ANTITRYPANOSOMAL ACTIVITIES OF *ACACIA NILOTICA* AND *KHAYA SENEGALENSIS* AND THE HAEMATOLOGICAL PROFILE OF *TRYPANOSOMA BRUCEI BRUCEI* INFECTED WISTAR RATS

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DEPARTMENT OF MICROBIOLOGY
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JULY, 2017

DECLARATION

I declare that the work in this dissertation entitled: '**Antitrypanosomal Activities of *Acacia nilotica* and *Khaya senegalensis* and the Haematological Profile of *Trypanosoma brucei brucei* infected *Wistar* Rats'** has been carried out by me in the Department of Microbiology Faculty of Live Sciences ABU Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Halimah Sudugi ABDULQADIR

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled 'Antitrypanosomal Activities of *Acacia nilotica* and *Khaya senegalensis* and the Haematological Profile of *Trypanosoma brucei brucei* infected *Wistar* Rats' by Halimah Sudugi ABDULQADIR meets the regulation governing the award of Master of Science Degree in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents Abdulqadir Iye Madihu and Aishatu Ope Zubairu.

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To my colleagues and friends for their support, thank you all.

ABSTRACT

This study was aimed at investigating antitrypanosomal activities and haematological profile of crude extract and fractions of the stem bark of *Acacia nilotica* and *Khaya senegalensis* plants against *Trypanosoma brucei brucei* infected Wistar rats with a view to determining the phytochemical constituents and LD_{50} of *Acacia nilotica* and *Khaya senegalensis*, antitrypanosomal activities of crude extract of *Acacia nilotica* and *Khaya senegalensis* against *Trypanosoma brucei brucei* infected Wistar rats, antitrypanosomal activities of the plant fractions administered to *Trypanosoma brucei brucei* infected Wistar rats and haematological profile of *Trypanosoma brucei brucei* infected Wistar rats, before and after administration of crude extract and fractions. The phytochemical constituents and toxicity of the stem bark of both plants were determined by the standard method and the LD_{50}respectively. The methanolic extracts and fractions of the plants was administered to the Wistar rats intraperitoneally daily and the parasitaemia count was determined using the rapid matching method. PCV, WBC and differential counts were determined before and after the administration to ascertain any significant differences. The phytochemical constituents of the stem barks of *Acacia nilotica* and *Khaya senegalensis* crude extracts and fractions revealed the following secondary metabolites; Alkaloids, tannins, glycosides, cardiac glycosides, saponins, triterpene, carbohydrates and flavonoids. The LD_{50} for the crude extract of the stem bark of Acacia nilotica was 707.1mg/kg body weight while the LD_{50} for the fractions (N-hexane, ethyl acetate and N-butane) was 547.7 mg/kg, 387.3 mg/kg and 707.1 mg/kg body weight respectively. The LD_{50} for the crude extract of the stem bark of *Khaya senegalensis* was 547.7mg/kg body weight while the LD_{50} for the fractions (N-hexane, ethyl acetate and N-butane) was 387.3 mg/kg, 547.7 mg/kg and 223.6 mg/kg body weight respectively. The stem barks of *Acacia nilotica* and *Khaya senegalensis* crude extracts (100, 200, 300 and 400mg/kg body weight) and fractions (50, 100, 150 and 200mg/kg body weight) had

antitrypanosomal activity. Parasites were cleared from circulation within 12 days of treatment. Haematological indices of *Acacia nilotica* and *Khaya senegalensis* in *Trypanosoma brucei brucei* infected Wistar rats showed that there was no statistical significant change in the packed cell volume, white blood cells and differential counts before and after treatment with all doses of the crude extracts and fractions. The findings in this study provide very useful source for biopharmaceutical industries for the development of antitrypanosomal agents from the stem bark of *Acacia nilotica* and *Khaya senegalensis* for therapeutic intervention in the control of African trypanosomiasis. There is need for further extensive work on these plants using different *Trypanosoma* species in the management of African trypanosomiasis.

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LIST OF ABBREVIATIONS

AAT -African Animal Trypanosomosis

WHO -World Health Organization

HAT -Human African Trypanosomiasis

VSG -Variant Surface Glycoprotein

NITR -Nigerian Institute for Trypanosomiasis Research

PBS -Phosphate Buffered Solution

PCV -Packed Cell Volume

rpm -Revolution per minute

WBC -White blood cell

bw -Body weight

IUCN -International Union for Conservation of Nature and Natural Resources

TDR Tropical Disease Research

OECD Organistion for Economic Cooperation and Development

CHAPTER ONE

1.0 INTRODUCTION

African Trypanosomiasis (African sleeping sickness) is caused by trypanosomes which is found in Sub-Saharan Africa and is threatening more than sixty million lives on daily basis (Abdullahi and Emmanuel, 2012). Trypanosomes are protozoan parasites in the family Trypanosomatidae. Most trypanosomes are transmitted by the vector, tsetse flies (*Glossinia* spp) which are found only in Sub-Saharan Africa, between latitudes 14^{o} N and 20^{o} S (Bernard and Alain, 2012). The parasites include *Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense*, (cause Human African Trypanosomiasis). Other trypanosomes primarily affect animals include *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma brucei brucei, Trypanosoma simiae and Trypanosoma godfreyi* (Bernard and Alain, 2012).

Nigeria's natural habitation is made up of both savannah and tropical rainforest, which falls within the endemic area in Africa i.e. between latitude 15^{0}Nand 29^{0} S. The diverse flora offers a wide spectrum of unique medicinal plants. There are varieties of studies of Nigerian plants used in the traditional management of trypanosomiasis, indicating significant anti-trypanosomal activity (*in-vitro/in-vivo*); some of which the metabolites responsible have been isolated and reported (Atawodi *et al*, 2003).

Trypanosoma brucei brucei are unicellular parasites transmitted by the tsetse fly. They are the causative agent of African animal trypanosomosis (AAT), also known as Nagana. *Trypanosoma brucei brucei* is the etiological agent for sleeping sickness which is one of the most serious protozoan diseases in Africa (Antia *et al.*, 2009; Simarro *et al.*, 2011).

The disease results in acute, sub acute or chronic disease characterized by intermittent fever, anaemia, occassional diarrhoea, rapid loss of consciousness and often terminates in death (Olukunle *et al.,* 2010). On the basis of mortality, Human African Trypanosomiasis is ranked ninth among 25 infectious diseases in Africa (Bernard and Alain, 2012).

The current chemotherapy of the human trypanosomiasis relies on six drugs namely; Suramin, Pentamidine, Melarsoprol, Eflorinithine, Arsobal and Mel B, five of which were developed over 30 years ago (Steverding and Tyler, 2005). All of the current therapies are unsatisfactory for various reasons, including unacceptable toxicity, poor efficacy, undesirable route of administration, drug resistance and high cost (Fairlamb, 2003). Natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes (Hoet *et al.,* 2004). The local use of natural plants as primary health remedies is due to their pharmacological properties. Many plant extracts owe their potency to the presence of metabolites. These metabolites are usually found in various parts of the plants like roots, leaves, shoots and barks. Many plants have therefore become sources of important drugs and as such the pharmaceutical industries have exploited medicinal plants as a source of bioactive agents that can be used in the preparation of synthetic medicine (Kinghorn, 1994).

Acacia nilotica belongs to the family Leguminosae. It is called 'Bagaruwa' in Hausa, 'Gaude' in Fulfulde and 'Kangar' in Kanuri. It is commonly found in Guinea and Sudan savannah. Ethanol extract of the leaves have been reported to have anticancer and antioxidant activity (Kalaivani *et al.,* 2010). The plant parts are commonly used, particularly in the Northern and South-Western Nigeria, as broad spectrum antimicrobials, especially in the treatment of dysentery. *Khaya senegalensis* is a tree in the *Meliaceae* family. It is widely distributed in the Sub-Saharan savannah. It is called "madachi" in Hausa, "dalechi" in Fulani, "ogonwo" in Yoruba and "ono" in Igbo.

The bark is dark-grey to grey-brown. It is used for carpentry and construction. The bark is used for a variety of medical purposes such as malaria, stomach complaints and headaches. It is applied externally to skin rashes, wounds or any abnormality (Keay *et al.*, 1989).

1.1. Statement of the Research Problem

Trypanosomiasis, a disease of major importance in human and animals has continued to threaten human health and economical development (Kuzoe, 1993; WHO, 2014). The population at risk being about 69 million with one third of this number being at a 'very high' to 'moderate' risk and the remaining two thirds at a 'low' to 'very low' risk (WHO, 2014).

Trypanosomiasis is one of the most severe medical problems in Africa, infecting around 50,000 people every year (Ohaeri, 2010), and also affects 50-70 million animals (Ogbadoyi *et al.,* 2007). Trypanosomiasis affects millions of people in Sub-Saharan Africa and is responsible for the death of about half a million patients per year (Barrett, 1999; WHO, 2014).

Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis and also the primary cause of death (Bizimana *et al.*, 2006).

Chemotherapy is the most widely used means of controlling the trypanosomiasis. The few registered trypanocides are often associated with severe side effects and required lengthly parenteral administration, lack efficacy and are unaffordable for most of the patients (Legros *et al.*, 2002). Chemotherapy and chemoprophylaxis, which form the most important aspect of control and eradication of trypanosomiasis in African countries, are beset with problems. These include limited repertoire of compounds, resistance to drugs, drug toxicity and protracted treatment protocols (TDR, 1984). Poor clinical

efficiency, drug resistance and toxicity are some of the limitations facing programmes targeted at controlling the disease (Onyeyili and Egwu, 1995; Geerts and Holmes, 1998; Legros *et al.,* 2000; Kamuanga, 2003).

Previous studies have shown that the crude extracts of *Acacia nilotica* and *Khaya senegalensis* have antitrypanosomal effects (antitypanocidal or antitrypanostatic). The subject of this study is to show that fractions of these plant extracts could exhibit different and indeed more effective results.

1.2. Justification

The high costs and toxicity of synthetic drugs have stimulated renewed interest in plant substitutes. With the emergence of drug resistant trypanosomes, cost-effective new drugs in the treatment of sleeping sickness are required.

Medicinal plants are less expensive and less hazardous to the health than their synthetic counterpart hence medicinal plants with anti-trypanosomal properties have paramount importance for the treatment of trypanosomiasis.

It has been known that plant products play an important role in certain disease control; for example Hoet *et al.*, (2004), stated that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes.

Chemotherapy is the most widely used means of controlling the trypanosomiasis. The few registered trypanocides are often associated with severe side effects (Guttering, 1985) and require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Legros *et al.,* 2002). Thus there is the urgent need to source for new, cheap effective non hazardous plants products that can help control trypanosomiasis.

1.3. Aim

The aim of this study was to investigate the anti-trypanosomal activity of *Acacia nilotica* and *Khaya senegalensis* and the haematological profile of *Trypanosoma brucei brucei* infected Wistar rats.

1.4. Objectives

The specific objectives were to determine:

1. The phytochemical constituents and LD_{50} of *Acacia nilotica* and *Khaya senegalensis*.
2. The antitrypanosomal activities of crude extract of *Acacia nilotica* and *Khaya senegalensis* against *Trypanosoma brucei brucei* infected Wistar rats.
3. The antitrypanosomal activities of the plant fractions administered to *Trypanosoma brucei brucei* infected Wistar rats.
4. The haematological profile of *Trypanosoma brucei brucei* infected Wistar rats, before and after administration of crude extract and fractions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. The Parasite: *Trypanosoma brucei*

Human African Trypanosomiasis (HAT) is caused by protozoan, *Trypanosoma brucei*(Malvy and Chappuis, 2011), but only two subspecies of the*Trypanosoma brucei* group are responsible for sleeping sickness; *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. The third sub specie of the group, *Trypanosoma brucei brucei* is nonpathogenic for humans, but parasitizes domestic and wild animals and it is widely used in experimental models of Human African Trypanosomiasis. Trypanosomes are extracellular parasites and they can be clearly identified microscopically, but the two subspecies that are pathogenic for human beings are morphologically indistinguishable. They have a complex life cycle (Lindner and Priotto, 2010) with differentiated biological stages in both the insect vector and the mammalian host. The parasites are ingested as bloodstream trypomastigote forms and they move to the midgut of the tsetsefly (Simon *et al*., 2012).

However some of the trypomastigote forms in the insect's midgut may arrive to differentiate into procyclic forms, which replicate in situ and cross the peritrophic membrane to reach the proventriculus, where they become mesocyclic trypomastigotes and later epimastigote forms (Lindner and Priotto, 2010).They, then migrate via the esophagus, proboscis, and hypopharynx to the salivary gland, where they are able to multiply and some of them can transform into infectious metacyclic forms (Sanjeev, 2014). During this migration from the midgut to the salivary glands, the parasite population size experiences a pronounced reduction. The metacyclic form is the only stage that is infective to vertebrates, and it is characterized by the presence of the variant

surface glycoprotein (VSG) coat that will protect the parasite in order to survive on the host (Simon, *et al.*, 2012). The whole cycle in the vector takes 18–35 days, and once infected, a tsetse fly remains so for the rest of its lifespan. Nevertheless, in laboratory conditions it has been observed that the majority of ingested trypanosomes fail to develop, and only 2%–5% of the flies ingesting trypanosomes produce metacyclic forms (Sinha, *et al.*, 1999).

Metacyclic forms are injected subdermally in the mammalian host during a tsetse fly blood meal. They proliferate at the site of inoculation and they transform in long slender forms, as they are carried by the draining lymph nodes to the bloodstream where they replicate (Simon, *et al.*, 2012).

In the blood stream, the parasite can be found in its proliferative, long, slender bloodstream trypomastigote form, which is adapted to maintain the parasite in mammalian blood, or as the nonproliferative, short, stumpy bloodstream trypomastigote form, which is adapted to differentiate into the replicative procyclic form in the tsetse fly, and thus to ensure transmission (Simon, *et al.*, 2012).

There are also intermediate forms, which are transitional, as they are between the slender and stumpy forms. The bloodstream forms can enter into different body fluids, including lymph and cerebrospinal fluid, and they can also cross the placenta (Sanjeev, 2014).

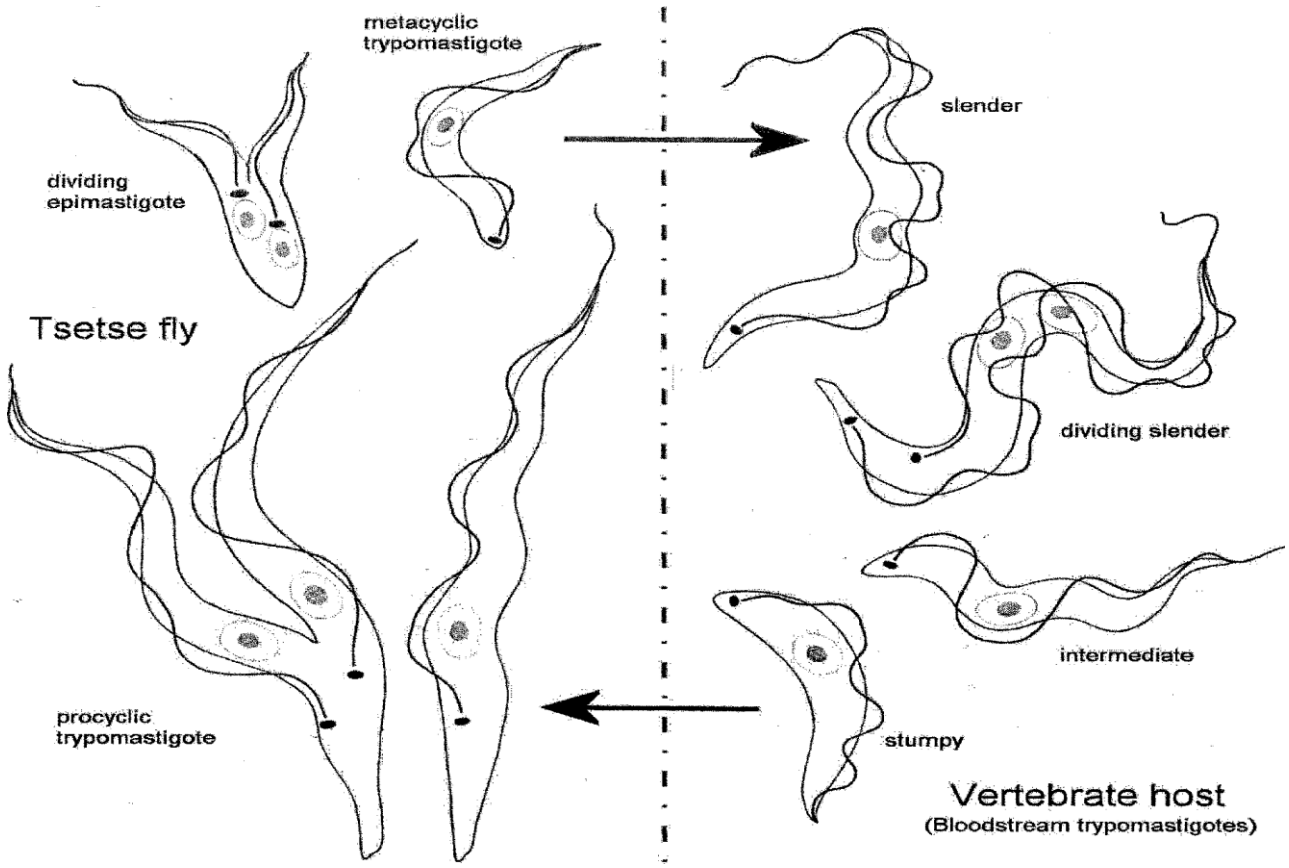


Fig 2.1 The life cycle of *Trypanosoma brucei brucei*(Source: Hoet et., al 2004)

The classification of trypanosomes is shown below:

Phylum: Protozoan

Sub-phylum: Sarcomastigophora

Super class: Mastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Family: Trypanosomatidae

Genus: *Trypanosoma*

2.2. The vector: *Glossina* spp:

Human African Trypanosomiasis is transmitted through the bite of an infected tsetse fly (Genus *Glossina*). Thirty-one species and subspecies of tsetse flies have been described and classified in three groups or subgenera, (Sanjeev, 2014) which are generally related to different habitats. The Subgenus *Nemorhina*, or the palpalis group, found in Western and Central Africa, live in vegetation close to a water source, such as forests, gallery forests, riverbanks and lakes, swamps and mangroves, coffee or cocoa plantations, and they can even adapt to environmental changes, surviving in the peri urban areas of medium and large townsand areas of intensive agriculture. *Glossina palpalis* is distributed in the Atlantic coast from Senegal to Angola. *G. fuscipes* is present in Central Africa from Cameroon and Congo to the Rift Valley (Malvy and Chappuis, 2011).

Tsetse flies are restricted to sub-Saharan Africa, between the latitudes of 14^{o}N and 29°S, south of the Sahel desert, and north of the Namibian and Kalahari deserts (Vreysen, 2001). Tsetse flies need a particular temperature (16°C–38°C) and humidity (50%–80% of relative humidity) to survive.

Therefore, they are linked to the presence of water that increases the local humidity, allowing for the growth of vegetation that protects them from direct sunlight and wind, and attracts the animals to where tsetse feed (Malvy and Chappuis, 2011).



Plate I. Photogragh of Tsetsefly (Source: Despommier, *et.,al* 2006)

2.3. Infection and symptoms of Trypanosomiasis

The disease is mostly transmitted through the bite of an infected tsetse fly but there are other ways in which people are infected with sleeping sickness.

1. Mother-to-child infection: the trypanosome can cross the placenta and infect the fetus.
2. Mechanical transmission through other blood sucking insects is possible. However, it is difficult to assess the epidemiological impact of transmission.
3. Accidental infections have occurred in laboratories due to pricks from contaminated needles.

In the first stage, the trypanosomes multiply in subcutaneous tissues, blood and lymph. This is known as a first stage or haemolymphatic phase, which entails bouts of fever, headaches, joint pains and itching (WHO, 2014).

In the second stage the parasites cross the blood-brain barrier to infect the central nervous system. This is known as the neurological or meningoencephalic phase. In general this is when more obvious signs and symptoms of the disease appear: changes of behaviour, confusion, sensory disturbances and poor coordination. Disturbance of the sleep cycle, which gives the disease its name, is an important feature of the second stage of the disease. Without treatment, sleeping sickness is considered fatal although cases of healthy carriers have been reported (WHO, 2014).

2.4. Disease Management

Disease management is made in three steps.

1. Screening for potential infection. This involves using serological tests (only available for *Trypanosoma brucei gambiense*) and checking for clinical signs - generally swollen cervical glands.
2. Diagnosing whether the parasite is present.
3. Staging to determine the state of disease progression. This entails examining cerebrospinal fluid obtained by lumbar puncture and is also used to determine the outcome of treatment.

Diagnosis must be made as early as possible to avoid progressing to the neurological stage in order to elude complicated, difficult and risky treatment procedures. The long, relatively asymptomatic first stage of *Trypanosoma brucei gambiense* sleeping sickness is one of the reasons for an exhaustive, active screening of the population at risk is recommended, in order to identify patients at an early stage and reduce transmission(WHO, 2014).

Exhaustive screenings require a major investment in human and material resources. In Africa such resources are often scarce, particularly in remote areas where the disease is mostly found. As a result, some infected individuals may die before they can ever be diagnosed and treated (WHO, 2014).

2.5. Treatment

The type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease are of lower toxicity and easier to administer. The earlier the disease is identified, the better the prospect of a cure. Treatment success in the second stage depends on a drug that can cross the blood-brain barrier to reach the parasite. Such drugs are toxic and complicated to administer. Four drugs are registered for the treatment of

sleeping sickness. These drugs are donated to WHO by manufacturers and distributed free of charge to countries endemic for the disease (WHO, 2014).

First stage treatment:

1. **Pentamidine:** discovered in 1941, used for the treatment of the first stage of *Trypanosoma brucei gambiense* sleeping sickness. Despite non-negligible undesirable effects, it is in general well tolerated by patients (WHO, 2014).
2. **Suramin:** discovered in 1921, used for the treatment of the first stage of *Trypanosoma brucei rhodesiense*. It provokes certain undesirable effects, in the urinary tract and allergic reactions (WHO, 2014).

Second stage treatment:

1. **Melarsoprol:** This is discovered in 1949, it is used in both forms of infection. It is derived from arsenic and has many undesirable side effects. The most dramatic is reactive encephalopathy (encephalopathic syndrome) which can be fatal (3% to 10%). An increase in resistance to the drug has been observed in several foci particularly in central Africa (WHO, 2014).

2. **Eflornithine:** This is less toxic than melarsoprol, it was registered in 1990. It is only effective against *Trypanosoma brucei gambiense*. The regimen is strict and difficult to apply (WHO, 2014).A combination treatment of nifurtimox and eflornithine was introduced in 2009. It simplifies the use of eflornithine in monotherapy, but unfortunately it is not effective for *Trypanosoma bruce. rhodesiense*. Nifurtimox is registered for the treatment of American trypanosomiasis but not for human African trypanosomiasis. Nevertheless, after safety and efficacy data provided by clinical

trials, its use in combination with eflornithine has been accepted and included in the WHO List of Essential Medicine, and it is provided free of charge for this purpose by WHO to endemic countries (WHO, 2014).

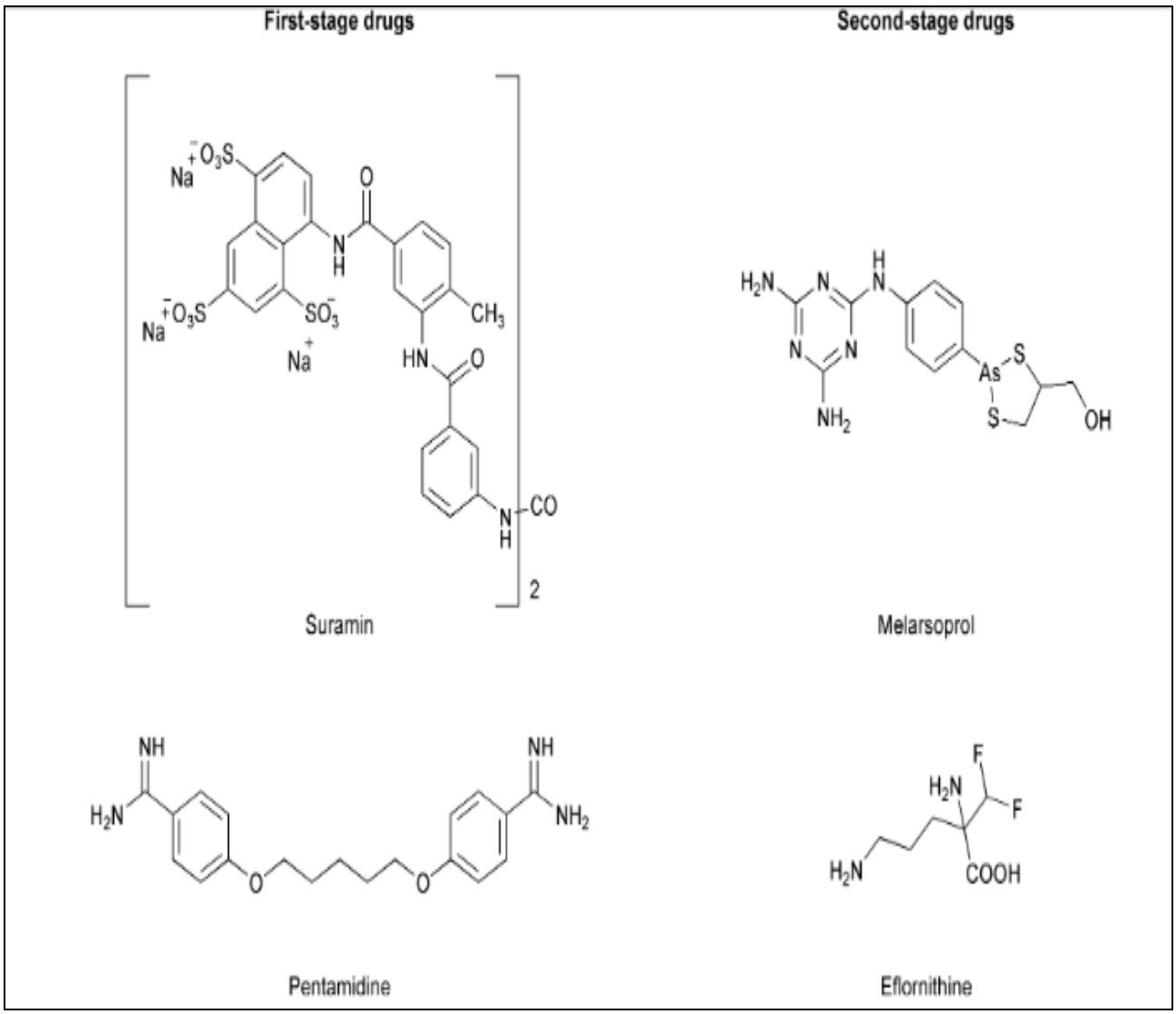


Fig2.2 Approved Drugs for First and Second Stage of Human African Trypanosomiasis (Source: Hoet *et al*., 2004)

2.6. Epidemiology and Control

The most important factors influencing transmission are reservoir hosts and the presence of suitable vectors and the environments necessary for these vectors to reproduce. Tsetseflies occupy 4.5 million square miles in Africa. *Glossina* species that transmit *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* occur in open country, pupating in dry, friable earth. Vectors of *Trypanosoma brucei gambiense* are riverline flies breeding in shady, moist areas along rivers. Transmission varies locally, depending on coincidence of the trypanosome proper fly species (Larry and John, 2010).

Control of trypanosomiasis is conducted along several lines, most of which involve vectors. Tsetseflies are larviparous and they deposit their young on the soil under bush. Owingtothis behavioral characteristic, bush removal and trimming are very successful means of control. Insecticide (DDT and benzene hexachloride) spraying from aircraft has also been used. One interesting approach to trypanosomiasis control is the development and use of trypanotolerant cattle stocks (Larry and John, 2010)

2.7. Taxonomic Classification of *Acacia nilotica*

Acacia nilotica belonging to the family *Leguminosae* and sub family *Mimosaceae* is a moderate sized tree that grows up to 20m, but this is attenuated by site. It has a flattish or umbrella shaped crown and is easily identified by its bright yellow, sweet-scented flower heads, its sweet-smelling gray pods and its paired whitish spines at the base of each leaf.

During the hot season the tree is in full leaf and its feathery foliage provides good shade. It is found throughout the drier parts of India (Bennison and Paterson, 1994).

Table 2.1 Taxonomic classification of *Acacia nilotica*

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Acacia*
Species	*nilotica*

Source: (Kyalangalilwa *etal.*, 2013)

2.7.1. Plant description

1. **Pods:** The pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance.
2. **Seeds:** The seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa (Iman *et al.*, 2007).
3. **Leaves:** The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long (Beniwal *et al.*, 1992).
4. **Stem:** Stems are usually dark to black coloured, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum (Brenan, 1983).
5. **Flowers:** Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow colour, develop either in axillary or whorly pattern on peduncles 2-3 cm long located at the end of branches (Bargali and Bargali, 2009).
6. **Bark:** The bark a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns (Khan *et al.*, 2009).
7. **Thorns:** Thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees.
8. **Roots:** The root is generally of brown colour in older and whitish in younger regions.
9. **Gum:** The gum varies in colour from very pale yellowish brown to dark reddish brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary (New, 1984).



Bark part



Root part



Seed part



Leaves part

Plate II. The stem bark, root, seeds and leaves of *Acacia nilotica*

2.7.2. Traditional claims

Acacia nilotica is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions; it is economically used as a source of tannins, gums, timber, fuel and fodder. *Acacia nilotica* used as anti-cancer, anti-tumours, antiscorbutic, astringent, anti-oxidant, natriuretic, antispasmodial, diuretic, intestinal pains and diarrhea, nerve stimulant, cold, congestion, coughs, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis (Malviya *et al.,* 2011).

Seeds have antimalarial, antidiabetic, antihypertensive and antispasmodic activities. The leaves and pods are an excellent fodder with anti inflammatory properties, rich in protein. The pods have molluscicidal and algicidal properties. The bark is used in the treatment of hemorrhages, cold, diarrhea, tuberculosis and leprosy. The root is used as an aphrodisiac and the flowers for treating syphilis lesions. The gum from the tree is pharmaceutically used as suspending and emulsifying agent and in preparation of many formulations. Its resins repel insects and water (Malviya *et al.,* 2011).

2.7.3. Medicinal uses of different parts of *Acacia nilotica*

1. **Root**: The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen(Agrawal *et al.,* 2010).
2. **Leaf**: This is used in chemo-preventive, anti-mutagenic, anti-bacterial, anti-cancer, astringent and anti-microbial activity. The tender leaves are used to treat diarrhea, aphrodisiac, dressing of ulcers,anti-inflammatory and Alzheimer's diseases (Atif *et al.,* 2012; Kalaivani and Mathew, 2010; Shittu, 2010; Kalaivani *et al.,* 2010).

3. **Gum**: Anti bacterial, antioxidant, anti-mutagenic (Atif *et al.,* 2012; Baravkar *et al.,* 2008).
4. **Stem bark**: The Bark is used as astringent, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, burning sensation, toothache and dysentery. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, bleeding piles and leucoderma (Abdullahi and Emmanuel 2012; Agrawal *et al.,* 2010; Del, 2009; Kalaivani and Mathew, 2010a; Kaur *et al.,* 2005; Khan *et al.,* 2009; Singh *et al.,* 2009a ; Singh *et al.,* 2009b).
5. **Seeds**: This is used in spasmogenic activity and antiplasmodial activity (Amos *et al.,* 1999; El-Tahir *et al.,* 1999).
6. **Pods**: This is used as Anti-hypertensive and anti-spasmodic, anti-diarrhoerial, astringent and against HIV-1 PR. It inhibits HIV-1 induced cythopathogenicity, antiplatelet aggregatory activity and anti oxidant (Gilani *et al.,* 1999; Asres *et al.,* 2005; Singh *et al.,* 2009b).

2.8. Taxonomic Classification of *Khaya senegalensis*

Khaya senegalensis is a deciduous evergreen tree, 15-30m high, up to 1m in diameter, with a clean bole to 8-16m, buttresses not prominent or absent; bark dark grey, with small, thin, reddish-tinged scales; slash dark pink to bright crimson, exuding a red sap.

The wood is valued for carpentry, joinery, furniture, cabinet work, ship building and decorative veneer. It is suitable for construction, flooring, interior trim, vehicle bodies, toys, novelties, railway sleepers, turnery and pulpwood. Traditionally, the wood is used for dug-out canoes, household implements such as mortars and spoons, and drums. It is also used as fuel wood and for charcoal production (Nikiema and Pasternak, 2008).

Table 2.2 Taxonomic classification of *Khaya senegalensis*

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Angiosperms
Class	Eudicots
Subclass	Rosidae
Order	Sapindales
Family	*Meliaceae*
Genus	*Khaya*
Species	*Senegalensis*

Source: (IUCN, 2016)

2.8.1. Plant description

1. **Leaves:** Theleaves are alternate, compound, stipules absent; petiole and rachis 13-33cm long; leaflets 3-4 (max. 7) usually opposite pairs, oblong to narrowly oblong-elliptic, 4-12 x 2-5cm, apex acute to shortly acuminate, base rounded, margins entire, pale green, lateral nerves 8-16, petiolules about 3.5cm long (Orwa *et al.*2009).
2. **Flowers:** The flowers are tetramerous, monoecious but with well-developed vestiges of those of the opposite sex with very little external differences between sexes. Calyx is pale green, lobed almost to the base, lobes subcircular, about 1 x 1mm, imbricate; petals cream, free, oblong-ovate, 4 x 2.5mm, contorted in bud; orange disk around the ovary(Orwa *et al.*2009).
3. **Fruit:** The fruit is an upright, almost spherical, woody capsule, 4-6cm in diameter, opening by 4 valves from the apex (a distinction from *K. ivorensis*, which is closely related but has 5 valves). The seeds are brown, 6 or more per cell, broadly transversely ellipsoid to flat, about 25 x 18mm, margins narrowly winged. *(*Orwa *et al.*2009*).*
4. **Bark:** The barkof this tree is very thick, scaly and dark brownish-gray color. In section it oozes reddish exudates (Takin *et al.*, 2013).



Bark part



Flower and Fruit part



Leaves and Seed part

Plate III: The stem bark, flower, fruit, leaves and seeds of *Khaya senegalensis*

2.8.2. Medicinal uses

The bitter-tasting bark is highly valued in traditional medicine. Bark decoctions or macerations are widely taken against fever caused by malaria, and against stomach complaints, diarrhoea, dysentery and anaemia, as anodyne in cases of rheumatism and headache, and as tonic, emmenagogue, antitrypanosomal and anthelmintic (Ogbadoyi et al., 2011; Nikiema and Pasternak, 2008).

They are also used as purgative, antidote and abortifacient, and to treat syphilis, leprosy, chickenpox and angina. The bark is applied externally as disinfectant in cases of inflammations and to treat skin diseases, rash, scabies, wounds, ulcers, boils, haemorrhoids, swellings and toothache (Nikiema and Pasternak, 2008).

The bark is commonly used in veterinary medicine, as anthelmintic, tonic and appetizer, and to treat trypanosomiasis, liver flukes, diarrhoea and ulcers. In Uganda it is used as fish poison. In Cameroon the bark is in demand as an additive in local beer brewing. Leaves are also used in traditional medicine, to treat skin complaints, wounds, jaundice, oedema, headache and depression, and as purgative. Roots are applied against jaundice, stomach-ache, oedema and amenorrhoea (Nikiema and Pasternak, 2008).

The roots and/or bark are an ingredient of complex arrow poisons of which *Strophanthus* roots or seeds are the main ingredients. Flowers are used in medicines against stomach complaints and syphilis. Seed oil is rubbed in to treat rheumatism and influenza, and it is taken to treat syphilis.Young twigs and roots are used as chewing sticks (Nikiema and Pasternak, 2008).

In Ghana the bark has been used for dyeing cloth brownish. The foliage is a common source of fodder, but it has a low fodder quality and is mainly used towards the end of

the dry season when better-quality forage is not available, or in mixtures with better fodders. The seed oil is used in cosmetics and for cooking.

The wood ash is added to stored grain to prevent insect attack. *Khaya senegalensis* is commonly planted as a roadside tree and ornamental shade tree, and sometimes for soil stabilization (Nikiema and Pasternak, 2008). The oil in combination with other substances to treat diseases such as syphilis, stomach upset and on cuts after making incisions on the body of patients.*Khaya.senegalensis* is used in treating sickle cell anaemia and is an analgesic (Mann *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Collection and Authentication of Plant materials

Plant materials were collected by the researcher at BZ, Ahmadu Bello Universitystaff quarters, Samaru Zaria and authenticated by a taxonomist in the department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with the voucher numbers;**6924** and **900181** for *Acacia nilotica* and *Khaya senegalensis* respectively.

3.2. Preliminary Processing and Preparation of Plant Materials

The stem barks of each plant (*Acacia nilotica and Khaya senegalensis*) were thoroughly washed and shade-dried. The dried stem barks were grounded first into coarse form using a pestle and mortar and then reduced to a powdery form using a mill.

3.3. Preparation of Crude Plants Extracts

The powdery plant materials (250g) were separately soaked in 500ml of 70% methanol each for 24hour with constant stirring. The extracts were filtered through a clean muslin cloth (2mm) and concentrated using a rotary evaporator. The concentrated extracts were dried under room temperature. The extracts were weighed, stored in specimen jars and used in the preparation of the stock extract for administration.

3.4. Preparation of Plant Fractions using Partitioning Fractionation (Solvent-Solvent Extraction).

The extract was dissolved in water and partitioned with N-hexane, N-hexane fraction was formed. The aqueous layer was partitioned with Ethyl Acetate, Ethyl Acetate fraction was formed. The aqueous layer was partitioned with n-Butanol, n-Butanol, fraction was

formed.

All the three fractions (N-hexane, Ethyl Acetate and N-Butanol,)were dried under room temperature and were tested for their antitrypanosomal activities using infected adult Wistar rats.

3.5. Experimental Animals

Adult Wistar rats (*Rattus norvegicus*) were purchased from Pharmacology Department, Ahmadu Bello University, Zaria, Nigeria. Adult Wistar rats bought were kept under laboratory conditions before being used in experiments. The Wistar rats were housed in laboratory cages fed with pellets and were watered *ad libitums*. The animals were allowed a 7-day period of acclimatization.

3.6. Trypanosome Stock

Trypanosoma brucei brucei strain (*Federe* strain) was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria and maintained in the laboratory by serial passage in adult Wistar rats (Herbert and Lumsden, 1976).

3.7. Phytochemical Screening of plant extracts

Phytochemical screening of the plant extracts were carried out to assess the concentration of each plant sample using standard methods (Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.1 Detection of Alkaloids

Mayer's test: A drop of Mayers reagent was added to a few milliliter of filtrate by the side of the test tube.A white or creamy precipitate indicates the test as positive(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

Wagner's test: Few drops of Wagner's reagent was added to a few milliliter of filtrate by the side of the test tube.A reddish brown precipitate confirms the test as positive(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

Hager's test: 2 ml of Hager's reagent was added to a few milliliter of filtrateby the side of the test tube. A prominent yellow precipitate indicates the test as positive(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.2 Detection of Tannins

Ferric chloride test: 50mg of the extract was dissolved in 5 ml of distilled water. Few drops of 5% Ferric chloride wasalso added. A dark green color indicates the presence of tannins(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.3 Detection of Glycosides

Sodium hydroxide reagent: Small amount of extract was dissolved in 1ml of water and 1ml of NaOH solution was added. A yellow colour indicates the presence of glycosides(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.4 Detection of Cardiac glycosides

Killer Kiliani test: 50mg of the extract was dissolved in 5ml of distilled water and then filtered. 1ml of glacial acetic acid, a drop of Ferric chloride and a drop of concentrated sulfuric acid was added to 2 ml of the filtrate.

Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.5 Detection of Saponins

Foam test: 50mg of the extract was dissolved in 20 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.6 Detection of Steroids

Lieberman Burchardt tests: 50mg of the extract was dissolve in 5ml of chloroform solution. Few drops of acetic anhydride and one ml of concentrated sulphuric acid was added from the sides of the test tube. Greenishcolor indicates the presence of steroids(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.7 Detection of Triterpenes

Salkowski test: 2ml of chloroform solution was added to the extract and shaken with few drops of concentrated sulphuric acid was added from the side of the test tube. The chloroform layer turns to yellow on standing. This indicates presence of triterpenes(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.8 Detection of Carbohydrates

Benedict's test: 0.5 ml of Benedict's reagent was added to 0.5 ml of filtrate. The mixture was heated on boiling water bath for 2 minutes. A characteristic red color indicates the presence of sugar(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.9 Detection of Flavonoids

Magnesium and hydrochloric acid reduction test: 50 mg of the extract was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5g ofmagnesium metal. Pink to Crimson colour develops which indicates the presence of flavonoids (Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.7.10 Detection of Anthraquinones

50mg of the extract was dissolved in 5 ml of distilled water. 1ml of dilute ammonia solution was added to 2 ml of the extract and shaken vigorously. Pink color layer indicates presence of anthraquinones(Harborne, 1989; Trease and Evans, 1989: Sofowora, 1993).

3.8 Determination of LD_{50}

The extracts of the plant were prepared in series of concentrations and were used to determine LD_{50}.The acute toxicity study was conducted in accordance with Lorke's method (Lorke, 1983). The study was conducted in two phases.

In the first phase, sixteen Wistar rats were divided into 4 groups of 4 Wistar rats each. Groups 1, 2 and 3 were given 10, 100 and 1000 mg/kg body weight of the extract, respectively to possibly establish the range of doses producing any toxic effect.

Group 4 was not given anything and served as control. The Wistar rats were placed under observation for 24 hours to monitor their behavior as well as mortality.

In the second phase, further specific doses (1500mg, 3000mg and 5000 mg/kg) of the extract were administered to twelve Wistar rats (four Wistar rats per dose) to further determine the LD_{50} value. The Wistar rats were placed under observation for 24 hours to monitor their behavior as well as mortality. Each of the extracts was dissolved in Phosphate buffered saline (PBS) solution and given via intraperitoneal route.

Recovery was indications of having survived the acute toxicity. LD_{50} were calculated using this formular:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

LD_{50} the dose that kills 50% of test animal population

D_0 is the highest dose that gave no mortality

D_{100} is the lowest dose that produced mortality

3.9 Determination of Antitrypanosomal Activities of the Crude Extract

Blood was collected by cardiac puncture with an EDTA coated syringe from the Wistar rat infected with *Trypanosoma brucei brucei* and immediately diluted with physiological saline to serve as the inoculum. Healthy Adult Wistar rats were infected intraperitoneally with 0.01ml of the inoculum. Development of parasitaemia in these adult Wistar rats was checked daily by wet blood film prepared from the tail blood and observed at x40 magnification as described by Adeyemi *et.,al*(2009).

Adult Wistar rats were grouped into six groups: A, B, C, D, E, F with each group containing five (5) adult Wistar rats. They were treated with various doses of 100-400mg/kg bodyweight of the crude extract.

KEY:

A-Infected but treated with 100mg/kg of the extract.

B- Infected but treated with 200mg/kg of the extract.

C- Infected but treated with 300mg/kg of the extract.

D- Infected but treated with 400mg/kg of the extract.

E-Not infected but treated with 400mg/kg of the extract.

F- Infected but not treated.

The average parasitaemia level of the Adult Wistar rats was checked at intervals of two days and recorded. The number of parasites seen per field under the microscope was counted as described by Herbert and Lumsden, (1976). This method involves counting of parasites per field of the microscope in blood diluted with buffered phosphate saline (PBS, pH 7.2).

3.10 Determination of the Antitrypanosomal Activities of the Plant Fractions using Partitioning Fractionation (Solvent-Solvent extraction).

Blood was collected by cardiac puncture with an EDTA coated syringe from the donor Wistar rat and immediately diluted with physiological saline to serve as the inoculum. Healthy adult Wistar rats were infected intraperitoneally with 0.01ml of the inoculum. Development of parasitaemia in these adult Wistar rats was checked daily by wet blood film prepared from the tail blood and observed at x40 magnification Adeyemi et.,al 2009.

Adult Wistar rats were grouped into six groups: A, B, C, D, E, F with each group containing five (5) adult Wistar rats. They were treated with various doses of 50-200mg/kg bodyweight of the fractions.

KEY

A-Infected but treated with 50mg/kg of the fraction.

B- Infected but treated with 100mg/kg of the fraction.

C- Infected but treated with 150mg/kg of the fraction.

D- Infected but treated with 200mg/kg of the fraction.

E-Not infected but treated with 200mg/kg of the fraction.

F- Infected but not treated

3.11 Determination of Haematological Profile

3.11.1 Packed Cell Volume (PCV)

Heparinized capillary tubes were ¾ filled with blood by capillary action. Excess blood was dabbed with a cotton wool. The dry end of the tube was sealed with a Bunsen

flame. It was centrifuged using a microhaematocrit centrifuge for 4minutes at 12,000 revolution per minute (rpm). The tubes were placed in a haematocrit reader to read the packed cell volume (PCV)(Adeoye, 2007).

3.11.2 White blood cell (WBC)

0.02ml of blood was added to 0.38ml of Turk's fluid (glacial acetic acid) to prepare a 1:20 dilution. The fluid was drawn to rinse the pipette and expelled back into the fluid for proper mixing. The quantity of the diluted blood was withdrawn and the Neubauer counting chamber was filled with the diluted blood.

The preparation was allowed to settle and the white cells present in the four corners ($1mm^2$) square were counted using the X10 objective. The white blood cell (WBC) was calculated using the following formula;

$$\text{Count (cells/L)} = \frac{N \times DF \times 10^6}{A \times D}$$

Where; N = total number of cells counted

DF = dilution factor

A = total area counted (in mm^2)

D = the depth of chamber (e.g 0.1mm)

10^6 = factor to convert count / µl to count/L (Adeoye, 2007).

3.11.3 Differential count

A thin film was prepared and allowed to dry. It was fixed in absolute methanol for 3minutes. The preparation was flooded with diluted Giemsa stain (1:10) and allowed to stand for 30 minutes. It was thoroughly rinsed, drained and allowed to air-dry. The preparation was examined microscopically and individual white blood cells such as Neutrophils, lymphocytes, monocytes, eosinophils and basophils were counted. (Adeoye, 2007).

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents of Crude and Fractions of *Acacia nilotica* (stem bark) and *Khaya senegalensis* (stem bark)

The result shown in Table 4.1indicates that Alkaloids, tannins, glycosides, cardiac glycosides, saponins, triterpene, carbohydrates and flavonoids were found in the crude extracts of *Acacia nilotica* (stem bark)and *Khaya senegalensis* (stem bark).

Result in Table 4.2, shows Alkaloids, tannins, glycosides, cardiac glycosides, triterpenes and carbohydrates were found in N-hexane, ethylacetate and N-butane fractions of Acacia *nilotica* while flavinoids were only found in N-butane fraction.Alkaloids, glycosides, cardiac glycosides, triterpenes and carbohydrates were found in N-hexane, ethylacetate and N-butane fractions of *Khaya senegalensis* (stem bark). Tannins, saponins, and flavinoids, were also found in N-butane fractions of *Khaya senegalensis* (stem bark).

Table 4.3 shows the physical characteristics of the stem bark extracts of *Acacia nilotica* and *Khaya senegalensis*.

Table 4.1: Phytochemical Constituents of Crude Extracts of the Stem bark of *Acacia nilotica* and *Khaya senegalensis*

Active components	*Acacia nilotica*	*Khaya senegalensis*
Alkaloids	+	+
Tannins	+	+
Glycosides	+	+
Cardiac glycosides	+	+
Saponins	+	+
Steroids	-	-
Triterpenes	+	+
Carbohydrates	+	+
Flavonoids	+	+
Anthraquinones	-	-

KEY: + = Present

- = absent

Table 4.2: Phytochemical Constituents of Fractions (N-hexane, Ethylacetate and N-butane) of Stem Bark of A*cacia nilotica* and *Khaya senegalensis*

Active components	***A nilotica***	***A nilotica***	***A nilotica***	***K senegalensis***	***K senegalensis***	***K senegalensis***
	N-hexane	Ethylacetate	N-butane	N-hexane	Ethylacetate	N-butane
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	-	-	+
Glycosides	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
Steroids	-	-	-	-	-	-
Triterpenes	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Flavonoids	-	-	+	-	-	+
Anthraquinones	-	-	-	-	-	-

KEY: + = Present

- = Absent

Table 4.3: Physical Characteristics of the Extracts of the Stem Bark of *Acacia nilotica* and *Khaya senegalensis*.

	Physical characteristics					
Solvent used	***Acacia nilotica***			***Khaya senegalensis***		
	Colour	**Odour**	**Consistency**	**Colour**	**Odour**	**Consistency**
Methanol	Brown	Agreeable	Dry crystalline	Reddish brown	Agreeable	Dry crystalline
N-hexane	Dark brown	Agreeable	Dry crystalline	Reddish brown	Agreeable	Dry crystalline
Ethyl acetate	Dark brown	Agreeable	Dry crystalline	Reddish brown	Agreeable	Dry crystalline
N- butane	Dark brown	Agreeable	Dry crystalline	Reddish brown	Agreeable	Dry crystalline

4.2 LD_{50} Crude and Fraction Extracts Analysis of *Acacia nilotica* and *Khaya senegalensis*

The result in Table 4.4 shows the lethal dose (LD_{50}) for crude extract of *Acacia nilotica*; which was calculated as 707.1mg/kg.

Table 4.5, 4.6 and 4.7 shows the lethal dose (LD_{50}) for fractions (N-Hexane, ethylacetate and N-butane) of *Acacia nilotica*which was calculated as 547.7 mg/kg, 387.3mg/kg and 707.1 mg/kg.

Table 4.8 shows the lethal dose (LD_{50}) for *Khaya senegalensis*which was calculated as547.72mg/kg for the crude extract

Table 4.9, 4.10 and 4.11 shows the lethal dose (LD_{50}) for fractions (N-Hexane, ethylacetate and N-butane) of*Khaya senegalensis*; which was calculated as 387.3mg/kg, 547.1 mg/kg and 223.6mg/kg.

4.3 Antitrypanosomal activities of crude extract and fractions of *Acacia nilotica* and *Khaya senegalensis* against *Trypanosoma brucei brucei* infected Wistar rats.

The result in) Table 4.12 shows the crude extracts of*Acacia nilotica* had antitrypanosomal activity at 100, 200,300 and 400 mg/kg body weight. Doses (100, 200,300 and 400 mg/kg body weight) were able to clear the parasites from circulation within 8 and 12 days of treatment.

The result in Table 4.13, the crude extract of *Khaya senegalensis* shows antitrypanosomal activity at 100, 200,300 and 400 mg/kg body weight. Doses (100, 200,300 and 400 mg/kg body weight) were able to clear the parasites from circulation within 10 and 14 days of treatment.

Table 4.14 shows the haematological profile of the crude extract of *Acacia nilotica* before and after administration. There was no significant difference in the haematological parameters with p values >0.05.

Table 4.4: LD_{50} of crude extract of *Acacia nilotica*

HOURS	Group 1AL	Group 2AL	Group 3AL	Group 4AL	Group 1AH	Group 2AH	Group3AH
0	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4
12	4	4	4	4	3	4	4
18	4	4	4	4	3	4	4
24	4	3	3	4	3	4	4

KEY

Group 1AL = 10mg/kg weight of *Acacia nilotica* (Low dose)
Group 2AL = 100mg/kg weight of *Acacia nilotica* (Low dose)
Group 3AL = 1000mg/kg weight of *Acacia nilotica* (Low dose)
Group 4AL = Control

Group 1AH = 1500mg/kg weight of *Acacia nilotica* (Highdose)
Group 2AH = 3000mg/kg weight of *Acacia nilotica* (High dose)
Group 3AH = 5000mg/kg weight of *Acacia nilotica* (High dose)

Table 4.5: LD_{50} of N-hexane fraction of *Acacia nilotica*

HOURS	Group 1AHL	Group 2AHL	Group 3AHL	Group 4AHL	Group 1AHH	Group2AHH	Group3AHH
0	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4
12	4	4	4	4	4	4	4
18	4	4	4	4	4	3	4
24	4	3	3	4	4	3	4

KEY
Group 1AHL = 10mg/kg weight of N-hexane fraction of *Acacia nilotica* (Low dose)
Group 2AHL = 100mg/kg weight of N-hexane fraction of Acacia *nilotica* (Low dose)
Group 3AHL = 1000mg/kg weight of N-hexane fraction of *Acacia nilotica* (Low dose)
Group 4AHL = Control

Group 1AHH = 1500mg/kg weight of N-hexane fraction of *Acacia nilotica* (Highdose)
Group 2AHH = 3000mg/kg weight of N-hexane fraction of *Acacia nilotica* (High dose)
Group 3AHH = 5000mg/kg weight of N-hexane fraction of *Acacia nilotica* (High dose)

Table 4.6: LD_{50} of ethyl acetate fraction of *Acacia nilotica*

HOURS	Group 1AEL	Group 2AEL	Group 3AEL	Group 4AEL	Group 1AEH	Group 2AEH	Group3AEH
0	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4
12	4	4	4	4	4	4	4
18	4	4	4	4	4	4	4
24	3	4	4	4	4	3	4

KEY
Group 1AEL = 10mg/kg weight of ethylacetate of *Acacia nilotica* (Low dose)
Group 2AEL = 100mg/kg weight of ethylacetate fraction of *Acacia nilotica* (Low dose)
Group 3AEL = 1000mg/kg weight of ethylacetate fraction of *Acacia nilotica* (Low dose)
Group 4AEL = Control

Group 1AEH = 1500mg/kg weight of ethylacetate fraction of *Acacia nilotica* (Highdose)
Group 2AEH = 3000mg/kg weight of ethylacetate fraction of *Acacia nilotica* (High dose)
Group 3AEH = 5000mg/kg weight of ethylacetate fraction of *Acacia nilotica* (High dose)

Table 4.7: LD_{50} of N-butane fraction of *Acacia nilotica*

HOURS	Group 1ABL	Group 2ABL	Group 3ABL	Group 4ABL	Group 1ABH	Group 2ABH	Group 3ABH
0	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4
12	4	4	3	4	4	4	4
18	4	4	3	4	4	4	4
24	4	3	3	4	4	4	4

KEY
Group 1ABL = 10mg/kg weight of N-butane fraction of*Acacia nilotica* (Low dose)
Group 2ABL = 100mg/kg weight of N-butane fraction of *Acacia nilotica* (Low dose)
Group 3ABL = 1000mg/kg weight of N-butane fraction of *Acacia nilotica* (Low dose)
Group 4ABL = Control

Group 1ABH = 1500mg/kg weight of N-butane fraction of *Acacia nilotica* (Highdose)
Group 2ABH = 3000mg/kg weight of N-butane fraction of *Acacia nilotica* (High dose)
Group 3ABH = 5000mg/kg weight of N-butane fraction of *Acacia nilotica* (High dose)

Table 4.8 LD_{50} of crude extract of *Khaya senegalensis*

HOURS	Group 1KL	Group 2KL	Group 3KL	Group 4KL	Group 1KH	Group 2KH	Group 3KH
0	4	4	4	4	4	4	4
6	4	4	4	4	3	4	3
12	4	4	4	4	3	4	2
18	4	3	3	4	2	4	2
24	4	3	2	4	2	4	1

KEY
Group 1KL = 10mg/kg weight of *Khaya senegalensis* (Low dose)
Group 2KL = 100mg/kg weight of *Khaya senegalensis* (Low dose)
Group 3KL = 1000mg/kg weight of *Khaya senegalensis* (Low dose)
Group 4KL = Control

Group 1KH = 1500mg/kg weight of *Khaya senegalensis* (High dose)
Group 2KH = 3000mg/kg weight of *Khaya senegalensis* (High dose)
Group 3KH = 5000mg/kg weight of *Khaya senegalensis* (High dose)

Table 4.9: LD_{50} of N-hexane fraction of *Khaya senegalensis*

HOURS	Group 1KHL	Group 2KHL	Group 3KHL	Group 4KHL	Group 1KHH	Group 2KHH	Group 3KHH
0	4	4	4	4	4	4	4
6	4	4	3	4	4	4	4
12	4	4	3	4	4	4	4
18	4	4	3	4	4	4	4
24	4	3	3	4	3	4	4

KEY
Group 1KHL = 10mg/kg weight of N-hexane fraction of *Khaya senegalensis* (Low dose)
Group 2KHL = 100mg/kg weight of N-hexane fraction of *Khaya senegalensis* (Low dose)
Group 3KHL = 1000mg/kg weight of N-hexane fraction of *Khaya senegalensis* (Low dose)
Group 4KHL = Control

Group 1KHH = 1500mg/kg weight of N-hexane fraction of *Khaya senegalensis* (High dose)
Group 2KHH = 3000mg/kg weight of N-hexane fraction of *Khaya senegalensis* (High dose)
Group 3KHH = 5000mg/kg weight of N-hexane fraction of *Khaya senegalensis* (High dose)

Table 4.10: LD_{50} of Ethyl acetate fraction of *Khaya senegalensis*

HOURS	Group 1KEL	Group 2KEL	Group 3KEL	Group 4KEL	Group 1KEH	Group 2KEH	Group 3KEH
0	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4
12	4	4	4	4	4	4	4
18	4	3	4	4	1	4	4
24	4	2	3	4	1	4	3

KEY
Group 1KEL = 10mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (Low dose)
Group 2KEL = 100mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (Low dose)
Group 3KEL = 1000mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (Low dose)
Group 4KEL = Control

Group 1KEH = 1500mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (High dose)
Group 2KEH = 3000mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (High dose)
Group 3KEH = 5000mg/kg weight of ethylacetate fraction of *Khaya senegalensis* (High dose)

Table 4.11: LD_{50} of N-butane fraction of *Khaya senegalensis*

HOURS	Group 1KBL	Group 2KBL	Group 3KBL	Group 4KBL	Group 1KBH	Group 2KBH	Group 3KBH
0	4	4	4	4	4	4	4
6	4	3	4	4	4	4	4
12	4	3	4	4	4	4	4
18	4	2	4	4	4	4	3
24	3	2	3	4	3	3	2

KEY
Group 1KBL = 10mg/kg weight of N-butane fraction of *Khaya senegalensis* (Low dose)
Group 2KBL = 100mg/kg weight of N-butane fraction of *Khaya senegalensis* (Low dose)
Group 3KBL = 1000mg/kg weight of N-butane fraction of *Khaya senegalensis* (Low dose)
Group 4KBL = Control

Group 1KBH = 1500mg/kg weight of N-butane fraction of *Khaya senegalensis* (High dose)
Group 2KBH = 3000mg/kg weight of N-butane fraction of *Khaya senegalensis* (High dose)
Group 3KBH = 5000mg/kg weight of N-butane fraction of *Khaya senegalensis* (High dose)

Table 4.12: Antitrypanosomal of activity *Acacia nilotica* (Crude Extract) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AA	Group BA	Group CA	Group DA	Group EA	Group FA
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	39	56	41	38	0	48
6	48	33	17	11	0	51
8	12	11	5	0	0	19
10	6	0	0	0	0	−
12	0	0	0	0	0	−
14	0	0	0	0	0	−

0 =No parasite count
= Rat died

KEY:

AA-Infected Wistar rats but treated with 100mg/kg of the crude extract of *Acacia nilotica*.

BA- Infected Wistar rats but treated with 200mg/kg of the crude extract of *Acacia nilotica*.

CA- Infected Wistar rats but treated with 300mg/kg of the crude extract of *Acacia nilotica*.

DA- Infected Wistar rats but treated with 400mg/kg of the crude extract of *Acacia nilotica*.

EA-Wistar rats not infected but treated with 400mg/kg of the crude extract of *Acacia nilotica*..

FA- Infected Wistar rats but not treated.

Table 4.13: Antitrypanosomal activity of *Khaya senegelensis* (Crude Extract) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AK	Group BK	Group CK	Group DK	Group EK	Group FK
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	71	60	49	44	0	29
6	51	43	18	10	0	52
8	35	24	7	2	0	38
10	19	11	0	0	0	25
12	8	2	0	0	0	–
14	0	0	0	0	0	–

0 = No parasite count

\- = rat died

KEY:

AK-Infected Wistar rats but treated with 100mg/kg of the crude extract of *Khaya senegelensis*.

BK- Infected Wistar rats but treated with 200mg/kg of the crude extract of *Khaya senegelensis*.

CK- Infected Wistar rats but treated with 300mg/kg of the crude extract of *Khaya senegelensis*.

DK- Infected Wistar rats but treated with 400mg/kg of the crude extract of *Khaya senegelensis*.

EK-Wistar rats not infected but treated with 400mg/kg of the crude extract of *Khaya senegelensis*.

FK- Infected Wistar rats but not treated.

Table 4.14: Effect of Administration of Stem Bark Crude Extract of *Acacia niloticа*on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	44.03	44.43	33.07	31.77	0.50
WBC ($x10^3/mm^3$)	8.56	8.59	0.55	0.55	0.83
Lymphocytes (%)	52.07	50.53	33.10	32.88	0.53
Neutrophils (%)	43.77	43.67	38.05	36.64	0.95
Eosinophils (%)	2.23	2.33	0.46	1.10	0.28
Monocytes (%)	1.33	1.43	0.37	0.39	0.52
Basophils (%)	1.83	1.80	0.73	0.72	0.86

The haematological profile of *Khaya senegalensis* crude extract before and after administration shows no significant difference in the haematological parameters with p values > 0.05 (Table 4.15).

The result in Table 4.16 shows N-hexane fraction of *Acacia nilotica* had antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 8 to 10 days of treatment.

Ethylacetate fraction of *Acacianilotica* had antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 6 to 8 days of treatment (Table 4.17).

The result in Table 4.18 shows that N-butane fraction of A*cacia nilotica* had antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 6 and 10 days of treatment.

N-hexane fraction of *Khaya senegalensis* shows antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 10 days of treatment (Table 4.19).

Ethylacetate fraction of *Khaya senegalensis* shows antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 10 and 12 days of treatment (Table 4.20).

Table 4.15: Effect of Administration of Stem Bark Crude Extract of *Khaya senegalensis*on Haematological Indices of Wistar Rats

.Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	43.43	45.2	31.70	25.55	0.90
WBC ($x10^3/mm^3$)	8.54	8.59	0.53	0.47	0.73
Lymphocytes (%)	52.30	50.53	27.00	30.12	0.21
Neutrophils (%)	42.83	43.47	32.76	25.91	0.56
Eosinophils (%)	2.27	2.30	0.41	0.42	0.86
Monocytes (%)	1.33	1.33	0.44	0.37	1.00
Basophils (%)	1.57	1.40	0.46	0.46	0.26

Table 4.16: Antitrypanosomal activity *Acacia nilotica* (N-hexane fraction) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AAH	Group BAH	Group CAH	Group DAH	Group EAH	Group FAH
0	0	0	0	0	0	0
2	13	2	0	0	0	2
4	54	26	38	57	0	40
6	49	19	11	17	0	45
8	14	6	0	0	0	42
10	0	0	0	0	0	40
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AAH-Infected Wistar rats but treated with 50mg/kg of N-hexane fraction of *Acacia nilotica*.

BAH- Infected Wistar rats but treated with 100mg/kg of N-hexane fraction of *Acacia nilotica*.

CAH- Infected Wistar rats but treated with 150mg/kg of N-hexane fraction of *Acacia nilotica*.

DAH- Infected Wistar rats but treated with 200mg/kg of N-hexane fraction of *Acacia nilotica*.

EAH-Wistar rats not infected but treated with 200mg/kg of N-hexane fraction of *Acacia nilotica*.

FAH- Infected Wistar rats but not treated.

Table 4.17: Antitrypanosomal activity of *Acacia nilotica* (Ethylacetate) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AAE	Group BAE	Group CAE	Group DAE	Group EAE	Group FAE
0	0	0	0	0	0	0
2	12	0	3	2	0	2
4	75	56	30	26	0	37
6	24	17	0	0	0	48
8	0	0	0	0	0	20
10	0	0	0	0	0	-
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AAE-Infected Wistar rats but treated with 50mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

BAE- Infected Wistar rats but treated with 100mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

CAE- Infected Wistar rats but treated with 150mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

DAE- Infected Wistar rats but treated with 200mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

EAE-Wistar rats not infected but treated with 200mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

FAE- Infected Wistar rats but not treated.

Table 4.18: Antitrypanosomal activity of *Acacia nilotica* (N-butane fraction) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AAB	Group BAB	Group CAB	Group DAB	Group EAB	Group FAB
0	0	0	0	0	0	0
2	16	2	11	18	0	3
4	58	40	38	22	0	54
6	30	16	0	0	0	66
8	13	0	0	0	0	67
10	0	0	0	0	0	26
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AAB-Infected Wistar rats but treated with 50mg/kg of N-butane fraction of *Acacia nilotica*.

BAB- Infected Wistar rats but treated with 100mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

CAB- Infected Wistar rats but treated with 150mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

DAB- Infected Wistar rats but treated with 200mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

EAB-Wistar rats not infected but treated with 200mg/kg of Ethyl acetate fraction of *Acacia nilotica*.

FAB- Infected Wistar rats but not treated.

Table 4.19: Antitrypanosomal activity t of *Khaya senegelensis* (N-hexane fraction) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AKH	Group BKH	Group CKH	Group DKH	Group EKH	Group FKH
0	0	0	0	0	0	0
2	4	0	2	0	0	0
4	55	43	60	39	0	51
6	34	27	42	28	0	50
8	20	16	20	9	0	42
10	9	3	8	0	0	34
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AKH-Infected Wistar rats but treated with 50mg/kg of N-hexane fraction of *Khaya senegalensis*.

BKH- Infected Wistar rats but treated with 100mg/kg of N-hexane fraction of *Khaya senegalensis*.

CKH- Infected Wistar rats but treated with 150mg/kg of N-hexane fraction of *Khaya senegalensis*.

DKH- Infected Wistar rats but treated with 200mg/kg of N-hexane fraction of *Khaya senegalensis*.

EKH-Wistar ratsnot infected but treated with 200mg/kg of N-hexane fraction of *Khaya senegalensis.*

FKH- Infected Wistar rats but not treated.

Table 4.20: Antitrypanosomal activity of *Khaya senegelensis* (Ethylacetate fraction) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AKE	Group BKE	Group CKE	Group DKE	Group EKE	Group FKE
0	0	0	0	0	0	0
2	5	3	0	0	0	0
4	64	43	44	66	0	45
6	43	37	36	40	0	39
8	17	12	10	12	0	34
10	8	2	2	0	0	25
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AKE-Infected Wistar rats but treated with 50mg/kg of ethyl acetate fraction of *Khaya senegalensis*.

BKE-Infected Wistar rats but treated with 100mg/kg of ethylacetate fraction of *Khaya senegalensis*.

CKE- Infected Wistar rats but treated with 150mg/kg of Ethylacetate fraction of *Khaya senegalensis*

DKE- Infected Wistar rats but treated with 200mg/kg of Ethylacetate fraction of *Khaya senegalensis*

EKE-Wistar rats not infected but treated with 200mg/kg of Ethyl acetate fraction of *Khaya senegalensis*.

FKE- Infected Wistar rats but not treated.

The result in Table 4.21shows N-butane fraction for *Khaya senegalensis* had antitrypanosomal activity at 50, 100,150 and 200 mg/kg body weight. Doses (50, 100,150 and 200 mg/kg body weight) were able to clear the parasites from circulation within 10 days of treatment.

The result in Table 4.22 shows the haematological profile of N-hexane fraction of Acacia *nilotica* before and after administration. There was no significant difference in the haematological parameters with p values > 0.05.

The haematological profile of Ethylacetate fraction of *Acacia nilotica* before and after administration shows no significant difference in the haematological parameters with p values > 0.05 (Table 4.23).

The haematological profile of N-butane fractionof *Acacia nilotica* before and after administration shows no significant difference in the haematological parameters with p values > 0.05 (Table 4.24).

Result in Table 4.25 shows the haematological profile of N-hexane fraction of *Khaya senegalensis* before and after administration. There was no significant difference in the haematological parameters with p values > 0.05.

Result in table 4.26 shows Haematological profile of Ethylacetate fraction of *Khaya senegalensis* before and after administration. There was no significant difference in the haematological parameters with p values > 0.05.

The haematological profile of N-butane fraction of *Khaya senegalensis* before and after administration. There was no significant difference in the haematological parameters with p values > 0.05 (Table 4.27).

Table 4.21: Antitrypanosomal activity of Khaya *senegelensis* (N-butane fraction) on Parasite Count in Wistar Rats infected with *Trypanosoma brucei brucei*

Post Treatment Days	Group AKB	Group BKB	Group CKB	Group DKB	Group EKB	Group FKB
0	0	0	0	0	0	0
2	1	0	0	0	0	3
4	46	36	52	48	0	55
6	32	32	33	29	0	56
8	27	13	18	7	0	45
10	2	2	5	0	0	33
12	0	0	0	0	0	-
14	0	0	0	0	0	-

KEY:

AKB-Infected Wistar rats but treated with 50mg/kg of N-butane fraction of *Khaya senegalensis*.

BKB- Infected Wistar rats but treated with 100mg/kg of N-butane fraction of *Khaya senegalensis*

CKB- Infected Wistar rats but treated with 150mg/kg of N-butane fraction of *Khaya senegalensis*

DKB- Infected Wistar rats but treated with 200mg/kg of N-butane fraction of *Khaya senegalensis*

EKB-Wistar rats not infected but treated with 200mg/kg of N-butane fraction of *Khaya senegalensis*

FKB- Infected Wistar rats but not treated.

Table 4.22: Effect of Administration of Stem Bark Extract of *Acacia nilotica* N-hexane fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	46.00	47.42	71.64	67.00	0.29
WBC ($x10^3/mm^3$)	8.33	8.40	1.11	0.76	0.62
Lymphocytes (%)	53.58	55.50	55.72	54.09	0.35
Neutrophils (%)	44.42	44.33	58.27	57.33	0. 90
Eosinophils (%)	1.50	1.48	1.18	0.63	0.80
Monocytes (%)	1.17	1.25	0.33	0.39	0.72
Basophils (%)	1.17	1.33	0.33	0.42	0.55

Table 4.23: Effect of Administration of Stem Bark Extract of *Acacia nilotica* Ethylacetate fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV	49.58	49.42	58.81	58.08	0.78
WBC ($x10^3/mm^3$)	8.07	8.15	1.28	1.27	0.62
Lymphocytes	53.58	55.08	57.17	58.08	0.22
Neutrophils	48.42	48.17	53.54	71.24	0.72
Eosinophils	1.50	1.50	0.82	0.82	1.00
Monocytes	1.25	1.17	0.39	0.33	0.72
Basophils	1.42	1.50	0.81	0.46	0.72

Table 4.24: Effect of Administration of Stem Bark Extract of *Acacia nilotica* N-butane fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	50.08	48.83	92.27	88.33	0.07
WBC ($x10^3/mm^3$)	8.43	8.34	1.51	1.48	0.21
Lymphocytes (%)	54.08	49.08	63.72	56.75	0.12
Neutrophils (%)	47.42	45.50	72.08	68.64	0.13
Eosinophils (%)	1.50	1.58	0.64	0.63	0.76
Monocytes (%)	1.17	1.00	0.33	0.37	0.44
Basophils (%)	1.42	1.33	0.63	0.69	0.72

Table 4.25: Effect of Administration of Stem Bark Extract of *Khaya senegalensis* N-hexane fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	49.92	48.42	60.08	54.36	0.26
WBC ($x10^3/mm^3$)	8.1	8.35	0.57	0.59	0.16
Lymphocytes (%)	50	49.08	77.64	75.90	0.21
Neutrophils (%)	49.92	46.58	62.45	69.54	0. 30
Eosinophils (%)	1.58	1.50	0.63	0.64	0.75
Monocytes (%)	1.17	1.08	0.33	0.45	0.72
Basophils (%)	1.42	1.25	0.63	0.57	0.50

Table 4.26: Effect of Administration of Stem Bark Extract of *Khaya senegalensis* Ethylacetate fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	48.92	47.67	76.81	68.42	0.13
WBC ($x10^3/mm^3$)	8.60	8.37	0.91	0.97	0.07
Lymphocytes (%)	55.00	52.33	63.09	57.33	0.12
Neutrophils (%)	50.08	48.00	38.45	37.45	0.14
Eosinophils (%)	1.48	1.50	0.63	0.64	0.72
Monocytes (%)	0.92	1.00	0.45	0.48	0.59
Basophils (%)	1.48	1.50	0.63	0.64	0.67

Table 4.27: Effect of Administration of Stem Bark Extract of *Khaya senegalensis* N-butane fraction on Haematological Indices of Wistar Rats

Haematological indices	Mean		Variance		P
	Before	After	Before	After	
PCV (%)	51.5	49.12	47.36	43.06	0.13
WBC ($x10^3/mm^3$)	8.46	8.48	0.96	1.23	0.26
Lymphocytes (%)	53.50	50.83	63.73	64.88	0.45
Neutrophils (%)	51.33	48.58	25.52	23.72	0.27
Eosinophils (%)	1.67	1.58	0.61	0.63	0.72
Monocytes (%)	0.92	1.08	0.45	0.47	0.44
Basophils (%)	1.67	1.67	0.61	0.61	1.00

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical Constituents of the Crude Extract and Fraction of the Stem Bark of *Acacia nilotica* and *Khaya senegalensis*

The phytochemical constituents of crude extracts and fractions from stem barks of *Acacia nilotica* and *Khaya senegalensis* in this study contains antitrypanosomal metabolites which include alkaloids, tannins, glycosides, cardiac glycosides, saponins, triterpene, carbohydrates and flavonoids. This is in agreement with the work of Hoet *et al*.(2004) and Hoet *et al.*(2007) where natural products with antitrypanosomal activities included alkaloids, tannins, flavonoids, anthraquinones, triterpenes, sterol and terpenes etc. this is also in consistent with the findings of Abdullahi and Emmanuel, (2012) andOkoro *et al.*(2014) who reported thattannins, glycosides, saponins, terpenes, sterols, carbohydrates, flavonoids and anthraquinones were present in the stem bark of *Acacia nilotica*.

Sawant *et al.* (2014), reported the presence of flavonoids , steroids, cardiac glycosides in Ethylacetate fraction of *Acacia nilotica* bark and alkaloids and resins N-hexane fraction;

Gbile (1986), reported the presence of Alkaloids, tannins, glycosides, cardiac glycosides, saponins, triterpene, carbohydrates and flavonoids in the stem bark of *Khaya senegalensis* which isin agreement with the result of this study. Also in the work of Kubmarawa *et al*. (2008), ethanol extract and water extract of *Khaya senegalensis* stem bark revealed the presence of saponins and tannins.

5.2 The LD50 of the Crude Extract and Fraction of the Stem Bark of *Acacia nilotica* and *Khaya senegalensis* against Adult Wistar rats.

The crude extract and fractions (N-hexane, ethylacetate and N-butane) of the stem bark of *Acacia nilotica*did not result in any obvious signs of toxicity. Ogbadoyi *et al.* (2011) reported that the LD_{50} of themethanol extract of stem bark of *Acacia nilotica*of the partially purified extract was found to be 2000mg/kg body weight which is higher compared to the result in this study.

The crude extract and fractions (N-hexane, ethylacetate and N-butane) of the stem bark of *Khaya senegalensis*did not result in any obvious signs of toxicity. Onu *et al.* (2013), found the LD_{50} of aqueous stem bark extract of *Khaya senegalensis* to be greater than 5000 mg/kg body weight.

5.3 The Antitrypanosomal activity of the Crude Extract and Fraction of the Stem Bark of *Acacia nilotica* and *Khaya senegalensis* against *Trypanosoma brucei brucei infected* Wistar rats

This study has provided evidence that the crude extracts and fractions of the stem bark of *Acacia nilotica* and *Khaya senegalensis* exhibits trypanocidal effect at various doses which is often associated with reduction in experimental African trypanosomiasis.

All infected rat became parasitaemic at day 4 post infection and the clinical signs observed were respiratory distress, raised hair coat, anorexia, pale ocular mucous membrane and weight loss. The increase in parasitaemia count may be due to antigenic variation that allows African trypanosomes to develop chronic infections which result from the alternative occurrence of transcriptional switching and DNA recombinations of variant-specific surface glycoprotein (VSG) (Larry and John, 2010 and David, 2014).

The prepatent period of 4 days observed in this study is in consistent with the findings of Umar *et al*(2007) in rats infected with *Trypanosoma brucei brucei*.

The crude extract of the stem bark of *Acacia nilotica* at various doses (100, 200, 300 and 400 mg/kg) was able to clear the parasites from the circulation; with 400mg/kg clearing the parasites at day 8 of post infection. The clearance of parasitaemia and survival of the Wistar rats may be linked to the ability of the extract to inhibit glycolysis which is the major source of energy for *Trypanosoma brucei brucei* which is in consistent with other findings (Umar *et al* 2000 and Ogbadoyi *et al.*(2011).

The crude extract of the stem bark of Khaya senegalensis at various doses (100, 200, 300 and 400 mg/kg) was able to clear the parasites from the circulation; with 300 mg/kg and 400mg/kg clearing the parasites from circulation at day 10 of post infection.

The clearance of parasitaemia and survival of the Wistar rats may also be linked to the ability of the extract to inhibit glycolysis which is the major source of energy for *Trypanosoma brucei brucei* which is in consistent with work of Umar *et al.,* (2010) that reported *Khaya senegalensis* topossess antitrypanosomal activitywhen use in treatment of trypanosome infected rats. It is also in consistent with the work of Abubakar *et al.,* (2005) that reported *Khaya senegalensis* reduces the parasite load and neutralizes the toxic metabolites produced by trypanosomes.

Khaya senegalensis bark extract was reported to have antitrypanosomal activity when a higher quantity was combined with the stem bark of *Anogeissus leiocarpus* (Awobode *et al.,* 2015).

The fractions of the stem bark of *Acacia nilotica and Khaya senegalensis* were more efficacious than the crude extract.

The fractions of the stem bark of *Acacia nilotica* at various doses (50, 100, 150 and 200 mg/kg) was able to clear the parasites from the circulation; with ethylacetate and N butane at doses of 150 mg/kg and 200mg/kg clearing the parasites at day 6 of post infection while the fractions of the stem bark of *Khaya senegalensis* at various doses (50, 100, 150 and 200 mg/kg) was able to clear the parasites from the circulation; with N hexane, ethylacetate and N butane at doses 200mg/kg clearing the parasites at day 10 of post infection. The clearance of parasitaemia and survival of the Wistar rats may be linked to the ability of the extract to inhibit glycolysis which is the major source of energy for *Trypanosoma brucei brucei* which is in consistent with work of Akanji *et.,al* (2009) and Hoet *et.,al* (2004) that reported many natural products exhibit their trypanocidal activity by acting on the cellular defenses against oxidative stress through interference with the redox balance of the trypanosomes. Natural products possess structures capable of releasing radicals that may cause peroxidative damage to enzymes that are sensitive to alterations in redox balance.

Death in groups not treated might be due to massive parasitaemia which is consistent with the findings of Umar *et al*(2000) due to hemolytic anaemia caused by *Trypanosoma brucei brucei infections.*

5.4 The Haematological profile for the *Trypanosoma brucei brucei infected*Wistar before and after crude extract and fraction of stem bark of *Acacia nilotica* and *Khaya senegalensis* administration

In this study, there was no significant change in the packed cell volume, white blood cells and differential counts in the crude extract of *Acacia nilotica* and *Khaya senegalensis* on haematological indices in *Trypanosoma brucei brucei* infected Wistar rats in all the groups before and after commencement of treatment which may be due to

some degree of maintenance of blood value parameters by the extracts since trypanosomiasis is associated with a rapid decline in PCV, haemoglobin concentration etc.Similarly in a study by Tanko *et al* (2015), the crude and aqueous fraction of *Acacia nilotica* were found tohave no significant changes on haematological parameters of Wistar rats.

There was also no significant change in the packed cell volume, white blood cells and differential counts in the fractions (N-hexane, ethyl acetate and N-butane) of *Acacia nilotica* and *Khaya senegalensis* on haematological indices in *Trypanosoma brucei brucei* infected Wistar rats in all the groups before and after commencement of treatment. This is in agreement with the work of Onu *et al*(2015) that reported the aqueous stem bark extract of *Khaya senegalensis* at different experimental doses to have no significant ($p > 0.05$) effect on the haematological indices.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The phytochemical constituents of the stem barks of *Acacia nilotica* and *Khaya senegalensis* crude extracts and fractions (N-hexane, ethyl acetate and N-butane) had antitrypanosomal activity. The metabolites include alkaloids, tannins, glycosides, cardiac glycosides, saponins, triterpene, carbohydrates and flavonoids.

The toxicity of the crude extracts of the stem bark of *Acacia nilotica* was found to be 707.1 mg/kg while that of the fractions (N-hexane, ethylacetate and N-butane) was found to be 547.7 mg/kg, 387.3 mg/kg and 707.1 kg/mg respectively.

The toxicity of the crude extracts of the stem bark of *Khaya senegalensis* was found to be 547.7 mg/kg while that of the fractions (N-hexane, ethylacetate and N-butane) was found to be 387.3 mg/kg, 547.7 mg/kg and 223.6 mg/kg respectively.

The administration of the crude extracts of stem barks of *Acacia nilotica* and *Khaya senegalensis* had antitrypanosomal activity at all doses (100, 200, 300 and 400 mg/kg) and the parasites were cleared from the circulation within 8 to 10 days of treatment.

The administration of the fractions (N-hexane, ethyl acetate and N-butane)of stem barks of *Acacia nilotica* and *Khaya senegalensis* had antitrypanosomal activity at all doses (50, 100, 150 and 200mg/kg) and the parasites were cleared from the circulation within 6 to 10 days of treatment.

There was no statistical significant change in the haematological profile before and after treatment with all doses of the crude extracts and fractions (N-hexane, ethylacetate and N-butane) of *Acacia nilotica* in *Trypanosoma brucei brucei* infected Wistar rats.

There was no statistical significant change in the haematological profile before and after treatment with all doses of the crude extracts and fractions (N-hexane, ethylacetate and N-butane) of *Khaya senegalensis*in*Trypanosoma brucei brucei* infected Wistar rats.

6.2 Recommendations

1. There is need for further extensive work on these plants using different *Trypanosoma* species, different administration and possible combination in order to exploit their full potential in the management of African trypanosomiasis.
2. These plants should be sourced from different geographical locations. Because of the existence of different variants, soils and climatic conditions which affects the phytochemical constituents and hence therapeutic actions.
3. There is need to improve on the extraction of the extractsas it increases the efficacy which will further allow its standardization and packaging.

6.3 Contribution to Knowledge

1. The LD_{50} for the crude extract of *Acacia nilotica* was found to be 707.1mg/Kg. The fractions (N-hexane, ethyl acetate and N-butane) were found to be 547.7mg/Kg, 387.3mg/Kg and 707.1mg/Kg respectively. The crude extract of *K. senegalensis* was found to be 547.72mg/Kg. The fractions (N-hexane ethylacatate and N-butane) were found to be 387.3mg/Kg, 547.1mg/Kg and 223.6mg/Kg respectively.

2. Ethylacetate and N-butane fractions of *Acacia nilotica* had antitrypansomal activity at 150mg/Kg and 200mg/Kg respectively.

3. Ethylacetate fraction of *Khaya senegalensis* had antitrypansomal activity 200mg/Kg.

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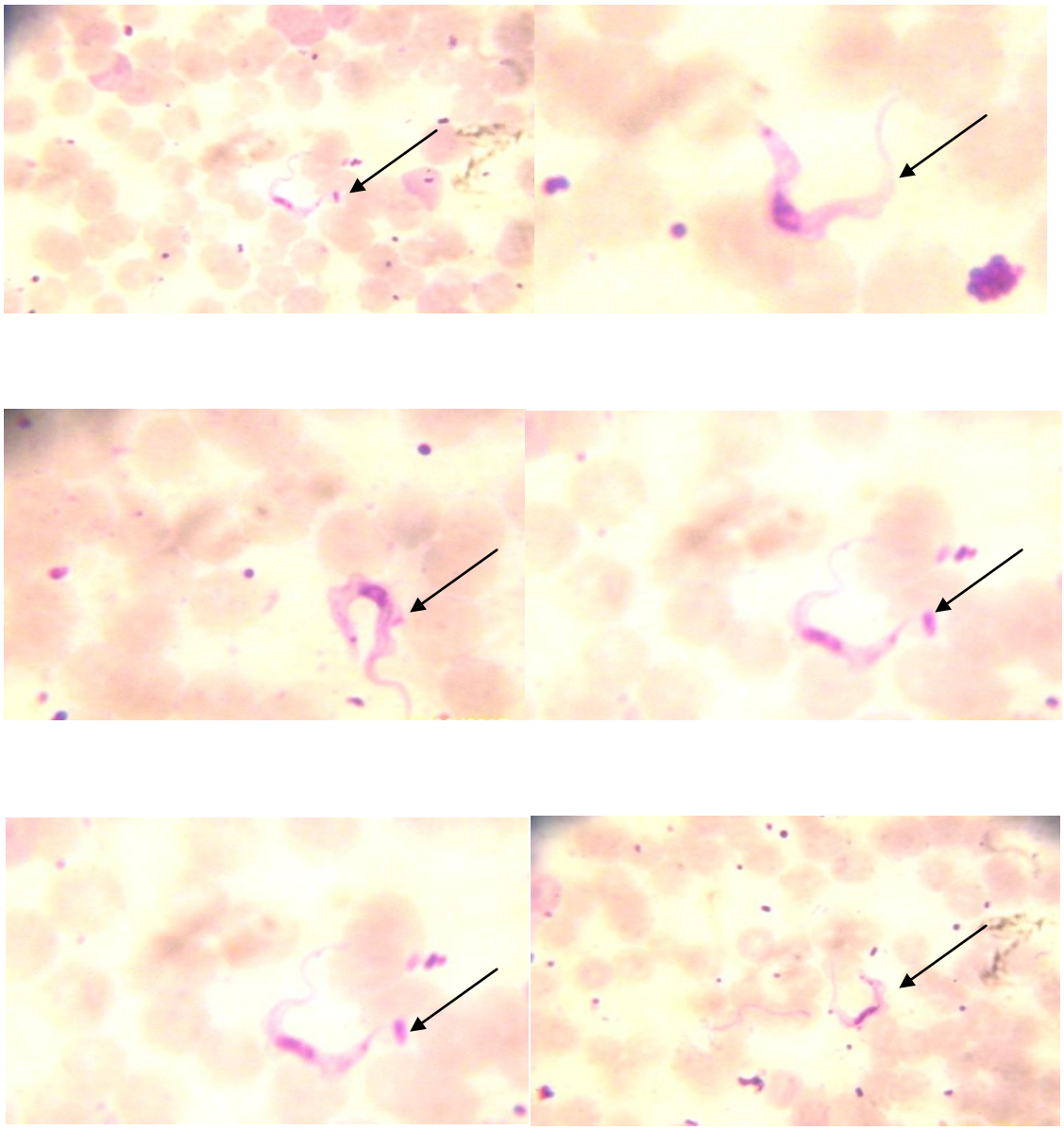
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APPENDICES

Appendix I. Photograph of *Trypanosoma brucei brucei* in blood film of Wistar Rats



Appendix II: Baseline for Haematology and Chemistry values for Charles River Wistar Rats

MALE Crl:(WI)BR Rats

	6-8 Weeks			19-21 Weeks			32-34 Weeks		
Test	$\bar{X}$	*Range ± 2S.D.*	*N*	$\bar{X}$	*Range ± 2S.D.*	*N*	$\bar{X}$	*Range ± 2S.D.*	*N*
HEMATOLOGY									
Hematocrit (%)	36	(32-40)	168	41	(36-46)	29	42.0	(39-48)	15
Hemoglobin (gm/dl)	13.5	(12.2-14.8)	170	16.0	(14.4-17.6)	28	15.5	(14.7-18.0)	15
RBCS (x10⁶/mm³)	6.46	(5.79-7.14)	166	8.31	(7.37-9.25)	29	8.4	(7.74-9.72)	15
WBCS (x10³/mm³)	8.66	(5.10-12.16)	162	9.37	(6.19-12.55)	30	7.8	(5.29-10.80)	15
DIFFERENTIAL (%)									
Segmented Neutrophils	12	(2-22)	167	15	(1-29)	26	18	(12-34)	15
Band Neutrophils	0	(0-2)	170	0	(0-2)	30	0	(0-0)	15
Lymphocytes	85	(76-98)	166	82	(70-99)	27	80	(65-86)	15
Monocytes	2	(0-6)	170	2	(0-6)	30	0	(0-0)	15
Eosinophils	1	(0-3)	170	1	(0-3)	30	2	(0-3)	15
Basophils	0	(0-2)	170	0	(0-2)	30	0	(0-0)	15
Atypicals	0	(0-2)	170	0	(0-2)	30	0	(0-0)	15
Nucleated RBCS (per 100 WBCS)	0	(0-2)	170	0	(0-2)	30	0	(0-1)	15
Platelets (x10⁵/mm³) (Estimated)	7.00	—	—	7.00	—	—	7.00		15
ERYTHROCYTE INDICES									
MCV (μ³)	56	(53-59)	167	50	(47-52)	30	50.1	(48-53)	15
MCHC (%)	37	(33-41)	169	39	(35-43)	29	37.0	(34-38)	15
MCH (μμgm)	20	(18-22)	170	19	(17-21)	30	18.5	(17-20)	15
CLINICAL CHEMISTRY									
Glucose (mg/dl)	130	(108-153)	170	115	(100-130)	28	118.1	(77-141)	15
Bun (mg/dl)	19	(15-23)	170	19	(16-23)	30	21.6	(18-25)	15
ALK PHOS. (IU/L)	238	(149-328)	166	137	(56-216)	29	103.1	(56-145)	15
Sgpt (IU/L)	24	(4-44)	166	31	(20-42)	30	38.7	(20-81)	15
Sgot (IU/L)	66	(37-94)	169	62	(39-84)	29	70.4	(41-115)	15
Sodium (mEq/L)	143	(140-146)	169	145	(141-149)	30	147.4	(145-150)	15
Potassium (mEq/L)	6.9	(6.1-7.7)	164	6.5	(5.2-7.8)	29	6.2	(5.7-7.1)	15
Chloride (mEq/L)	102	(99-106)	169	105	(102-109)	29	109.9	(105-126)	15
Total Protein (gm/dl)	6.0	(5.4-6.6)	168	7.2	(6.5-7.8)	29	6.9	(6.3-7.7)	15
Albumin (gm/dl)	3.7	(3.0-4.4)	168	4.0	(3.3-4.7)	29	3.9	(3.5-4.2)	15
Globulin (gm/dl)	2.3	(1.7-2.8)	170	3.2	(2.5-3.8)	29	3.0	(2.6-3.5)	15
A/G Ratio	1.6	(1.0-2.2)	169	1.3	(0.8-1.7)	29	1.3	(1.0-1.6)	15

N = Number of Animals Represented
$\bar{X}$ = Mean

FEMALE Crl:(WI)BR Rats

	6–8 Weeks			19–21 Weeks			32–34 Weeks		
Test	$\bar{X}$	*Range ± 2S.D.*	*N*	$\bar{X}$	*Range ± 2S.D.*	*N*	$\bar{X}$	*Range ± 2S.D.*	*N*
HEMATOLOGY									
Hematocrit (%)	38	(34–42)	167	40	(35–44)	28	40.7	(35–44)	15
Hemoglobin (gm/dl)	14.1	(12.9–15.3)	170	15.6	(14.1–17.1)	27	16.3	(15.2–19.3)	15
RBCS ($x10^6/mm^3$)	6.92	(6.20–7.64)	167	7.81	(6.86–8.75)	29	7.8	(6.92–8.78)	15
WBCS ($x10^3/mm^3$)	6.96	(4.19–9.73)	161	8.43	(4.77–12.08)	28	6	(4.60–13)	15
DIFFERENTIAL (%)									
Segmented Neutrophils	13	(1–25)	170	17	(1–32)	28	23	(10–40)	15
Band Neutrophils	0	(0–2)	170	0	(0–2)	29	0.07	(0–1)	15
Lymphocytes	84	(74–98)	168	80	(67–98)	29	75	(58–90)	15
Monocytes	2	(0–6)	170	2	(0–6)	29	0.07	(0–1)	15
Eosinophils	1	(0–3)	170	1	(0–3)	29	2	(0–6)	15
Basophils	0	(0–2)	170	0	(0–2)	29	0.00	(0–0)	15
Atypicals	0	(0–2)	170	0	(0–2)	29	0.00	(0–0)	15
Nucleated RBCS (per 100 WBCS)	0	(0–2)	170	0	(0–2)	29	0.00	(0–0)	15
Platelets ($x10^5/mm^3$) (Estimated)	7.00	—	—	7.00	—	—	7.00	—	—
ERYTHROCYTE INDICES									
MCV (μ^3)	55	(52–59)	168	53	(49–56)	28	52.40	(50–57)	15
MCHC (%)	36	(34–39)	169	38	(34–42)	27	40.27	(36–45)	15
MCH ($\mu\mu$gm)	20	(19–21)	170	20	(18–22)	28	21.00	(18–23)	15
CLINICAL CHEMISTRY									
Glucose (mg/dl)	131	(113–149)	170	109	(85–132)	29	117.1	(91–140)	15
Bun (mg/dl)	21	(16–27)	170	22	(17–26)	29	20.2	(18–24)	15
ALK PHOS. (IU/L)	172	(93–251)	170	108	(39–177)	28	56.9	(36–111)	15
Sgpt (IU/L)	22	(8–37)	169	33	(17–50)	29	53.6	(20–268)	15
Sgot (IU/L)	67	(47–88)	170	66	(40–92)	28	141.8	(43–1120)	15
Sodium (mEq/L)	143	(140–147)	170	145	(139–150)	28	146.4	(145–149)	15
Potassium (mEq/L)	6.5	(5.8–7.3)	165	6.0	(4.8–7.2)	29	5.6	(4.9–6.6)	15
Chloride (mEq/L)	104	(99–108)	169	106	(99–114)	29	107.1	(105–113)	15
Total Protein (gm/dl)	6.4	(6.0–6.9)	170	7.5	(6.3–8.6)	29	7.6	(6.8–8.4)	15
Albumin (gm/dl)	4.1	(3.6–4.6)	169	4.3	(3.7–4.9)	29	4.4	(4.1–4.7)	15
Globulin (gm/dl)	2.4	(1.9–2.9)	170	3.1	(2.4–3.9)	29	3.3	(2.5–4.1)	15
A/G Ratio	1.7	(1.2–2.2)	170	1.4	(1.1–1.7)	29	1.4	(1.0–1.7)	15