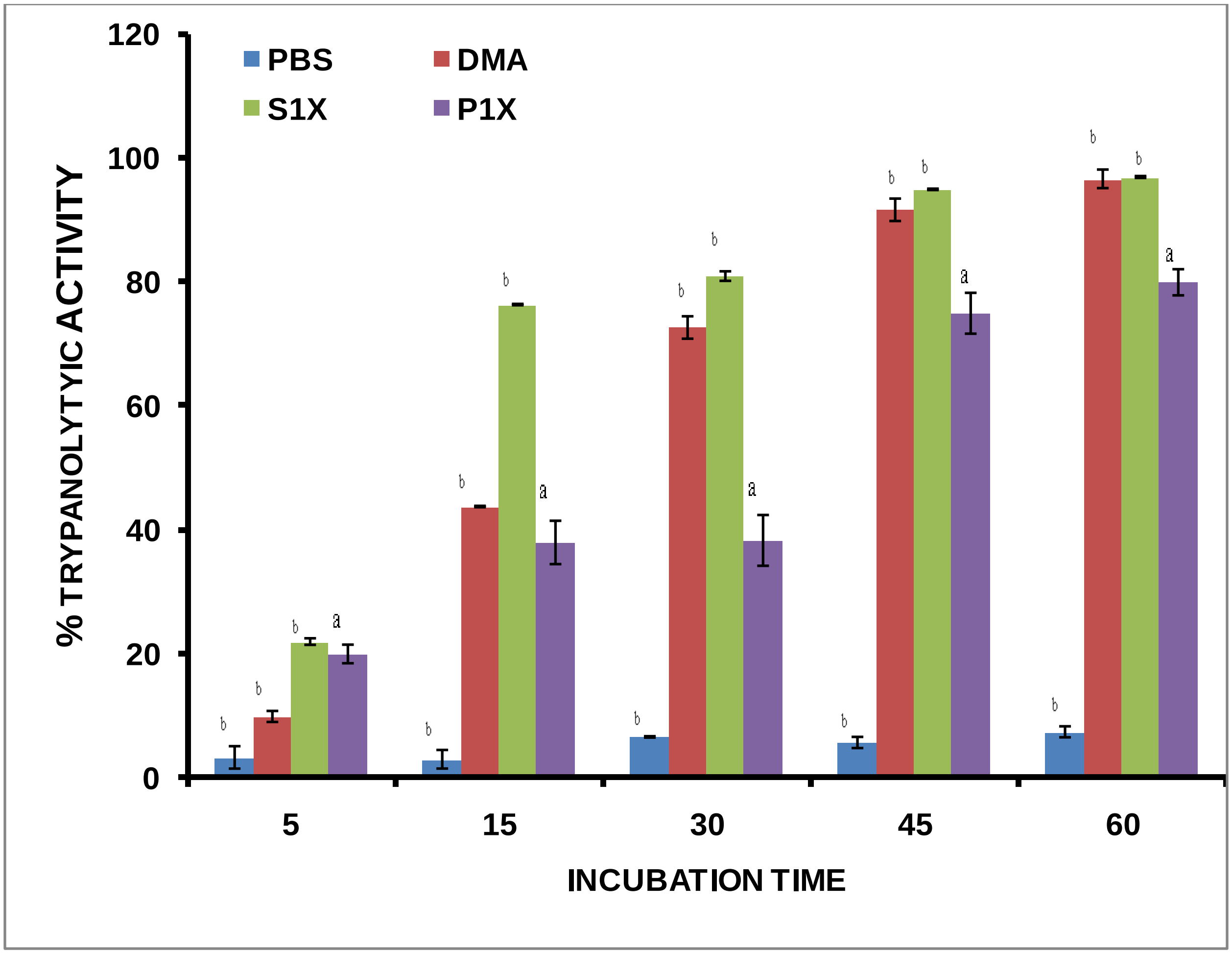


Fig: 4.4 Trypanolytic activity of the highest concentrations (1×) of both serum and plasma after one hour of incubation.

KEY PBS = Phosphate Buffered Saline DMA = Diminazene Aceturate

Note that a indicate significant difference ($P<0.05$) with serum 1× while b indicate significant difference ($P<0.05$) with plasma 1×, ab indicate significant difference to both a and b

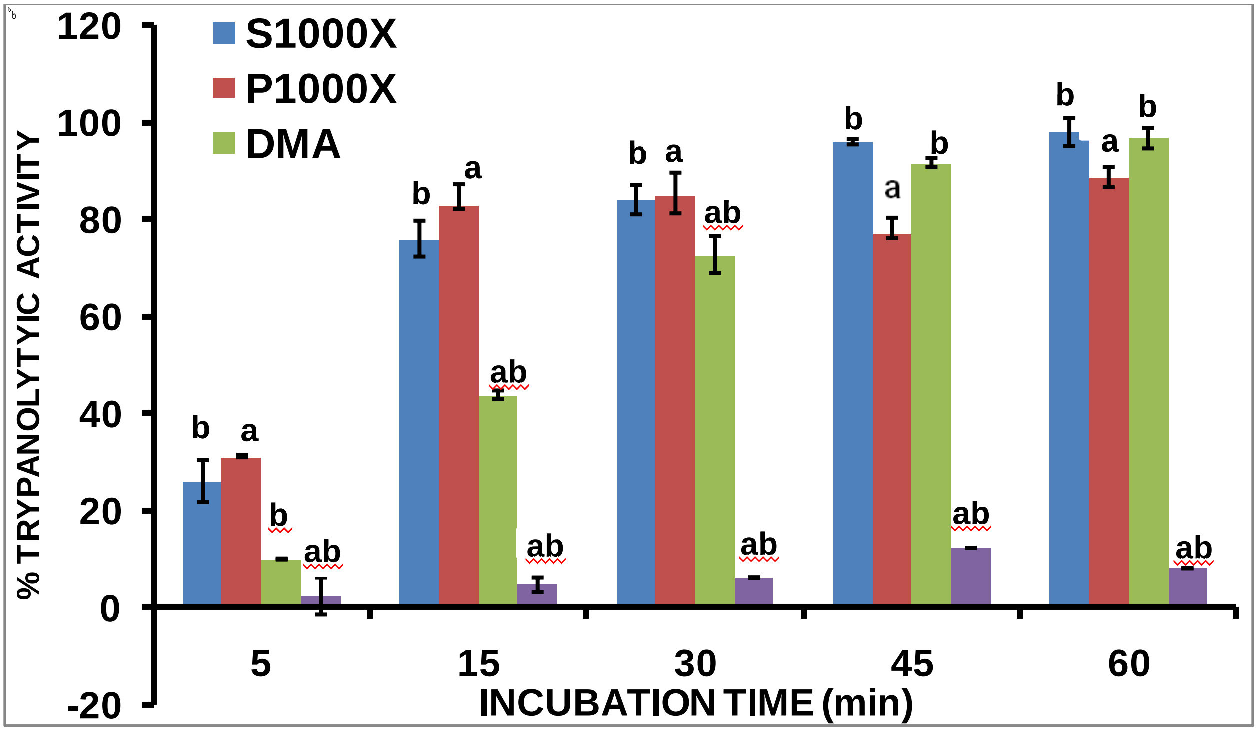


Fig. 4.5: Trypanolytic activity of the lowest concentration (1000×) of both serum and plasma

Note that a indicate significant difference (P<0.05) with serum 1× while b indicate significant difference (P<0.05) with plasma 1×, ab indicate significant difference to both a and b

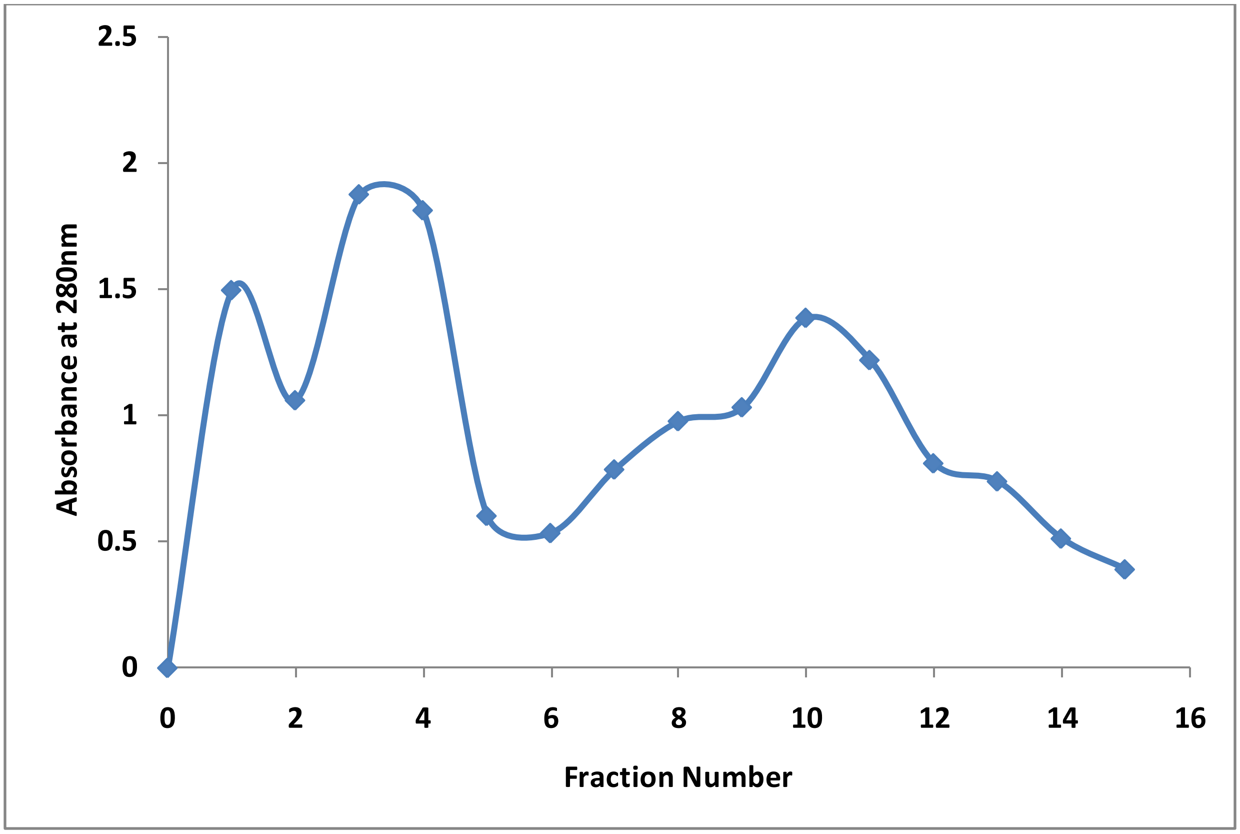


Figure 4.6: Ion exchange chromatography peaks on DEAE Cellulose column eluted with five concentrations (0.05-0.25M) of potassium phosphate buffer at flow rate of 1.0 ml/min.

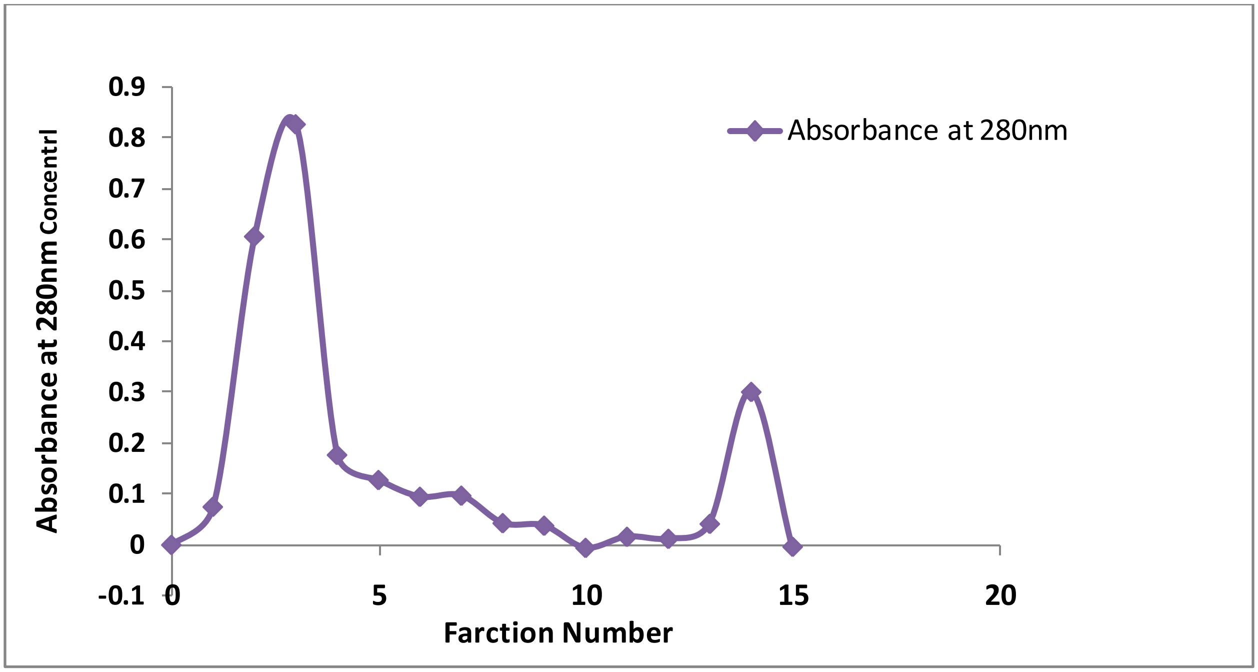


Figure 4.7: Gel filtration chromatography peaks on Sephadex G-50 column eluted with potassium phosphate buffer at flow rate of 1.0 ml/min.

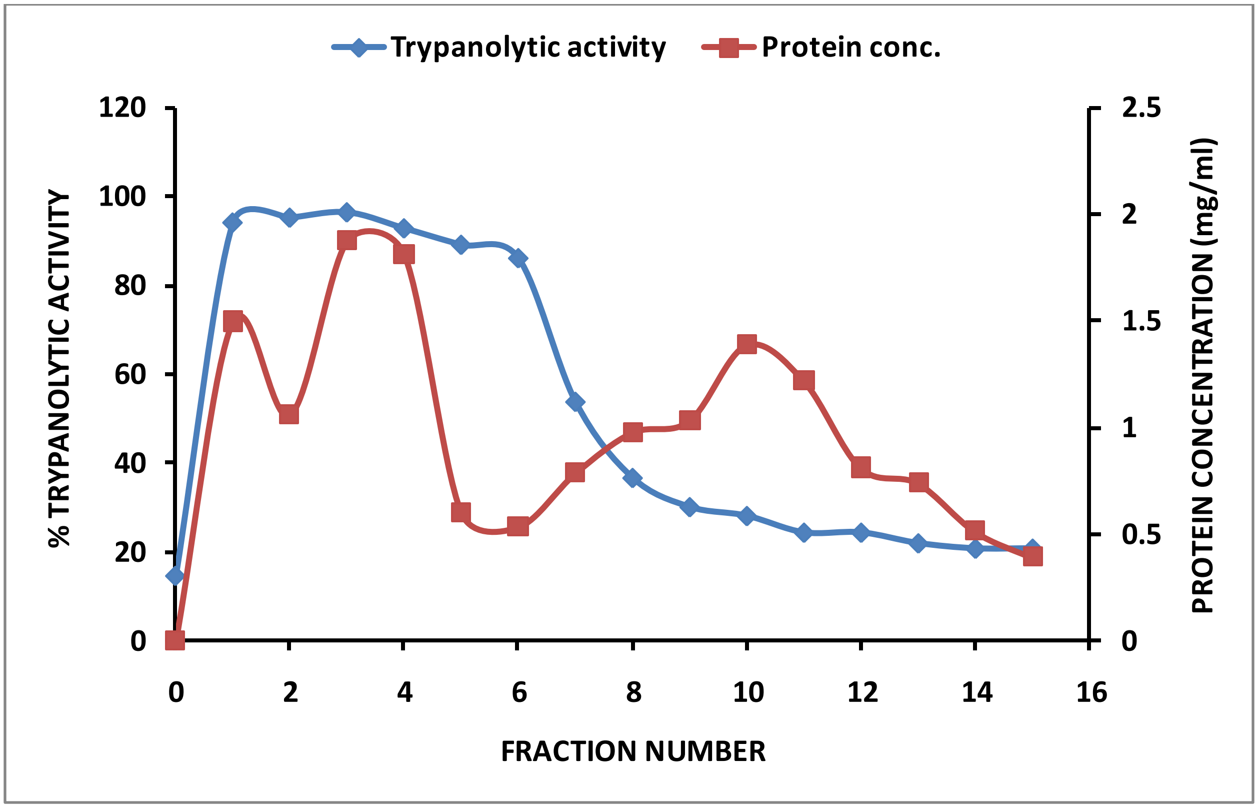


Figure 4.8:Elution profile of Crude Serum on Ion Exchange Chromatography (DEAE Cellulose) (15 × 1.5 cm). This shows the protein concentrations and biological activity of the fractions against *T. congolense* after 60 minutes of incubation

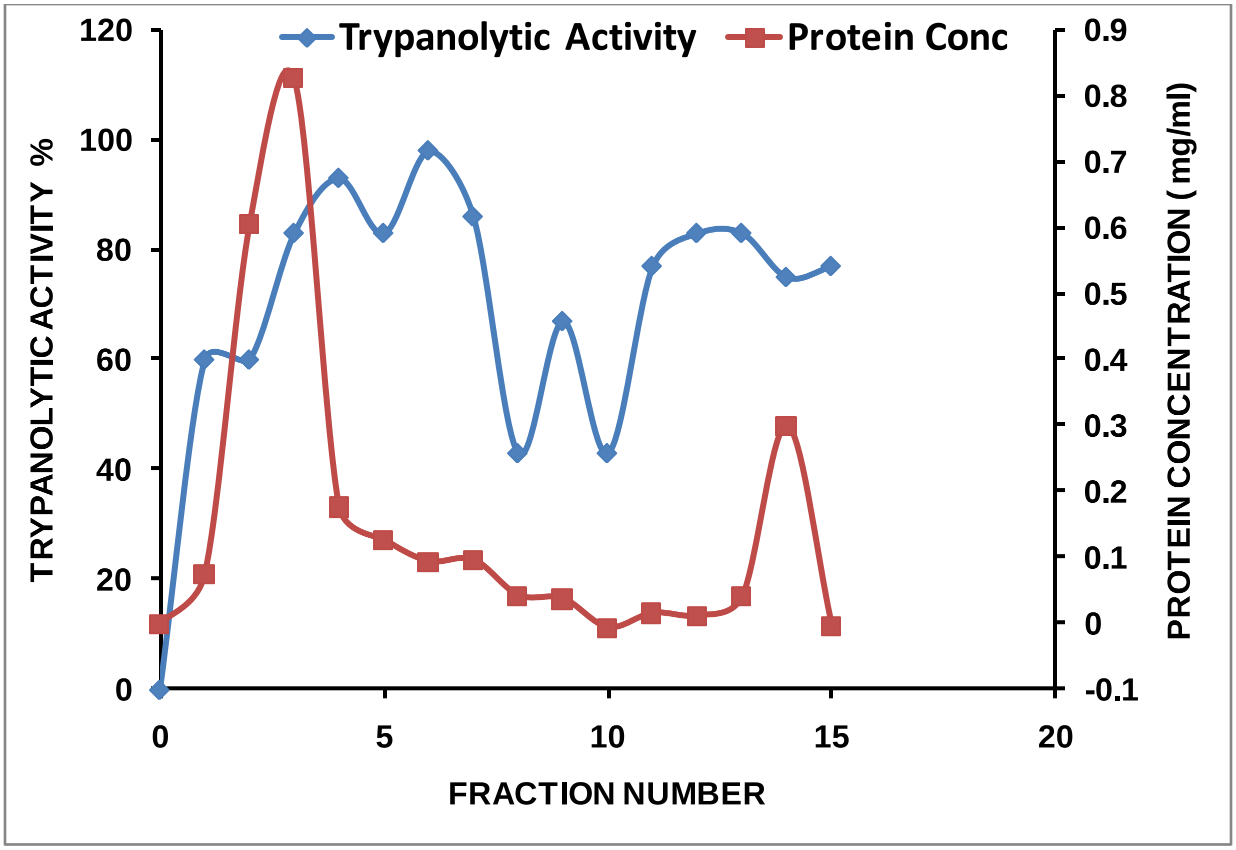


Figure: 4.8 Elution profile serum fractions on gel filtration (Sephadex G-50) column chromatography (15 × 1.5 cm). This shows the protein concentrations and biological activity of the fractions against *T. congolense* after 60 minutes of incubation.

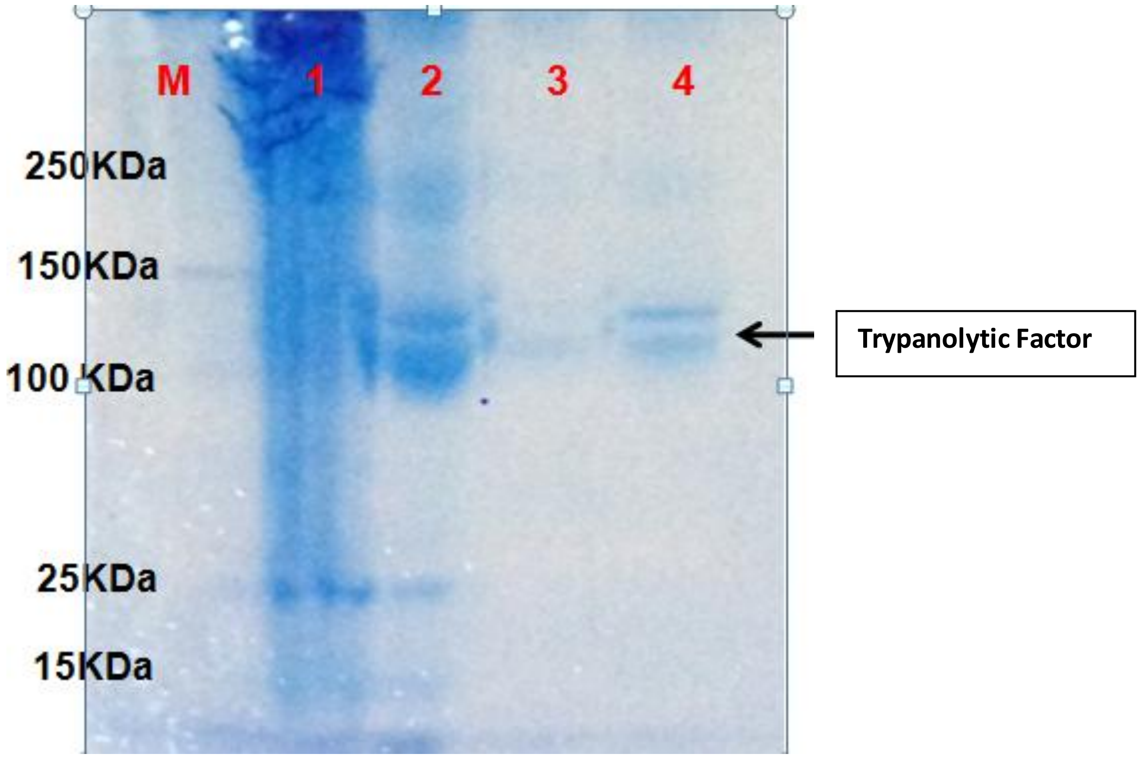


Plate: 4.2 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Electrophoregram under non reducing condition showing the characterized trypanolytic factor protein.

Lane M Marker, lane 1 Crude serum, lane 2 pooled fractions from ion exchange while lanes3 and 4 are eluates of gel filtration chromatography

CHAPTER FIVE

5.0 DISCUSSION

African Trypanosomosis remains an impediment to human and animal health and is responsible for considerable mortality and a major obstacle to socio-economic development of the rural areas afflicted. Available chemotherapies for the disease are unsatisfactory due to their toxicity and drug resistance by parasites. However, AAT is one of the major limitations to livestock production majorly in rural areas where the livestock farmers subsist about 1.24 USD per day (Perry, 2007). Therefore, control of AAT is imperative due to its massive impact on the livelihood of rural communities. Trypanosomes have been known to infect a wide variety of mammals; however some mammals are not susceptible to trypanosome infection, similarly it has been known for over a century that the serum of humans and other primates are highly toxic to most African trypanosome species (Cooper *et al.,* 2016). It was also reported that serum from Cape buffalo kills all *Trypanosome* species *in vitro* (Reduth *et al.*, 1994) or prevents their replication. This activity is absent in the serum of a wide variety of domestic animals (Reduth *et al.*, 1994).

PCR is known to posse's high sensitivity and specificity in term of parasite identification (Barticadors *et al.*, 2011). Therefore our findings confirmed the identity of the test parasite to be *Trypanosoma congolense kilifi* with 560bp. This is in agreements with the findings of Adams *et al.*, (2006) who reported that the band size from 560 to 640bp corresponds to *T. congolense* depending on the sub-species using internally transcribed space region ITS-1 primers. This PCR method has been used to improve sensitivity and detection of trypanosomes in animal blood as reported by (Salim *et al.*, 2014; Regassa *et al.,* 2016). The observation that trypanosome posses ITS-1 region of unique size and sequence provides independent support for its recognition as a separate species (Njiru *et al.,* 2004; Cox *et al.,* 2005). As a result of the introduction of molecular based taxonomic methods, three new species were found *T. congolense kilifi, T. godfreyi* and *T. simiae tsavo* all have ITS-1 regions of unique size and sequence that distinguished them from the other tsetse- transmitted trypanosomes (Ravel *et al.*, 2004).

The empirical knowledge that African hedgehog is resistant to animal trypanosome was validated in this study by experimental infection and the findings revealed that up to 14 days post infection no single parasite was detected and none of the hedgehogs were died, whereas the mice were all died at 12 days post infection following massive parasitemia. Several studies have shown that different mammals respond differently to trypanosome infection (Kaushik *et al.*, 2000; Chukunonso *et al.,* 2014). The reason for the hedgehog resistance to trypanosome infection could be attributed to the report by Janpeta *et al.*, (2010) that some mammals are genetically resistant to trypanosome infection both in natural and experimental conditions. Also, Sparrow *et al.*, (1995) reported that hedgehog possess apoA1 which is homologous to that of humans and human, apoA1 is confirmed to be part of the trypanolytic factors as reported by (Pays *et al.,* 2006; Philippe *et al.*, 2013).

These findings indicate that both the serum and plasma of African hedgehog are trypanolytic against *T. congolense* when compared with a standard control (DMA) after 60 minutes of incubation. It was also observed that 10× diluted serum had significant trypanolytic activity when compared with the standard (DMA). This result is similar to the findings of Vanhollebeke and Pays, 2010) who reported on the trypanolytic activity of human serum.

The trypanolytic activity is higher in serum than in plasma at all dilutions evaluated. This finding is in agreement with the findings of Madhavi *et al.,* (1997) who reported that both plasma and serum from Cape buffalo (*Syncerus caffer*) killed bloodstream stages of all species of African trypanosomes *in vitro.* The decrease in trypanolytic activity in plasma concentrations could be attributed to the use of EDTA as an anticoagulant when obtaining the plasma which could reduce the bioavailabity of the trypanolytic factor in the plasma. Also EDTA is known to chelate ions that could serve as cofactor to the protein that might likely be the trypanolytic factor as reported by Bartimaeus *et al.,* (2017). Similarly, (Guder, 2002; Raffick *et al.,* 2010; Oddoze *et al.,* 2012) reported that in terms of storage and stability, serum is more stable than the plasma once separated. This current results that shows hedgehog serum is trypanolytic corresponds with several reports that confirmed sera from human and some primate such as baboon, to be trypanolytic to most African trypanosomes(Vanhollebeke and Pays, 2010).

Bioassay guided fractionation was performed using ion exchange and gel filtration chromatography. Elution profile of ion exchange chromatography revealed that fractions 2, 4, 5 and 6 has the highest trypanolytic activity, this could be attributed to the fact that they have significant trypanolytic factors are in minute quantities although very potent. However, the gel filtration chromatography fractions 4-7 shown to be the most active, this findings is in agreement with the findings of (Freiburghaus *et al.*, 1998; Bizimana *et al*., 2006; Atawodi *et al.,* 2011) reported the use of bioassay guided fractionation in order to choose the most active fractions with trypanocidal effects.

In this current study the trypanolytic factor was found to be a protein with a molecular mass of 110kDa as revealed from SDS-PAGE under non reducing conditions. This finding is in agreement with the findings of Muranjan *et al.*, 1998 who reported the trypanolytic factor protein in human serum to be 110kDa size. The mechanism of trypanolytic activity in hedgehog serum is not fully understood but it is speculated to have the same mechanism with that of humans and other primates. The trypanolytic factors in human were shown to be associated with high density lipoprotein (HDL) particles (ApoA1, Hpr and ApoL-1).Vanhollebeke and Pays *et al.*,2010).

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CHAPTER SIX

6.0 CONCLUSION AND RECOMENDATIONS

6.1 Conclusion

Both Hedgehog serum and plasma had trypanolytic activity; however the serum displayed 10 to 20% higher significant trypanolytic potency than the plasma. The trypanolytic factor in hedgehog serum was partially purified. The protomer of the trypanolytic factor in hedgehog serum is a 110 kDa protein thus the information provided in this study may be exploited for the development of a new therapy for Trypanosomosis in future although its mechanism of action needs to be fully understood.

6.2 Recommendations

It is recommended that from this study, further studies to ascertain the trypanolytic activity of hedgehog serum against different species of trypanosome both *in vitro* and *in vivo* be carried out.

Also the amino acid sequencing of the trypanolytic factor in hedgehog serum and the identification of the gene for the trypanolytic factor is also suggested.

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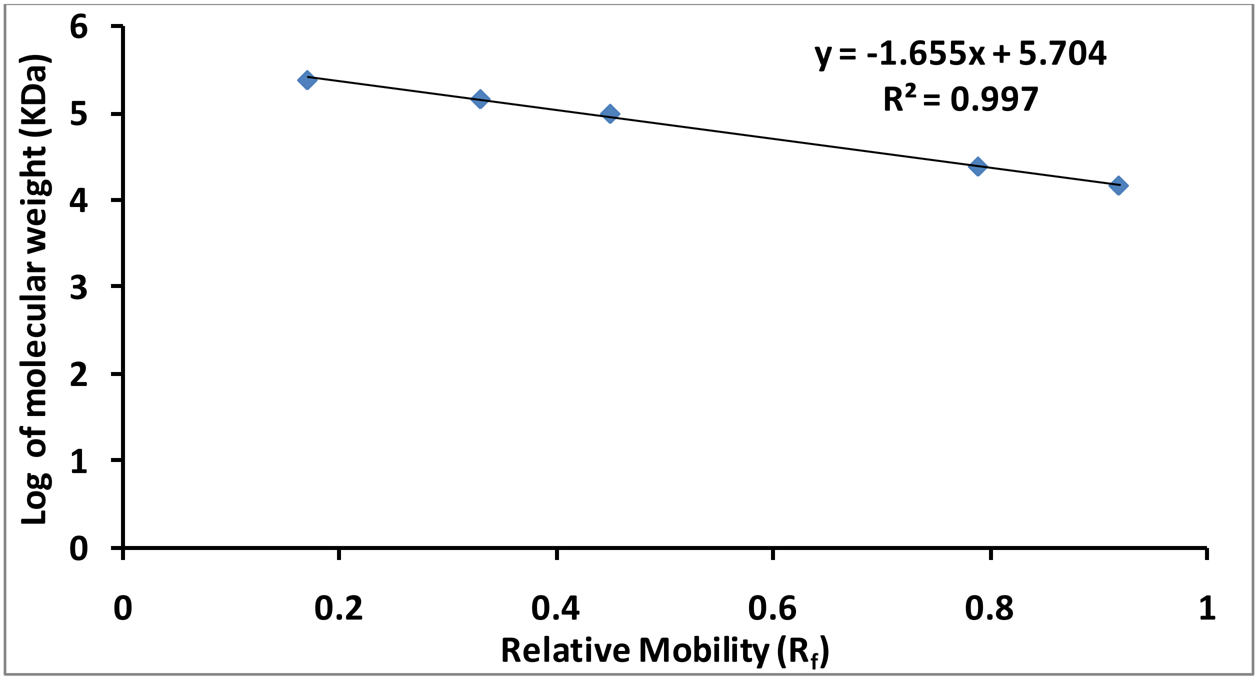
APPENDIX I



y = -1.655x + 5.704
R² = 0.997
Log of molecular weight (KDa)
Relative Mobility (Rf)
0
1
2
3
4
5
6
0
0.2
0.4
0.6
0.8
1

APPENDIX II

DEPARTMENT OF BIOLOGY
FACULTY OF LIFE SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

[illegible] February 2018

The Head,
Department of Biochemistry,
Ahmadu Bello University, Zaria.

RE: REQUEST FOR IDENTIFICATION OF SPECIMEN

With reference to your application dated 28th November 2017 requesting the identification of one specimen of [illegible] Hedgehog, deposited by Abubakar [illegible] ABDULLAHI (M.Sc. Biotechnology – [illegible]) at the Museum of Natural History, Department of Biological Sciences, I write to inform you that the specimen has been identified as follows:

1. **English Name:** Four-toed Hedgehog
2. **Order:** Erinaceomorpha
3. **Family:** Erinaceidae
4. **Scientific Name:** *Atelerix albiventris*
5. **Habitat:** Terrestrial and local distribution in and around Zaria, Nigeria

Thank you.

Yours,

Dr. Gadzama, Ibrahim Madu.

Department of Biology,
Ahmadu Bello University, Zaria.