EVALUATION OF ANTITRYPANOSOMAL EFFECT OF STEM-BARK EXTRACTS OF *SECURIDACA LONGEPEDUNCULATA* (FRES. HOLL) AGAINST *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN WISTAR RATS

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SEPTEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled Evaluation of antitrypanosomal effect of stem-bark extracts of *Securidaca longepedunculata* (Fres. Holl) against *Trypanosoma brucei brucei* infection in Wistar rats has been carried out by me in the Department of Veterinary Pharmacology and Toxicology. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other university.

Mohammad TAUHEED

| --- | --- | --- |
| Name | Signature | Date |

CERTIFICATION

This thesis, entitled EVALUATION OF ANTITRYPANOSOMAL EFFECT OF STEM-BARK EXTRACTS OF SECURIDACA LONGEPEDUNCULATA (FRES. HOLL) AGAINST TRYPANOSOMA BRUCEI BRUCEI INFECTION IN WISTAR RATS by Mohammad TAUHEED meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my beloved mother, Mallama Khadijah Muhammad.

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Glory be to Almighty Allah, Who has given me the strength, wisdom and sound health to enable mecarry out this work. May His peace and blessings be on prophet Muhammad (SAW), his household and his companions.

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ABSTRACT

Due to numerous problems associated with current treatment of trypanosomosis, there is increasing need to find effective drug against trypanosomosis ravaging sub-Saharan Africa from traditional medicinal herbs. This work is aimed at evaluating efficacy of stem-bark extracts of *S. longepedunculata* against *T. brucei brucei* infected Wistar rats. The *in vitro* study and drug incubation infectivity test were carried out in duplicates. One hundred microlitre of blood containing 30-35 parasites per field was mixed with 50 µl of extract at 3 mg/ml and 6 mg/ml of crude methanol, ethyl acetate and aqueous methanol extracts of *S. longepedunculata* and incubated at 37 ºC for 90 minutes. Similarly, diminazene aceturate, normal saline and parasite-laden blood served as controls. Motility of the parasite was monitored under microscope at 5 minutes interval throughout 90 minutes observation period. The mixtures were subsequently inoculated into rats that were not previously infected with trypanosomes. In the *in vivo* studies, 40 adult Wistar rats of both sexes were divided into eight groups of 5 rats each and were individually infected intraperitoneally (*i.p*) with 10^6*T. brucei brucei* per ml of blood. Animals in groups I and II were treated with crude methanol extract at 0.7 and 0.35 mg/kg *i.p*, groups III and IV were treated with ethyl acetate fraction at 0.7 and 0.35 mg/kg, while groups V and VI were treated with 0.9 and 0.45 mg/kg of aqueous methanol fraction, respectively. Animals in groups VII and VIII were treated with diminazene aceturate at 3.5 mg/kg and normal saline (2 ml/kg), respectively. Four animals in group IX were neither infected nor treated and served as neutral control. In the prophylactic studies, 25 rats of both sexes were divided into V groups of 5 rats each. Animals in groups I, II, and III were pre-treated with crude methanol extract at 0.35 mg/kg IP for 3, 5 and 7 days, respectively; while group IV received normal saline and served as

negative control. The animals were then individually infected with 10^6 parasites per ml of blood. Animals in group V served as neutral control. Phytochemical screening of the extracts revealed the presence of carbohydrates, cardiac glycosides, saponins, steroid, triterpenes, flavonoids and tannins. However, aqueous and ethyl acetate fractions were devoid of flavonoids. In the *in vitro* study, crude methanol extract immobilized parasite within 75 minutes while ethyl acetate and aqueous extracts induced slight reduction in motility at 90 minutes. However, inoculated rats developed infection and succumbed to the infection. The stem-bark extracts could not eliminate the parasites in all the groups tested. Nonetheless, crude methanol extract prolonged the survival period of rats when compared to other groups, though not statistically significant ($P<0.05$). Pre-treatment of animals with crude methanol extract before challenge with the parasite could not prevent infection. Thus, stem-bark of the plant could be said to possess trypanostatic activity against the parasite.

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LIST OF ABBREVIATIONS

µl	mircolitre
A	adenine
AAT	African Animal Trypanosomosis
AM	aqueous methanol
ANOVA	analysis of variance
B	*Babesia*
CM	crude methanol
DA	diminazene aceturate
EA	ethyl acetate
eg	for example
g	gram
H_2SO_4	tetraoxosulphate VI acid
ie	that is
i.m	intramuscular
i.p	intraperitoneal
i.v.	intravenous
kDNA	kinetoplast deoxyribonucleic acid

kg	kilogram
km^2	kilometre square
LD_{50}	median lethal dose
m	metre
mg	milligram
MIF	Migratory Inhibitory Factor
min	minutes
ml	millilitre
mm	millimetre
N	north pole
NC	normal control
NS	normal saline
RBC	red blood cells
S	south pole
S	*Securidaca*
SEM	standard error of mean
SLE	*Securidaca longepedunculata* extract

T	thymine
T. b.	*Trypanosoma brucei*
USA	United States of America
VSG	variable surface glycoprotein
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

Trypanosomosis is a debilitating disease of man, domestic and wild animals characterised by parasitaemia, pyrexia, anaemia, loss of condition, reduced productivity and high mortality (Losos and Ikede, 1972; Anosa, 1983; Murray *et al*., 1983; Nwosu and Ikeme, 1992; Igbokwe, 1994). In livestock, *Trypanosoma congolense, T. vivax* and, to a lesser extent, *T.brucei brucei* are the causal agents. An infection with one of the trypanosomes may result in a chronic, debilitating and often fatal disease (Connor and Van den Bossche, 2004).

The disease in domestic livestock has negative impact on food production and economic growth in many parts of the world, particularly in sub-Saharan Africa (Taylor, 1998). In regions where human African trypanosomosis is endemic, several other trypanosome species, including *T. vivax* and *T.congolense*, are prevalent which affect animal health and production (Piccozzi *et al*., 2002).

The greatest impact of the disease across the continent is on the nutrition of millions of people living in the most highly endemic areas and on the agricultural economies of their countries because it renders vast areas of semi-arid savannah land in Africa unsuitable for raising livestock that are the source of dairy and meat products (Donelson, 2003). It is estimated that over 60 million people (WHO, 1998), and 50-70 million animals are exposed to the infection (Geerts and Holmes, 1998; Geerts *et al*., 2001).

Control of trypanosomosis is aimed at the destruction of the arthropod vectors especially the insect; *Glossina species* and the elimination of the parasite in infected individuals by

treatment with trypanocidal drugs. Several chemical agents are currently available for the treatment and prophylaxis of trypanosomosis (Mbaya *et al*., 2007). The toxicity associated with the use of these drugs and the resistance developed against them by trypanosomes are some of the problems associated with the disease. Besides, the development of vaccine against the disease has not been successful (ILRAD, 1990; Murray *et al*., 1990). These factors coupled with the scarcity and high cost of available trypanocides has increased the need to exploit the use of alternative methods for the control of trypanosomosis (Asuzu and Chineme, 1990).

In traditional medicine practice in Nigeria and many countries in Africa, many plants provide very useful clues for potential anti-parasitic compounds. It is estimated that 66-85% of the world population depends directly on plants as medicines (Terry, 2000; Tagboto and Townson, 2001). Plants have a long history in medicine with a number of recorded successes, the most recent one being the anti-malarial, artemisinin which is obtained from the plant, *Artemisia annua* (Ogbadoyi *et al*., 2007). Nigeria is blessed with abundant medicinal plants and traditional medicine is officially recognised as an integral part of, or complementary to the nation's healthcare delivery system (Ogbadoyi *et al*., 2007).

Securidaca longepedunculata is widely used in African traditional medicine as a general remedy for several ailments such as cough, cold, fever, backache, toothache, sleeping sickness, venereal disease, malaria, inflammation, rheumatism, snakebite, tuberculosis, ulcers and pneunmonia (Watt and Breyer-Brandwijik, 1962; Neuwinger, 1964; Galeffi *et al*., 1990; Ojewole *et al*., 2001; Ajali and Chukwurah, 2004; Rakuambo *et al*., 2004; Maiga *et al*., 2005; Okoli *et al*., 2005; Lino and Deogracious, 2006; Dapar *et al*., 2007; Meyer *et al*., 2008; Pallant and Steenkamp, 2008). This has earned the plant a popular name in Hausa

language of Nigeria "*uwar magunguna*", meaning mother of all medicines (Adebiyi *et al*., 2006).Recent studies have reported the anti-microbial activities of the plant against pathogenic protozoa, bacteria, and fungi (Ajali and Chukwurah, 2004; Maiga *et al*., 2005; Lino and Deogracious, 2006; Pallant and Steenkamp, 2008). The alkaloid, securine obtained from the plant has activity against *Plasmodium falciparum*; the causative agent of malaria (Maiga *et al*., 2005).

The xanthones obtained from the root-bark have also shown promising result in the treatment of erectile dysfunction (Rakuambo *et al*., 2004; Marion Meyer *et al*., 2008). In addition, the root bark is used by farmers as a pesticide in stored grain (Belmain and Stevenson, 2001; Boeke *et al*., 2004; Steveson *et al*., 2009). The efficacy of this has been validated in laboratory studies (Jayasekara *et al*., 2005; Stevenson *et al*., 2009). Molluscicidal properties of the root extract has also been recently demonstrated (Olofintoye, 2010). The methanol, chloroform and aqueous extracts of the root of the plant showed trypanocidal activity against both *T. brucei* and *T. congolense* (Atawodi *et al*., 2003; Atawodi, 2005).

1.1 Statement of Research Problem

Tsetse-transmitted trypanosomosis is an important constraint to livestock development in sub-Saharan Africa with estimated direct annual losses to producers and consumers exceeding US$1 billion (Kristjanson *et al*., 1999; Simukoko *et al*., 2007). This estimate does not include losses due to unavailability of livestock manure and draught power in crop systems in areas where cattle cannot be kept because of the threat of trypanosomosis. (Taylor, 1998). Trypanosomosis is ranked among the top 10 global cattle diseases affecting livestock production in sub-Saharan Africa. (Perry *et al*., 2002).

Ten million square kilometres of land that could be fertile is not being used for arable farming due to the effect of the tsetse-transmitted disease. This region includes land in 32 of the world's poorest countries (Aderbauer *et al*., 2008). Within this region, some 46.62 million head of cattle and other livestock species are at risk of the disease (Swallow, 1998). Up to 80% of the African population still relies on traditional medicine for their primary health care. In traditional medicine, the use of medicinal plants plays an important role. Twenty-five percent of modern medicines are made from plants that were first used traditionally (WHO, 2003).

Currently, only three trypanocides are available for controlling tsetse-transmitted trypanosomosis in domestic ruminants. These are isometamidium and homidium which have both prophylactic and therapeutic effects, and diminazene aceturate which has only therapeutic properties. It is estimated that 35 million doses of these drugs are used in Africa annually for the control of the disease (Geerts *et al*., 2001).

Drug-screening activities from plants have started decades back, and an emerging number of studies have now been developed and reported so far to discover drugs from medicinal plants that can help to combat trypanosomosis. The main aim has been geared towards alternatives to conventional drugs with fewer side effects but greater effectiveness. A plethora of studies has been conducted to investigate the effect of some traditionally used medicinal plants in alleviating the cellular changes produced during *T. brucei brucei* infections of rats (Etet and Mahomoodally, 2012).

1.2 Justification

The prevalence of African trypanosomosis is now as high as it was in the 1920s and that, in the almost complete absence of large-scale vector control programmes or the prospect of a vaccine, the only available option is chemotherapy (Delespaux and de Koning, 2007). However, our chemotherapeutic armoury is outdated and blunted by overuse. Improvement in trypanosomosis control in Africa will carry a huge benefit, especially in animal production to areas where there is poverty and chronic malnutrition. Current control measures include the use of trypanocidal drugs and management of the vector, but resistance to trypanocidal drugs and pyrethroids combined with high costs reduce their success rate and sustainability (Naessens *et al*., 2002).

Recent surveys in Eastern and Southern Africa (Ndung'u *et al*., 1999) and in West Africa (McDermott *et al*., 1999) have shown that the prevalence of trypanocidal drug resistance might even be higher than hitherto suspected. Because of the relatively limited market in Africa and high costs of developing and licensing of new drugs, International pharmaceutical companies have shown little interest in the development of new trypanocides for use in either animals or humans (Geerts *et al*., 2001).

Ethnobotanically used plants in African medicine can be a good source of new drugs in the treatment of trypanosomosis (Aderbauer *et al*., 2008). Natural products are important sources of new drugs because their derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity (Sülsen *et al*., 2007).

Trypanocidal drug treatment will probably remain the mainstay of control of African bovine trypanosomosis in the foreseeable future, and development of resistance to the small number of available compounds is generally regarded as a cause of considerable concern (Geerts and Holmes, 1998). Most of the drugs in current use for the treatment of trypanosomosis are

closely related chemically (Peregrine and Mamman, 1993), so resistance to one drug often results in cross-resistance to others (Aliu, 2006),thus, necessitating the research into newer alternative drug for the treatment of trypanosomosis.

1.4 Aim

The aim of this research is to evaluate *in vitro* and *in vivo* anti-trypanosomal effects of the stem-bark extracts of *Securidaca longepedunculata* against *T. brucei brucei* experimental infection in Wistar rats.

1.4 Objectives

The specific objectives are therefore, to investigate:

- *In vitro* anti-trypanosomal effect of the stem-bark extracts of *Securidaca longepedunculata* against *T. brucei brucei*.
- Therapeutic anti-trypanosomal effect of the stem-bark extracts of *Securidaca longepedunculata* against *T. brucei brucei* infection in rats.
- Prophylactic activity of extract of stem-bark of *Securidaca longepedunculata* against *T. brucei brucei* infection in rats.

1.5 Null Hypothesis:

Extract of *Securidaca longepedunculata* does not have *in vitro* or *in vivo* anti-trypanosomal effect against *T. brucei brucei* experimental infection in rats.

CHARPTER TWO

LITERATURE REVIEW

2.1 Overview

African trypanosomosisis a disease of humans and livestock caused by several species of flagellated single-celled protozoan parasites belonging to the genus Trypanosoma. Cyclic forms of trypanosomes are transmitted from infected to uninfected animals by different species of tsetse fly during regular blood meals (Kuriasko *et al*., 2012).Tsetse fly-transmitted trypanosomosis is an important constraint to livestock development in sub-Saharan Africa with estimated annual losses owing to the direct and indirect effects of the disease exceeding US$1billion (Simukoko *et al*., 2007). The disease is found between latitude 14ºN and 29ºS covering 10 Million km² stretching across over 37 countries in Africa (Seifert, 1996; WHO, 1998; Mulumba, 2003). Approximately 9 million km^2 of land in sub-Saharan Africa, representing about one-third of the total land is endemic to tsetse flies that are vectors of the disease. Within this region, some 46–62 million head of cattle and other livestock species are at risk of the disease (Swallow, 2000). African trypanosomosis is responsible for 3 million livestock death and 55,000 people death annually in agriculture and mixed farming (ILRAD, 1990; Abenga *et al*., 2003; Mulumba, 2003). Trypanosomosis is controlled by strategic intervention against the vector or the parasite, or a combination of both. Over the years, a large arsenal of vector-control tools has been developed. Nevertheless, the control of animal trypanosomosis in poor rural communities has and will continue to rely heavily on the use of trypanocidal drugs. This is due to the ease of such treatments and the difficulties in maintaining cleared areas in the absence of barriers to re-invasion by tsetse flies. Only a small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use and new compounds is unlikely to become available in the near future (Barrett, 2003).

Chemotherapeutic agents used for the treatment of animal trypanosomosis include diminazene aceturate, isometamidium, homidium and suramin (Kuriasko *et al*., 2012). As a result of inappropriate trypanocidal drug-use practices, there is growing concern that the effectiveness of chemotherapy will be severely reduced by the widespread development of resistance in trypanosomes (Van den Bossche *et al*., 2000; Delespaux and de Koning, 2007).

In countries where sleeping sickness occurs, plants have been used traditionally for centuries and are still widely used to treat this illness and other parasitic diseases. It is estimated that two-third of the world population relies on traditional medical remedies due to the limited availability and high cost of pharmaceutical products (Tagboto and Townson, 2001). The recent search for new drugs for the treatment of African trypanosomosis is towards traditional medicinal plants. Plant drugs have been shown to contain biologically active ingredients (Mbaya *et al*., 2010). Furthermore, several well-known drugs, such as quinine and artemesinin used as antiprotozoan agents have their origin from plants (Kirby, 1996; Camacho *et al*., 2000; Tagboto and Townson, 2001). Very limited chemotherapeutic drugs against trypanosomosis exist and the few that are available have shortcomings such as high levels of toxicity to the host and perhaps most importantly increasing levels of treatment failure due to drug resistance (Teka *et al*., 2011). The insufficiency of current therapies for the treatment and management of trypanosomosis, combined with both conventional medical treatment and high cost of pharmaceuticals, have created a growing public interest in alternative natural drugs from botanicals (Etet and Mahomoodally, 2012). Thus, the development of cost-effective new drugs for the treatment of the disease is required for effective control of the disease.

2.2 Aetiology of Trypanosomosis

Trypanosoma congolense, T. vivax and T. brucei have been reported to cause *nagana* in cattle while *T. evansi* causes *surra* in camels (*Camelus dromedarieus*) (Mbaya *et al*., 2010). The disease in equine is caused by *Trypanosoma equiperdum*.(Teka *et al*., 2011).In humans, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodeseinse* are responsible for human sleeping sickness in West and East Africa respectively, while *T. cruzi*, transmitted by triatomid bugs (*Triatoma magista*) is responsible for transmitting chagas diseases to humans in South America (Solano *et al*., 2003). The *T. brucei* group of trypanosomes (*T. brucei*, *T. b. gambiense*, *T. b. rhodesiense* and *T. evansi*) mostly invade tissues (humoral) whereas, *T. congolense* and to a lesser extent *T. vivax* and *T. cruzi* predominantly are restricted to the blood circulation (haemic) (Igbokwe, 1994; Mbaya *et al*., 2011). The infection is known as sleeping sickness in humans, nagana in cattle, dourine in horses, and surra in camels and other high-value livestock such as buffalo (Teka *et al*., 2011).

2.3 Transmission of Trypanosomosis

Trypanosomosis is transmitted primarily by the bite of infected tsetse fly (*Glossina*) which is responsible for biological (cyclical) transmission while haematophagus arthropod vectors of the family, *Tabanidae*, *Stomoxynae* and *Hippoboscidae* are responsible for its mechanical transmission. *T. equiperdum* is transmitted sexually (Soulsby, 1982) Transplacental transmission of *T. brucei* has also been reported in cattle (Ogwu *et al*., 1992).

2.4 Antigenic Variation in Trypanosomosis

African trypanosomes are protozoan parasites that reside in the mammalian bloodstream asextracellular parasites and are constantly exposed to the host's immune system. As a result, African trypanosomes have developed sophisticated immune evasion mechanisms associated

with antigenic variation (Pan *et al*., 2006; Magez *et al*., 2008), excessive activation of the complement system leading to hypocomplementemia (Tomlinson and Raper, 1998; Pan *et al*., 2006), polyclonal B cell activation (Taylor *et al*., 1996) and immunosuppression (Tabel *et al*., 2008). Trypanosomes keep one step ahead of the immune system by continually switching from the expression of one variant surface glycoprotein (VSG) on their surface to the expression of another immunologically distinct VSG, a phenomenon called antigenic variation (Borst, 1991; Cross *et al*., 1998; Donelson, 2003). Antigenic variation is an immune evasion strategy that has evolved in viral, bacterial and protistan pathogens(Morrison *et al*., 2009). In the African trypanosome this involves stochastic switches in the composition of a VSG coat, using a massive archive of silent VSG genes to change the identity of the single VSG expressed at a time. VSG switching is driven primarily by recombination reactions that move it into specialized expression sites, though transcription-based switching can also occur (Morrison *et al*., 2009).

Antigenic variation is a result of complex interaction between the host immune responses and parasite survival strategies, which enable African trypanosomes to develop very sophisticated mechanisms to evade immune killing, survive in the chronically infected host and, hence, allow transmission to the next host, via the tsetse vector. VSG is a dense immunogenic surface coat (12–15 nm thick) of a single polypeptide species that completely surrounds the parasites (Cross, 1990; Borst and Rudenko, 1994) which shields invariant surface antigens from immune recognition. Moreover, trypanosomes constantly modify their VSG by the process of antigenic variation, resulting in the fluctuating waves of parasitaemia that characterizes African trypanosomosis (Van-der-Ploeg *et al*., 1992; Borst, 2002; Pays, 2006). Antigenic variation remains one of the most spectacular adaptive mechanisms exhibited by

African trypanosomes and is the central most important immune escape mechanism by these parasites (Cross *et al*., 1998; Barry and McCulloch, 2001; Borst, 2002).

By switching VSG genes and expressing a new variant antigenic type, trypanosomes evade B- and T-cell mediated immune responses (Mansfield and Paulnock, 2005). Furthermore, expression of VSG is central to the process of antigenic variation that eventually leads to exhaustion of the host immune system for the benefit of the trypanosome. With this phenomenon, parasites evade immune responses of mammalian hosts by: the population growth to avoid eradication by specific, potent immune responses (Barry and Carrington, 2004), and proliferation of this variant produces a new parasite population which in turn, is subject to new, specific immune responses (Wakelin, 1995). Regardless of whether they are mainly tissue-fluid dwelling (*T. brucei*), or blood dwelling (*T. congolense* and *T. vivax*), the level of trypanosomes in the blood fluctuates with time due to antigenic variation (Benjamin and Leskowitz, 1994; Barry and Carrington, 2004).

2.5 Anaemia in Trypanosomosis

Anaemia is an important symptom in animals suffering from trypanosomosis (Trail *et al*., 1992). The haematological changes of African trypanosomosis influence the pathogenesis of the disease. The disease is associated with a rapid decline in red blood cell counts, haemoglobin concentration and packed cell volume and pallor in mucous membranes in the infected hosts. The anaemia in livestock has been described as either normocytic normochromic, or macrocytic normochromic (Lorne, 1986).

The pathophysiology of anaemia in trypanosomosis is complex and multifactorial in origin (Naessens *et al*., 2005). It initiates a cascade of events leading to haemolytic anaemia and

cardiovascular collapse (Anosa, 1988). Most common among these factors are erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition (Murray and Morrison, 1978; Morrison *et al*., 1981; Saror, 1982; Igbokwe, 1994). The production of cytokines, such as interferon-c, interleukin 12, and tumor necrosis factor α are increased by *Trypanosoma brucei brucei* infection in the rat (Nishimura *et al*., 2006). These cytokines have an inhibitory effect on erythropoiesis in the bone marrow (McDougall and Cooper, 2002; Saini *et al*., 2006). Furthermore, macrophage migration inhibitory factor (MIF) was also increased in *T. brucei brucei*-infected rats (Nishimura *et al*., 2006) and MIF is reported to inhibit hematopoiesis of the bone marrow during malaria infection (McDevitt *et al*., 2006). Some reports suggested that proliferation of African trypanosomes in blood vessels causes fragility of red blood cells which can result in haemolytic anaemia (Esievo *et al*., 1982; Igbokwe, 1994; Mabbott and Sternberg, 1995; Stijlemans *et al*., 2008).

Trypanosomes produce a potent enzyme, sialidase (Esievo, 1979; Esievo, 1983; Nok and Balogun, 2003) which cleaves off the membrane surface of sialic acid of erythrocytes. This exposes the galactose of the cell membrane (Janick *et al*., 1978) which then binds to lextin on the surface of kupffer cells and other macrophages with resultant erythrophagocytosis (Kuster and Schauer, 1981; Muller *et al*., 1983; Kelm *et al*., 1986). Esievo and Saror (1991) reported that binding of trypanosome antigen to the surface of erythrocytes results in sequestration of these red blood cells (RBC) in the reticuloendothelial system; thus contributing to anaemia. The anaemia in trypanosomosis occurs in two distinct phases, though overlapping. The initial phase corresponds to the first parasitaemia with the packed cell volume dropping markedly and splenomegaly that later leads to destruction of RBC (Behnke, 1990). The second phase (clinical phase) is mediated by toxic substances released by the trypanosomes after their

destruction in the circulatory system. There is damage to the lining of blood vessels at this phase (Witola and Lovelace, 2001). There is no significant drop in RBC at this stage but there is marked haemosiderosis of the spleen, liver and bone marrow (Murray *et al*., 1983).

2.6 Epidemiology of Trypanosomosis

The epidemiology of trypanosomosis and its impact on livestock production varies from one area to another and depends largely on the level of interaction between tsetse, domestic and game animals. The nature of this interaction is subject to spatial and temporal variations. Within the tsetse belts of southern Africa four distinct epidemiological situations can be distinguished: wildlife zones where livestock is absent, areas where livestock have been recently introduced into wildlife zones, areas where livestock are present at the edge of wildlife zones (interfaces) and areas where livestock are kept in tsetse-infested zones and where large game animals are absent (Van den Bossche, 2001). Areas where livestock are kept in a tsetse-infested zone and where livestock constitutes the major host of tsetse are of particular economic importance. Such an epidemiological circumstance is usually the consequence of the gradual encroachment of people and their livestock into tsetse-infested areas and the subsequent disappearance of large game animals as a result of human interference and the clearing of vegetation for cultivation (Simukoko *et al*., 2007).

2.7 Economic Importance of Trypanosomosis

African animal trypanosomosis (AAT) is a parasitic disease that causes serious economic losses in livestock from anaemia, loss of condition and emaciation. Many untreated cases are fatal. AAT is found mainly in those regions of Africa where its biological vector, the tsetse fly, exists (Anon, 2013). Trypanosomosis in domestic livestock has negative impact on food production and economic growth in many parts of the world, particularly in sub-Saharan

Africa (Taylor, 1998). The greatest impact of the disease across the continent is on the nutrition of millions of people living in the most highly endemic areas and on the agricultural economies of their countries because it renders vast areas of semi-arid savannah land in Africa unsuitable for raising livestock that are the source of dairy and meat products (Donelson, 2003). It is estimated that over 60 million people (WHO, 1998), and 50-70 million animals are exposed to the infection (Geerts and Holmes, 1998; Geerts *et al*., 2001).

2.7.1 Direct economic losses

The disease has direct impact on livestock productivity, reducing meat and milk production by 20%, calving rate by 20% and increases calf mortality by 20%.The disease decreases both lambing and kidding rates in sheep and goat, and livestock management especially the number of livestock kept by farmers, the breed and species composition of the livestock herd, the way the livestock are grazed, cost of trypanocidal drugs and cost of insecticides (Swallow, 2000). It also has direct impacts on human settlement in a considerable part of sub-Saharan Africa including Nigeria (Takele and Abebe, 1988). The occurrence of trypanosomosis in Nigeria has directly affected the productivity of livestock population especially cattle, sheep, goats and pigs (Samdi *et al*., 2010).

2.7.2 Indirect economic losses

Indirect impact of trypanosomosis mostly lies on crop production through the availability and cost of animals that provide traction power (Swallow, 2000). Animal trypanosomosis reduces work efficiency of Oxen for cultivation, reducing access to animal traction or discourages the introduction of drought animals into crop farming (Omotainse *et al*., 2004). Evaluation on effect of trypanosomosis incidence on the productivity of oxen used for traction showed that relative inefficiency in the high risk area was 38% less efficient than oxen in the low risk area

(Leak *et al*.,1995; Swallow *et al*., 1995). The risk is most serious in the sub-humid zone and wetter parts of the semi-arid zone, which hold the continent's greatest potential for agricultural expansion (Anon, 2013).

2.8 Control of Trypanosomosis

The control strategies over the years have been directed against both the parasites and the vectors. These include surveillance and treatment, use of trypano-tolerant breeds and vector control (Seed, 2000). The main approach to controlling the parasite in the host has been by chemotherapy and chemoprophylaxis (Onyiah, 1997). Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries (Geerts and Holmes1998). Current control measures depend on the application of relatively new, environmentally friendly insecticides including synthetic pyrethroid, daltamethrimand cypermethrim (Seed, 2000). A more recent control measure involves the use of sterile insect technique (Seed 2000; Kamuanga, 2003).

2.9 Chemotherapy of Animal Trypanososmosis

Over the years, chemotherapy of animal trypanosomosis has depended largely on drugs belonging to the four principal classes: the aromatic diamidines, *eg*, diminazene and pentamindine; the phenanthridine, *eg*, homidium; the phenanthridine aromatic amidine, *eg*, isometamidium and the quinoline pyrimidine, *eg*, quinapyramine (Aliu, 2006).

2.9.1 Quinapyramine

Quinapyramine is soluble in water and has been used as a curative at a dose of 5 mg/kg intramuscularly (Davey, 1957). A prosalt of quinapyamine in a mixture of dimethylsulphate and chloride in 3:2 w/w confers prophylaxis for up to 6 months, depending on the degree of

challenge (Robson, 1958). Quinapyramine acts by reducing the growth rate of flagellated protozoans leading to suppression of the parasites to a level sufficient enough for the defence mechanisms of the body to control the infection (Newton, 1980). It also interferes with ribosomal functions, protein synthesis and polyamine function (Bacchi, 1981).

2.9.2 Phenanthridines

Phenanthridines came to limelight following a report by Ehrlich that the activity of trypanosan was due to the presence of an acridine compound present in the material as an impurity (Williamson, 1962). This lead to the synthesis of phenidium chloride against *Trypanosoma congolense* (Browning *et al*., 1938). However, phenidium exhibited delayed photosensitization and has since been replaced with the less toxic homidium with curative activity and isometamidium with curative and prophylactic activities (Aliu, 2006). Phenanthridines act by binding avidly to kinetoplast DNA and interfere with glycosomal functions and replication of kinetoplast minicircles. They may also cleave kDNA-topoisomerase complexes, giving rise to dyskinetoplastic trypanosomes (Aliu, 2006).

2.9.3 Diminazene aceturate

2.9.3.1 *Description*

Diminazene aceturate is an aromatic diamidine developed from "congasin" and other aminoquinaldines that were active against trypanosomes and babesias (Fussgänger and Bauer, 1962). Diminazene was first developed in 1944 (Fussgänger, 1995). It has significant quantitative differences from other diamidines, presumably resulting from the nature of the bridge that joins the two benzimidazine rings in the molecule (Hawking, 1956). The diaceturate is more soluble in water. Diminazine acetruate is N-acetylglycine compound chemically described as 4-4 (diazoamino) dibenamidine diaceturate. It is highly soluble in

water, poorly soluble in alcohol and only sparingly soluble in ether or chloroform (Wien, 1943).

2.9.3.2 *Mechanism of action*

Diminazene aceturate (Berenil®) has been widely used as a chemotherapeutic agent for trypanosomosis in livestock since 1955. The main biochemical mechanism of Berenil's trypanocidal actions is thought to be via its binding to kinetoplast DNA (Uzonna *et al*., 1998) thereby inducing complete and irreversible loss of kDNA in certain strains of trypanosomes (Riou and Benard, 1980; Homeida *et al*., 1981). Due to its molecular structure, Berenil has particular affinity for A-T base pairs in circular DNA and kinetoplast DNA (Mahler and Perlman, 1973; Gonzalez *et al*., 1997 and Uzonna *et al*., 1998).

2.9.3.3 *Spectrum of activity*

Diminazene aceturate is the most widely used therapeutic drug against trypanosomosis in food animals (Peregrine and Mamman, 1993). Besides its efficacy against trypanosomes, diminazene is highly effective against babesia species (Taylor *et al*., 1956). Intramuscular injection of 3-8 mg/kg was effective in controlling *B. bigemina, B. bovis* and *B. divergens* infections in cattle (Kuttler, 1981). *B. caballi* is sensitive to treatment with diminazene aceturate when given at 5 mg/kg twice within 24-hour interval (Kirkham, 1969). For *B. gibsoni* however, higher dose of 10 mg/kg is required but with attendant toxicity. Therefore, two doses of 3 mg/kg body weight 24 hours apart were effective against *B. canis* and *B gibsoni* in dogs and were devoid of toxic effects. However, administrations of the same dosage regimen to 3-week-old puppies were associated with acute toxicity in some animals (Harvey *et al*., 1988). These results suggest that maximum tolerated dose of diminazene is

reduced in young animals. The diminazene administered *i.m* has a fast initial phase followed by an intermediate second phase of distribution (Aliu *et al*., 1993).

2.9.3.4 *Mechanism of resistance to diminazene*

Treatment of many infectious diseases is under threat from infectious agents developing resistance against drugs (Bridges *et al*., 2007). Cellular uptake of melaminophenyl arsenical compounds and diamidines classes of trypanocide has been shown to occur via the P2 adenosine/adenine transport activity in *T. brucei* bloodstream forms, and loss of P2 has been implicated in drug resistance (Carter and Fairlamb, 1993; Carter *et al*., 1995). *T. brucei* AT1/P2 purine transporter is crucial for the accumulation of diamidine to attain and maintain therapeutic concentration. (Carter and Fairlamb, 1993; M□ser *et al*., 1999; de Koning, 2001; Delespaux and de Koning, 2007). Loss of TbAT1/P2 alone was shown to be sufficient for high levels of diminazene aceturate resistance in *T. brucei brucei* and *Trypanosoma evansi* (de Koning *et al*., 2004; Witola *et al*., 2004).

2.9.3.5 *Side effects and toxicity*

Most human trypanocidal compounds have low margin of safety (Peregrine and Mamman, 1993). Diminazene lowers blood pressure for a few minutes when administered intravenously to dogs and cats. This may result from peripheral vasodilatation (Wien, 1943). A fall in blood pressure was not observed after intramuscular injection (Van Heerden, 1995). Parenteral administration of diminazene aceturate more often than not resulted in acute clinical signs, with vomiting and diarrhoea being the consistent signs observed (Naude *et al*., 1970). Intramuscular administration of rabbit resulted in erythema formation after 24 hours which was cleared within 8 days. Bleeding and malacia in the mesencephalon and diencephalon were the predominant post-mortem lesions (Gilbert, 1983). Treatment of healthy dogs with

15 mg/kg of diminazene intramuscularly induced brain lesions similar to that seen in dogs that died from natural infection of babesiosis (Losos and Crockett, 1969). Tremors, itching, sweating, convulsions, dyspnea, recumbency and vomiting in camels (Riou and Benard, 1980) and decreased blood pressure (Joubert *et al*., 2003) and diarrhoea in dogs have been observed. Miller (2005) reported that the highest tolerated dose of diminazene aceturate in healthy dogs was 20 mg/kg and 12.5 mg/kg for intramuscular and intravenous administration, respectively.

2.10 Strategic Use of Trypanocidal Drugs

To circumvent the disturbing challenges of trypanocidal drug resistance, certain strategies for efficient use of available trypanocides have been developed, which include:

2.10.1 Monitoring and treatment of infected animals

This involves the use of standard parasitological methods, *eg*, wet mount, haematocrit centrifugation technique or buffy coat technique to ascertain clinical status of the animal. Thereafter the infected animals are treated with therapeutic trypanocide (Holmes *et al*., 2004).

2.10.2. Monitoring and treatment of clinical cases

Treatment is instituted in this case when the packed cell volume is subnormal or when the clinical signs of the disease are evident. The rationale for this is to prevent interference with the passive immunity acquired from dam (Holmes *et al*., 2004).

2.10.3 Routine block treatment

The principle of *sanative pair* is employed in this method. All the animals in the herd or a group of valuable or at-risk animals are treated at predetermined intervals with prophylactic drugs based on the plasma half-life of the drugs (Whiteside, 1962).*Sanative pair* involves administration of homidium for 6 months during the rainy season (May-October) and then switch diminazene for another 6 months during the dry season (November-April). Alternatively, diminazene can be used until resistant strains emerge and homidium is introduced (Aliu, 2006).

2.10.4 Strategic block treatment

All the animals in a herd or group of valuable or at-risk animals are treated after challenge at predetermined threshold (Holmes *et al*., 2004).

2.11 Role of Medicinal Plants as Potential Sources of new Drugs

2.11.1 Prospect of medicinal plants as source of alternative drugs

Interest in plant extracts exhibiting antimicrobial activity has increased in recent years. The use of and search for drugs derived from plants have accelerated in recent times(Matthews *et al*., 1999; Hamdan and Afifi, 2004; Ravi Kumar *et al*., 2011),whereby ethnopharmacologists, botanists, microbiologists, and natural-product chemists are combing the earth surface for phytochemicals and "leads" which could be developed for the treatment of various ailments (Etet and Mahomoodally, 2012). WHO (2005; 2007) has estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. It is obvious that modern pharmacopoeia still contains at least 25% drug derived from plants, and many others, which are synthetic analogues, built on prototype compounds isolated from plants (Hostettmann, 1998; Mahomoodally *et al*., 2005).

Despite the availability of different approaches for the discovery of therapeuticals, natural plant products still remain as one of the best reservoirs of new structural types. Concurrently, many people in developing countries have begun to turn to alternative therapies as cheap sources of complex bioactive compounds and evidence of the beneficial therapeutic effects of these medicinal herbs is seen in their continued use (Patwardhan *et al*., 2005; Rangasamy *et al*., 2007; Bent, 2008; Ranilla *et al*., 2010). In addition to the important pharmacologic or chemotherapeutic effects of medicine of natural products origin, they also serve as template molecules for theproduction of new drug molecules.

Knowledge gained from the use of medicinal herb sand their active ingredients has served as the foundation for much of modern pharmacology, and many modern drugs have their origin from ethnopharmacology. The development of modern chemistry has permitted the isolation of chemicals from medicinal herbs which have served as drugs or starting materials for the synthesis of many important commercially important compounds used today (Patwardhan *et al*., 2005; Pieters and Vlietinck, 2005). Drugs such as aspirin, digitalis, morphine, metformin, and quinine amongst others were all originally isolated or synthesized from materials derived from plants (Yeh *et al*., 2003; Goldman, 2001).

2.11.2 Plants with antitrypanosomal effects

Previous research work showed the activity of plants against trypanosomes (Atawodi *et al*., 2003; Aderbauer *et al*., 2008; Mbaya *et al*., 2010; Habila *et al*., 2011; Mann and Ogbadoyi, 2012; Ogbadoyi *et al*., 2011).Similarly, extracts of *Hymenocardia acida* stem bark exhibited significant trypanocidal activity. *In vitro* evaluation of extracts of *Khaya senegalensis*, *Piliostigma reticulatum*, *Securidaca longepedunculata*, and *Terminalia avicennoides* showed trypanocidal activity against *T. brucei and T. congolense*, while *in vitro* study of extracts of

Anchomanes difformis, *Cassytha spp*, *Lannea kerstingii*, *Parkia clappertioniana*, *Striga spp*, *Adansonia digitata*, *Gardenia erubescens*, *Lophira lanceolata* and *Prosopis africana* were trypanocidal to either *T.b. brucei* or *T. congolense*. *Kigelia africana*, was efficacious against *T. brucei*, *T. congolense* or both. Also, dichloromethane fruits extract of *K. africana* was effective against *T.b. rhodesiense*-infected mice (Atawodi *et al*., 2003; Abu *et al*., 2009; Peter *et al*., 2009).

2.11.3 Putative antitrypanosomal mechanism of action of phytochemicals

Traditional medicinal plants, unlike conventional drugs, sometimes contain several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined action of these substances increases the activity of the main, medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary substances from plant origins might increase the stability of the active compound(s) or phytochemicals, minimize the rate of undesired side effects, and have an additive, potentiating, or antagonistic effect (Mahomoodally *et al*., 2005; 2010). The composition of these secondary metabolites in turn varies from species to species, climatic conditions, and the physiological state of developments of the endemic plants (Hussain and Deeni, 1991). Available reports tend to show that alkaloids and flavonoids are the responsible compounds for the antimicrobial activities, and antitrypanosomal effects in higher plants (Cordell *et al*., 2001).

Plant parts differ significantly in their activity. The differences observed in the antimicrobial evaluation suggest the susceptibility of the test microorganism to various secondary metabolites present in these medicinal plants. In general, discussions pertaining to anti-trypanosomal agents from plants centre on plant secondary metabolites, that is, nonubiquitous

constituents with no known essential role in the plant's metabolism. However, it has been postulated that bioactive plant secondary metabolites may play a role in chemical defense mechanisms and are the likely molecules possessing antiparasitic activities in these plants (Mahomoodally *et al*., 2005; 2010).

It has been suggested that different phytochemicals could be responsible and operate in a synergistic effect for the observed antitrypanosomal activities. Interestingly, preliminary phytochemical screening of potent plants against trypanosome showed the presence of biological known active compounds such as saponins, tannins, flavonoids, and alkaloids in the crude plant extracts tested. Several authors have also identified or isolated tannins and phenolic compounds, flavonoids, and alkaloids in plants that showed significant trypanocidal activities (Atawodi *et al*., 2011). Accumulated evidence also suggest that many natural products exhibit their antitrypanosomal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defences against oxidative stress. On the other hand, Freiburghaus *et al*. (1996) indicated that different solvent extracts of the same plant may exhibit different trypanocidal activity just as extracts of different parts of the same plants.

2.12 *Securidaca Longepedunculata*

2.12.1 Botanical description

Securidaca longepedunculata (Fresen.) Polygalaceae is a shrub of 8-9 m height with sweetly scented violet flowers, alternate leaves of variable sizes and shapes and pale grey smooth bark. It is common in the savannah woodland from Senegal to Northern and Southern Nigeria, and is generally widespread in tropical Africa (Booth and Wickens, 1988; Burkill, 1997). It is commonly called violet tree (English). Flowers are abundant at the beginning of

rainy season. They are rather small, 10 mm long, pink to lilac or purple, sweetly scented, on long slender stalks produced tin beautiful profusion in terminal axillary sprays 3-5 cm long, appearing with the very young leaves. The fruit is more or less a round nut, somewhat heavily veined, occasionally smooth, bearing a single, oblong rather curved, membranous wing up to 4 cm long; purplish-green when young, becoming pale, straw-coloured when mature (Orwa *et al*., 2009).

2.12.2 Nomenclature

The generic name comes from a Latin word securis, meaning hatchet, referring to the shape of the nut with its curved membranous wing; and longepedunculata which refers to the long peduncle (Anon, 2012).

2.12.2.1*Common names*

The plant is commonly called violet tree or fibre tree or rhodesian violet in English; uwar magunguna orsanya in Hausa; ipeta orkyiritoo or ofodo in Yoruba; ezeogwu ortshi-venda or mpesu in Igbo and Jechi in Nupe (Mann *et al*., 2003; Anon, 2012).

2.12.3 Ecology

S. longepedunculata is found in a wide range of climates, from subtropical, hot and arid climate to summer rainfall and equatorial humid. It is resistant to bush fires and is frost sensitive. It is attractive to birds, butterflies and insects especially when in flower (Orwa *et al*., 2009).

2.12.4 Conservation status

S. longepedunculata is a threatened and protected species. The violet tree is used for medicinal purposes and other uses in rural areas. What makes it so threatened is the fact that roots are the target for people using this plant, which makes it difficult for the plant to survive constant harvesting (Anon, 2012).

2.12.5 Traditional medicinal uses

Neuwinger (1964) reported that the roots, stem bark, and leaves of *S. longepedunculata* are used medicinally for treating a plethora of human and animal ailments in Nigeria, and some other African countries. The roots and bark are taken orally either powdered or as infusions for treating chest pain, headache, inflammation, abortion, ritual suicide, rheumatism, tuberculosis, infertility, venereal diseases and constipation (Neuwinger, 1996; Van Wyk and Gericke, 2000). The powdered roots are reportedly rubbed on scarification marks made on the forehead to treat headaches, and roots are chewed to relieve toothache; powdered root of the plant has been applied externally to wounds and sores, and around the joints for relief of rheumatism (Watt and Breyer-Brandwijk, 1962). In north western states of Nigeria, fresh pungent root bark is reportedly applied topically over the incision with sharp object at the site of snake bite or scorpion stings, and the roots are also used to treat cold and meningitis (Hambali, 1990). *S. longepedunculata* has been used traditionally for the management of pain, inflammation, epilepsy and other ailments. (Bodeker, 1994; Neuwinger, 1996; Burkill, 1997).

2.12.6 Pharmacological effects

In vitro study of methanol root extract was effective against *T. brucei brucei* (Atawodi *et al*., 2003). Also, *in vitro* result of petroleum ether, chloroform, methanol and aqueous extracts of

stem-bark and root-bark of *S. longepedunculata* exhibited trypanocidal effect against *T. brucei brucei*. The root extract of the plant has been shown to exhibit antinociceptive, anticonvulsant, anxiolytic, antidepressant and sedative effects in mice (Adebiyi *et al*., 2006; Adeyemi *et al*., 2010). Marion-Meyer *et al*. (2008) reported the efficacy of acetone extract of *S. Longepedunculata* against erectile dysfunction. The root- bark extract of the plant reduced the number of *Sitophilus zeamis* and *Callosobrochus maculate* in infected maize and cowpea 9 months post-treatment (Stevenson *et al*., 2009). Jayasekara *et al*. (2005) reported that the extract of the root powder of *S. longepedunculata* exhibited repellent and toxic effect against *S. zeamais* adults.

2.12.7 Bioactive metabolites

Phytochemical investigations of the root bark of *S. longepedunculata* revealed the presence of flavonoids, alkaloids, saponins, triterpenoids and volatile oils (Ekpendu*et al*., 2000). Other secondary metabolites like sapogenins; presenegenin, indole alkaloid; securinine and some ergot alkaloids have also been reported to be present in the root of this plant (Van Wyk and Gericke, 2000). Also, Prista and Correira (1958) isolated methyl salicylate from the root of the plant. Dugan *et al*. (1964) obtained the aglycone, presenegenin. Costa *et al*. (1992) isolated ergot alkaloids as psychoactive compounds from the root of the plant. Elymoclavine and dehydroelymoclavine were identified along with a novel ergoline alkaloid A. Mahmood *et al*. (1993) showed the presence of 3,4,5-tri-*o*-caffeoylquinic acid, 4,5-di-*o*-caffeic acid and sinapic acid from the roots and demonstrated that 3,4,5-tri-*o*-caffeoylquinic acid has anti-HIV activity. Meli Lannang *et al*. (2006) recently isolated securidaca xanthone A. Securine, an alkaloid was isolated from the stem-bark of the plant (Kogan *et al*., 1968). Odebiyi (1978) and Kamwendo *et al*. (1985) reported the presence of saponines, tannins anthraquinones, sterols and terpenes in the plant.



Plate I: *Securidaca longepedunculata*



Plate II: Stem-bark of *Securidaca longepedunculata*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the Experiment

The experiment was carried out at the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

3.2 Plant Materials

3.2.1 Plant collection and identification

Fresh stem-bark of *Securidaca longepedunculata* was collected in Zaria, Nigeria. The leaves, flower and seeds of the plant were sent to the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. It was identified and a voucher number 900213 was allotted for reference purpose. The identified stem-bark was dried to constant weights under shade in an open air in the laboratory. The dried sample was kept in polythene bags until required for preparation of the extract.

3.2.2 Plant extraction and concentration

One kilogram of the dried stem-bark was ground to powder and 790 g of the pulverised form was extracted with absolute methanol in soxhlet. The liquid extracts were pooled together and concentrated to dryness on a water-bath (HH-S McDonal Scientific International, USA).

3.3 Fractionation

About 72.99 g of the methanol extract was dissolved in 40 ml of distilled water and then made up to 300 ml with distilled water. The solution was transferred to 1 L separating funnel and 600 ml of ethyl acetate was added. The funnel was agitated gently and then allowed to

stand for 8 hours undisturbed. The tap was then gradually opened and the lower denser aqueous portion was collected into a conical flask. The remaining upper portion was dispensed into a clean conical flask to obtain the ethyl acetate fraction. The procedure was repeated two more times and ethyl acetate fractions were pooled together. The remaining aqueous portion is termed aqueous methanol. Similarly, the liquid fractions were pooled together and concentrated to dryness over a water bath (HH-S McDonald Scientific International, USA).

3.4 Phytochemical Screening

The extracts of *S. longepedunculata* was evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes using standard procedures (Trease and Evans, 1983).

3.4.1 Test for Carbohydrates (Molisch's test)

Five drops of molisch reagent was added to a 9 ml of extract in a test tube and 3 ml of concentrated sulphuric acid was allowed to run down the side of the test tube. A lower purple colour was formed at the interface, indicating presence of carbohydrates.

3.4.2 Test for cardiac glycosides (Kella-killiani test)

Zero point one gram of extract was dissolved in glacial acetic acid containing traces of ferric chloride. With the test tube held at an angle of 45 degree, 1 ml of concentrated sulphuric acid was added down the side. A purple ring colour was observed at the interface, indicating presence of cardiac glycosides.

3.4.3 Test for free anthraquinone (Borntrager's test)

Fifteen ml of the extract was shaken with 10 ml of benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. No colour change was observed, indicating absence of anthraquinone.

3.4.4 Test for saponins (Frothing test)

One gram of the extract was dissolved in 10 ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb was formed which persisted for up to 30 minutes indicated presence of saponins.

3.4.5 Test for steroids and triterpenes (Lieberman-Burchards test)

Three ml of acetic anhydride was added to 8 ml of the extract in a test-tube and 1 ml of concentrated sulphuric acid was added down the side of the test-tube. The colour change was observed immediately and after 3 min. Red and blue-green colours were observed, respectively, indicating presence of steroids and triterpenes, respectively.

3.4.6 Test for flavonoids (Shinoda test)

Zero point one gram of extract was dissolved in 2 ml of 50% methanol in the heat. Metallic magnesium and four drops of concentrated hydrochloric acid were added. An orange colour was observed, indicating presence of flavonoids.

3.4.7 Test for tannins (ferric chloride test)

Zero point one gram of extract was dissolved in 10 ml of distilled water, and then filtered. 4 drops of ferric chloride solution were added to the filtrate. Formation of a green precipitate was observed, indicating presence of tannins.

3.4.8 Test for alkaloids (Meyer's test)

Four drops of Mayer's reagent was added to sample of the extract in a test tube. A cream precipitate was observed which indicated absence of alkaloids.

3.5 Thin Layer Chromatography

The thin layer chromatographic plate was cut into 6 cm wide and 10 cm tall. A horizontal line was drawn 2 cm from the base which was divided into 5 spots of 1 cm apart with pencil for inoculation of crude, ethyl acetate, and aqueous extracts, respectively. A solution of each extract was prepared for inoculation onto the plate. With the aid of a capillary tube (one for each extract), one drop of extract was inoculated onto each spot already labelled on the plate. At the top of each plate, the plate was properly labelled with the solvent system used. Meanwhile, chromatographic tank was rinsed with respective solvent to be used and then filled with 10 ml of the solvent. It was allowed to stand for 30 min for the tank to get saturated. The prepared spotted plates were inserted into the tank until solvent move to a predetermined point, 7 cm from the horizontal line on the plates; after which they were removed and air dried. The plates were placed in fume cupboard and sprayed with 10% H_2SO_4. Another set of plates was prepared as above and sprayed with $FeCl_3$.

3.6 Experimental Animals

The rats were obtained from the Animal House, Department of Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, and were maintained on commercial grower poultry feed, maize bran (*dusa*) and groundnut cake (*kuli-kuli*) compounded in a ratio 3: 2: 1. Water was provided *ad libitum*. The animals were kept in clean iron cages at room temperature throughout the study. The beddings were changed twice in a week.

3.7 Acute Toxicity Studies of the Extracts

The determination of the median lethal dose (LD_{50}) of extracts was carried out by the method described by Lorke (1983) using rats. In the initial phase, 9 rats of both sexes were randomly divided into three groups of 3 rats each. Groups 1, 2 and 3 were treated intraperitoneally with crude methanol extract at 10, 100 and 1000 mg/kg, respectively. The rats were then observed over 72 hours for signs of toxicity and mortality. The procedure was repeated for ethyl acetate and aqueous methanol fractions. In the second phase of the study, 3 rats for each extract were assigned into 3 groups of 1 rat each. The animals were individually administered 2, 6, and 8 mg/kg of crude methanol extract and ethyl acetate fraction, while animals for aqueous methanol fraction were administered 6, 8, and 10 mg/kg.

All animals that died during the experiment were autopsied *in-situ* grossly for any lesion. The liver, kidneys, heart, lungs spleen and brain were removed and samples were taken for histopathological examination.

3.8 Test Organism

Trypanosoma brucei brucei was obtained from Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The parasite was maintained in the laboratory by continuous passage in rat until required. Each cycle of passage was done when parasitaemia was in the range of 35 – 40 parasites per field, which corresponded to an interval of 6 days-post infection in rats. For several passages, about 3 ml of blood was obtained from an infected rat by cardiac puncture after light chloroform anaesthesia into 5 ml syringe and emptied into a vial containing 9 ml of normal saline (NS). About 1×10^6 parasites in 0.2 ml blood/PSS solution was injected intraperitoneally into a rat previously unexposed to trypanosomal infection.

3.9 Determination of Parasitaemia in Experimental Rats

Parasitaemia was monitored daily in blood obtained from tail vein by nipping the tail and a drop of blood was placed on a clean microscope slide, covered with cover slip and observed under a light microscope at ×400 magnification. The number of parasites was determined microscopically at ×400 magnification using the "Rapid Matching" method of Herbert and Lumsden (1976). The method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with NS. Logarithm values of these counts were matched by those on the table and were converted to antilog to provide absolute number of trypanosomes per ml of blood.

3.10 *In Vitro* Screening

In vitro antitrypanosomal screening test was performed in duplicates with 48-well microtitre plates using the rapid matching method (Herbert and Lumsden, 1976) and the drug incubation infectivity test (Vincent *et al*., 2008). Briefly, 100 µl of blood containing 8.1 $\times 10^6$trypanosome was mixed with 50 µl of extract solutions of 3 and 6 mg/ml *Securidaca longepedunculata* extracts (SLE), respectively. The mixture was rocked gently and then incubated in an incubator at 37 ˚C for 5 minutes. Similarly,100 µl of blood containing 8.1 $\times 10^6$trypanosome was mixed with diminazeneaceturate 10 µg/ml and NS (50 µl); and blood (100 µl)containing trypanosome only served as treated, negative and untreated controls, respectively. At the end of incubation, 2 µl of individual test mixture was placed on microscope slides and covered with cover slips and observed under a light microscope at ×400 magnification. The parasites were observed at 5 minutes interval for a total duration of 90 minutes. A reduction in motility or cessation of the parasites in SLE-treated blood compared to that of untreated control was taken as a measure of antitrypanosomal activity.

3.11 Drug Incubation Infectivity Test

About 0.2 ml of the concentration of the extract that was observed to have immobilised the parasites was taken into a clean 1 ml insulin syringe with a 25 gauge needle and inoculated into 2 rats each not previously infected with the trypanosome. Those extracts and concentrations that neither reduced the motility of nor immobilised the parasites at the end of 90 minutes observation were also inoculated *i.p* into 2 rats each not previously exposed to trypanosomal infection. The rats were observed daily for development of parasitaemia for a period of 3 weeks.

3.12 *In Vivo* Therapeutic Screening

A total of 44 rats of both sexes were used for this study. Forty of them were infected *i.p* each with 10^6*T. brucei brucei* and then observed daily for development of parasitaemia. Forty trypanosome-infected rats were divided into 8 groups of 5 rats each. One tenth and one twentieth of LD_{50} were used, respectively for evaluation. Rats in groups I and II received the crude methanol extract at 0.7 and 0.35 mg/kg, respectively, while rats in groups III and IV were administered ethyl acetate fraction of the extract at 0.7 and 0.35 mg/kg, respectively. Similarly, rats in groups V and VI received 0.9 and 0.45 mg/kg of aqueous methanol fraction of the extract, respectively, while animals in groups VII and VIII were treated with diminazene aceturate (3.5 mg/kg) once and NS (2 ml/kg) and served as treated and untreated control animals, respectively. Four uninfected and untreated rats were set aside and served as neutral control animals. The treatments in the extract and NS groups were administered once daily for 4-7 days, *i.p*. Level of parasitaemia and temperature were monitored daily for a period of 21 days. *In vivo* evaluation of the extracts is shown in table 3.1 below.

Table 3.1: *In vivo* therapeutic evaluation of *S. longepedunculata* extracts against *T. brucei brucei* in rats

Drug	Group	N	Dose (mg/kg)	Route of administration	No of trypanosome
Crude methanol	1	5	0.7	Intraperitoneal	10^6*T. brucei brucei*
	2	5	0.35		
Ethyl acetate	3	5	0.7	Intraperitoneal	10^6*T. brucei brucei*
	4	5	0.35		
Aqueous methanol	5	5	0.9	Intraperitoneal	10^6*T. brucei brucei*
	6	5	0.45		
Diminazine aceturate	7	5	3.5	Intraperitoneal	10^6*T. brucei brucei*
Normal saline	8	5	2 ml/kg	Intraperitoneal	10^6*T. brucei brucei*
Neutral control	9	4	Nil	Nil	Nil
Total			44		

N= Number of rats

3.13 *In Vivo* Prophylactic Study

Twenty-five rats of both sexes were randomly divided into five groups of 5 rats each. All animals in groups 1, 2, and 3 received the crude methanol extract at 0.7 mg/kg intraperitoneally and treatment was administered for 3, 5, and 7 days for groups 1, 2 and 3, respectively, while animals in group 4were administered NS and served as untreated control. Animals in each group were then challenged intraperitoneally with 10^6*T. brucei brucei* and observed for parasitaemia. Rats in group 5 were neither infected with trypanosomes nor treated and served as neutral control. The summary of prophylactic study is shown in table 3.2 below.

Table 3.2: Prophylactic study of *S. longepedunculata* extracts against *T. brucei brucei* in rats

Drug	Group	N	Dose (mg/kg)	Duration of treatment (Days)	Route of administration
Crude methanol	1	5	0.7	3	
	2	5	0.7	5	Intraperitoneal
	3	5	0.7	7	
Normal saline	4	5	2 ml/kg	7	Intraperitoneal
Neutral control	5	5	Nil	Nil	Intraperitoneal
Total		25			

N= Number of rats

3.14 Data Analysis

Data was expressed as mean ± standard error of mean (±SEM). Analysis of variance was used, followed by Tukey's post-hoc test for multiple comparisons of groups. Values of $p < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 Plant Yield

About 986 g of the pulverized stem-bark of *S. Longepedunculata* (SL) gave 109.49 g (13.9%) of crude methanol extract. Upon partitioning with different solvents, the crude methanol yielded 4.83 g(6.7%) and 18.44 g(25.4%) of ethyl acetate and aqueous methanol fractions, respectively.

4.2 Phytochemical Screening of the Extracts

Table 4.1: Phytochemical screening of the stem-bark extracts of *S. longepedunculata*

Extract	C	CG	Aq	S	ST	F	T	A
Crude methanol								
	+	+	-	+	+	+	+	-
Ethyl acetate								
	+	+	-	+	+	-	+	-
Aqueous methanol								
	+	+	-	+	+	-	+	-

Key: C =Carbohydrate, CG = Cardiac glycosides, Aq = Anthraquinone, S=Saponin, ST = Steroid and triterpene, F = Flavonoid, T = Tannins and A = Alkaloids
+ = present, - = absent

4.3 Acute toxicity Test

4.3.1 Median lethal dose (LD_{50}) of crude methanol extract

All animals administered with 10, 100 and 1000 mg/kg of crude methanol extract of *S. longepedunculata* died. In addition, rough hair coat, severe depression and anorexia were observed before the death of the rats. In the second phase, the rats survived at 6 mg/kg of crude methanol extract of *S. longepedunculata*. The LD_{50} was then computed as geometric mean of highest dose that did not kill the rat and lowest dose that killed the rat.

$$LD_{50} = \sqrt{6 \text{ X } 8}$$

$$LD_{50} = 6.9$$

$$\approx 7 \text{ mg/kg}$$

4.3.2 LD_{50} of ethyl acetate fraction

All animals administered with 10, 100 and 1000 mg/kg of ethyl acetate fraction of *S. longepedunculata* died. In addition, rough hair coat, severe depression and anorexia were observed before the death of the rats. In the second phase, the rats survived at 6 mg/kg of ethyl acetate of *S. longepedunculata*. The LD_{50} was then computed as geometric mean of highest dose that did not kill the rat and by lowest dose that killed the rat.

$$LD_{50} = \sqrt{6 \text{ X } 8}$$

$$LD_{50} = 6.9$$

$$\approx 7 \text{ mg/kg}$$

4.3.3 LD_{50} of aqueous methanol extract

All rats administered with10, 100 and 1000 mg/kg of aqueous methanol fraction of *S. longepedunculata* died within 72 hours of observation. The animals exhibited severe depression and anorexia prior to death. In the second phase, rats survived at 8 mg/kg of aqueous methanol fraction of *S. longepedunculata*and the LD_{50} was computed as thus:

$$LD_{50} = \sqrt{8} \text{ X } 10$$

$$LD_{50} = 8.94$$

$$\approx 9 \text{ mg/kg}$$

4.3.4 Post-mortem Findings

Post-mortem examination revealed congested liver and haemorrhagic lungs in rats administered the crude methanol extract, ethyl acetate fraction and aqueous methanol fraction of *S. longepedunculata* at doses of 10, 100 and 1000 mg/kg.

4.4 *In* Vitro Study

Complete cessation of motility was observed in trypanosomes that were treated with crude methanol extract of SL at concentrations of 3 and 6 mg/ml after 75 minutes of observation. The ethyl acetate and aqueous methanol fractions at concentrations of 3 and 6 mg/ml slightly reduced parasites motility after90minutes of observation. However, trypanosomes that were treated with diminazene aceturate (10 µg/ml) were immobilised within 15 minutes of treatment.

4.5 Drug Incubation Infectivity Test

There was an increase in prepatent period of rats in crude methanol treated group, though not statistically significant (P> 0.05). It took 8 days before animals were patent for the parasite post-inoculation. Animals in ethyl acetate and aqueous methanol groups were patent on day 6 post-inoculation, while normal saline and whole blood were also patent on day 6 post-infection. However, diminazene aceturate treated rats remained aparasitic for 21 days. The differences in time course of the disease were not statistically significant (P> 0.05) for all the groups.

4.6 Effect of Treatment on the Level of Parasitaemia in Infected Rats

The pattern of parasitaemia is shown in figure 4.1.Parasitaemia occurred in all the infected groups 4 days after inoculation. The level of parasitaemia increased progressively in rats treated with ethyl acetate fractions (EA) 0.7 mg/kg and NS 2 ml/kg. Similarly, animals that received crude methanol extract (CM) at 0.7 and 0.35 mg/kg and EA at 0.35 mg/kg showed undulating parasitaemia. Also, rats that were treated with aqueous methanol fraction (AM) initially showed low level of undulating parasitaemia at day 2 of treatment but later followed by progressive increase in the level of parasitaemia at day 3 post-treatment. The parasites were not seen in the blood of rats treated with diminazene aceturate (3.5 mg/kg) 24 hours after treatment and remained aparasitic for 3 weeks. There was significant (P<0.05) reduction in the level of parasites in CM (0.35 mg/kg) 3 days after treatment when compared with animals in NS group. No significant (P>0.05) difference was observed in the level of parasitaemia in the remaining groups when compared to animals in NS group.

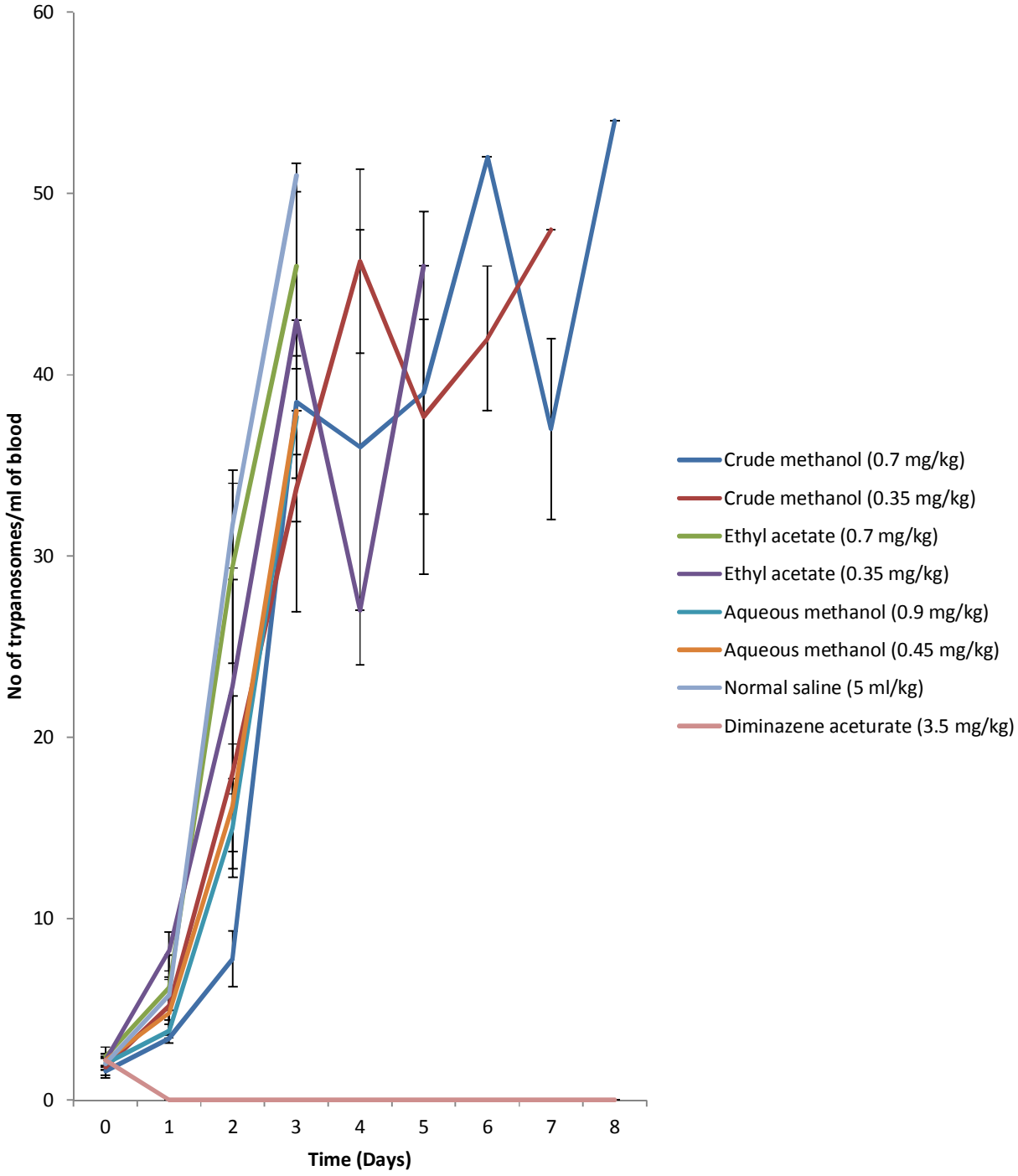


Figure4.1: Effect of treatment with stem-bark extracts of *S. longepedunculata* on the level of parasitaemia in *T. brucei brucei*-infected Wistar rats.

4.7 Effect of Treatment on Daily Rectal Temperature

The effect of treatment on daily rectal temperature of rats infected with *T. brucei brucei* is shown on figure 4.2.There was a significant ($P<0.05$) increase in rectal temperature of rats treated with ethyl acetate extract of *S. longepedunculata* (0.35 mg/kg) on days4 and 5 of treatment. Similarly, rats treated with the aqueous methanol extract (0.9 mg/kg) showed a significant ($P<0.05$) increase in rectal temperature on days 2 and 3 of treatment. Rats treated with crude methanol (0.7 mg/kg) exhibited significant ($P<0.001$) reduction in mean daily rectal temperature when compared with those treated with crude methanol (0.35 mg/kg), ethyl acetate (0.35 mg/kg) and aqueous methanol(0.7 mg/kg) extracts at day 2 of treatment.

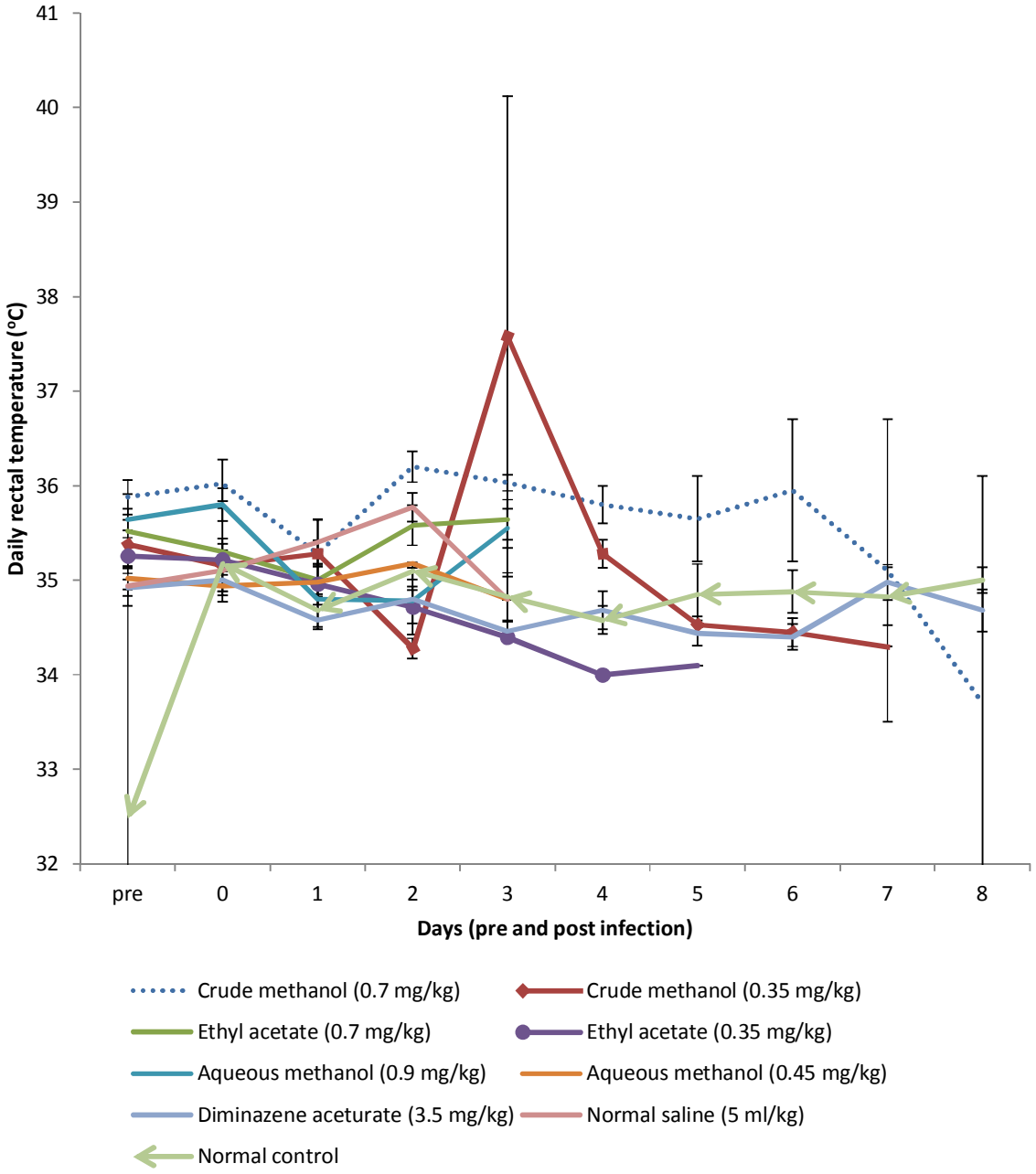


Figure4.2: Effect of treatment with stem-bark extracts of *S. longepedunculata* on daily rectal temperature in *T. brucei brucei* infection in Wistar rats.

4.8 Effect of Treatment on Survival Period of Rats

The effect of treatment with *S. Longepedunculata* extracts on the survival period of rats infected with *T. brucei brucei* is shown in figure 4.3. The animals in CM (0.7 mg/kg) group survived longer than other extract treated groups and NS treated group. Mortality of animals in extract treated groups started on day 3 post-administration of extract while NS began on day 2 of commencement of treatment. Only 2 animals in CM (0.7 mg/kg) group completed dosage regimen of daily treatment for 7 days. None of the animals in DA and NC died throughout the period of observation. Only one animal in NS lived for 4 days during treatment. Overall, CM (0.7 mg/kg) and CM (0.35 mg/kg) animals lived longer than other extract treated groups.

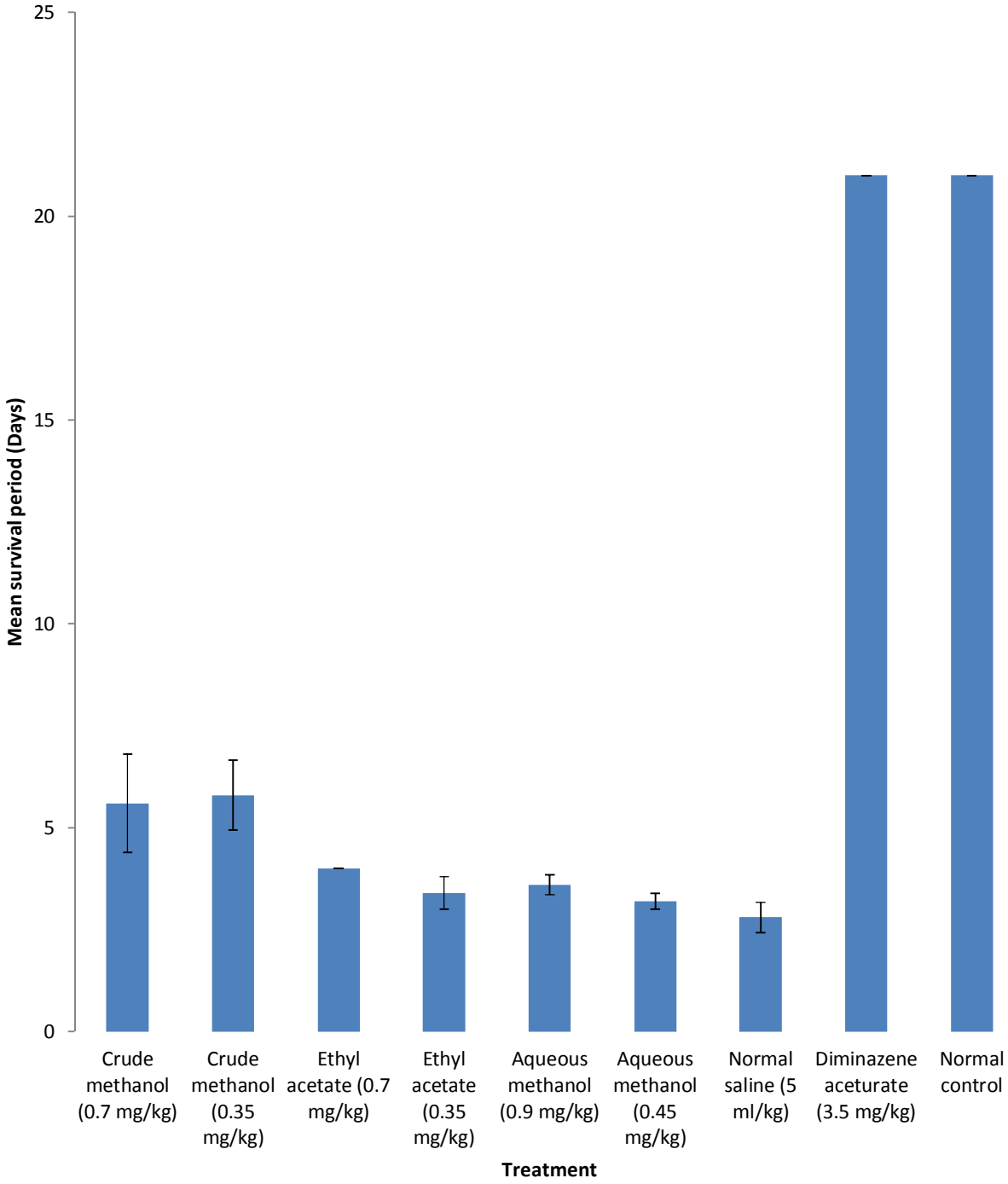


Fig 4.3: Effect of treatment with stem-bark extracts of *S. longepedunculata* on mean survival period of Wistar rats.

CHAPTER FIVE

DISCUSSION

In vitro evaluation of plant extract for antitrypanosomal effect is a rapid and cheap test for evaluating the efficacy of plants against trypanosomes. Some plants have been reported to exhibit *in vitro* trypanocidal effect measured by cessation of motility of the parasites (Atawodi and Shehu, 2010; Habila *et al*., 2011), minimum inhibitory concentration (Freiburghaus *et al*., 1996; Aderbauer *et al*., 2008) while some are reported to exhibit trypanostatic effect measured by reduction in motility of the parasites(Atawodi *et al*., 2003; Atawodi, 2005). The result of this study showed that crude methanol extract of *S. longepedunculata* was able to immobilise the parasite completely within 75 minutes. This is in agreement with the report of Atawodi (2005) who reported cessation of motility of trypanosomes treated with *S. longepedunculata*. In the present study however, it took a longer time for the stem-bark crude methanol extract of *S. longepedunculata* to inhibit parasite motility. Furthermore, ethyl acetate and aqueous methanol fractions showed only slight reduction in parasite motility. This agrees with the findings of Atawodi and Shehu (2010) for root-bark of *S. longepedunculata*.

Drug incubation infectivity test (DIIT)employs both *in vitro* and *in vivo* evaluations of the effect of the plant extract and fractions on *T. brucei brucei*. This test is done to confirm hitherto a known substance that possesses *in vitro* trypanocidal effect. In this study, parasites that were immobilised as an indication of trypanocidal activity were shown to be infective. It follows therefore, that DIIT can be used to evaluate or confirm the effect of a plant extract for trypanosomal effect. This study in a way failed to show the anti-trypanosomal effect of the plant since the parasite was able to infect experimental rats despite being incubated with the

extracts. In addition, the course of the disease in the rats used in DIIT was only slightly longer than that of the rats from *in vivo* study, indicating that the virulence of the pathogen was not affected. This is very important because hitherto cessation of parasite motility has been taken as trypanocidal effect (Freiburghaus *et al*., 1996; Aderbauer *et al*., 2008; Atawodi and Shehu, 2010; Habila *et al*., 2011), while reduction in trypanosome motility was taken as trypanostatic (Atawodi *et al*., 2003; Atawodi, 2005). This conclusion should be made with caution when *in vitro*model is used to assess trypanocidal or trypanostatic effect of plants. Suffice to mention that *T. brucei brucei* maintains its infectivity for up to 30 hours in blood sample maintained at room temperature (Lawal, *et al*., 2004). It may be inferred that under *in vitro* conditions, there is initial stage of parasite paralysis before clinical death of parasite. It is possible that cessation of motility in this study was at the point of paralysis of the parasite.

One factor that could be responsible for the low-efficacy of the extractis the relatively low dose of the extract administered to the animals. This was done due to the apparently high toxicity of the extracts (7 – 9 mg/kg).This resulted in low concentrations administered to the animals which may have resulted in low plasma concentration. The higher concentration of extract shows better efficacy than the extract with lower concentration since an increase in concentration results in a supplementary input of different active compound (Wabo *et al*., 2011). However, Mamman *et al.* (1993) reported that neither stages of infection nor presence or absence of trypanosome influenced the concentration of diminazene in the plasma. It is possible that the low doses used in this study could not support the attainment and maintenance of adequate plasma concentration that could produce therapeutic effect. Plant with higher concentration of saponins than other secondary metabolites tend to be more toxic (Williams, 1978). The saponins content of the stem bark of *S. longepedunculata* may have

been responsible for toxicity of the extract and thus low safety margin of the extract used in this study.

Another factor that may be responsible for apparent lack of efficacy may be the plant itself. It is well established that age, species, location, season of collection of plant and plant part evaluated affect efficacy of an extract on trypanosomal infection (Raskin *et al*., 2002). Plant cultivated under different environmental conditions produce different phytochemical profiles or different amounts of individual components (Stevens *et al*., 2000; Taiz and Zeiger, 2006). Besides, different cultivars within species may produce different levels of bioactive components or other constituent which enhance or counteract their medicinal activities (Juliani *et al*., 2006). The stem-bark of *S. longepedunculata* used in this study may not contain active metabolites against *T. brucei brucei*. McCorkle *et al*.(1996) had earlier reported that chemical constituents of plant vary within the parts of the plant, and between plants with regard to location, age and stage of growth. In the same vein, Atawodi *et al*. (2011) have reported better efficacy of leaves of *Bozwellia dalzielii* against trypanosome than root bark and stem-bark of the same plant. Phytochemical screening of the different parts of *B. dalzielii* revealed that they have different constituents (Atawodi *et al*., 2011). In another study, enhanced survival rate of rats treated with root-bark of *S. longepedunculata* was observed. However, the parasite was not cleared from the blood of infected rats treated with root-bark of *S. longepedunculata* (Haruna *et al*., 2013). Stem-bark used in this study may contain more toxic compounds than other parts of the plant due to the high toxicity observed in this study for the extracts. Dapar *et al*. (2007) reported an LD_{50} of 36.74 mg/kg for root bark of the plant. The apparently low toxic effect of the root-bark of *S. longepedunculata* could explain differences in the biologically active metabolites contained in different parts of the plant. Anon (2012) reported that *S. longepedunculata* is being threatened because local

folks use more of root bark than other parts of the plant. This may further underpin the observation that stem bark may lack efficacy and possibly its toxic effect.

It has been established that method of extraction affects composition of plant extract (Handa *et al*., 2008). Dapar *et al*. (2007) reported higher LD_{50} of 36.74 mg/kg using percolation of root-bark of *S. longepedunculata* whereas soxhlet was employed in this study. Extraction using heat tends to produce higher yield than cold extraction (Handa *et al*., 2008). It is possible that hot extraction used in this study resulted in greater penetration of the solvent into the plant tissue leading to extraction of more of the toxic metabolites. Furthermore, hot extraction has inherent tendency of destroying active metabolites in an extract (Youan *et al*., 1997; Handa *et al*., 2008). Thus, the proportion of bioactive secondary metabolites may have been shifted in favour of toxic metabolite leading to higher toxicity of the plant. Perhaps this could have affected its pharmacological effect as well. In contrast, Youan *et al*. (1997) reported that there is no difference in the efficacy of extracts obtained from cold and hot extraction against experimental trypanosome infection in mice. *S. longepedunculata* has been used as ritual suicide (Neuwinger, 1996) and as a pesticide in stored grains (Steveson *et al*., 2009). Therefore, the plant may contain potent toxic secondary metabolites which further explain the selection of low doses for therapeutic evaluation in this study. Recent reports showed that *S. longepedunculata* is toxic to rats, with variable levels of toxicity on vital organs (Ajiboye *et al*., 2010).

Besides, geographical location and season affect the composition of bioactive secondary metabolites in a plant (Schneider and Segre, 2009). The plant was collected in July 2012, which falls within the rainy season. This period may have affected bioactive metabolites in

the plant resulting in reduced efficacy. Chemical defence against predators or pathogens affects efficacy of plant extract (Cock, 2011). Williams (1978) reported a marked increase in the concentrations of soluble oxalates of *Drymaria arenarioides* in August (4%) when compared to 2% obtained in April of the same year.

Different solvents used for extraction of plant materials also influence composition of bioactive metabolites in an extract. Atawodi *et al*. (2011) reported varied efficacy of the extracts of *B. dalzielii* when solvents were used for extraction. In this study, extract of the plant extracted using different solvent systems did not produce any significant pharmacological action. However, crude methanol prolonged the survival period of infected rats when compared to other extracts treated rats. This agrees with the findings of Youan *et al*. (1997) and Wabo *et al*. (2013) who reported that extracts from various plants when tested for pharmacological activity did not show any significant difference in their effects.

Virulence of the parasite may have been a contributing factor for the apparent lack of activity of the plant against *T. brucei brucei* infection in this study. Recent studies have shown that the course of disease of rats infected with *T. brucei brucei*was12 days (Ehizibolo, 2012; Kobo, 2012), while Haruna *et al*. (2013) reported 14 days for the same *T. brucei brucei*. In this study, 4 days was observed as course of the disease for the parasite. Different strains of the same parasite have different virulence (Masumu *et al*.,2006). It may be inferred that the strain of this parasite is more virulent than the strain used in the earlier reports. The short course of disease which prevented animals from receiving sufficient amount of extract may have been a contributing factor for the inability of the extracts to either clear or at least lower the parasite burden.

Species variation in response to drug therapy has been reported by several workers. Extracts of *Chenopodium ambrosinoides* were observed to have no anthelmintic effect when tested against *Syphacia obvelata* and *Aspiculuris tetraptera*; however, the plant possesses activity against the helminth *Strongyloides venezuelensis* (Borba and Amorim, 2004). Furthermore, aqueous and alcoholic extracts of *Myrsine african* fruits were ineffective against *Oesophagostomum columbianum* but alcoholic extracts were active against *Bunostomum trigonocephalalum* and *Taenia solium* (Kakrani and Kalyani 1983; Desta, 1995). It will therefore be reasonable to assume that extracts of *S. longepedunculata* could have activity against other species of trypanosomes. Variation in susceptibility of different strains of a particular parasite is an important factor that could affect the response of parasites to drug therapy and perhaps lead to treatment failure (Filardi and Brener, 1987).

The increase in body temperature has been a characteristic feature of trypanosomosis in susceptible animals (Uza *et al*., 1998). In this study, all treated animals had no significant rise in rectal temperature. Although undulating parasitaemia was observed, there was no corresponding undulating pyrexia. Anosa (1980) observed that increase in parasitaemia and pyrexia did not occur concurrently.

In this work, administration of crude methanol extract of *S. longepedunculata* prior to inoculation of *T. brucei brucei* did not prevent infection and did not alter the outcome of infection as the course of the disease was not different from that of the therapeutic evaluation. Similar incidence was observed by Ogbadoyi *et al*. (2011) who showed that *Accacia nilotica* exhibited therapeutic effect but lacked prophylactic effect.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From the foregoing discussion, it can be concluded that:

- ✓ The stem-bark extract of *Securidaca longepedunculata* could be said to possess trypansotatic effect against *Trypanosoma brucei brucei*.
- ✓ Drug incubation infectivity test (DIIT) has been shown to be more sensitive in evaluating *in vivo* antitrypanosomal effect of plant extracts when compared with *in vitro* screening test.
- ✓ The stem-bark of *S. longepedunculata* did not show significant antitrypanosomal effect against the tested stock of *T. brucei brucei* in rats.
- ✓ Similarly, the plant did not protect rats against infection with the trypanosome used in this study.
- ✓ Crude methanol extract exhibited better antitrypanosomal effect than other fractions.
- ✓ The extracts of *S. longepedunculata* are relatively toxic when administered to rats intraperitoneally.

6.2 Recommendations

- ✓ Stem-bark extracts of *S. longepedunculata* should be evaluated for activity against other species of trypanosomes.
- ✓ Detailed toxicity studies of the plant should be conducted in rats to establish its safety.
- ✓ Finally, work should be carried out to identify the mechanism of trypanostatic effect of the plant extract.

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Appendix 1: Mean parasitaemia per field of rats infected with *T. brucei brucei* and treated with *Securidaca longepedunculata* extracts.

Day	CM1	CM2	EA1	EA2	AM1	AM2	NS	DA
0	1.6±0.25	1.8±0.58	2.4±0.51	2.2±0.37	2±0.32	2.2±0.37	2±0.36	2.2±0.37
1	3.4±0.25	5.2±1.59	6.2±1.77	8.2±1.07	3.8±0.37	4.8±1.2	5.8±0.86	0±0.00
2	7.8 1.53	18±4.28	29.4±5.325	22.8±5.91	15±2.70	16.2±3.43	31.67±2.33	0±0.00
3	38.5±11.59	33.75±1.84	46±5.67	43±0.00	37.67±3.38	38±0.00	51±0.00	0±0.00
4	36±12.00	46.25±5.07		27±0.00				0±0.00
5	39±10.00	37.67±5.36		46±0.00				0±0.00
6	52±0.00	42±4.00						0±0.00
7	37±5.00	48±0.00						0±0.00
8	54±0.00							0±0.00

CM= Crude methanol, EA= Ethyl acetate, AM= Aqueous methanol, NS= Normal Saline and DA= Diminazene aceturate.

Appendix 2: Mean log parasitaemia of rats infected with *T. brucei brucei* and treated with *Securidaca longepedunculata* extracts.

Day	CM1	CM2	EA1	EA2	AM1	AM2	DA	NS
0	6.72±0.07	6.78±0.12	6.88±0.08	6.87±0.09	6.88±0.09	6.92±0.09	2.2±0.37	2±0.32
1	7.12±0.04	7.24±0.12	7.32±0.12	7.5±0.05	7.18±0.04	7.22±0.10	0±0.00	5.8±0.86
2	7.56±0.04	7.78±0.12	8.04±0.08	7.85±0.04	7.74±0.10	7.74±0.10	0±0.00	31.67±2.33
3	8±0.27	8.1±0.04	8.24±0.05	8.2±0.00	8.17±0.00	8.2±0.00	0±0.00	51±0.00
4	8.15±0.15	8.23±0.05		8±0.00			0±0.00	
5	8.15±0.15	8.13±0.07		8.2±0.00			0±0.00	
6	8.3±0.00	8.3±0.00					0±0.00	
7	8.25±0.05						0±0.00	

CM= Crude methanol, EA= Ethyl acetate, AM= Aqueous methanol, NS= Normal Saline and DA= Diminazene aceturate.

Appendix 3: Mean rectal temperature of rats infected with *T. brucei brucei* and treated with *Securidaca longepedunculata* extracts.

Day	CM1	CM2	EA1	EA2	AM1	AM2	DA	NS	NC
Preinf	35.88±0.18	35.38±0.26	35.52±0.39	35.26±0.19	35.64±0.12	35.02±0.21	34.92±0.09	34.92±0.25	32.48±2.43
0	36.02±0.26	35.16±0.28	35.3±0.53	35.22±0.17	35.8±0.18	34.98±0.22	35±0.16	35.1±0.27	35.18±0.09
1	35.28±0.14	35.28±0.36	35±0.17	34.96±0.22	34.8±0.11	35.18±0.24	34.58±0.10	35.4±0.30	34.68±0.17
2	36.2±0.16	34.28±011	35.58±0.21	34.72±0.18	34.78±0.35	34.8±0.15	34.8±0.13	35.77±0.41	35.1±0.09
3	36.03±0.09	37.58±2.55	35.64±0.21	34.4±0.00	35.55±0.21		34.46±0.10	34.8±0.00	34.83±0.25
4	35.8±0.20	35.28±0.15		34±0.00			34.68±0.20		34.58±0.15
5	35.65±0.45	34.53±0.09		34.1±0.00			34.44±0.13		34.85±0.32
6	35.95±0.75	34.45±0.15					34.4±0.14		34.88±0.23
7	35.1±1.60	34.3±0.00					34.98±0.19		34.83±0.31
8	33.7±2.40						34.68±0.22		35±0.14

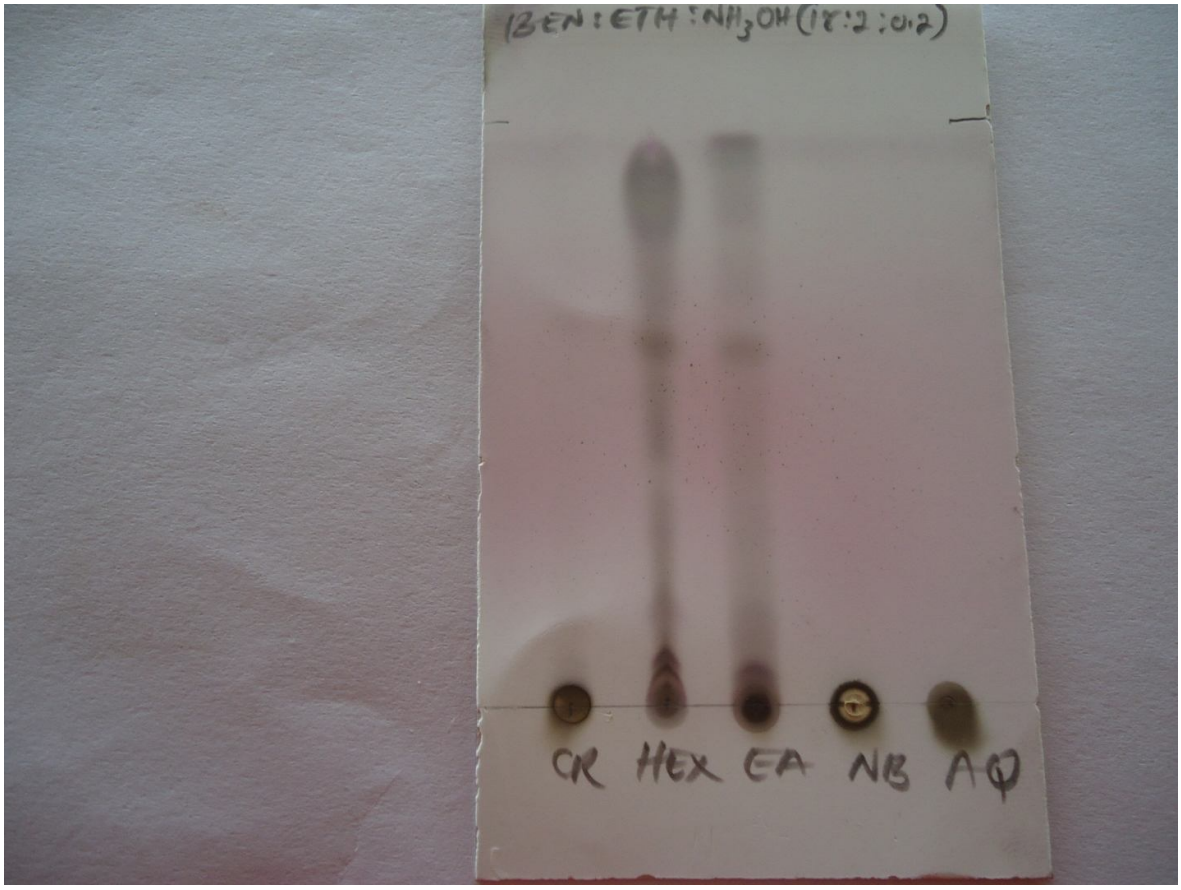
CM= Crude methanol, EA= Ethyl acetate, AM= Aqueous methanol, NS= Normal Saline, NC= Normal control and DA= Diminazene aceturate.

Appendix 4: Survival periods of experimental rats

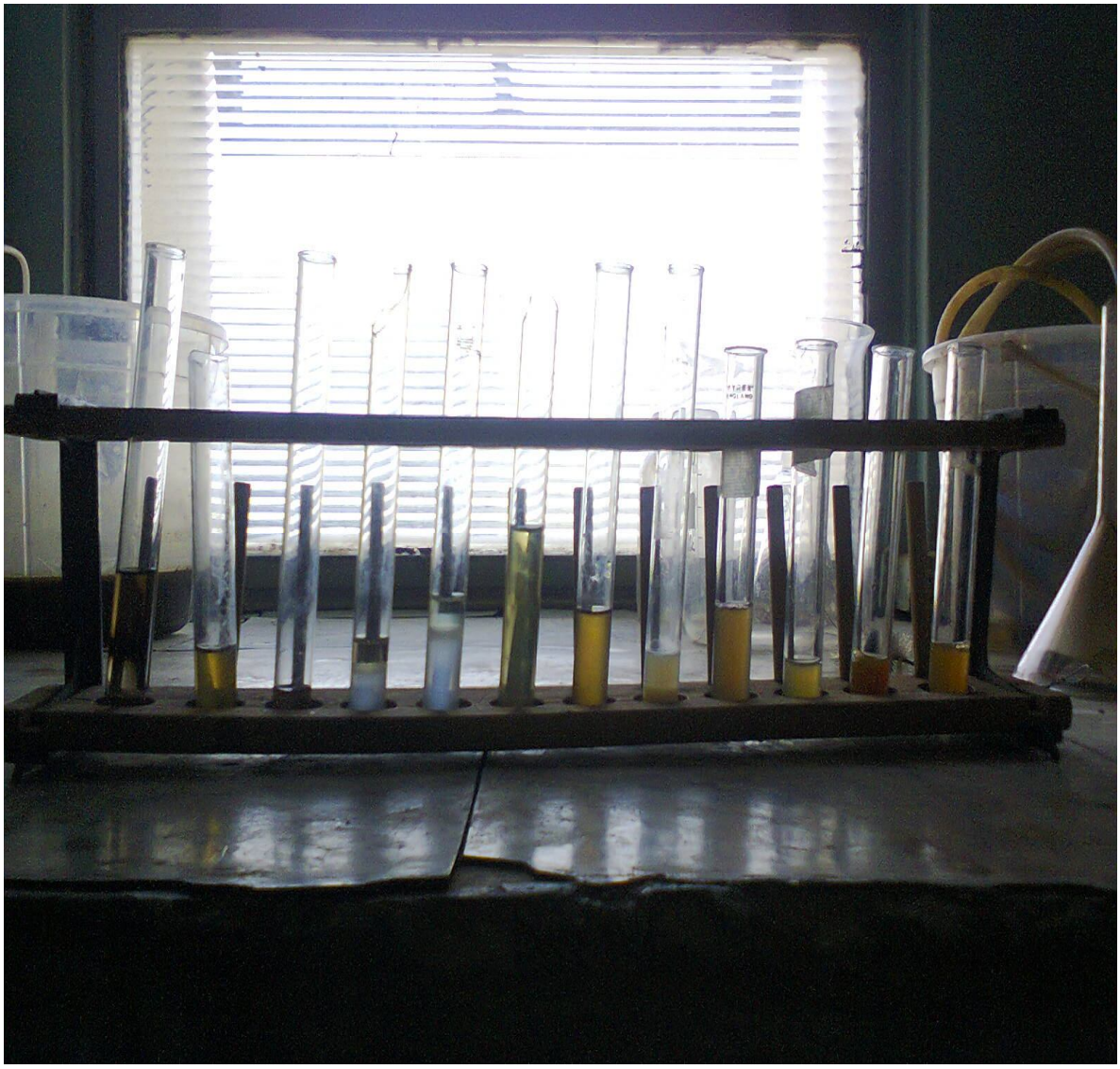
Rats	CM1	CM2	EA1	EA2	AM1	AM2	DA	NS	NC
1	4	5	4	3	4	4	21	3	21
2	8	7	4	5	3	3	21	3	21
3	9	6	4	3	4	3	21	4	21
4	3	8	4	3	4	3	21	2	21
5	4	3	4	3	3	3	21	2	

CM= Crude methanol, EA= Ethyl acetate, AM= Aqueous methanol, NS= Normal Saline, Normal control and DA= Diminazene aceturate.

Appendix 5: Thin layer chromatographic plate.



Appendix 6: Phytochemistry



Appendix 7: Soxhlet apparatus during extraction



Appendix 8: Equipment used for drug incubation infectivity test

