PHYTOCHEMICAL ANALYSIS AND *IN VIVO* ANTITRYPANOSOMAL ACTIVITY OF METHANOL AND AQUEOUS EXTRACTS OF *PERISTROPHE BICALYCULATA* IN MICE INFECTED WITH *TRYPANOSOMA EVANSI.*

BY

AWULU, EMMANUEL ADUOJO

DEPARTMENT OF BIOLOGICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

AUGUST, 2012

PHYTOCHEMICAL ANALYSIS AND *IN VIVO* ANTITRYPANOSOMAL PROPERTIES OF METHANOL AND AQUEOUS EXTRACTS OF *PERISTROPHE BICALYCULATA* IN MICE INFECTED WITH *TRYPANOSOMA EVANSI*

BY

AWULU, EMMANUEL ADUOJO (B. Sc ABU 2007)
MSC/ SCIE/00679/09-10

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOLOGICAL SCIENCES

DEPARTMENT OF BIOLOGICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

AUGUST, 2012

DECLARATION

I declare that the work in the thesis entitled 'Phytochemical screening and *in vivo* antitrypanosomal activity of methanol and aqueous extracts of *Peristrophe bicalyculata* in mice infected with *Trypanosoma evansi*' has been performed by me in the Department of Biological Sciences under the supervision of Prof. S. J. Oniye and Dr. M. A. Adelanwa.

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

AWULU, EMMANUEL ADUOJO ---------- ----------
NAME OF STUDENT SIGN DATE

CERTIFICATION

The thesis entitled "Phytochemical analysis and *in vivo* antitrypanosomal properties of methanol and aqueous extracts of *Peristrophe bicalyculata* in mice infected with *Trypanosoma evansi*" by Awulu, Emmanuel Aduojo meets the regulations governing the award of Masters of Science degree in Biological Sciences of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

---------- ----------

Prof. S. J. Oniye Date
Chairman, supervisory committee

---------- ----------

Dr. M. A. Adelanwa Date
Member, supervisory committee

---------- ----------

Dean of postgraduate school Date

DEDICATION

I dedicate this thesis to God Almighty for the life of my late sibling Miss. Awulu, Ojima Glory. May her soul continue to rest in peace, Amen.

ACKNOWLEDGEMENT

I appreciate God for life, good health, knowledge, wisdom and understanding to come up with the topic for this dissertation.

My supervisors, Prof. S. J. Oniye and Dr. M. A. Adelanwa who have contributed immensely by supervising this thesis, God bless you abundantly in Jesus name.

The Head of Department of Pharmacognosy and their staff, Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, staff of National Research Institute of Chemical Technology (NARICT), I thank you for the opportunity to make use of your laboratories.

I will never forget the Head of Department of Biological Sciences, Prof. J. Auta, staff, colleagues and friends for the role they play towards the accomplishment of this work. I thank you.

My beloved Sister, Late Awulu, Ojima Glory who longed to witness my defense and graduation but God in his mighty way has called her to rest. I appreciate you even in death and also for your prayers before your demise. May God continue to grant you rest. Amen.

I appreciate my parents, Mr. & Mrs. S. A. Awulu and siblings; I thank especially Dr. S. J. Awulu, and Engr. D. O. Awulu for their financial support and encouragement. The sacrifices you have made for me will never be forgotten. My bosom friend, Obinna, Jessica Chinenye, thanks for been patient with me during the course of this work.

Finally, to my brothers and friends Daniel Yaro, Evang. (Engr) and Mrs. Ezekiel Oiganji, Azizat Mohammed, Mrs. Okanigbe, Mrs. E. C. Akpa, Evang. and Mrs. Dauda, the Praise and Worship ministry of the Immaculate Heart Catholic Charismatic Renewal of Nigeria

and the parish choir of Our Lady Queen of Peace Parish, ABU, Zaria. I thank you for your prayers. God Bless you all.

TABLE OF CONTENT

PRELIMINARY PAGES

Topic **Page**

Cover page----------i

Declaration----------iii

Certification----------iv

Dedication----------v

Acknowledgement----------vi

Table of content----------viii

Abstract----------xv

CHAPTER ONE

INTRODUCTION

1.1: Background information----------1

1.2: Taxonomy of *Peristrophe bicalyculata*----------2

1.3: Research problems----------3

1.4: Justification----------4

1.5: Aim----------5

1.6: Objectives----------5

1.7: Hypothesis----------5

CHAPTER TWO

LITERATURE REVIEW

2.1: Introduction----------6

2.2: Morphology----------7

2.3: Life cycle and transmission----------7

Topic **Page**

2.4: Host----------10

2.5: Sialidases----------11

2.6: Parasite, antigen variation----------12

2.7: Clinical signs and pathology----------14

2.8: Histopathological effects----------16

2.9: Symptoms----------16

2.10: Diagnosis----------18

2.11: Treatment----------19

2.12: Epidemiology----------21

CHAPTER THREE

MATERIALS AND METHOD

3.1: Collection and identification of plant material----------25

3.2: Drying and size reduction of plant material----------25

3.3: Methanol extraction process----------25

3.4: Aqueous extraction process----------25

3.5: Phytochemical analysis of plant extract----------26

3.5.1: Qualitative analysis----------26

3.5.1.1: Test for carbohydrates----------26

3.5.1.2: Test for alkaloids----------26

3.5.1.3: Test for cyanogenic glycoside----------27

3.5.1.4: Test for saponins----------27

3.5.1.5: Test for anthraquinones----------27

3.5.1.6: Test for tannins----------28

Topic **Page**

3.5.1.8: Test for sterols and terpenes----------28

3.5.1.8: Test for flavonoids----------28

3.5.2: Quantitative analysis of secondary metabolites----------29

3.5.2.1: Determination of cyanogenic glycosides----------29

3.5.2.2: Alkaloids determination----------29

3.5.2.3: Tannin determination----------29

3.5.2.4: Saponin determination----------30

3.5.2.5: Flavonoid determination----------30

3.5.2.6: Oxalate determination----------31

3.6: Experimental animal----------31

3.7: Survival period of the trypanosome infected mice----------32

3.8: Passaging of mice and parasite development----------32

3.9: Safe dose and acute toxicity----------32

3.10: Suppressive test----------33

3.11: Curative test----------34

3.12: Histopathological studies----------34

CHAPTER FOUR

RESULTS

4.1: Methanol and aqueous extract of *P. bicalyculata*----------36

4.2: Qualitative analysis----------36

4.3: Quantitative analysis----------36

4.4: Suppressive test conducted using methanol and aqueous

extract of *Peristrophe bicalyculata* on *T. evansi*----------41

Topic **Page**

4.5: Curative test using methanol and aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*----------44

4.6: Histopathology of the Liver----------47

4.7: Histopathology of the spleen----------54

4.8: Morphohaematological variation----------60

CHAPTER FIVE

DISCUSSION

5.1: Phytochemical analysis----------66

5.2: Acute toxicity----------68

5.3: Antitrypanosomal activity----------69

5.4: Histopathology----------71

5.5: Conclusion----------73

5.6: Recommendation----------73

References----------74

Appendix----------85

LIST OF TABLES

Topic **Page**

Table 4.1: Comparative qualitative analysis of the methanol and aqueous extract of *Peristrophe bicalyculata*----------37

Table 4.2: Comparative quantitative analysis of the methanol and aqueous extract of *Peristrophe bicalyculata*----------38

Table 4.3: The first phase of the lethal dosage (LD_{50}) test for methanol extract using mice as test animal----------39

Table 4.4: The second phase of the lethal dosage (LD_{50}) test for methanol extract using mice as test animal----------=----------39

Table 4.5: The first phase of the lethal dosage (LD_{50}) test for aqueous extract using mice as test animal----------40

Table 4.6: The second phase of the lethal dosage (LD_{50}) test for aqueous extract using mice as test animal----------40

LIST OF FIGURES

Topic **Page**

Figure 1: Fine structure of *Trypanosoma evansi* as revealed by electron microscope----------8

Figure 2: Generalized life cycle of a fly-transmitted trypanosome that reproduces by binary fission in the host----------10

Figure 3: Curative bioassay of the methanol extract of *Peristrophe bicalyculata* on *T. evansi*----------42

Figure 4: Curative bioassay of the aqueous extract of *Peristrophe bicalyculata* on *T. evansi*----------43

Figure 5: Suppressive bioassay of aqueous extract of *Peristrophe bicalyculata* on *T. evansi*----------45

Figure 6: Suppressive bioassay of methanol extract of *Peristrophe bicalyculata* on *T. evansi*----------46

LIST OF PLATES

Topic **Page**

Plate i: Pictorial presentation of *Peristrophe bicalyculata*----------3

Plate ii: Histopathology of the liver of mice without infection and treatment----------49

Plate iii: Histopathology of the liver of mice infected with
T. evansi and treated with diminazine aceturate----------50

Plate iv: Histopathology of the liver of mice infected with
T. evansi and not treated----------51

Plate v: Histopathology of the liver of mice infected with
T. evansi and treated with 500mg/kg bw of *Peristrophe bicalyculata*----------52

Plate vi: Histopathology of the liver of mice infected and
treated with 400mg/kg bw of *Peristrophe bicalyculata* ----------53

Plate vii: Histopathology of the spleen without infection and treatment----------55

Plate viii: Histopathology of the spleen infected with *T. evansi*
and treated with diminazine aceturate ----------56

Plate ix: Histopathology of the spleen infected with *T. evansi* without treatment---------57

Plate x: Histopathology of the spleen infected with *T. evansi* and
treated with 500mg/kg bw of *Peristrophe bicalyculata*----------58

Plate xi: Histopathology of the spleen infected with *T. evansi* and
treated with 400mg/kg bw of *Peristrophe bicalyculata*----------59

Plate xii: Morphohaematological variations in a blood film prepared
from uninfected mice----------61

Plate xiii: The variation in colour and size of the red blood cells
in mice treated with diminazine aceturate----------62

Topic **Page**

Plate xiv: The variation in colour and size of red blood cells infected and treated with 500mg/kg bw of *Peristrophe bicalyculata*----------63

Plate xv: The variation in colour and size of red blood cells Infected and treated with 400mg/kg bw of *Peristrophe bicalyculata*----------64

Plate xvi: The variation in colour and size of red blood cells infected and not treated----------65

ABSTRACT

Phytochemical analysis and the *in vivo* antitrypanosomal activity of methanol and aqueous extract of *Peristrophe bicalyculata* were carried out in mice infected with *Trypanosoma evansi.* A total of 90 mice were used for the study. Qualitative and quantitative tests of the methanol extracts of *Peristrophe bicalyculata* revealed the presence of the following secondary metabolites, oxalates (0.12mg/100g), phytates (59.5mg/100g), saponins (2%), alkaloids (15%), flavonoids (0.42%), cyanogenic glycosides (960mg/100g) while the aqueous extract revealed the presence of oxalates (0.13mg/100g), phytate (47.6mg/100g) saponins (14%), cyanogenic glycosides (1216mg/100g), alkaloids (16%) and flavonoids (0.24%). The differences in quantities were significant ($P < 0.05$). The LD_{50} for the methanol extract was 1131mg/kg body weight and 1265mg/kg body weight for the aqueous extract. Both extracts showed significant antitrypanosomal activity against *Trypanosoma evansi*, however, the methanol extract displayed more potency. The results of the study suggest that *P. bicalyculata* has potential in the treatment of trypanosomiasis caused by *T. evansi.* The histopathology of the liver and spleen revealed that *P. bicalyculata* has hepatoprotective and spleenoprotective property.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Trypanosomiasis is a complex debilitating, zoonotic protozoan disease of man and animal (WHO, 1998), which has been a major factor limiting livestock productivity in large areas of humid and sub-humid Africa (Mihret and Mano, 2007). Tsetse fly (*Glossina spp.*) which are responsible for the transmission of African trypanosomiasis are found between latitude 14^0N and 29^0S covering 10 million Km^2 stretching across over 37 countries in Africa (Seifert, 1996; WHO, 1998; Molumba, 2003).

Trypanosoma is a genus of kinetoplastids, a monophyletic group of obligate, unicellular parasitic flagellate protozoa. The most important trypanosome species in Nigeria are *Trypanosoma brucei, T. congolense, T. vivax*, and *T. evansi* in livestock, while *T. gambiense* infect human (Samdi *et al.,* 2010). *Trypanosoma evansi* is responsible for a disease known as 'surra', and is the most widespread pathogenic trypanosome globally (Luckins, 1998; Cadioli *et al.,* 2006).

Medicinal plants have been used for centuries as remedies for human diseases and other organisms because they contain certain components of therapeutic value. There are more than 35, 000 plant species being used in various human cultures around the world for medicinal purposes (Philip *et al.,* 2009). According to World Health Organization (WHO, 1998), medicinal plants will be the best choice to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which involves the use of plants that are yet to be scientifically investigated. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

The plant chosen for this study, *Peristrophe bicalyculata* belong to the family Acanthaceae. They are erect, hispid herb or under shrub, 60-120 cm height found in the forest undergrowth, hedges and waste band almost throughout India. The local name in Hausa and Fulfude is *Tubannin dawaki* and *fureya pucci* respectively. It is commonly known as *kali agheli* in Hindi and *kakajangha* in Sanskrit. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomach ache, anticancer, fertility, diuretic, diarrhea and other skin related infection. It is also used as an antidote for snake poison when macerated in an infusion of rice, and as insect repellant. This plant is also used for horse feed and ploughed into the soil as green manure (Dwivedi, 2008).

1.2 Taxonomy of *Peristrophe bicalyculata*

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Scrophulariales

Family: Acanthaceae

Genus: *Peristrophe*

Species: *Peristrophe bicalyculata*

The plant is widely distributed cutting across continents of Africa and Asia. Countries where *P. bicalyculata* is found includes Ethiopia, Mali, Nigeria, India, Benin, Chad, Senegal, Niger, Cameroon, Burkina Faso, Sudan, Gambia, Burundi, Democratic Republic of the Congo, Tanzania, Madagascar, Somalia, Nepal etc.



Plate 1: *Peristrophe bicalyculata* taken from the field at Kuru-Kuru village of Giwa Local Government Area of Kaduna state.

1.3: Research Problems

About 35 million dosages of trypanocidal drugs are administered annually in Africa, to combat the scourge of trypanosomiasis (Geerts and Holmes, 1998). Irrespective of this, 60 million people and 48 million cattle are estimated to be at risk of contracting Africa trypanosomiasis transmitted by 22 species and 33 sub-species of tsetse flies in Africa (Seifert, 1996; WHO, 1998; Kristjason *et al.,* 1999). African trypanosomiasis is responsible for 3 million livestock deaths and 55, 000 people die annually in Africa (ILRAD, 1990; Abenga *et al.,* 2003; Molumba, 2003).

The socioeconomic impact of trypanosomiasis is extremely difficult to analyze, however loses have been estimated to amount to over 500 million US dollars in meat, milk, lost in traction power, and control programmed (ILRI, 1997).

In Nigeria, eleven of the twenty-two species of tsetse flies are known to infest over 80% of the 928, 300 km^2 of landmass, and are widely distributed from latitude 4^0N

and 13^0N in the country (NITR, 1989; Onyiah, 1997). Trypanosomes have a wide range of hosts and are pathogenic to many species of domestic and wild animals (Franke *et al.,* 1994a; Herrera *et al.,* 2004). It is primarily transmitted mechanically by biting flies from the species like *Tabanus, Stomoxys, Haematopota, Lyparosia*, and *Chrysops* spp (Fraser, 1909; Luckins, 1999b) as subclinical infections have been reported in sheep and goats from Sudan and Brazil (Luckins, 1999a).

In Africa, camels are the most important host (Dia *et al.,* 1997), but cattle have also been reported as the next most highly susceptible animals (Mahmoud and Gray, 1980). In Nigeria, experimental studies have shown that donkeys, cattle, sheep, and goats are susceptible to *Trypanosoma evansi* but undergo a protracted course of the disease (IIemobade, 1971; Audu *et al.,* 1999; Shehu *et al.,* 2006). There are considerable differences in the severity of symptoms caused by *T. evansi* infection in different geographical areas of its occurrence, depending on the virulence of the strain and the susceptibility of the host (Herrera *et al.,* 2004). The pre-patent period of the disease varies according to the animal species and the immune status of such infected animals (IIemobade, 1971). Although *T. evansi* is primarily a parasite of camels, there is a likelihood that animals herded together with camels especially small ruminants may become infected by *T. evansi* (Ngeranwa *et al.,* 1993).

1.4: Justification

A large proportion of the world population depends on traditional medicine because of the scarcity and high cost of orthodox medicine (Tagboto and Townson, 2001; Hudaib *et al.,* 2008). Medicinal plants have provided modern medicine with numerous plant derived therapeutic agents (Evans, 2000; Oladunmoye *et al.,* 2009). The need to

screen plants for pharmaceuticals is particularly urgent in the light of rapid deforestation and the concurrent loss of biodiversity throughout the world (Lynch and Berry, 2007; Sawalha *et al.,* 2008). More so, the rapid increase of the resistance to most antitrypanosomal drugs as well as resistance of the vectors to insecticides has brought about increased cases of the disease (Ridley, 2002; Zirihi *et al.*, 2005). Therefore, it is imperative to explore other alternative treatments that are cheaper, less toxic and insignificant side effects. The antineoplastic, anti-inflammatory and anti-allergic role of *Peristrophe bicalyculata* has been established (Abdulazeez *et al.,* 2010), however, the antitrypanosomal effect has not been carried out hence the reason for this research.

1.5: Aim

The aim of this work is to analyze qualitatively and quantitatively the secondary metabolites of *Peristrophe bicalyculata* and to test its antitrypanosomal activity against *Trypanosoma evansi* using the curative and suppressive design.

1.6: Objectives

- To determine qualitatively and quantitatively the secondary metabolites present in the methanol and aqueous extract of *P. bicalyculata*
- To determine the curative and suppressive effects of methanol and aqueous extracts of *P. bicalyculata* in mice infected with *T. evansi*

1.7: Hypotheses

- There is no difference in the secondary metabolites of methanol and aqueous extract of *Peristrophe bicalyculata*
- The methanol and aqueous extracts of *Peristrophe bicalyculata* has no curative effects and suppressive properties on the mice infected with *Trypanosoma evansi*

CHAPTER TWO

LITERATURE REVIEW

2.1: Introduction

The protozoan parasite *Trypanosoma evansi* causes a disease known as 'Surra', is said to be the first pathogenic trypanosome discovered (Hoare, 1972). The disease is known in India since time immemorial, and was given the name 'surra' denoting anything rotten (Indrakamhang, 1998). The etiology of surra was first described in 1880 by Griffith Evans (Hoare, 1972), who discovered motile spirillum-like organism in the blood of equines and camels affected by this disease in Punjab (Hoare, 1972). John Henry Steel made similar observation during an outbreak in 1885 (Mahmoud and Gray, 1980). Surra is endemic in great parts of South-East Asia, but has been found in Africa, America and Europe. Different mammalian species such as water buffaloes, cattle, camels, and pigs are susceptible to this disease (Womack, 2001). All blood sucking flies can transmit the disease, all though the 'horse' flies, *Tabanus*, stable flies, *Stomoxys* are vectors in South-East Asia and increased time between blood meals reduces the survival of the parasite. (Womack, 2001) while the tsetse flies are the most common vector in different countries in Africa and south and central America, vampire bats (*Desmodus rotondus*) may serve as vector and reservoir of the parasite (Hoare, 1972; Womack, 2001). It has been proposed that *Trypanosoma evansi* like *T. equiperdum,* are sub genus of *T. brucei brucei*, because of the loss of the mitochondrial DNA. *Trypanosoma evansi* is not capable of infecting invertebrate vector and establishing the subsequent life stages (Womack, 2001).

Infection with *T. evansi* has a negative effect on fertility, growth rates and feed conversion, also the immune system is depressed (Womack, 2001). Surra as an acute disease is not a problem for the pigs but the endemic state of the disease causes immunosuppressive effects. The parasite is covered by a thick layer of glycoprotein (or variable surface antigen) that is the primary immunogen eliciting antibodies formation; periodically, the organism alters the glycoprotein coating and evades the host's defensive responses (Luckins, 2002). This characteristic has made development of vaccine against this organism to be difficult, because the parasite has the ability to vary antigens during the course of infection (Womack, 2001).

2.2: Morphology

Trypanosoma evansi is a monomorphic haemoflagellate protozoan that has an elongated nucleus and a small circular terminal kinetoplast, which is sometimes missing in mutated wild strains that have followed drug treatment. This flagellate (Fig. 1) is long and slender, it has length that varies between 15-34µm and a width between 1.5 to 2.2µm; like all parasitic trypanosomes, *T. evansi* is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic variation (Goday *et al.,* 1993).

2.3: Life Cycle and Transmission

It was initially suspected that transmission of trypanosomes were purely mechanical (as in the case of *T. evansi*, which is transferred by biting flies or when carnivores bite their prey and get wounded in the mouth). In 1909, Kleine carried out

transmission experiments from human patients to apes and monkeys. He showed that infected tsetse flies were vectors and that the parasite had to be present in the fly for a minimum period before it could cause infection. He subsequently showed that only the metacyclic forms in the salivary glands of the insects were infectious. When tsetse fly bites an infected human patient, it can ingest the parasites. After their arrival in the insect's stomach, many (99%) die because of interactions with specific lectins in the insect stomach; lectins are proteins that bind specifically to certain saccharides, such are present on glycoproteins (Holland, 2003).

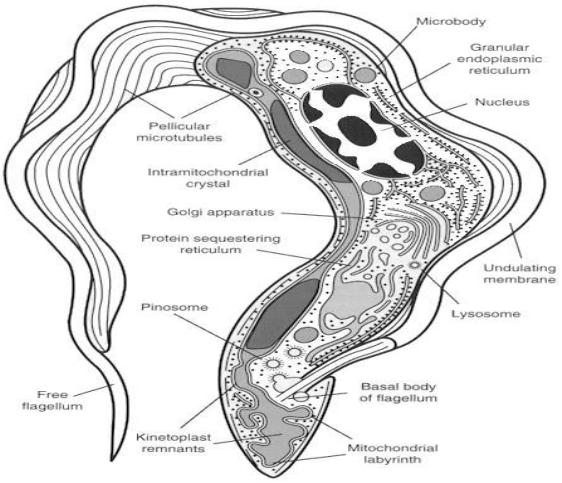


Figure 1: Fine structure of a protozoan parasite, *Trypanosoma evansi*, as revealed by transmission electron microscopy of thin sections. (Gardiner *et al.*, 1988)

The presence of bacteria endosymbionts in the insect possibly plays a role in whether or not an infection is produced in the fly. If the parasite survives, they become slender again ('procyclic trypanosome'). They must then manage to get into the salivary glands. It was initially thought that there were two ways for the parasites to do this. They may first pierce the intestinal wall and then penetrate directly into the salivary glands (this is no longer accepted as a hypothesis), or they may undertake a complex migration in the insect. They would then migrate distally in the intestine around the posterior free

extremity of the peritrophic membrane, and then swim back between the intestinal wall and the peritrophic membrane. When they reach the level of the proventricular valve, they penetrate the peritrophic membrane that is being formed and swim up the oesophagus into the proboscis. Finally, they penetrate the hypopharynx and pass into the salivary glands. During this migration they change morphologically into 'mesocyclic forms'. In the salivary glands they replicate as 'epimastigotes'. These then become infectious 'metacyclic trypanosomes'. After about 3 weeks the fly can transmit parasites into its next victim when taking a blood meal (Holland, 2003).

In man, the parasite multiply outside the cells, namely in blood, Lymph and cerebrospinal fluid. Others can survive in certain areas like the plexus choroideus in the brain, from whence they cause flare-ups (WHO, 2012).

Trypanosoma evansi is spread mechanically by an arthropod vector, which varies geographically. All blood sucking flies can transmit the disease, although the horse fly, *Tabanus*, Stable flies, *Stomoxys* are the most common vectors in South-East Asia with Tsetse fly being the most common in different countries in Africa (Womack, 2001). Trypanosome species have limited survival time in the mouth parts of a vector, therefore, time between feedings have to be short. It is not only spread by mechanical transmission, but also during nursing, copulation or ingestion of infected tissues by carnivores. Equids and camelids are commonly infected with *T. evansi* but other mammals such as buffalo, cattle, deer, dogs and elephants are also susceptible. The severity of illness depends upon the protozoan strain, concurrent infections, stress and environmental factors (Womack, 2001).

Unlike other trypanosome species, whose life cycle needs an intermediate host before reaching the adult state, *Trypanosoma evansi* does not undergo development in an intermediate host. *Trypanosoma evansi* multiplies in a mammalian host by the process of longitudinal binary fission in the trypomastigote stage, as seen below. It rarely infects humans, but a case was reported from India of a human infection however, it was successfully cured using suramin (Joshi, 2007).

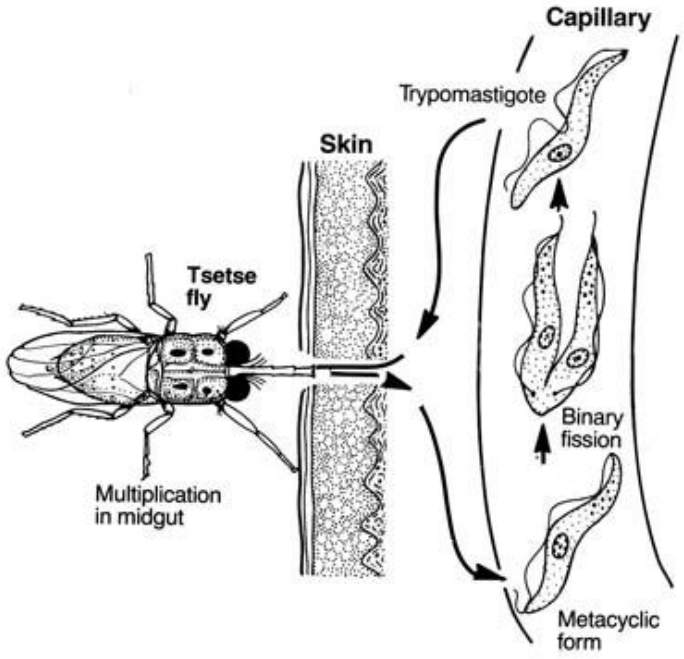


Figure 2: Generalized life cycle of a fly-transmitted trypanosome that reproduces by binary fission in the host (Gardiner *et al.,* 1988)

2.4: Host

All species of domesticated livestock can be infected with *T. evansi* although the principal host varies geographically. In Africa, beyond the northern-most limits of the tsetse fly zone and in parts of eastern Africa, camels are most important host (Dia *et al.,* 1997), whilst in central South America horses are principally affected (Monzon *et al.,* 1995; Silva *et al.,* 1995). In Asia, a much wider range of hosts are involved including

bacterian and dromedarian camels, cattle, buffaloes, horses, and pigs (Tuntasuvan *et al.,* 1992; Pathak *et al.,* 1993; Partoutomo *et al.,* 1994). In Africa and South America, there are very little evidences to suggest that domesticated livestock, other than camels and horses, are clinically affected or indeed infected with *T. evansi*. However, there are reports of serological evidence of infection in goats and sheep from Sudan (Boid *et al.,* 1981) and in cattle from Brazil (Franke *et al.,* 1994a).

2.5: Sialidases

Sialidases have been called neuraminidases or receptor-destroying enzymes because they were first described for viruses. They are the key enzymes of sialic acid catabolism, hydrolysing the glycosidic linkage between the sialic acid molecules and the penultimate sugar of the carbohydrate chains of oligosaccharides and glycoconjugates (Traving and Schauer, 1998) the enzymes occur in higher animals of the deuterostomate lineage that possess the corresponding sialic acid metabolism. They are also found in different varieties of micro-organisms such as viruses, bacteria and protozoa (Saito and Yu, 1993; Traving and Schauer, 1998). For some microbial pathogens, sialidases are believed to act as virulence factors, allowing successful competition with the host by facilitating their spread to host tissue (Goday *et al.,* 1993). A possible role for sialidases is that of spreading factors which facilitate the propagation of bacteria and their invasion of host tissue (Nee *et al.,* 1975; Corfield, 1992). Another effect of sialidases overflow is in anaemia; red blood cells are covered by dense coat of sialic acid molecules that is removed by this dysregulated sialidase action, as a consequence, the galactose residues are de-masked on the blood cell surface after removal of sialic acids, preventing a signal for degradation by liver hepatocytes (Schauer, 1985). Another possible role of the

sialidases from parasites is the de-masking of sub-terminal host cell structure, which then serves as receptors for the parasites and toxins, as in the case of cholera (Galen *et al.,* 1992; Traving and Schauer, 1998).

A special sialidase subgroup is represented by trans-sialidase, which combines the factors of sialidase and sialyl transferases. These enzymes preferably transfer the sialic acid molecule directly from one glycosidic linkage onto another sugar residue instead of a water molecule (Schauer and Kamerling, 1997).

2.6: Parasite, Antigenic Variation

When the parasite is present in an individual, it is covered with a thick monotonous layer of a single type of glycoprotein, Variant Surface Glycoprotein (VSG). These glycoproteins consist of 400-500 amino acids and various saccharide groups and are anchored into the cell membrane with a so called GPI-anchor (glycosyl-phosphatidyl-inositol). Ten percent of the proteins of the trypanosome consist of VSG. The entire VSG surface of a trypanosome is recycled every seven minutes by a process of VSG endocytocysis and exocytosis. When the parasite is transferred to the tsetse fly, the VSG coating disappears within 4 hours and is replaced by an invariant glycoprotein. After the parasite has completed its cycle in the fly and arrives into the latter's salivary glands, the VSG coating reappears. The VSG coating is of vital importance for the parasite when it is in the vertebrate host. This explains why only metacyclic trypanosomes (the mature forms in the salivary glands of the insect) are infectious (Kobayashi and Tizard, 1976).

Trypanosoma evansi does not undergo any morphological change in the invertebrate host hence, its wide host range. When an antigenically homogenous

population of parasites is in the human body, antibodies against the VSG of this population are produced. The immune system lyses the parasites (fever episode). Infections with trypanosomes would be cured quickly, if the parasite population could not constantly change its surface antigens (Gardiner *et al.,* 1988).

The parasite has about 1000 genes that code for different VSGs and thus has a vast repertoire of surface antigens. Most of these are located on some hundred minichromosomes in the nucleus of the parasite. The parasite also has about twenty chromosomes of normal size. These do not condense during mitosis. At any one time only one VSG gene per parasite is active. A few trypanosomes in a population have different VSG (heterologous variant). After destruction of the first, dominant population by immune system, the heterologous parasites increase in number until the variant VSG has induced antibodies and a new cycle of destruction begins. A third population of minority variants then emerges. This antigenic variation is a very important factor in the development of the disease and explains various symptoms, including its chronic course, fluctuating parasitaemia and fever episodes (Holland, 2003).

The order in which antigenic variants appear is partially pre-determined. Alteration of surface antigens is not induced by antibodies but occurs spontaneously. The genetic mechanism that the parasite uses for this is very complex. The genes that need to be expressed are duplicated from a locus on one of the chromosomes to a subtelomeric locus (close to one end of the chromosomes). The mRNA originating from the gene on this locus is subsequently coupled to a small mRNA fragment that is coded elsewhere. This small fragment is the same for all VSGs. The mechanism for mutually exclusive activation of the VSG genes is still not known. Some other genetic mechanisms are

linked to this. An unusual DNA-pyrimidine base 'J' compares with A, T, G and C is present in small quantities in the trypanosome genome and occurs more frequently in the telomeres. The unusual nucleotide is present only in the blood form. Not in the procyclic form. The significance of this is not yet known (Kobayashi and Tizard, 1976).

It is important for the parasite to keep the population in the host as homogenous as possible in order to use the VSGs economically. However, when parasites infect a host, diversity is advantageous. Hence, some 20 different variant can be present in the saliva of a fly. Any one of these variants is capable of infecting a host provided there are no antibodies present from a previous infection. If the host is immunologically naive to the trypanosome antigens, the same VSG eventually dominated the first parasitic population (Holland, 2003).

Different attempt has been made in the development of the vaccine against trypanosomosis. This includes the use of anti-idiotypic antibodies (David *et al.,* 1982). Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, rescent research into the biology of B cells has indicated that the problems might go further than that (Magez *et al.,* 2010)

2.7: Clinical Signs and Pathology

There is a variation in the pathogenicity of different strains of *Trypanosoma* and the susceptibility of different host species. Animals which are subjected to stress,

malnutrition, pregnancy or work are more susceptible to diseases. The first sign of infection is swelling of the skin where the parasites are multiplying. This is followed by fever, which is directly associated with the parasitaemia (Luckins, 2002).

Another important clinical sign is progressive anaemia, which is caused by extra vascular haemolysis. The haemolysis is triggered by an auto-immune reaction against the erythrocytes which is often catalysed by the action of the enzyme sialidase which hydrolyses the sialic acid on the surface membrane of the erythrocytes (Rickman, 1983). Anaemia is a reliable indicator of trypanosome infection, but it is not itself pathognomonic. On the other hand animals with mild subclinical infection can have parasitaemia without evidence of anaemia (Boid *et al.,* 1981)

Recurrent periods of fever and parasitaemia occur during the course of the disease, also there is a loss of condition and lassitude. Late in the infection, there can be oedema, particularly on the lower parts of the body. Also urticarial plagues and petechial haemorrhages of the serous membranes are often observed. Skin rashes on ears and the lateral side of the body can be seen. Abortion is seen in an early stage of pregnancy (Teeraprasert *et al.,* 1984). The disease manifests itself as an acute or chronic condition and will then persist for many months. Severe clinical signs of *Trypanosoma evansi* in naturally infected pigs are rarely seen (Stephen, 1986). Secondary infections can be expected because of the immunosuppression. The clinical signs caused by *Trypanosoma evansi*, are indicative but not specific enough to proof infection. Therefore the disease has to be confirmed by laboratory methods.

2.8. Histopathological Effects

Studies conducted using *Trypanosoma congolense, T. vivax*, and *T. brucei* on Yankassa rams showed that the infection has some pathological effects on the liver, spleen, and lymph nodes. Pathological changes in the infected animals include splenomegaly and hepatomegaly which are more pronounced in acute than in chronic conditions (Omotainse and Anosa, 2009). Similar result was reported by Ohaeri (2010) however, there was infiltration of the kidney by the macrophages and lymphocytes around the glomeruli and blood vessels. These observations of cell infiltrations in the organs suggest that trypanosome infection is associated with histopathological changes that contribute to chronic debilitation.

2.9: Symptoms

Researchers have reported that animals infected with trypanosomosis show a progressive loss of weight. This was attributed to decline of dry matter intake and catabolisation of body reserves to meet the increased requirements for maintenance (Cadioli *et al.,* 2006). Therefore adequate nutrition will prevent alternative utilization of fat for energy requirement by the infected animal. Other symptoms noted include increase in total plasma protein concentration (Boid *et al.,* 1980; Adekola and Durotoye, 2004 and Cadioli *et al.,* 2006).

In the first stage (the haemolymphatic phase), the parasites multiply in subcutaneous tissues, blood and lymph. Symptoms at this stage include fever, headache, joints pains and itching. The second phase (Neurological phase), the parasites cross the blood brain barrier to infect the central nervous system. The symptoms include

disturbance of the sleep cycle, changes of behaviour, confusion, sensory disturbances, and poor coordination. Diagnosis must be made as early as possible in order to avoid complicated, difficult and risky treatment procedures (Cadioli *et al.,* 2006).

Excessive haemlolysis of erythrocytes may be intravascular or extravascular. In *T. congolense* or *T. vivax* infected ruminants, Fiennes (1954) observed severe intravascular haemolysis in the acute and relapsing stages and demonstrated the presence of haemolysin in the plasma. Similar observations have been made by Wellde *et al.* (1974) and Huan *et al.* (1975). On the other extravascular haemolysis was the main feature in *T. congolense* infected sheep and in *T. brucei* infected rats and mice (Jennings *et al.,* 1974).

It has been reported that increased rate of destruction of erythrocytes may be mediated by immunologic mechanisms (Kantzier *et al.,* 1974; Kobayashi and Tizard, 1976). Reports have shown that intravenous injections of rats with *T. evansi* antigen resulted in moderately severe and probably, antibody mediated haemolytic anaemia. It is then hypothesised that development of anaemia associated with *T. evansi* infection might be immunologically mediated. This was supported by the presence of trypanosome antigen on erythrocytes of calves infected with *T. congolense* leading to speculation that immune complexes caused the elimination of affected erythrocyte resulting in clinical anaemia (Kobayashi and Tizard, 1976).

It may be concluded that immunoglobulin complement opsonises desialylated erythrocytes (Muller and Shauer, 1983) produced in acute bovine trypanosomosis (Esievo 1978; Esievo *et al.,* 1982) are the causative factors of erythrophagocytosis. The role of lectin in erythrophagocytosis by Kupffer cells was demonstrated in *T. vivax* infected

cattle, contributing to the pathogenesis of the anaemia in bovine trypanosomosis. Jennings *et al.,* (1974) suggested that the rapid onset of anaemia in trypanosomosis might be due to damage to erythrocytes as a direct or indirect result of trypanosome infection.

Esievo (1979; 1983) reported that *T. vivax* hydrolysed sialic acid *in vitro* and the activity of neuraminidase is proportional to the concentration of trypanosomes. The decline in erythrocyte surface sialic acid and increase of free serum sialic acid concentrations in *T. vivax*- infected cattle corresponded to the time of appearance of circulating trypanosomes with lowest values of red cell sialic acid at the initial peak of parasitaemia, and that the early anemia observed in infected animals may be attributed to the activities of circulating trypanosomes which produce neuraminidase that cleaves off the surface sialic acid from erythrocytes, thus rendering them more prone to erythrophagocytosis (Esievo *et al.,*1982).

2.10: Diagnosis

The general clinical signs of *T. evansi* infection are not sufficiently pathognomonic for diagnosis. Laboratory methods for detecting the parasite are required. In early infection or acute cases, when the parasitaemia is high, examination of wet blood films, stained blood smears or lymph node materials might reveal the trypanosomes. In more chronic cases, or more generally when the parasitaemia is low, the examination of thick blood smears as well as methods of parasite concentration and the inoculation of laboratory rodents are required. In apparently healthy carriers (animals without clinical signs), parasites are rarely observed and mouse inoculation gives the best results. Several primer pairs targeting the subgenus (trypanozoon) or the species-specific (*T. evansi*)

parasitic DNA sequences are available for diagnosis by polymerase chain reaction (PCR). PCR is more sensitive than microscopic parasitological examination. But it may give false-negative results when parasitaemia is very low; in these cases, suspicion of potential carriers can only be confirmed by serological examination (Luckins, 2002).

Serological test gives rise to specific antibody responses and a variety of antibody detection tests have been introduced for laboratory and field use. Some have been partially validated, but await large scale evaluation and standardization. The most relevant are immunofluorescence test, enzyme linked immunosorbent assays (ELISA) and card agglutination test. However, PCR has been reported to represent the most viable tool for wide epidemiological studies, which is used to report the true prevalence of the trypanosome infection (EL-Metanawey *et al.,* 2009).

Other techniques used in the identification of the trypanosomes are the concentration technique and this involves the microhaematocrit centrifugation technique, dark-ground/phase- contrast buffy coat technique (Murray method), anion exchange method and in-vitro cultivation (www.oie.int).

2.11: Treatment

Reduction of the global economic impact of surra on animal health requires dependable diagnostic test, efficacious treatment, and diligent control programs. Historically, three drugs include diminazine, suramin, and quinpyramine. Cymelarsan is the newest drug to be introduced in the last decade. The choice of drug, dosage, and route of administration vary by species affected, local preference, and presence or absence of trypanosome drug resistance (Brun *et al.,* 1998). In one study, diminazine cleared the

parasitaemia of horses following natural infection. However, parasitaemia returned in many animals within one week of the first treatment with diminazine or within 24 hours of the second treatment with diminazine. These show that diminazine affords little protective effect against repeated *T. evansi* infection or the drug is ineffective in totally eliminating the initial *T. evansi* infection (Tuntasuvan *et al.,* 2003). Relapses have also been observed following treatment with quinapyramine sulphate (Monzon *et al.,* 2003). There is growing body of evidence that *T. evansi* is becoming resistant to currently available drugs and may restrict their use or retard their effectiveness in the future. However, *in vitro* assays may help predict the effectiveness of drug treatment to given isolates of *T. evansi* (Brun *et al.,* 1998).

In summary, the decision to treat an animal usually is based on the presence and severity of clinical signs of disease. Given the occasional low sensitivity of certain diagnostic tests, subclinical infections may present a dilemma when attempting to control *T. evansi* infections in herd outbreaks (Luckins, 1999a). Other measures (besides trypanocidal drug administration) such as minimizing exposure to fly populations may help control surra. Stables with suitable netting can be used to exclude fly populations. Smudge fires may also be used to repel flies (Brun *et al.,* 1998). Fortunately, *T. evansi* cannot survive very long outside of its host. Therefore, animal carcasses from surra fatalities pose little threat of disease spread. Finally, it is always prudent to restrict the movement of infected animals (Holland, 2003).

2.12: Epidemiology

It has been proposed that *T. evansi* originated from Africa as variant of *T. brucei* (Hoare, 1972). It has been suggested that the camel was probably infected with *T. brucei* in the marginal zones of tsetse fly infected areas, and then spread the infection throughout North-Africa and Asia by camel caravans and military campaigns. In a similar manner, horses were probably the means by which the disease reached South America; many animals were moved from West Africa in the 16th century. Evidence from iso-enzyme and genetics studies suggests only a limited evolutionary origin of *T. evansi* (Holland, 2003). The parasite is widely distributed geographically and infects many animal host species.

Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. Many of the affected populations live in remote areas with limited access to adequate health services, which hampers the surveillance and therefore the diagnosis and treatment of cases. In addition, displacement of populations, war and poverty are important factors leading to increased transmission and this alters the distribution of the disease due to weakened or non-existent health systems (WHO, 2012). According to WHO, (2012) the distribution of the disease has been facilitated by the following:

- In 1986, it was estimated that some 70 million people lived in areas where disease transmission could take place.
- In 1998, almost 40, 000 cases were reported, but estimates were that 300, 000 cases were undiagnosed and therefore untreated.

- During epidemic periods prevalence reached 50% in several villages in the Democratic Republic of Congo, Angola and South Sudan. Sleeping sickness was first or second greatest cause of mortality in those communities.
- By 2005, surveillance was reinforced and the number of new cases reported on the continent was reduced; between 1998 and 2004 the number of both forms of the disease fell from 37,991 to 17, 616. The estimated number of actual cases was between 50,000 and 70, 000.
- In 2009, after continued control efforts, the number of cases reported has dropped below 10, 000 (9878) for first time in 50 years. This trend has been maintained in 2010 with 7139 new cases reported. The estimate number of actual cases is currently 30,000 (WHO, 2012).

The prevalence of the disease differs from one country to another as well as in different parts of a single country. In the last 10 years, over 70% of reported cases occurred in the Democratic Republic of Congo (DRC). In 2010, only the DRC declare over 500 new cases per year. Angola, Central African Republic, Chad, the Sudan and Uganda declared 100 to 500 new cases per year. Countries such as Cameroun, the Congo, Cote d'lvoire, Equatorial Guinea, Gabon, guinea, Malawi, Nigeria, united republic of Tanzania, Zambia and Zimbabwe are reporting fewer than 100 new cases per year. Countries like Benin, Botswana, Burkina Faso, Burundi, Ethiopia, the Gambia, Ghana, Guinea Bissau, Kenya, Mali, Mozambique, Namibia, Niger, Rwanda, Senegal, Sierra Leone, Swaziland and Togo have not reported any new cases for over a decade. Transmission of the disease seems to have stopped but there are still some areas where it is difficult to assess the exact situation because the

unstable social circumstances and/or remote accessibility hinder surveillance and diagnostic activities (WHO, 2012).

Many researches have been conducted using *Peristrophe bicalyculata* for different physiological regulatory properties. These properties include antihypertensive, antipyretic and anti-inflammatory (Abdulazeex *et al.,* 2010). The plant is used also in bone fracture, sprain, fever, cold, cough, and for eye and ear treatments (Rashmi *et al.,* 2010). Abdulazeez, (2011) reported that *Peristrophe bicalyculata* has phytoprotective, hypolipidemic and antiatherogenic properties after exposing Wister rats to P-407-induced hyperlidemic drug. Furthermore, the work of Esenowo *et al.* (2010), revealed that *P. bicalyculata* increased the packed cell volume (PCV), haemoglobin concentration (Hb conc.) and Red blood cell count (RBC) in albino rats. However, the antitrypanosomal properties of *Peristrophe bicalyculata* has never been reported, hence the basis for this research. The antibacterial effects of the *Peristrophe bicalyculata* have been reported by Janakiraman *et al.* (2012). They tested the ethanolic, acetone and chloroform extract of *Peristrophe bicalyculata* on *E. coli, B. cereus* and *S. typhi.* The outcome of the work revealed the antibacterial properties of *Peristrophe bicalyculata.* The work of Giwa *et al.* (2010) also pointed to the fact that *Peristrophe bicalyculata* has antibacterial properties. The plant was tested against *Klebsiella spp, Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa.* Significant growth inhibition ($p < 0.05$) was recorded for all the treatments.

Plants whose anti-*Trypanosoma evansi* properties have been reported in Nigeria are *Khaya senegalensis* (Adeiza *et al.,* 2010; Umar, *et al.*, 2010), *Eupatorium*

adenophorum (Shaba *et al.*, 2012). In these cases respectively, the plant extract were reported to have significant therapeutic properties against *Trypanosoma evansi*. The effects were reported to be dose-dependent. Other plants with anti-*Trypanosoma evansi* activity in other countries such as include *Tinospora bakis, Argemone maxicana* and *Aristolachia brateolata* in the Sudan (Abdelrahman, 2011).

CHAPTER THREE

MATERIALS AND METHOD

3.1: Collection and Identification of Plant Material

The leaves of this plant *Peristrophe bicalyculata* was collected at Kuru-Kuru village, Giwa Local Government, Kaduna State. It was transported to the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria for identification and authentication.

3.2: Processing of Plant Material

The plant was washed and air dried under laboratory condition for a week. The leaves were collected from the dried plant, crushed into powder using mortar and pestle, transferred into a sterile plastic container and stored in a refrigerator until required.

3.3: Methanol Extraction Process

The method of extraction employed was maceration using separating funnel. Two hundred and twenty-five grams of the pulverized plant material was weighed using digital weighing scale. The weighed pulverized plant material was transferred into conical flask. 775ml of 70% methanol was poured into the conical flask. This was allowed to stand for 72 hours. Thereafter, the liquid was drained into a conical flask and transferred into an evaporating dish placed on a water bath. The temperature of the water bath was maintained at a range of 45±5^{o}C. This was maintained for 24 hours to allow evaporation of the solvent. The residue was scrapped gently using a sharpened spatula, and weighed.

3.4: Aqueous Extraction Process

Ten grams of pulverized plant material was extracted in distilled water for 6 hours at slow heat at a range of (45±5^{0}C). The mixture was filtered through 8 layers of muslin

cloth and centrifuged at 5000rpm for 5mins. The supernatant was collected. This procedure was repeated twice and after 6 hours the supernatant was collected and transferred into a water bath to allow for evaporation and the residue weighed thereafter.

In both cases (methanol and aqueous extraction process), the colour of the extract observed was light brown, bitter when tasted and aromatic from the scent perceived.

3.5: Phytochemical Analysis of the Plant Extract

3.5.1: Qualitative Analysis

Five grams of the plant extract was dissolved in 5ml of distilled water to form the solution that will be used throughout the qualitative bioassay. The bioassay covers for both methanol and aqueous extracts of *Peristrophe bicalyculata.*

3.5.1.1: Test for carbohydrates.

This was carried out as described by Honeyman and Guthrie (1968):

Molisch's test

To 1ml of the extract, 3 drops of Molisch's reagent were added and allowed to stand for 2 minutes. The presence of red or dull violet coloration at interphase indicated the presence of carbohydrate.

3.5.1.2: Test for alkaloids

This was carried out as described by Rafauf (1970)

Dragendorff's test

To 1ml of the extract, 1ml of HCl and 3 drops of Dragendorff's reagent were added. The formation of orange precipitates indicated the presence of alkaloids.

Wagner's test

To 1ml of the extract, 1ml of HCl and 3 drops of Wegner's reagent were added. The formation of a brown precipitate indicated the presence of alkaloids.

3.5.1.3: Test for Glycosides

This was carried out as described by Evans (1996):

To 1ml of the extract, 2 drops of conc. H_2SO_4 were added and placed in water bath for about 15 minutes. Twenty percent of KOH was added to make the solution alkaline. To this solution, few drops of $FeCl_2$ were added. The formation of brick red precipitate indicates the presence of glycosides.

3.5.1.4: Test for Saponins

To 1ml of extract, 3ml of water was added, shaken and allowed to stand for about 3 minutes. The presence of persistent froth denoted the presence of saponins (Evans, 1996).

3.5.1.5: Test for Anthraquinones

This was carried out as described Tyler *et al.* (1988)

Combine Anthraquinones (Bontrager's Test)

To 1ml of extract, 5ml of 10% HCl was added and allowed to stand for 5 minutes. The solution was filtered. The filtrate was decanted into a test tube and shaken with 5ml of benzene. The upper benzene layer was pipetted and transferred into a test tube containing 5ml of 10% ammonium hydroxide. Production of pink, red or violet colouration in the lower ammonia layer confirms the presence of combined anthraquinones.

Free Anthraquinones

To 1ml of the extract, a few drops of benzene were added and shaken. The content of the test tube was filtered. Five milliliters of 10% ammonia solution was added to the filtrate. The presence of pink, red or violet colour in the lower ammoniacal layer indicated the presence of free anthraquinones.

3.5.1.6: Test for Tannins

Gelatin Test

To 1ml of the extract, 5ml of 1% gelatin containing NaCl was added. Formation of a yellow precipitate denotes the presence of tannins.

3.5.1.7: Test for Sterols and Terpenes (Liebermann-Burchard's Test)

To 1ml of the extract, few drops of conc. H_2SO_4 acid in a slanty position was added and left standing for an hour. The formation of brown ring at interphase indicated the presence of sterols and terpenes (Briggs, 1970).

3.5.1.8: Test for flavonoids

This was carried out as described by Dermarderosian and Liberti (1988):

Sodium hydroxide test

To 1ml of the estract, 3ml of 10% sodium hydroxide was added followed by 3ml of 10% HCl. The formation of a yellow colour on addition of NaOH, which disappeared on addition of the HCl, indicated the presence of flavonoids.

Shinoda's test

To 1ml of the extract, 3 chips of magnesium and the few drops of conc. H_2SO_4 acid was added. A pink or red colouration on addition of the acid indicated the presence of flavonoids.

3.5.2: Quantitative Analysis

3.5.2.1: Determination of Cyanogenic glycoside

The extraction process as described by Onwuka (2005) was adopted. Twenty-five grams of the extract was dissolved in 50ml of distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1ml of the sample filterate, 4ml of alkaline pocrate were added and absorbence was recorded at 550nm. The cyanide content was determined from a cyanide standard curve.

$$\text{Cyanide (mg/g)} = \frac{\text{Absorbance x GF x DF}}{\text{Sample weight.}}$$

Where GF= gradient factor and

DF= dilution factor

3.5.2.2: Alkaloid determination using Harbourne (1973) method

Five grams of the dried extract was weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on the water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and then weighed.

3.5.2.3: Tannin determination by Van-Burden and Robinson (1981)

Five hundred milligrams of the extract were weighed into a 50ml plastic bottle. Fifty milliliter of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pippetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCL

and 0.008M Potassium ferrocyanide. The absorbance was measured at 120nm within 10min.

3.5.2.4: Saponin determination

The method used is that of Obadoni and Ochuko (2001). Twenty grams of the extract were put into a conical flask and 100cm^3 of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4hrs with continuous stirring at about 55^{0}C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts will be reduced to 40ml over ether bath at about 90^{0}C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight; the saponin content was calculated as percentage of the extract.

3.5.2.5: Flavonoid determination by the method of Boham and Kocipai-Abyazan (1994):

Ten grams (W_1) of the pulverized plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whitman filter paper. The filtrate was later transferred into a crucible that had been weighed (W_2). The content of the crucible (filterate) was evaporated into dryness over a water bath and weighed to a constant weight (W_3) and the quantity of flavonoids

was calculated as a percentage of the mass of the pulverized plant material as shown below.

$$\% \text{ Flavonoids} = \frac{W_3 - W_2}{W_1} \times 100$$

3.5.2.6: Determination of oxalate

The titration method as described by Omale and Omajali, (2010) was employed to determine the oxalate content. 1 gram of the sample was weighed into 100ml conical flask and 75ml 3N H_2SO_4 was added and stirred intermittently with a negative stirrer for 1hr. it was then filtered using whatmann filter paper. From the filtrate, 25ml was taken and titrated while hot 80-90^0 against 0.1N $KMnO_4$ solution until a faint pink colour persisted for at least 30secs. The overall reaction is

$$MnO_4^- + Cr_2O_4^{2-} + 8H^+ \longrightarrow Mn^{2+} + 4H_2O + 2CO_2$$

$$\text{Oxalate (mg/100g)} = \frac{T \times Vme \times DF}{Me \times Ms\ (g)}$$

Where T is the titer value of $KMnO_4$ (ml), Vme is the volume mass equivalent ($1cm^3$ of 0.05M $KMnO_4$ solution) is equivalent to 0.00225g anhydrous oxalic acid. DF is the dilution factor (Vt/A) where Vt is the total volume of titrate (filtrate) and A is the aliquot used, Me is the molar equivalent of $KMnO_4$ in oxalate.

3.6: Experimental Animals

Eighty Swiss albino mice of either sex, about 4-6 weeks old were obtained from the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria for the research. These animals were acclimated in the laboratory for two weeks. During this period, the rodents were fed *ad libitum* with commercially pelleted diet and water. Mice were pre-screened by microscopy of wet mount, thin and

thick tail tip blood smears to exclude the possibility of test animals harboring rodent *Trypanosoma evansi* species.

3.7: Survival period of the trypanosome infected mice

A pilot study was carried out to determine the survival period of mice infected with *Trypanosoma evansi*. 0.2ml of blood from a sacrificed (passaged) mouse, diluted with normal saline was inoculated into four mice. The parasitaemia was first noticed on the fourth day post inoculation in all the mice and on the tenth and twelfth day, the level of parasitaemia became massive. On the thirteenth and fourteenth day, 100% mortality was recorded.

3.8: Passaging of Mice and Parasite Development

Trypanosoma evansi was obtained from the Department of Parasitology and Entomology in Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, and was maintained in the laboratory by serial passaging in mice. The massively parasitaemic mice were sacrificed and the blood was collected using a sterilized syringe diluted in normal saline in the ratio of 1 to 3. A wet mount was prepared and the estimated number of trypanosomes was 1.585×10^7 per ml of blood at magnifications x400. 0.2ml of the blood was inoculated into thirty two (32) mice for antitrypanosomal bioassay (16 mice each for curative test using methanol and aqueous extracts respectively). Daily monitoring of the parasitaemia development was carried out.

3.9: Safe Dose and Acute Toxicity (LD_{50})

Lethal dosage test was carried out as described by Lorke (1983). The toxicity test was conducted in two phases. Nine mice were used in the first phase (Three groups of three mice each). Each group was inoculated with doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight respectively. The outcome of the first dosage test conducted

determined the range of doses that were administered in the second phase of the toxicity test. In the second phase, four mice were used, one for each group. The ranges of doses used were 200, 400, 800, 1600mg/kg body weight respectively. A total of 13 mice each were used in the toxicity bioassay for both the methanol and aqueous extract. The toxicity bioassay of the methanol and aqueous extract of *P. bicalyculata* using laboratory mice showed that the methanol and aqueous extracts lethal dosage (LD_{50}) was 1333mg/kg and 1400mg/kg body weight respectively. Therefore, the lethal dosage is less than 1500mg/kg body weight. Consequently, the dosage of the extract used for the curative bioassay are 400mg/kg and 500mg/kg body weight and 300mg/kg body weight and 400mg/kg body weight for the suppressive bioassay (this represents 30% the lethal dosage value).

The LD_{50} was computed using the formulae:

$LD_{50} = \sqrt{minimum\ toxic\ dose} \times maximum\ tolerated\ dose.$

3.10: Suppressive Test

The method of Fidock *et al.,* (2004) was employed. This involves treatment with the extract immediately after the mice were inoculated (early infection) with the parasite. Sixteen mice of both sexes were divided into four groups of four each in duplicate (a total of 32 mice). The mouse infected with the *T. evansi* (passaged mice) was anaesthetized with chloroform and its blood (3ml) was collected by cardiac puncture with sterilized syringe and needle and diluted using normal saline in the ration of 1 to 3. Each of the sixteen mice was inoculated intraperitoneally with 0.2ml diluted blood.

The first group of mice was treated with normal saline, in the second group, the mice were treated with 400mg/kg body weight of the extracts, the third group, the mice were treated 300mg/kg body weight and in the fourth group, the mice were treated with

5mg/kg body weight of diminazine aceturate. All drugs were administered intraperitoneally. All blood were collected for microscopy between 10am and 2pm daily.

Thick and thin films were made from the tail blood. A drop of blood was placed on a clean slide and spread evenly on the slide. The blood was allowed to dry under laboratory condition. The slide was fixed in methanol for 5 minutes and stained with 4% Giemsa (pH 7.2) for 25 minutes before examination under a microscope using glycerol. Five fields were examined per slide and the estimated number of parasite was recorded as described by Herbert and Lumsden, (1978).

3.11: Curative test

The curative test is similar to that of the suppressive test but treatment in this case began from the sixth day post infection. A duplicate of four groups of four mice each (a total of thirty-two mice) were used for the curative test. All the mice were inoculated with 0.2ml of the *T. evansi* infected blood. In the first group, the mice were infected and treated with normal saline. In the second group, the mice were treated with 500mg/kg body weight of the extract, in the third group, the mice were treated with 400mg/kg body weight and in the fourth group, and the mice were treated with 5mg/kg body weight diminazine aceturate (Berenil). The parasitaemic level was monitored daily by preparing a thin smear slide using Giemsa's stain. The slide was examined under the microscope to estimate the level of parasitaemia at x400 magnification according to the method of Herbert and Lumsden, (1978).

3.12: Histopathological studies

The liver and the spleen of each test animal were excised and preserved in 70% formal saline to preserve the tissue from degradation and to maintain the structure of the

cell and sub-cellular components for histopathological studies to determine the extent to which the infection affected these organs. The organs were later passed through baths of graded series of ethanol (30%, 50%, 70% and 100%) for dehydration to occur. These were cleared in xylene to remove the alcohol and were finally infiltrated with molten paraffin wax, the infiltration agent replaced the xylene. Each embedded organ was made into a block, and was sectioned using microtome set at 5-7µm. The sectioned sample was mounted on a glass slide for observation under the microscope and subsequent analysis. The sections prepared from all the treatments were compared to determine the pathological effect of the parasite.

CHAPTER FOUR

RESULTS

4.1: Methanol and Aqueous Extraction of *Peristrophe bicalyculata*

The weight of the residue obtained from the methanol and aqueous extract of *Peristrophe bicalyculata* were 55.17g from 367.28g of the methanol extract and 33.2g from 212.1g of the aqueous extract. These represent 15% and 15.6% of methanol and aqueous extract respectively. The result showed that there was no much difference in the quantity of the extract obtained using aqueous and methanol.

4.2 Qualitative analysis

The result of the qualitative test of methanol and aqueous extracts shows the presence of carbohydrate, alkaloids, cardiac glycosides, saponins, tannins, flavonoids, sterols and terpenes. Anthraquinones were absent (Table 4.1).

4.3 Quantitative analysis

Quantitatively, the methanol and aqueous extracts of *P. bicalyculata* had 0.12mg/100g and 0.13mg/100g of oxalate respectively. Saponins was lower (2%) in methanol extract compared with aqueous (14%). Flavonoid was found to be 0.42% and 0.24% in methanol and aqueous extracts respectively. Other secondary metabolites analyzed include phytate, cyanogenic glycosides and alkaloids as indicated in Table 4.2

Table 4.1: Comparative qualitative analysis of the phytochemical components of the methanol and aqueous extracts of *Peristrophe bicalyculata*

Sample/ secondary metabolites	Methanol extract	Aqueous extract
Oxalate	+	+
Phytate	+	+
Saponins	+	+
Cyanogenic glycosides	+	+
Alkaloids	+	+
Flavonoids	+	+
Sterols	+	+
Terpenes	+	+
Anthraquinones	–	–

+ = present - = absent

Table 4.2: Comparative quantitative analysis of the methanol and aqueous extract of *Peristrophe bicalyculata*

Sample / secondary metabolites	Methanol extract	Aqueous extract
Oxalate	0.12mg/100g	0.13mg/100g
Phytate	59.5mg/100g	47.6mg/100g
Saponins	2%	14%
Cyanogenic glycosides	960mg/100g	1216mg/100g
Alkaloid	15%	16%
Flavonoids	0.42%	0.24%

Tables 4.3: The first phase of the lethal dosage (LD_{50}) test (mortality expressed in %) for methanol extract using mice as test animal.

10 mg/kg body weight (%)	100 mg/kg body weight (%)	1000 mg/kg body weight (%)
0/3 (0%)	0/3 (0%)	2/3 (66.67%)

Table 4.4: The second phase of lethal dosage (LD_{50}) test (mortality expressed in %) for methanol extract using mice as test animal.

200mg/kg body weight (%)	400mg/kg body weight (%)	600mg/kg body weight (%)	1600mg/kg body weight (%)
0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)

NB: The numerator denotes number of animal which died while the denominator is the number of animals used.

Table 4.5: The first phase of the lethal dosage (LD_{50}) test (mortality expressed in %) for aqueous extract using mice as test animal.

10 mg/kg body weight (%)	100 mg/kg body weight (%)	1000 mg/kg body weight (%)
0/3 (0%)	0/3 (0%)	1/3 (33.3%)

Table 4.6: The second phase of lethal dosage (LD_{50}) test (mortality expressed in %) for aqueous extract using mice as test animal

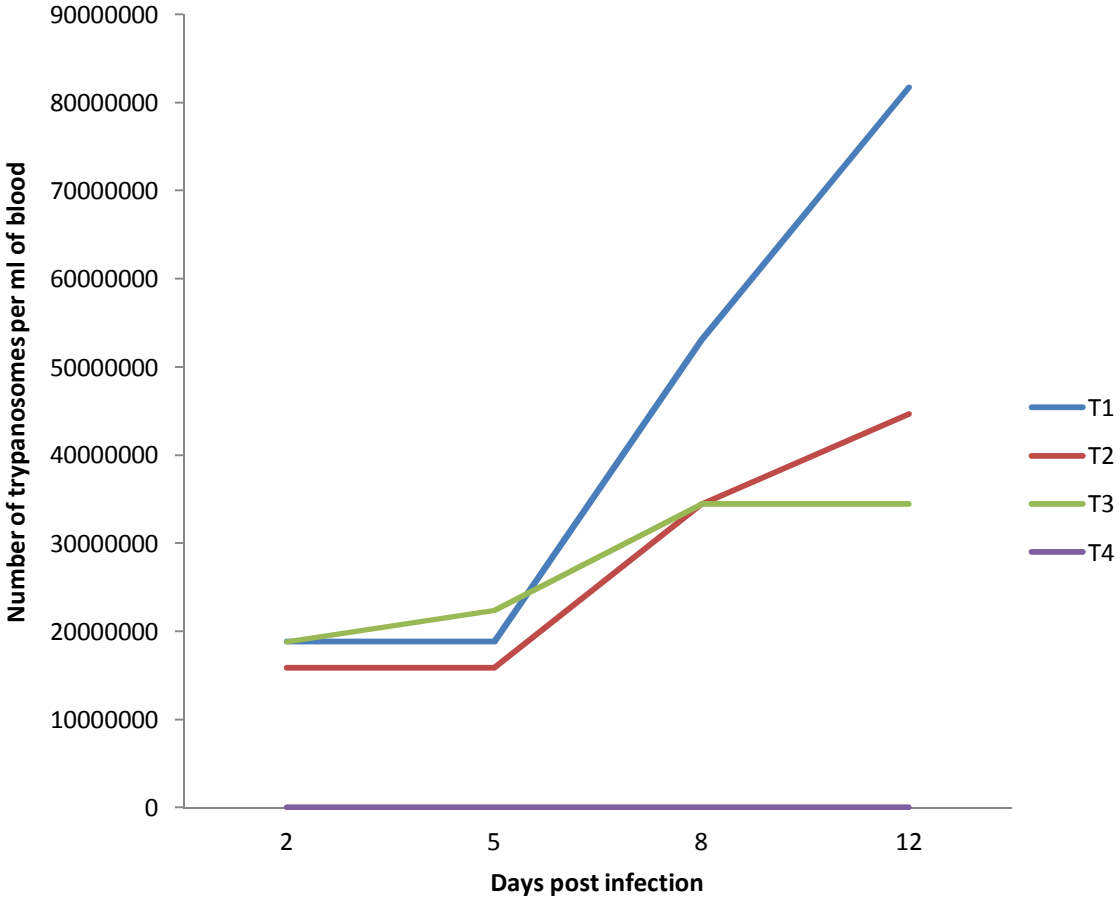
600mg/kg body weight (%)	1000mg/kg body weight (%)	1600mg/kg body weight (%)	2900mg/kg body weight (%)
0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)

NB: The numerator denotes number of animal which died while the denominator is the number of animals used.

4.4: Suppressive Test Conducted Using Methanol and Aqueous Extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*

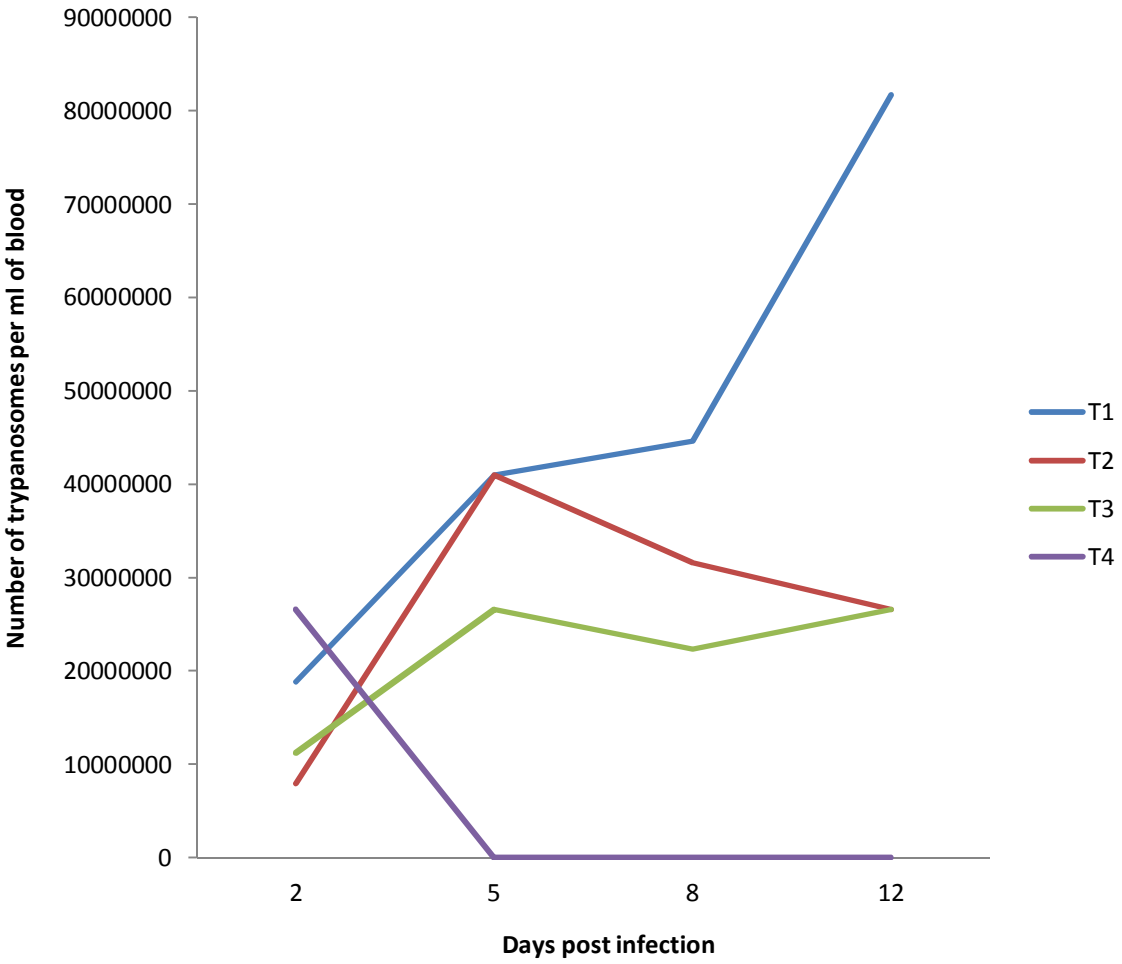
In the suppressive bioassay, the group treated with normal saline showed a progressive increase in the level of parasitaemia while group treated with diminazine aceturate showed complete suppressive effect. The other groups treated with aqueous extract at dose level of 400 and 500mg/kg body weight of *P. bicalyculata* respectively showed significant suppressive effect which is dose dependent as seen in figure 5.

The group treated with diminazine aceturate showed complete suppressive effect while the group treated with normal saline showed a continuous increase in the level of parasitaemia. The group treated with methanol extract of *P. bicalyculata* showed significant suppressive effect at doses of 300 and 400mg/kg body weight respectively and this effect is dose dependent as seen in figure 6



Where T1= Mice infected with *T. evansi* and treated with Normal saline, T2= Mice infected with *T. evansi* and treated with 300mg/kg body weight of *Peristrophe bicalyculata,* T3= Mice infected with *T. evansi* and treated with 400mg/kg body weight of *Peristrophe bicalyculata*, T4= Mice infected with *T. evansi* and treated with 5mg/kg body weight Diminazine aceturate

Figure 3: Suppressive bioassay of aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*



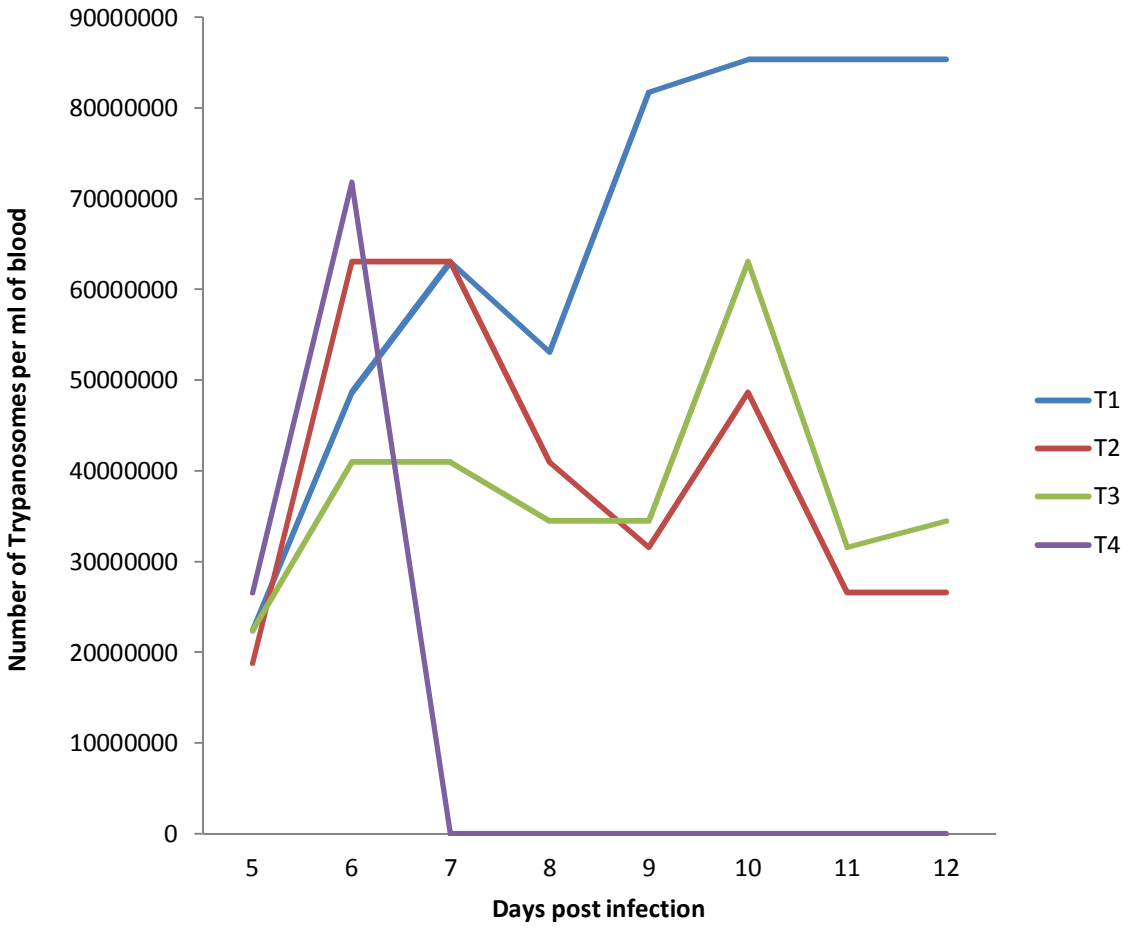
Where T1= Mice infected with *T. evansi* and treated with Normal saline, T2= Mice infected with *T. evansi* and treated with 300mg/kg body weight of *Peristrophe bicalyculata,* T3= Mice infected with *T. evansi* and treated with 400mg/kg body weight of *Peristrophe bicalyculata*, T4= Mice infected with *T. evansi* and treated with 5mg/kg body weight Diminazine aceturate

Figure 4: Suppressive bioassay of methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*

4.5: Curative Test Using Methanol and Aqueous Extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*.

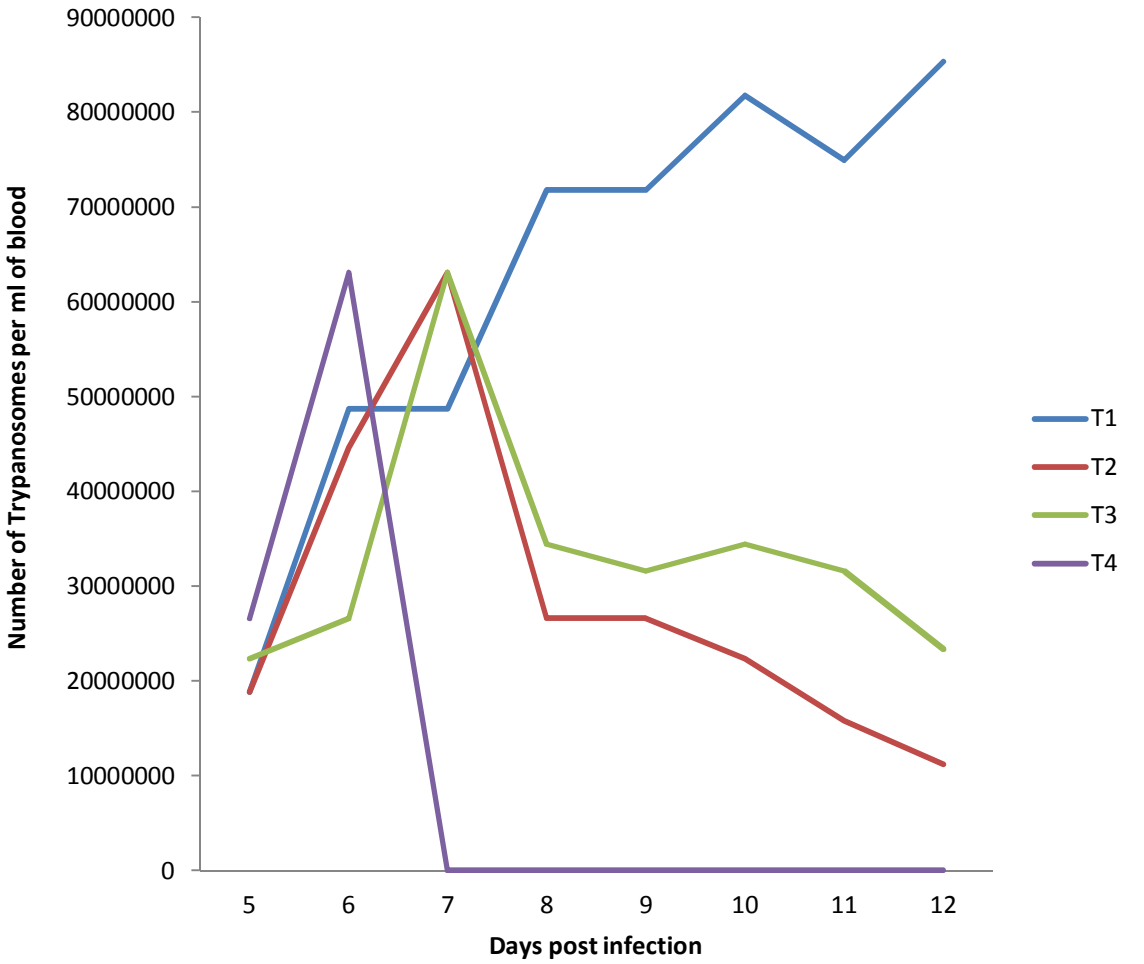
On the fourth day post inoculation, the average parasitaemia was 1.585 x 10^7 trypanosomes per ml of blood. The treatment began on the sixth day post inoculation after the level of parasitaemia was determined and ended on the twelfths day. Wet mounts and thin slides of blood smear were prepared accordingly and the level of parasitaemia was recorded on daily basis using a mathematical quick matching method as described by Herbert and Lumsden, (1978). These data obtained were subjected to analysis of variance (ANOVA). Both methanol and aqueous extract observed were significant at 5% level of significance and the effect is dose dependent.

The pretreatment period of the curative bioassay showed a progressive increase in the level of parasitaemia from the first day to the sixth day. No parasite was recorded in the group treated with diminazine aceturate while the group treated with normal saline showed a continuous increase in the level of parasitaemia, reaching their peak on the twelfth day. The group treated with the methanol extract at doses of 400 and 500mg/kg body weight respectively showed a significant decrease in the level of parasitaemia ($P<0.05$) and the treatment of diminazine aceturate (Berenil) cured the infection (figure 3).



Where T1= Mice infected with *T. evansi* and treated with Normal saline, T2= Mice infected with *T. evansi* and treated with 300mg/kg body weight of *Peristrophe bicalyculata,* T3= Mice infected with *T. evansi* and treated with 400mg/kg body weight of *Peristrophe bicalyculata*, T4= Mice infected with *T. evansi* and treated with 5mg/kg body weight Diminazine aceturate

Figure 5: Curative bioassay of the methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*



Where T1= Mice infected with *T. evansi* and treated with Normal saline, T2= Mice infected with *T. evansi* and treated with 300mg/kg body weight of *Peristrophe bicalyculata,* T3= Mice infected with *T. evansi* and treated with 400mg/kg body weight of *Peristrophe bicalyculata*, T4= Mice infected with *T. evansi* and treated with 5mg/kg body weight Diminazine aceturate

Figure 6: Curative bioassay of aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*

A progressive increase in the level of parasitaemia was noticed in the group treated with normal saline and reached its peak on the twelfth day. The group treated with diminazine aceturate was most effective while the group treated with aqueous extract of *P. bicalyculata* had significant reduction in the level of parasitaemia ($P < 0.05$) as seen in figure 4

4.6: Histopathology of the liver.

The histopathology of the liver of mouse without infection and treatment showed well outlined and compacted hepatocytes (H), central veins and minor arteries well defined (C), absence of inflammatory cells which precede necrosis. This shows a normal feature of an uninfected liver (plate II).

Plate III is the liver of an infected mouse treated Berenil. It showed the central hepatovein (C) with red blood cells (RBC) and sparse inflammatory cells (I), fatty appearance of the hepatocytes (H). The liver architecture remains compacted and intact signifying a healing process.

The histopathology of the liver in mouse treated with normal saline (Plate IV). It showed a total inflammation around the central portal vein (C), the presence of bridged central veins and portal areas by inflammatory cells (I) and detachment of hepatic cords (H).

The mouse treated with 500mg/kg body weight of the methanol extract of *Peristrophe bicalyculata* (Plate V) showed mild inflammation (I) around the central vein fewer bridges of the hepatic cords (H), spongy appearance and pigmentation of some nuclei (P). These features are characteristics of healing process initiated by the methanol extract of *P. bicalyculata.*

The liver of the mouse treated with 400mg/kg body weight of the methanol extract of *Peristrophe bicalyculata* showed some levels of denaturing of the hepatocytes contents, nuclei enlargement with scanty chromatin material, multiple nucleoli and spongy appearance of the hepatocytes (H), reduced inflammatory cells (I) around the central vein and reduced bridged blood vessels (M).

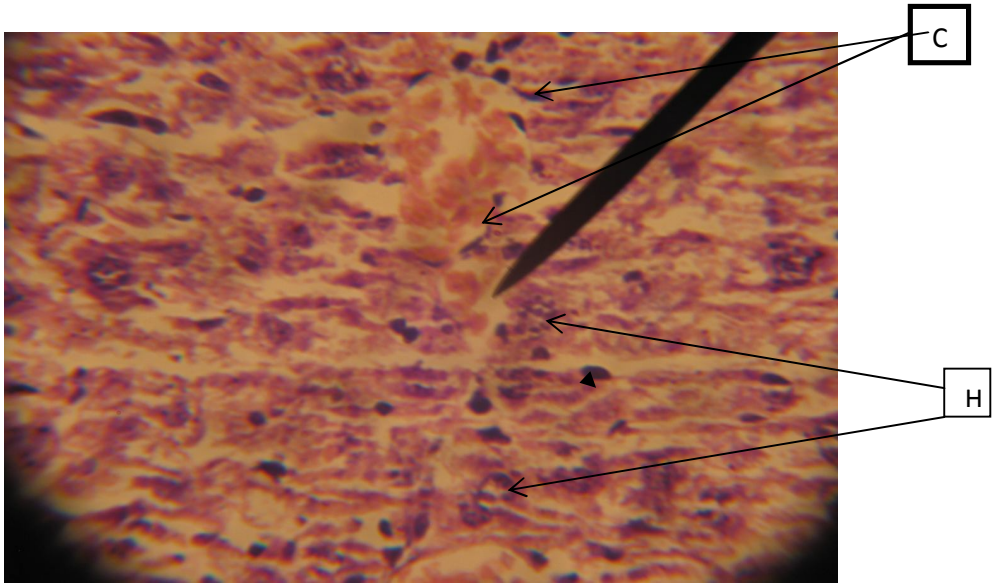


Plate II: Histopathology of the liver of mouse without infection and treatment. Compacted hepatocytes (H), Central vein and minor artery (C) (x400).

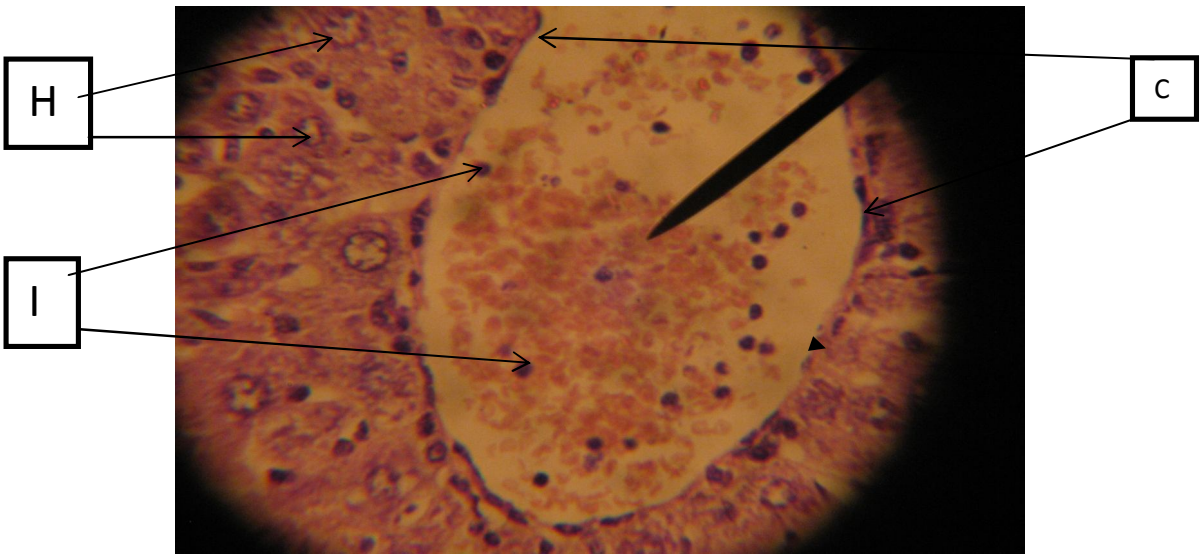


Plate III: Histopathology of the liver of mouse infected with *T. evansi* and treated with Diminazine aceturate. Central hepatovein (C), sparse inflammatory cells (I), Hepatocytes (H) (x400).

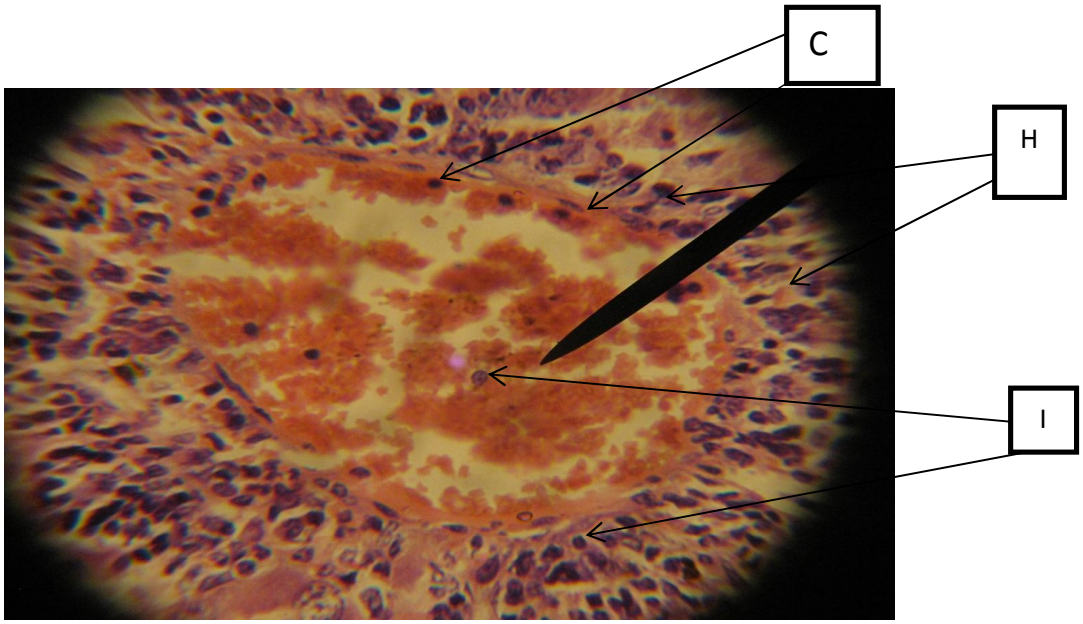


Plate IV: Histopathology of the liver of mouse infected with *T. evansi* and not treated. Central portal vein (C), inflammatory cells (I), detached hepatic cords (H) (x400).

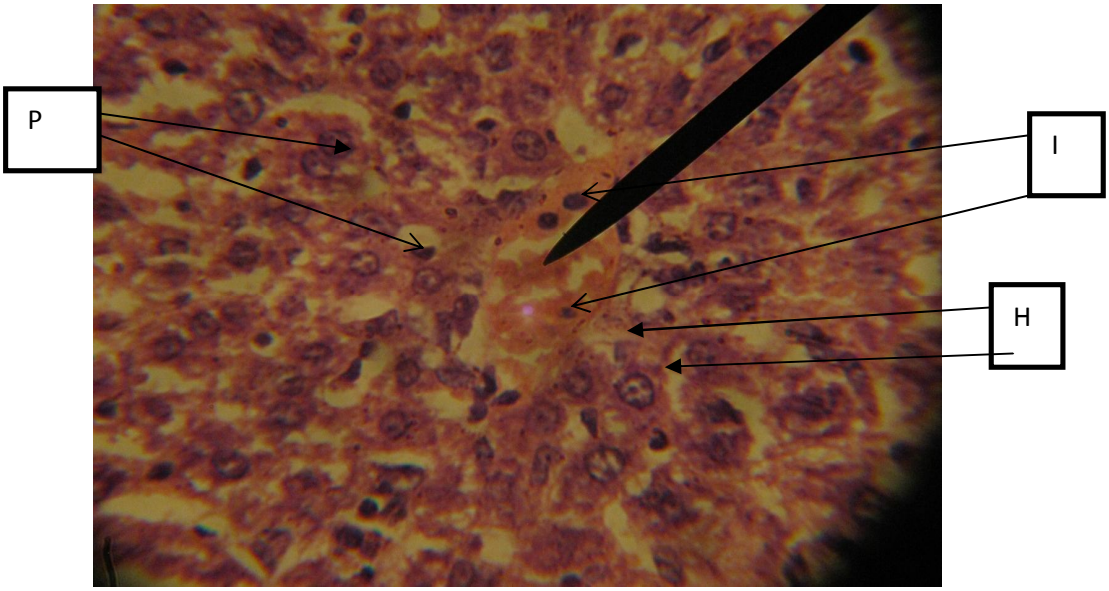


Plate V: Histopathology of the liver of mouse infected with *T. evansi* and treated with 500mg/kg body weight methanol extract of *Peristrophe bicalyculata*.
Inflammation of central vein (I), Bridges of Hepatic cords (H), spongy appearance and pigmentation of nuclei (P) (x 400).

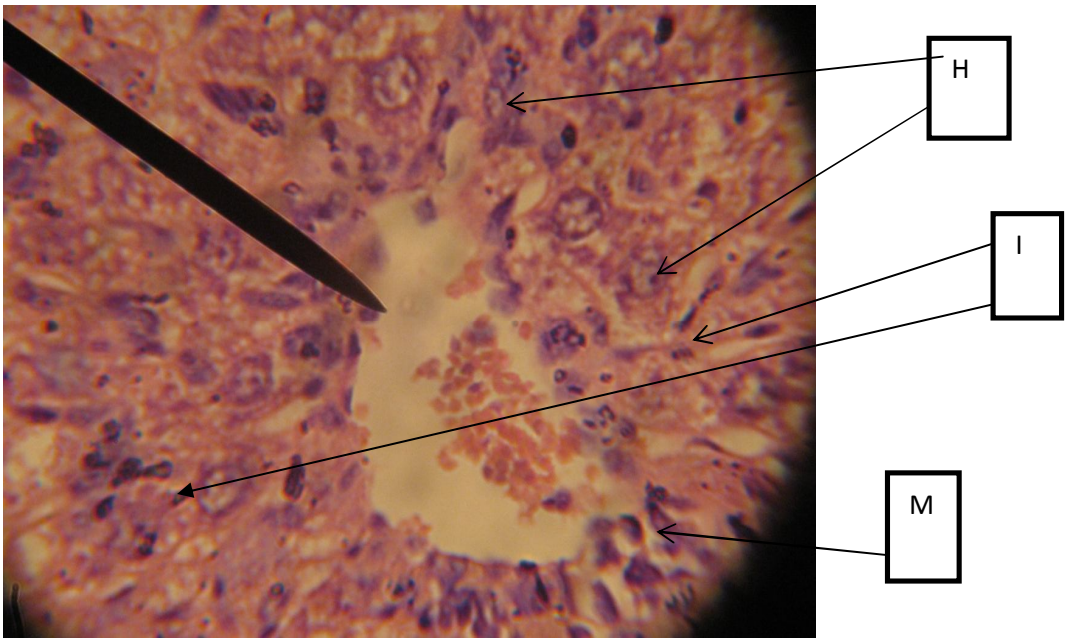


Plate VI: Histopathology of the liver of mice infected with *T. evansi* and treated with 400mg/kg body weight methanol extract of *Peristrophe bicalyculata.* spongy hepatocytes (H), reduced inflammatory cells (I), reduced bridged blood vessels (M) (x400).

4.7: Histopathology of the Spleen

The spleen of the infected mice (Plate VII) showed a normal histology with distinct white pulp (WP) and red pulp (RP) with distinct cuticle. The white pulp acts as reservoir for white blood cells (WBC) while the red pulp acts as a reservoir of the red blood cell (RBC).

The histopathology of the spleen in the mice treated with diminazine aceturate (Berenil) revealed reduced number of white pulp (WP) and less compacted red pulp (RP) as seen in plate VIII. The reduced number of the white pulp can be attributed to their defensive role in combating infection. The red pulps are less evenly distributed compared to the normal without infection and treatment. This change is to augment the red blood cells destroyed due to infection.

The spleen of the mouse infected with *Trypanosoma evansi* and not treated showed the absence of the white pulp (WP) and less accumulation of the red pulp (RP) as seen in Plate IX.

The spleen of the mouse infected and treated with 500mg/kg body weight methanol extract of *P. bicalyculata* (Plate X) showed the presence of white pulp (WP) and relatively compacted red pulp (RP). This view suggests that the methanol extract has some defensive role against the infection.

The histopathology of the spleen of mouse infected with *T. evansi* treated with 400mg/kg body weight methanol extract of *Peristrophe bicalyculata* (Plate XI) showed highly compacted red pulp (RP) and sparse white pulp (WP).

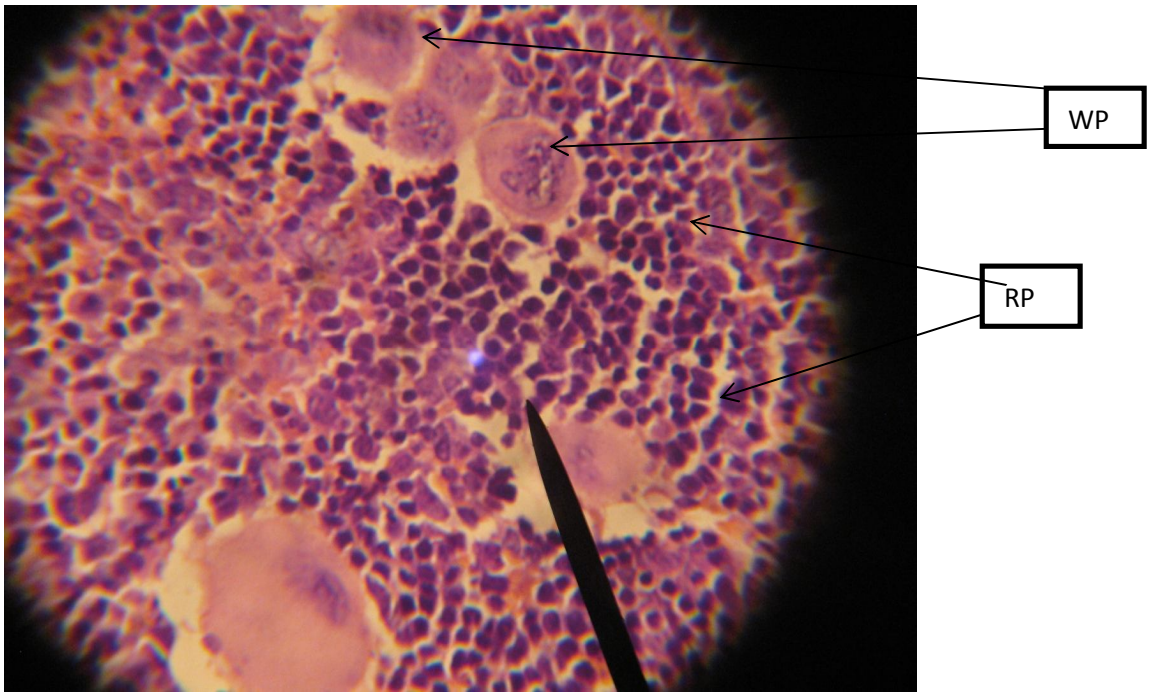


Plate VII: Histopathology of the spleen without infection and treatment. Distinct white pulp (WP), red pulp (RP) (x400)

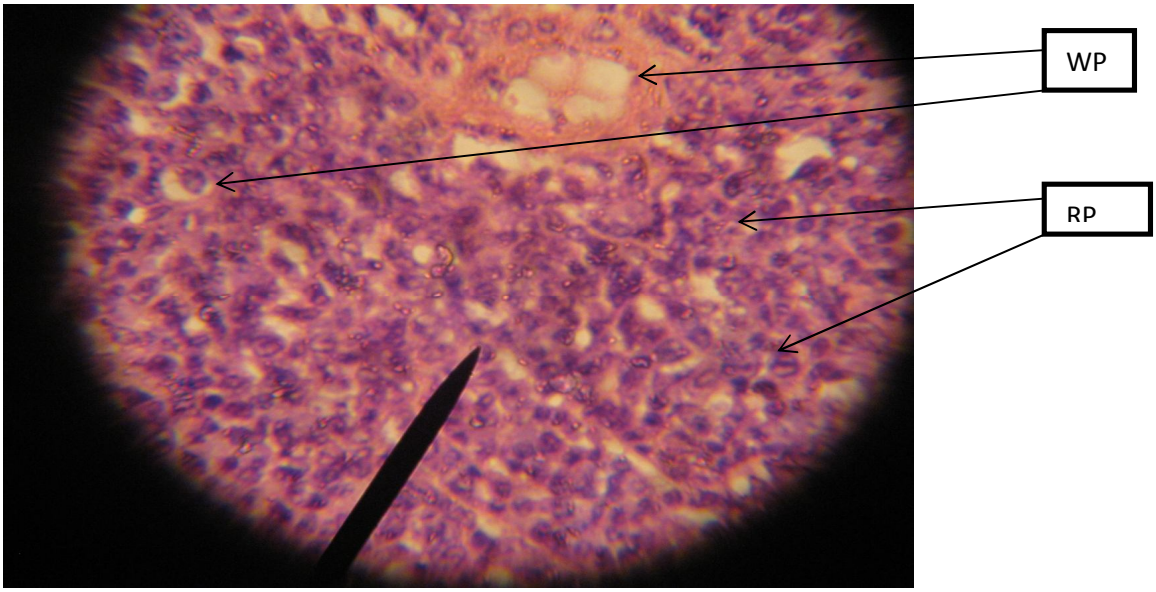


Plate VIII: Histopathology of the spleen of mouse infected with *T. evansi* and treated with Diminazine aceturate. Reduced white pulp (WP), less compacted red pulp (RP) (x400).

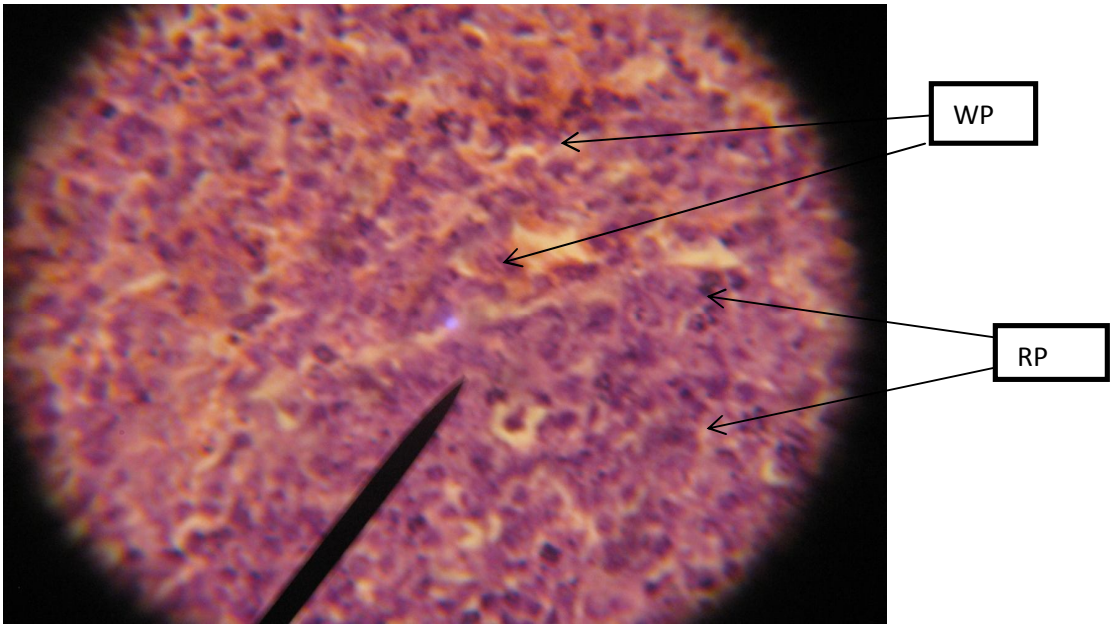


Plate IX: Spleen of mice infected with *T. evansi* without treatment. Absence of white pulp (WP) to support the leucocytes in their defensive role against infection. Reduced red pulp (RP). (x400)

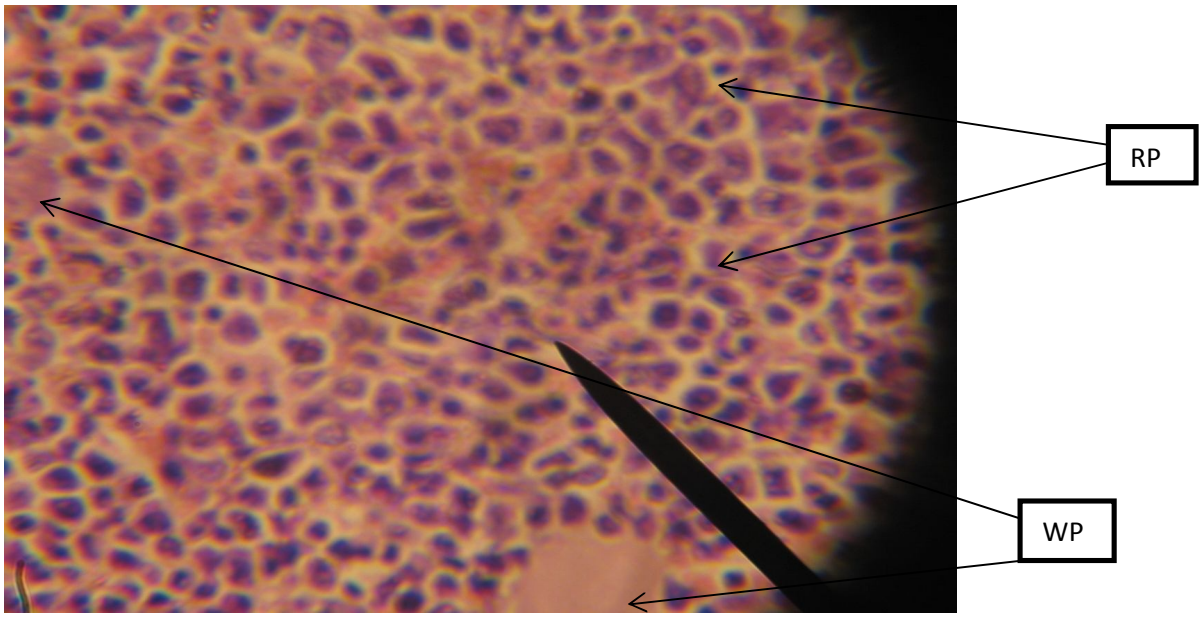


Plate X: Spleen of mice infected with *T. evansi* and treated with 500mg/kg body weight methanol extract of *Peristrophe bicalyculata.* White pulp (WP), compacted red pulp (RP) (x400).

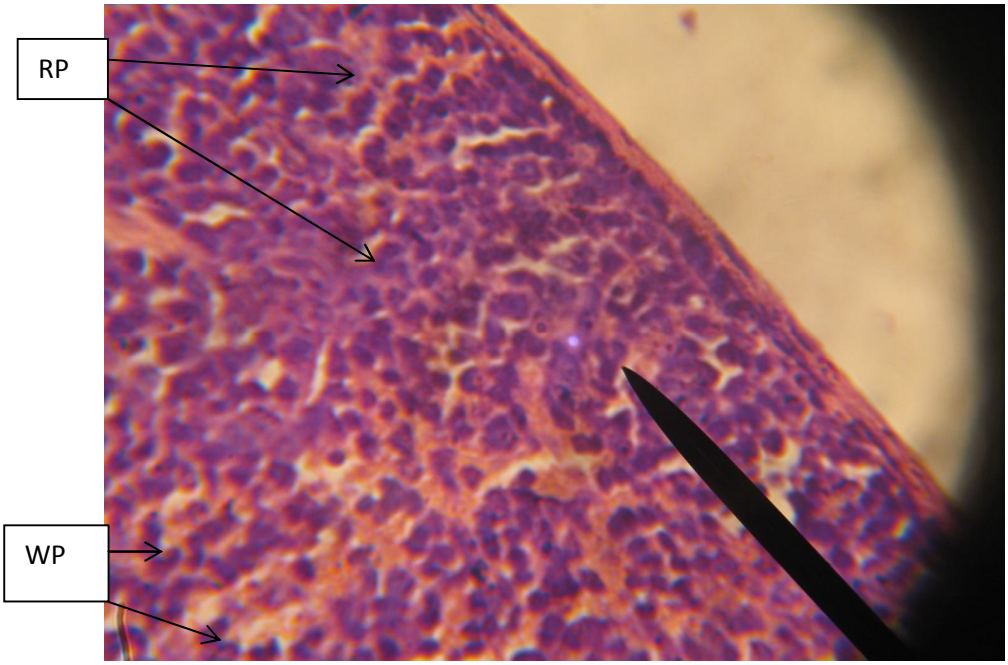


Plate XI: Histopathology of spleen infected with *T. evansi* and treated with 400mg/kg body weight methanol extract of *Peristrophe bicalyculata.* Compacted red pulp (RP), sparse white pulp (WP) (x400).

4.8: Morphohaematological Variation

Some morphological characteristics observed during the course of the bioassay include polychromasia and anisocytosis (PA), a stage which precede phagocytosis leading to destruction of the red blood cells resulting in anaemia in the infected mice.

Plate XII shows a normal morphology of the red blood cells (RBC) of healthy mice (mice without infection).

The group of mice infected with *Trypanosoma evansi* and treated with diminazine aceturate (Berenil) showed some variations in colour (Polychromasia) and size (anisocytosis) of the red blood cells (RBC) and total absence of the parasite as seen in plate XIII.

The group of mice infected with *Trypanosoma evansi* and treated with 500mg/kg bw methanol extract of *Peristrophe bicalyculata* (Plate XIV) showed low level of parasitaemia, variation in colour (Polychromasia) and size (anisocytosis) of the red blood cells (RBC).

The mice infected with *Trypanosoma evansi* and treated with 400mg/kg body weight of *Peristrophe bicalyculata* showed a higher level of parasitaemia, polychromasia and anisocytosis as seen in plate XV.

The highest level of parasitaemia was seen in the group of mice infected with *Trypanosoma evansi* and treated with normal saline. Anisocytosis and polychromasia was also noticed in this group as seen in plate XVI.

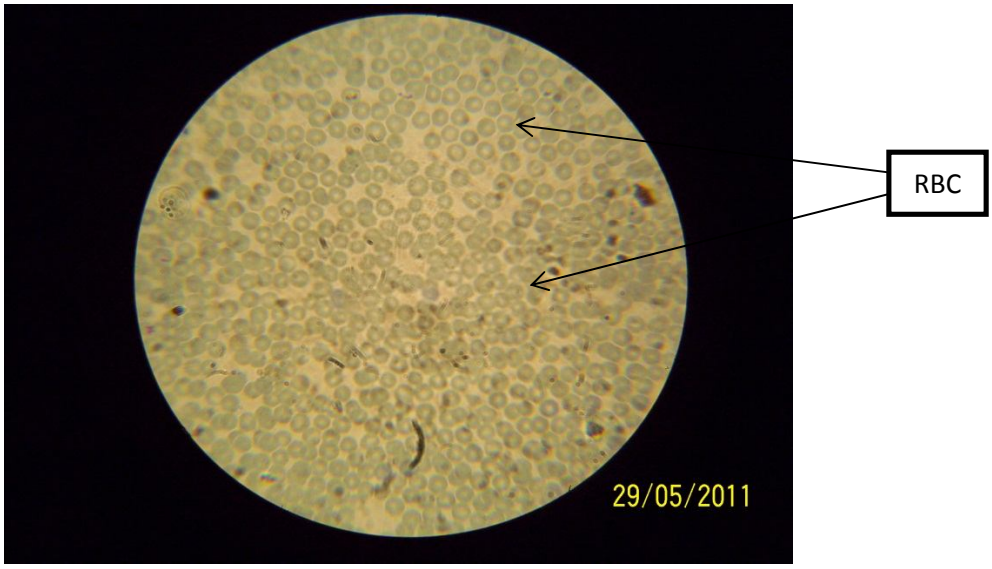


Plate XII: Morphohaematological variations in a blood film prepared from uninfected mouse. Normal red blood cells (RBC) (x400).

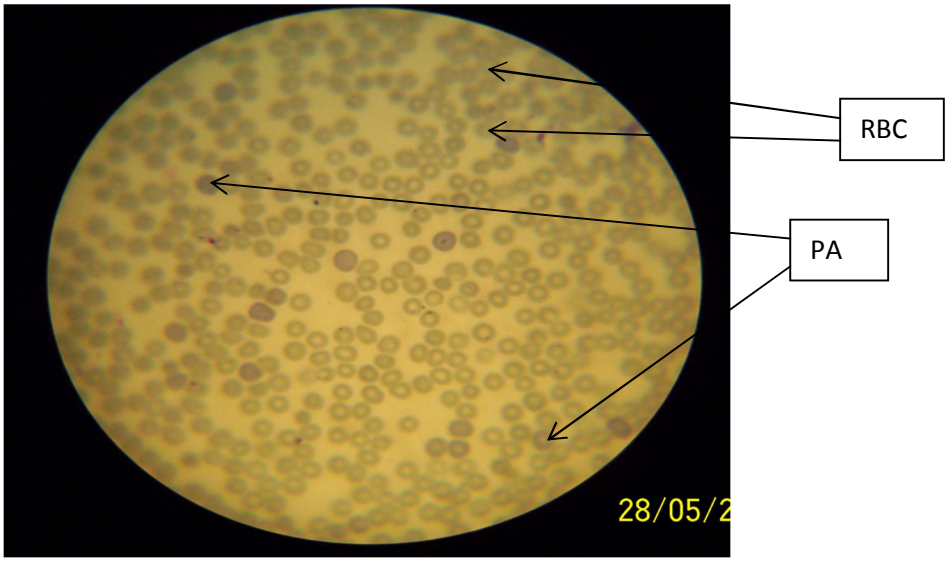


Plate XIII: The variation in the colour (polychromasia) and size (anisocytosis) of the red blood cells (PA) in mouse treated with Diminazine aceturate (Berenil). RBC (Red Blood Cells) (x400).

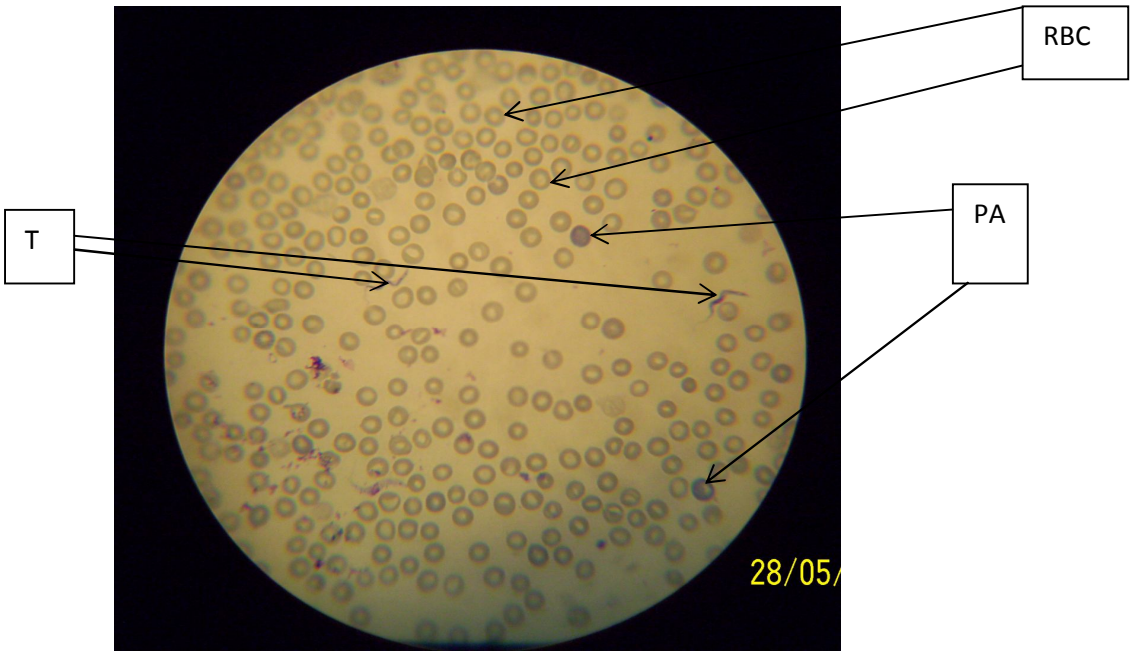


Plate XIV: Presence of *T. evansi* (T), polychromasia and anisocytosis (PA) of red blood cells (RBC) in mouse infected and treated with 500mg/kg body weight methanol extract of *P. bicalyculata* (x400).

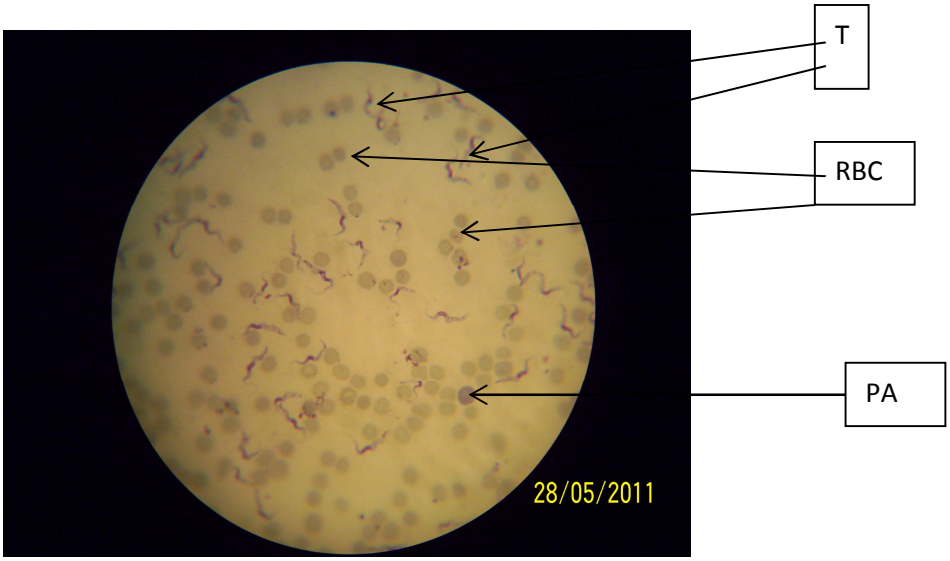


Plate XV: Presence of *T. evansi* (T), polychromasia and anisocytosis (PA) in red blood cells (RBC) of mice infected and treated with 400mg/kg body weight methanol extract of *P. bicalyculata* (x400).

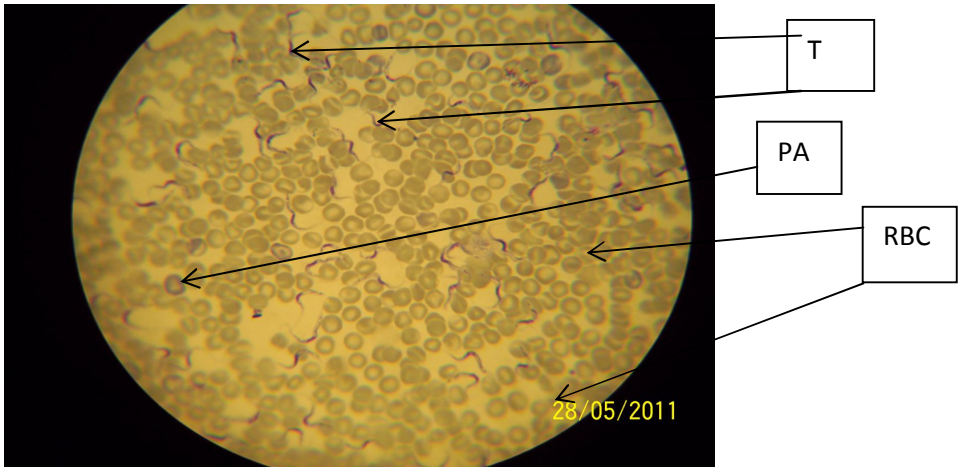


Plate XVI: Polychromasia and anisocytosis (PA) in red blood cells (RBC) in mice infected with *Trypanosoma evansi* (T) but treated with normal saline (x400).

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical analysis

The qualitative analysis of the methanol and aqueous extract of *Peristrophe bicalyculata* shows the presence of the following secondary metabolites; saponins, cyanogenic glycosides, flavonoids, terpenes, oxalate, phytate, alkaloids and sterols. A similar finding was reported by Esievo *et al.,* (2010) on the same plant except for the presence of cyanogenic glycoside. The absence of anthraquinones in this plant is in agreement with the findings of Abdulazeez *et al.,* (2010).

The quantitative analysis of the secondary metabolites shows varying compositions in the methanol and aqueous extract of *P. bicalyculata* which is significantly different at ($P<0.05$). This difference could be as a result of the solvent used in the extraction processes. The presence and the relative composition of secondary metabolites contribute to the medicinal value of most plants as well as physiological activity (Sofowora, 1993). The quantity of flavonoids in methanol (0.42%) was found to be higher than in aqueous extract (0.24%). For instance, flavonoids are responsible for the yellow or orange colours in herbs (Hoffman, 2000). Many flavonoids have a diuretic action, antibacterial, anti-inflammatory, anti-allergic, antiviral, antineoplastic activity (Alan and Miller, 1996). Many of these allergic effects have been linked to their known functions as strong antioxidant, free radical scavengers and metal chellators (Sofowora, 1993; Burda and Oleszek 2001). Bioflavonoids are part of plants that contain vitamin C and they act with vitamin C to enhance its absorption and metabolism in the body. They have a strengthening and healing effect on the blood vessels, they are used to treat

conditions such as capillary fragility, tendency to bruising and nosebleeds and high blood pressure (Hoffman, 2000).

Saponins are present in *P. bicalyculata*. They are glycosides found in many medicinal plants; they lather like soap when they are mixed with water. Saponins have a number of different effects on the body including an expectorant effect, diuretic effects, beneficial effects on the circulatory system, reducing the fragility of the blood vessel walls (McGrath, 1991). Saponins have been reported to show tumor inhibiting activity on some experimental animals such as *Rattus novergicus* (Akindahunsi and Salawu, 2005)

Steroidal compounds are of importance in pharmacy because of their relationship with compounds used as sex hormones (Okwu, 2001). Steroidal compounds have similar structure and function to the human sex hormones produced by the ovaries, adrenal glands and in men the testes. They are used most widely in the treatment of women's problems. They are regulating and as such are called 'adaptogens', and the most known of these is ginseng (McGrath, 1991).

The average alkaloid composition was 15% and 16% in the methanol extract and aqueous extracts respectively. These differences can be considered to be negligible nevertheless alkaloids contribute to plant species fitness of survival and have pharmacological effects; they are used as medication and recreational drugs and produce bitter taste that repels insects from feeding on the plant leaves (Roger and Wink, 1998).

In this study methanolic extraction of phytate is higher (59.5mg/100g) in methanol extract than in aqueous extraction (47.6mg/100mg). This probably indicates higher activity of phytate to alcohol than water. Phytate has chelating effect, inhibits or even cure some cancers by depriving those cells of mineral (especially iron) as broad

treatment for cancer (Hunell, 2003). Phytate can also be used as preservative and also food additive (Malleshi and Desikachar, 1980).

Cyanogenic glycoside concentration in *Peristrophe bicalyculata* in this study appears to be higher (1216mg/100g) in aqueous extract than (960mg/100g) the methanol extract. This variation in the composition of the cyanogenic glycoside could be due to the differences in the processes of preparation and the solvent used in the extraction (Siegler, 1998). Cyanogenic compound is a group of the aglycone. It is often very important in the field of biochemistry and pharmacology. In this group, aglysone contains this glycoside in the vacuole, but if the plant is attacked, they are released and become activated by enzymes in the cytoplasm. These remove the sugar part of the molecule and release the toxin hydrogen cyanide. Storing them in inactive forms in the cytoplasm prevents them from damaging the plant under normal conditions (Marco, 2007). The positive effects of glycoside and cyanogenic glycoside are not common but their toxic effects include decreased heart rate, sympathetic activity and systematic vascular resistance (Siegler, 1998).

5.2 Acute toxicity

The lethal dosage test (LD_{50}) of *P. bicalyculata* was determined to be less than 1500mg/kg body weight of mice. The lethal dosage LD_{50} of the methanol extract of *Peristrophe bicalyculata* was determined to be 1131mg/kg body weight while that of the aqueous extract of the same plant was determined to be 1265mg/kg body weight. In most researches conducted, rats were used as the experimental animal and the LD_{50} determined vary. For instance, in the work of Abdulhazeez *et al.,* (2010) showed that the LD_{50} of *Peristrophe bicalyculata* was above 5000mg/kg body weight in his work titled "the

effects of *Peristrophe bicalyculata* on the lipid profile of rats". These differences in the lethal dose values can be attributed to differences in weight and physiological processes of the mice and rats. Rats are generally of bigger body size compared to mice hence, their ability to withstand toxins compared to mice (Jigam *et al.,* 2009).

5.3 Antitrypanosomal activity

The antitrypanosomal test conducted for both the methanol and aqueous extracts of *Peristrophe bicalyculata* using *Trypanosoma evansi* as test parasite in laboratory mice had significant ($P < 0.05$) curative and suppressive activity against *T. evansi*. These effects are dose-dependent. The specific secondary metabolite(s) with the antitrypanosomal properties have not been ascertained, however, from the curative and suppressive bioassay, methanol extract was found to be more potent. This action can be attributed to the flavonoid content. However, it is suspected that synergistic effects of the secondary metabolites such as flavonoids, cyanogenic glycosides, alkaloids, saponins and phytates content of the plant produced the resultant antitrypanosomal effects.

In the group of mice treated with Diminazine aceturate, there was no parasite development, suggesting the high efficacy of the drug against the parasite, hence, the parasites were completely suppressed in the standard drug treatment while in the treatments with methanol and aqueous extracts there was progressive suppressive effect which is dose dependent. It is possible that the active compounds might not be present in enough concentrations to effect rapid clearance of the target parasites (Jigam *et al.,* 2009).

The antitrypanosomal activity of *P. bicalyculata* investigated in mice infected with *T. evansi* showed significant positive activity *in vivo* compared to the untreated

group. Its curative and suppressive effects were moderate. It has been suggested that crude plant extracts tends to have better trypanostatic than trypanocidal effects because unpurified bioactive compounds may require initial conversions which time lag allows for parasite proliferation (Noedl *et al.,* 2003; Jigam *et al.*, 2009). Similarly, on the antitrypanocidal properties of neem (*Azadirachta indica)* revealed that the antitrypanosomal effect could be attributed to the active secondary metabolites such as condensed tannins, gallic acids, gallocatechin, epicatechin, catechin and epigallocatechin, three trycyclic diterpenoids: margolone, margolonone and isomargolone, and polysaccharides isolates from neem bark (Udenyi, 1993). Polyphenols have been reported to have the ability to complex with extra-cellular and soluble proteins and also parasite cell wall and thereby disrupting the parasite cell membrane. The site and number of hydroxyl groups on polyphenols are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in toxicity (Christiana *et al.,* 2004; Tasdemir *et al.,* 2006; Karori *et al.,* 2008). This explains why polyphenols present *P. bicalyculata* are thought to be more effective in parasite reduction. The ability of the plant extract to lower the levels of parasitaemia can therefore be attributed to the toxic activity of bioactive compounds, working in synergy to bring about the antitrypanosomal effects (Paveto *et al.,* 2004; Tasdemir *et al.,* 2006).

The improved survival of trypanosome infected mice could be due to the effect of polyphenols in the *P. bicalyculata* plant extract. Polyphenols in tea have been shown to attenuate cytokine induced (Karori *et al.,* 2008) and also nitric oxide inflammation (Chen *et al.,* 2004). At the same time tea protects against nitric oxide toxicity due to catechins (Paquay *et al.,* 2000).

The variation in size (anisocytosis) and colour (polychromasia) of the red blood cells may be as a result of the action of sialidase enzyme secreted by the parasites which acts on the sialic acid of the red blood cells thereby altering the chemical orientation of the cell membrane thus, exposing the red blood cells to phagocytic action (Schauer, 1985).

5.4 Histopathology

The biopsies of the liver reveal some variations which differ markedly between the normal mice (non- infected mice) and the treatments. The variations observed among the treated mice ranged from the presence of inflammatory cells around the central veins which cut across all the treatments, through enlarged hepatocytes, vacuolation, congestion of the central veins, spongy appearance of hepatocytes to the detachments of the hepatic cords (degeneration), these features define hepatomegaly. These observations are innate response to the invading parasites but some level of hepatoprotective action was observed in mice treated with methanol and aqueous extracts. This finding is in agreement with that of Abdulazeez *et al.* (2010) who made similar observation while working on the antihypertensive and antiprotective action of *P. bicalyculata.*

Similarly, the biopsy of the spleen shows some variations between the treatments and normal (non-infected) mice. Although, the spleen plays a role in boosting the immune system but it is not indispensible. The gross view of the spleen when compared to the normal shows some level of the degeneration of the white pulp and varying compactness of the red pulp. The white pulps are known to contain cells that are defensive therefore, their reduced degeneration in an indication that they may likely be released into the circulatory system to aid the body immune system in their defensive

responsibility. Other changes observed in the spleen are high proliferation of plane nuclear chromatin, less accumulation of red blood cells, fibroblast formation, and high infiltration of the leucocytes. These were more observed in the negative control without treatment. It is very likely that this was a response to the foreign antigen of the parasites which probably has resulted to such conditions when compared to the normal (without infection).

In both liver and spleen biopsy, it was noticed that the features of the histopathology of those mice treated with methanol and aqueous extracts of *P. bicalyculata* at 500mg/kg body weight and diminazine aceturate did not differ much with the normal. These observations can be attributed to the hepatoprotective and spleenoprotective properties of *P. bicalyculata* however, the negative treatments showed the highest damage. In the mice treated with 400mg/kg body weight, the damage was more compared to the treatments with 500mg/kg body weight of *P. bicalyculata*. These observations suggest that the extent of the damage is dose dependent.

Other observations include variation in colour (polychromasia) and size (anisocytosis) as seen in plates XIII, XIV, XV and XVI. It is suspected that the changes observed is due to the action of the sialidase enzyme secreted by the parasites which react with the sialic acid found on the membrane of the red blood cells thereby changing the chemical orientation of the red blood cell membrane exposing them to phagocytic action (Nees *et al.,* 1975; Corfield, 1992). These observations were made on both the methanol and aqueous extracts of *P. bicalyculata* and the positive treatment. This action may play a significant role in the invasive action of the parasite in the host resulting in anemia.

5.5 Conclusion

This study showed that the secondary metabolites present in the methanol and aqueous extract of *Peristrophe bicalyculata* are oxalate, phytate, saponins, cyanogenic glycosides, alkaloids and flavonoids with compositions 0.12mg/100g, 59.5mg/100g, 2%, 960mg/100g, 15% and 42% respectively for the methanol extract and 0.13mg/100g, 47.6mg/100g, 14%, 1216mg/100g, 16% and 0.24% respectively for the aqueous extract. The qualitative test showed similar results of the secondary metabolites in methanol and aqueous extract; however, quantitatively aqueous extract of *P. bicalyculata* had more of the quantity of the secondary metabolites compared with the methanol extract ($P<0.05$). The test plant *Peristrophe bicalyculata* has significant curative and suppressive activity against *Trypanosoma evansi* and also had hepatoprotective and spleenoprotective activity. These activities were dose-dependent.

5.6 Recommendation

Further studies should be carried out on the antitrypanosomal activity of *P bicalyculata* using oral route of drug administration to ascertain its activity *in vitro* using *T. evansi* as the test parasite. The plant could be well integrated into Nigerian medicine considering the results of this investigation. However, more work is needed on isolation and characterization of the phytoconstituents to justify biological activities.

REFERENCES

Abdulazeez, A. M., Awasun, C. A., Dogo, Y. S. and Abiayi, P. N. (2010). Effect of *Peristrophe bicalyculata* on blood pressure, kidney and liver functions of the two kidney one clip (2K1C) hypertensive Rats. *British Journal of Pharmacology and Toxicology,* 1 (2): 101-107.

Abdelrahman, S. H.(2011). Trypanocidal activity of some Sudanese plants against experimental *Trypanosoma evansi* infection. *Research Journal of Medicinal Plant,* 5(6):756-763

Abdulazeez, M. (2011). Effect of *Peristrophe bicalyculata* on lipid profile of P-407-induced hyperlipidemic Wister rats. *Journal of Medicinal Plants Research,* 5(4):490-494

Abenga, J. N., Enwezor, F. A., Lawani, C. Ezebuiro, J. S. and K.M. David. (2003). Prevalence of trypanosomiasis in trade cattle at slaughter in Kaduna. *Nigerian Journal of Parasitology*, 23: 107-110.

Adeiza, A. A., Mohammed, A. and Mamman, M. (2010). Comparative *in vivo* evaluation of the trypanocidal activities of aqueous leaf, stem-bark and root extracts of *Khaya senegalensis* on *Trypanosoma evansi*. *Journal of Medicinal Plants Research* 4(17):1770-1777.

Adekola, A. Y. and Durotoye, L. A. (2004). Haematological study during reparum and postpartum periods in brown savanna does in zaria, Nigeria. Proceeding of the 38th annual conference of the Agricultural society of Nigeria. Pp. 538-540.

Akindahunsi, A. A. and Salawu, S. O. (2005). Phytochemical screening and nutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology* 4:497-501.

Alan, L. and Miller, N. D. (1996). Antioxidant flavonoids: structure, function and chemical usage. *Alternative Medicine Revolution,* 1:103-111.

Audu, P. A., Esievo, K. A. Mohammed, G. and Ajanusi, J. O. (1999). Studies on the infectivity of an isolate of *Trypanosoma evansi* in Yankassa sheep. *Veterinary Parasitology,* 86: 185-190.

Boham, B. A. and Kocipai-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium*. *Pacific Science*, 48: 458-463.

Boid, R., Luckins, A.G., Ray, P. F., Gray, A. R., Manhood, M. M. and Malik, K. H. (1980). Serum immunoglobulin levels and electrophoretic patterns of serum proteins in camels infected with *Trypanosoma evansi. Veterinary Parasitology*, 6: 333-345

Boid, D., Elamin, E. A., Mahmoud, M. M., and Luckins, A. G. (1981). *Trypanosoma evansi* Infections and antibodies in goats, sheep and camels In the Sudan. *Tropical Animal Health Production,* 13: 141-146.

Briggs, M. H. (1970). *Advances in Steroid Biochemistry and Pharmacology*. New York, USA: Academic Press, Pg. 24

.

Bruce, D. (1911). Morphology of *Trypanosoma evansi*. Report on sleeping sickness. *Communiqué of the Royal Society*. 1912, 12, 13.

Brun, R., Hecker, H. and Lun, Z. R. (1998). *Trypanosoma evansi* and *T. equiperdum*: Distribution, Biology treatment and phylogenetic relationship (a review). *Veterinary Parasitology*. 79: 95-107.

Burda, S. and Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agriculture Food Chemistry*, 49: 2774-2779.

Cadioli, F. A., Marques, L. C., Machado, R. Z., Alessi, A. C., Aquino, L. P. and Barnade, P. A. (2006). Experimental *Trypanosoma evansi* infection in donkeys' haematological, biochemical and hispathological changes. *Medical Veterinary Zootechnology,* 58(5): 746-756.

Chen, J., Tipoe, G. L. and Liong, E. C. (2004). Green tea polyphenols prevents toxins-induced hepatotoxicity in mice by down-regulating inducible nitric oxide derived pro-oxidants. *American Journal of Clinical Nutrition*, 80:742-751.

Christian, P., Guida, M. C. and Esteva, V. M. (2004). Antitrypanosomal activity of green tea (*Camellia sinensis*) catechins. *Journal of Antimicrobial agents and chemotherapy*, 48:69-74

Corfield, T. (1992). Bacterial sialidase roles in pathogenicity and nutrition. *Glycobiology*, 2:509-521.

Courtion, B., Boda, C. and Vatunga G. (2006). A link between chemokine levels and disease severity in human African trypanosomiasis. *International Journal of Parasitology,* 36:1057-1066.

David L. S., Klaus M. E., and Alan S. (1982): Vaccine development. http//www.jem.rupress.org. Pp.2-4.

Dermarderosian, A. and Liberti, L. E. (1988). Natural products medicine: *A scientific guide to foods, drugs and cosmetics*, 1st Edition, Philadelphia, USA: Geo. F. Stickley Co., Pp.46-66.

Dia, M.I., Van Mervenne, N, Magnus, E., Luckins, A. G., Diop, C., Thiam, J. P. and Hammers, R. (1997). Evaluation de quarte test de diagnostic. Frottis sanguains, CATT, iflet al ELISA-Ag dans L' fetude de I' fepidemiologic des trypanosomes cameline a *Trypanosoma evansi* en Mauritanie. *Revue D'Elevage et al de Medicine Veterinaire de pays tropicanx,* 50: 29-36.

Dwivedi S. (2008). Ethnomedicinal uses of some plants species by ethnic and rural peoples of indore district of Madhya Pradesh India (online) Pp.23 Available from: http://pharmainfo.net

El-Metanawey, T. M., Nadia, M. E., Abdel-Aziz, M. M., Hassanane, M. S. and Abdul El-Aziz T.H., (2009). Comparative studies on diagnosis of *Trypanosoma evansi* in experimentally infected goats. *International Journal of Parasitology,*12(2):223-225

Esenowo, G. J., Sam, S. M., Bala, D. N., Ekpo, B. A. and Edung, E. M. (2010). Phytochemocal screening and the haematological effects of *Peristrophe bicalyculata* (Retz) Nees Diet preparation in Albino rats. *World Journal of Applied Science and Technology,* 2:277-281.

Esievo, K. A. (1978). *In vitro* production of neuraminidase (sialidase) by *Trypanosoma vivax.* In proceedings of the 6th meeting of the OAU/STRC international scientific council for trypanosomiasis research and control 29th October- 3rd November, Younde, Cameroun. Pg. 65

Esievo, K. A. (1979). Studies on an aspect of anaemia of bovine trypanosomia (*Trypanosoma vivax*) PhD. Thesis Ahmadu Bello University, Zaria. Nigeria. Pp.35-40

Esievo, K. A., Saror, D. I., IIemobade, A. A. and Hallaway, M. H. (1982).Variation in the erythrocyte surface and free serum sialic acid concentrations during experimental *T. vivax* infection in cattle. *Research In Veterinary Science*, 32:1-5.

Esievo, K. A. (1983). *Trypanosoma vivax* stock Va 53: Inhibitory effect of type A influenza Virus anti HAV 8 serum on *in vitro* neuraminidase (sialidase) activity. *Journal of Parasitology,* 69: 491-495.

Esievo, K. A. and Saror, D. I. (1983). Leucocyte response in experimental *T. vivax* infection in cattle. *Journal of Comparative Pathology*, 93: 165-169.111

Esievo, G. J., Sam, S. M. Bala, D. N., Ekpo, B. A. and Edung, E. M. (2010). Phytochemical screening and the haematological effect of *Peristrophe bicalyculata* diet preparation in albino rats. *World Journal of Applied Science and Technology*. 2: 277-281

Evans, W.C. (1996). *Trease and Evans' Pharmacognosy,* 14th Edition. W.B. Saunders \Ltd. Pp.105-766.

Evans W.C. (2000). *Trease and Evans Pharmacognosy*, 14th Edition. W.B. Saunders Company Ltd., London, Pp. 19-20.

Fidock, D.A., Rosenthal, P.J., Croft, S.L., Brun, R. and Nwaka S. (2004). Antimalarial drug discovery: Efficacy Models for Compound Screening Supplementary Documents. *Trends in Parasitology.* **15:** 19-29

Fiennes, R. N. (1954). Haematological studies in trypanosomiasis of cattle. *Veterinary record*, 66: 423-434

Franke, C. R., Greiner, M. and Mehlitz, D. (1994b). Investigation in horses, cattles, dogs and Capybaras (*Hydrochaeris hydrochaeris*) in pantanal de pecone mato grosso Brazil, *Acta Tropical*, 58: 159-169.

Fraser, H. (1909). Surra in the Federated Malay States. *Journal of Tropical Veterinary Science*, 4:345-386

Franke, C. R., Greiner, M. and Melhlitz D., (1994a). Investigations on naturally occurring *Trypanosoma evansi* infections in horse, cattle, dogs and capybaras (*Hydrochaeris hydrochaeris*) in pantanl de pocone (mato grosso.) *Acta Tropica,* 58: 159-169

Galen, J. E., Ketley, J. M., Fasano, A., Richardson, S. H., Wasserman, S. S. and Kaper, J. B. (1992). Role of *Vibrio cholerae* neuraminidase in the function of cholera toxin. *Infection Immunology,* 60:406-415

Gardiner, C. H., Fayer, R. and Dubey, J. P. (1988). An atlas of protozoan parasites in animal tissues. *Agriculture Handbook.* Pp.3
(Online source:http://www.vet.uga.edu/VPP/clerk/womack/index.php)

Geerts, S. and Holmes P. H. (1998). Drugs management and parasite resistance in bovine trypanosomiasis in Africa. PAAT Technical and Scientific Series 1, A Part Information Service Publications FAO. P. 44

Giwa, O. E., Seyifunmi, O. E., Adewumi, B. L. Adebote, V. T. and Aldejimokun (2010). Screening of antimicrobial ethanolic extract of *Peristrophe bicalyculata. Ethnobotanical Leaflets,* 14:766-773

Goday, V. G., Miller, D. M., Russo, T. A., and Malamy, M. H. (1993). A role for *Bacteriodes fragilis* neuraminidase in bacterial growth in two model systems. *International Journal of Infection and immunlogy*, 61: 4415-4426

Harbourne J. B. (1973). *Phytochemical methods*, London. Chapman and Hall, Ltd. Pp. 49-188

Herbert, W. J. and Lumsden, W. H. R. (1976). *Trypanosoma brucei*: A Rapid "Matching" Method for Estimating the Host's Parasitaemia. *Experimental Biology*. Pp 427-431

Herrera, H. M. Aquino, L. P. and Menezes, R. F. (2004). *Trypanosoma evansi* experimental infection in the South American coati (*Nasuna nasua*); Haematological, biochemical and histopathological changes. *Acta Tropica*, 81:203-210

Hoare, C. A. (1972). *Trypanosoma evansi*: In trypanosomes of mammals, a zoological monograph. Blackwell scientific publication Pp. 555-593

Hoffman, D. (2000). *The New Holistic Herbal.* Element publication. 3rd Edition. Pp 23

Holland, W. G. (2003). The Diagnosis of *Trypanosoma evansi* and its Immuno-suppressive effect in water buffaloes and pigs. *International Journal of Parasitology,* 5(2):3-16

Honeyman J. and Guthrie, R. D. (1988). An Introduction to Chemistry of Carbohydrates, 3rd edition. Clanderon, Oxford, UK: Oxford University Press. Pp 73.

Hudaib, M., Mohammad, M., Bustanji, Y., Tayyem, R., Yousef, M., Aburjaie, M., and Aburjai,T. (2008). Ethnopharmacological survey of medicinal plants in Jordan, Mujib nature reserve and surrounding area. *Journal of Ethnopharmacology*. 120: 63-71.

Hunell R. F. (2003). Influence of vegetable protein sources in trace element and mineral bioavailability. *Journal of Nutrition.* 133 (a) 29735-75

Huan, C. N., Webb, L., Lambert, R. H. and Miescher, P. A. (1975). Pathogenesis of anaemia in African trypanosomiasis: characterization and purification of haemolytic factor. *Schweiz medicine* 105: 1582-1583

Indrakamhang, P. (1998). Trypanosome infection in livestock in Thailand. *Journal of Protozoology Research.* 8, 153-161.

IIemobade, A. A. (1971). Studies on the incidence and pathogenicity of *Trypanosoma evansi* for equine and bovine species, ISCTRC,OAU/STRC Publication. 105:107-114

ILRAD (1990). Annual report of the international laboratory for research on animal diseases. International Livestock Research Institute Development (1997). Livestock, people and the environment. ILRID, Nairobi, Kenya. P. 23

International livestock research institute (ILRI) monograph (1997). Disease Resistance and protecting the environment. In; *Livestock, People and the Environment*. Pp.10-11

Janakiraman, N., Sathish, S. S. and Johnson, M. (2012). Antibacterial studies on *Peristrophe bicalyculata* (Retz.) Nees. *Asian Pacific Journal of Tropical Biomedicine,* 2:147-150

Jennings, F. W., Murray, M. and Urquhart, Q. M. (1974). Anaemia in trypanosomiasis: studies in rats and mice infected with *T. brucei*. *Research in Veterinary Science*, 16: 70-76

Jigam, A. A., Helmina, O. A., Ogbadoyi, E. O. Dauda, B. E. and Egwin, C. E. (2009). *In vivo* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extracts of *Lippia multiflora* mold. *Journal of Medicinal Plants Resources,* 3(3): 148 – 154.

Joshi, P.P. (2007). Treatment and follow-up of the first case of human trypanosomiasis caused by *Trypanosoma evansi* in India. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* Published by Elsevier Ltd. Pp 77-80

Kantzier, G. B., Lauteria, S. F., Cusimano, C. L., Lee, J. D., Ganguly, R. and Waldman, R. H. (1974). Immunosuppression during influenza virus infection. *Infection and Immunity*,
10: 996-1002.

Karori, S. M., Ngure, R. M. and Wachira F. N. (2008). Different types of tea products attenuate inflammation induced in *Trypanosoma brucei* infected mice. *International Journal of Parasitology,* 57:325-333

Kobayashi, A. and Tizard I. R. (1976). The response to *T. congolense* infection in calves determination of immunological IgGi IgG2 and C3 levels and the complement fixing antibodies titre during the course of infection. *Tropen Medizin Parasitologies,* 27: 411-417

Kristjason, P. M., Swallow B. M., Rowland, G. J., Kruska, R. L., and Delew, P. P. (1999). Measuring the cost of A. A. T. the potential benefit of control and return to research. *Agricultural Systems,* 59(1): 79-98.

Lorke, D. (1983). A New Approach to Practical Acute toxicity Testing. *Archives of Toxicity*, 54: 275-287

Luckins, A. G. (1998). Epidemiology of surra unanswered question. *Journal of Protozoology Research*, 8: 106-119

Luckins, A. G. (1999a). Trypanomiasis caused by *Trypanosoma evansi* in Indonesia. *Journal of Protozooology research*, 144-152

Luckins A. G. (1999b). Epidemiology of non-tsetse transmitted trypanosomiasis- *Trypanosoma evansi* in perspective. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors.* Pp 34-45.

Luckins A. G. (2002). Surra. Office International Epizootics (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Pp 33

Lynch N. and Berry D. (2007). Differences in perceived risks and benefits of herbal, over-the-counter and prescribed conventional medicine and the implications of this for the safe and effective use of herbal products. *Complementary Therapy in Medicine*, 15: 84-91.

Magez, S., Caljon G., Tran, T., Stijlemans B. and Radwanska M. (2010). Current issues of vaccination against African trypanomosis. *Journal of Cell Biology,* 3:36

Mahmoud, M. M. and Gray A. R. (1980). Trypanomosis due to *Trypanosoma evansi* (Steel, 1885). Balbani 1888. A review of rescent *Research Tropical Animal Health and Production.* 12:35-47

Marco, B. (2007). *Synthesis and Characterization of Glycosides*. Springer.P. 123

McGrath, W. R. (1991). Common Herbs for Common Illness. *American Survival Guide.* p.34

McIntyre, A. (1995). The complete woman's herbal. Henry Holt Reference Books. P 23

Molumba, K. (2003). Socio-economic and agricultural factors in the research and control of Trypanosomiasis. PAAT Technical and Scientific Series4. FAO, Rome. Pp 43-44

Monzon, C. M., Hoyos, C. B., and Jara, G. A. (1995). Outbreak of equine trypanosomiasis caused by *Trypanosoma evansi* in Formosa province, Argentina. *Review of Science and Technology*. Office International Epizootics (OIE) 14: 747-752

Monzon, C. M., Mancebo, O. A. and Russo, A. M. (2003). Antibody levels by indirect ELISA test in *Trypanosoma evansi* infected horses following treatment with quinapyramine sulphate. *Veterinary Parasitology*, 111: 59-63

Mihret, A. and Mano, G. (2007). Bovine trypanosomosis in three districts of East Gojjan zone bordering the blue, Nile River in Ethiopia. *Journal of Infected Developing Countries*,1(3): 321-325

Muller, H. E. (1974). Die pathogenetiche Bedeutung micro neuraminidase (as quoted by Traving and Schauer, 1998)

Muller, E., and Schauer, R. (1983). Factors influencing binding and phagocytosis of sialidase-treated erythrocytes by homologous rat peritoneal macrophages proceedings of the 17th symposium on glycoconjugate held at lund-Runneby. 17-23rd July, P. 667

Nee, S. S., Veh, R. W. and Shauer, R. (1975). Purification and characterization of neuraminidase from *Clostridium perfringes*. *Journal of Biotechnology,* 4:22-25

Ngeranwa, J. J., Gathumbi, P. K., Mutiga, E. R. and Agumkah, G. J., (1993). Pathogenesis of *Trypanosoma evansi* in small East African goats. *Research in Veterinary Research Communications*, 54: 283-289.

Nigerian Institute for Trypanosomiasis Research (NITR) (1989). Annual Report. P 22

Noedl, H., Wongsrichanalai, C. and Wernsdorfer, W. H. (2003). Malaria drug sensitivity testing: new assays, new perspective. *Trend in Parasitology*. 19: 175-181

Obadoni, B. O. and Ochuko P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta states of Nigeria. *Global Journal of Pure Applied Sciences,* 8b: 203-208.

Ohaeri, C. C. (2010). Histological changes in organs of rats infected with *Trypanosoma congolense*. *Research Journal of Animal Science*. 4:50-52

Okwu , D. E. (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure and Applied Sciences.* 7:455-459

Oladunmoye, M. K., Adetuyi, F. C. and Akinyosoye, F.A. (2009). Effect of *Cassia hirsuta* (L) extract on DNA profile of some microorganisms. *African Journal of Biotechnology.* 8(3): 447-450.

Omale, J. and Omajali, J. B. (2010). Studies on some nutritional characteristics of the fruits and leaf of *Saba florida* (benth) from Ibaji forest. *International Journal of Nutrition Metabolism*, 2(1):11-26

Omotainse, S. O. and Anosa, V. O. (2009). Comparative histopathology of the lymph nodes, spleen, liver and kidney in experimental ovine trypanosomosis. *Onderstepoort Journal of Veterinary Research*. Pp 125

Onyiah, J. A. (1997). African trypanosomiasis: An overview of the current status in Nigeria. *Tropical Veterinarian,* 15: 111-116

Onwuka, B. (2005). *Food Analysis and Instrumentation Theory and Practical*. Naphtali prints, lagos, Nigeria. P. 148

Partoutomo, S., Soleh, M., Politedly, F., Day, A., Stevenson, P., Wilson, A. J., Copeson, D. B. and Owe, L. (1994). The epidemiology of *Trypanosoma evansi* and *Trypanosoma theileri* in cattle and buffalo in small holder farms Java. *Penyakit Hewan*. 26: 48: 41-46.

Pathak, K. M., Arona, J. K. and Kapoor, M. (1993). Camel trypanosomiasis in rajasthan. *Indian Veterinary Parasitology*, 49: 319-323

Paquay, J. B., Haenen G. R., Stender G., Wiseman, S. A., Tijburg, L. B. and Bast, A. (2000). Protection against nitric oxide toxicity by tea. *Journal of Agriculture and Food Chemistry,* 48: 5788-5772

Paveto C., Maria, C. G. and Estiva, M. I. (2004). Anti-trypanosomal cruzi activity of green tea (*Camelis sinensis*) catechin. *Journal of Antimicrobial Chemotherapy*, 48: 69-74

Philip, K., Sri Nurestri A. M., Wirakarnain S., Sim K. S. and Saravana K. (2009). Antibacterial activity of some medicinal plants from Malaysia. *American Journal of Applied sciences* **6:** 1613-1617

Rafauf, R. (1970). *Handbook of alkaloids and alkaloid containing plants.* Wiley, New York USA: 2nd Edition, Academic Press, P 6.

Rashmi, G., Jaya, P., Hardik, P., Bhumi, M. and Shivani, A. (2010). *Peristrophe bicalyculata*-a review. *Pharmacognosy Journal,* 2:39-46

Ridley, R.G. (2002). Medical need, scientific opportunity and the drive for antimalarial drugs**.** *Nature*, 415:686-693.

Rickman, W. J. and Cox, H. W. (1983). Trypansome antigen-antibody complexes and immunoconglutinin interaction in African trypanosomiasis. *International Journal of Parasitology*, 13: 389-392

Roger, M. F. and Wink, M. (1998). Alkaloids: Biochemistry, Ecology and Medicine applications. Plenum press, Pp. 2-3.

Saito, M. and Yu, R. K. (1993). Possible role of myelin associated neuraminidase in membrane adhesion. *Journal of Neurological Science Research*, 36: 127-132

Sawalha, A. F., Sweileh, W. M., Zyoud, S. H. and Jabi, S. W. (2008). Self-therapy practices among university students in Palestine: Focus on herbal remedies. *Complementary Therapy in Medicine*, 16: 343-349.

Samdi, S. M., Abenga, J. N., Attahir, A., Haruna, M. K., Wayo, B. M., Fajinmi, A. O., Sumayin, H. M., Usman, A. O., Hussaina, J. Z., Muhammed, H., Yarnap, J. E., Ovbagbedia, R. P. and Abdullahi, R. A. (2010). Impact of trypanosomiasis on food security in Nigeria: A Review in *International Journal of Animal and Veterinary Advances,* 2(2): 47-50.

Schauer, R. (1985). Sialic acids and their roles as biological masks. *Trends in Biochemistry Science,* 10: 357-360.

Schauer, R. and Kamerling, J. P. (1997). Chemistry, biochemistry and biology of sialic acid. *Glycoproteins*. Elsevier Amsterdam. 11: 241-400

Seifert, H. S. (1996). *Tropical Animal Kluwer Academic Publishers*. Dorrech, Bonston, London, 2nd Edition. Pp. 53-260

Shaba, P., Pandey, N. N., Sharma, O. P., Rao, J. R., Mishra, A. K. and Singh, R. K. (2012). Therapeutic activity of methanolic extract of *Eupatorium adenophorum* leaves against *Trypanosoma evansi*. *International Journal of Basic and Applied Medical Sciences,* 2(1):77-82

Shehu, S. A, Ibrahim, N. D., Esievo, K. A. and Mohammed, G. (2006). Role of erythrocyte surface sialic acid in inducing anaemia in savannah brown bucks experimentally infected with *Trypanosoma evansi*. *Vesterinarski Archive*. 76 (6): 521-530

Siegler, D. S. (1998). Plants with saponins and cardiac glycosides. www.lifwe.Vinc.ed/plant/biol/363/saponinsslides.

Silva, R. A., Arosemena, N. A., Herrera, H. M., Sahib, C. A. and Ferreira, M. S. (1995). Outbreak of *Trypanosoma evansi* in horses of panatela Mato-Grossence, Brazil. *Veterinary Parasitology.* 43: 167-180.

Stephen, L. E. (1986). *Trypanosomiasis: A veterinary perspective*. 4th Edition. Pergamon Press, Oxford, Pp. 184-215.

Sofowora, E. S. (1993). *Medicinal plants and traditional medicine in Africa.* Spectrum books. 2nd Edition.Ltd, Ibadan, Pp. 55-71.

Tagboto S. and Townson S. (2001). Antiparasitic properties of medicinal plants and other naturally occurring products. *Advanced Parasitology.* 50:199-295.

Tasdemir, D., Kaiser, M. and Brun, R. (2006). Antitrypanosomal and anti-leishmanial activities of flavonoids and their analogues. *In vitro, in vivo,* structural-activity relationship and quantitative structure activity relationship studies. *Journal of Antimicrobial agents and Chemotherapy,* 50:1352-1364

Teeraprasert, E. (1984). A case report of *Trypanosoma evansi* in pigs, 11th Annual Veterinary Science Conference. Thailand Veterinary Medicinal Association. 12-14 Dec. 1984. Pp 53-64

Traving, P. and Schauer, R. (1998). Structure, function and metabolism of sialic acids. C. M. L. S. *Cellular Molecular Life Science* 54: 1330-1349

Tuntasuvan, D., Jarabrun, W. and Viseshakul, N. (2003). Chemotherapy of surra in horses and mules with diminazene aceturate. *Veterinary Parasitology*; 110222-223

Tuntasuvan, D., Chompoochan, T., Vongpakorn, M. and Mohkaew, K. (1992). Detection of *Trypanosoma evansi* antibodies in pigs using enzyme linked immunoabsorbent assay. *Journal of Thailand Veterinary Medical Association*. 47: 45-53.

Tyler, V. E., Brady, L. R. and Robbers, J. E. (1988). *Pharmacognosy,* 9th Edition. Philadelphia, USA: Lea and Febiger. Pp 29, 47-50, 123, 202.

Udenyi, I. (1993). Anti-malarial activity of Nigerian neem leaves. *Transactions of the Transcorp Royal Society of Tropical Medicine*.3:427-431

Umar, I. A., Ibrahim, M. A. Fari, N. A., Isah, S. and Balogun, D. A. (2010). *In vitro* and *in vivo* anti-*Trypanosoma evansi* activities of extracts from different parts of *Khaya senegalensis*. *Journal of Cell and Animal Biology,* 4 (6):92-95

Van-Burden, T. P. and Robinson, W. C. (1981). Formation of complexes between protein and tannin acid. *Journal of Agriculture and Food Chemistry*, 1:77

Wellde, B., Lotzsch, R., Deindl, G., Sadun, E., Williams, J. and Warui, G. (1974). *T. congolense*: clinical observations of experimentally infected cattle. *Experimental Parasitology.* 36: 6-19

World Health Organization (1998). Control and surveillance of African trypanosomiasis. A report of WHO expert committee. WHO Technical Report Series, No. 881.

World Health Organisation (2012). Human African Trypanosomiasis (sleeping sickness). P 445

Womack, S. (2001). *Trypanosoma evansi* in Horses, 2nd Edition. University of Georgia Pp. 1-2

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B. and Grellier, P. (2005). *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria**.** *Journal of Ethnopharmacology,* 98:281-285.

APPENDIX

The estimated number of trypanosomes per ml of blood in the curative test using the methanol extract of *Peristrophe bicalyculata* against *Trypanosoma evansi* using the methods as described by Herbert and Lumsden, 1978.

Treatments/ days	5	6	7	8	9	10	11	
T1 (Normal saline)	22387211	48696752	63095734	53088444	81752303	85359133	85359133	85
T2 (400mg/kg body weight)	18836490	63095734	63095734	40973210	31620000	48696752	26607250	26
T3 (500mg/kg body weight	22387211	40973210	40973210	34474660	34474660	63095734	31620000	34
T4 (5mg/kg body weight of Diminazine aceturate)	26607250	71820760	0	0	0	0	0	0

The curative effects of methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* was shown to be significant at $P < 0.05$

The estimate of the number of trypanosomes per ml of blood in the curative test using the aqueous extract of *Peristrophe bicalyculata* against *Trypanosoma evansi* using the methods as described by Herbert and Lumsden, 1978.

Treatments/days	5	6	7	8	9	10	11	1
T1(Normal saline)	18836490	48696752	48696752	71820760	71820760	81752303	74989420	83
T2(400mg/kg body weight of *Peristrophe bicalyculata*)	18836490	44668359	63095734	26607250	26607250	22387211	15850000	14
T3(500mg/kg body weight	22387211	26607250	63100000	34474660	31620000	34474660	31620000	21
T4(5mg/kg body weight of Diminazine aceturate)	26607250	63095734	0	0	0	0	0	0

The curative effect of aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* was shown to be significant at $P < 0.05$

The number of trypanosomes per of blood in the suppressive bioassay of aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi.*

Treatment/days	2	5	8	12
T1 (Normal saline)	18836490	18836490	53088444	81752303
T2 (300mg/kg body weight of *Peristrophe bicalyculata*)	15850000	15850000	34474660	44668359
T3 (400mg/kg body weight *Peristrophe bicalyculata)*	18836490	22387211	34474660	34474660
T4 (5mg/kg body weight of Diminazine aceturate)	0	0	0	0

The number of trypanosomes per ml of blood in the suppressive bioassay of methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi*

Treatment/days	2	5	8	12
T1 (Normal saline)	18836490	40973210	44668359	81752303
T2 (300mg/kg body weight of *Peristrophe bicalyculata*)	7943000	40973210	31620000	26607250
T3 (400mg/kg body weight *Peristrophe bicalycualta)*	11220184	26607250	22387211	26607250
T4 (5mg/kg body weight of Diminazine aceturate)	26607250	0	0	0

ANOVA for curative test using methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* using mice as test animal.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.1E+16	3	3.67E+15	9.861862	0.000132	2.946685
Within Groups	1.04E+16	28	3.72E+14			
Total	2.14E+16	31				

ANOVA for curative test using aqueous extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* using mice as test animal.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.11E+16	4	5.27E+15	16.20001	1.34E-07	2.641465
Within Groups	1.14E+16	35	3.25E+14			
Total	3.25E+16	39				

ANOVA for suppressive test using Aquoeus extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* using mice as test animal.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.33E+15	4	1.33E+15	6.033718	0.004233	3.055568
Within Groups	3.31E+15	15	2.21E+14			
Total	8.64E+15	19				

ANOVA for suppressive test using methanol extract of *Peristrophe bicalyculata* on *Trypanosoma evansi* using mice as test animal.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.80303E+15	4	1.45076E+15	6.071216	0.004121	3.055568
Within Groups	3.58435E+15	15	2.38957E+14			
Total	9.38738E+15	19				

T-test for the Quantitative analysis of methanol and aqueous extract of *Peristrophe bicalyculata*

	Variable 1	*Variable 2*
Mean	172.84	215.6617
Variance	149225.5	240462.8
Observations	6	6
Pearson Correlation	0.999649	
Hypothesized Mean Difference	0	
Df	5	
t Stat	-1.00173	
P(T<=t) one-tail	0.03233	
t Critical one-tail	1.2346	
P(T<=t) two-tail	0.04234	
t Critical two-tail	2.570582	

LETHAL DOSAGE (LD_{50}) COMPUTATION

Calculations for lethal dosage (LD_{50}) for methanol extract of *Peristrophe bicalyculata* Using mice as test animal
Minimum toxic dose= 800mg/kg body weight, Maximum tolerated dose=1600mg/kg body weight.

Solution
LD_{50} = √ Minimum toxic dose x Maximum tolerated dose
=√ 800 x 1600
=√1280000
=1131.3708498985
≈1131mg/kg body weight

Calculations for lethal dosage (LD_{50}) for Aqueous extract of *Peristrophe bicalyculata* Using mice as test animal

Solution
Minimum toxic dose= 1000mg/kg body weight, Maximum tolerated dose=1600mg/kg body weight.
LD_{50} = √ Minimum toxic dose x Maximum tolerated dose
=√ 1000 x 1600
=√1600000
=1264.9110640674
≈1265mg/kg body weight