ANTITRYPANOSOMAL AND BIOCHEMICAL ACTIVITIES OF *CAESALPINIA BONDUC, ANTHOCLEISTA NOBILIS* AND *KIGELIA AFRICANA* COMBINED CRUDE EXTRACTS AND FRACTIONS IN RATS

BY

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MTECH/SLS/2018/8406

THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY

SEPTEMBER, 2023

DECLARATION

I hereby declare that this thesis titled: **"Antitrypanosomal and Biochemical Activities of C*aesalpinia bonduc, Anthocleista nobilis* and *Kigelia africana* Combined Crude Extracts and Fractions in Rats"** is a collection of my original research work and has not been submitted or presented in part or full for any other diploma or degree in this or any other university. Information from other sources (published or unpublished) have been duly acknowledged.

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CERTIFICATION

This thesis titled: **"Antitrypanosomal and Biochemical Activities of C*aesalpinia bonduc, Anthocleista nobilis* and *Kigelia africana* Combined Crude Extracts and Fractions in Rats"** by ADESUYI Temitope Ojo (MTech/SLS/2018/2021) meets the regulations governing the award of the degree of MTech of the Federal University, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Herbal medicine is an alternative approach to reduce the downsides associated with conventional medicine and major limitations that beset current drugs against trypanosomiasis. This study was carried out to screen for antitrypanosomal activities of combined crude extract and fractions of the leaves of *Caesalpinia bonduc, Anthocleista nobilis* root back and *Kigelia africana* stem back. These plant samples were first extracted in aqueous medium. Then the extracts were fractionated in hexane, ethyl acetate and methanol in order of polarity. The phytochemical components of the crude extracts and fractions were analysed, and thereafter, the extracts and fractions were combined in ratio 1:1:1. The experimental animals were grouped into A-G of five rats each. All the animals were inoculated with *Trypanosoma, brucei brucei* except those in group G by intraparietal. With the establishment of infection, animals in Group A were treated with crude extracts, group B were treated with partition fractions of n-hexane, group C were treated with ethyl acetate, group D were treated with methanol and group E were treated with berenil standard, while group F served as negative control, group G were uninfected untreated. All treatment were by oral administration at 200mg/kg body weight for 10 days consecutively. Parasitemia and body-weight were monitored at 2 days intervals while packed cell volume (PCV) and biochemical parameters such as glucose level, albumin, total protein and globulin as well as the percentage organ body-weight were taken after day six post treatment. The result showed a significant decrease in parasitemia ($p<0.05$) for all treated groups as compared to the uninfected untreated control. There was a significant increase in PCV and biochemical parameters analyzed as well as the percentage organ body-weight ratio. The result of the organ body weight ratio showed non enlargement of organs such as lungs, heart, kidney, liver and spleen of treated groups. There was also a total clearance of parasitaemia in all the extract treated groups with group A clearing fastest after day 6 post treatment. All the phytochemicals analyzed were present in appreciable amount (20.6 % saponin, 11.9 % flavonoid, 26.9 % alkaloid, 0.031 % phenol and 6.46 % tannins) except in few combinations where glycosides were not detected, while alkaloid had the highest in the crude extracts. Consequently, the combination of the plant various parts has proved to be anti-trypanosoma as used in traditional medicine and the abundant presence of these phytochemicals could be responsible for the observed activities.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Natural products are biochemical found in plants and animals. They are used in medicine to treat different kinds of diseases. These biochemical are phytochemicals effective to mediate several biological activities. The discovery of these phytochemicals has enhanced the production of several drugs (Hong, 2011). In the past, the multidimensional structures of the phytochemicals have been linked to their functions in biological system. The phytochemicals have been able to exhibit their pharmacological activities on target cells, therefore, alleviating the diseases. Artemisinin was obtained from plant phytochemical which is in current use as anti-malaria (Muschietti *et al.,* 2013; Cragg and Newman, 2013).

Caesalpinia bonduc is plant found in several regions of Africa. It is from the family *Caesalpiniaceae* and genus *Fabaceae.* Its common name in English Bondoc nut. This plant has several important phytochemicals that enables its usefulness in herbal medicine. These phytochemicals are found in several parts of the plant effective in treatment of disease (Valiathan, 1998). The therapeutic activities showed success against diabetes, bacteria and viral infections (Singh and Paghav, 2012).

Anthocleista nobilis (A. nobilis) is plant belonging to the family *Gentianaceae* (formerly in *Lonaniaceae*). It is fondly called saposapo in Yoruba and kwani in Hausa. It is commonly known as cabbage palm. This plant is naturally found in forest damp site. *Anthocleista nobilis* (*A. nobilis*) has several bioactive phytochemicals on various parts that is medicinal. The phytochemicals are effective against wide range of diseases both in man and animals (Burkill, 2000 and Iwu, 2014).

Kigelia africana (synonyms *Kigelia pinnata*) is a plant found in several places in Africa. Its family name is *Bignoniaceae* popularly known as cucumber tree. Its natural habitat wet area. This plant possesses several phytochemicals in its various parts. The phytochemicals are effective against a wide range of infections inside and outside the body (Gill, 1992).

1.2 Statement of the Research Problem

World Health Organization (2015) identified trypanosome infection has of the major cause of mortality and morbidity. Although, several drugs have been discovered against this infection but there existed some side effects such as drug accessibility, parasite resistant strain and other notable limitations to the drugs which include drug metabolism and absorption.

This infection is caused by trypanosome and transmitted via tsetse-fly. The increased proliferation and differentiation in host circulatory system within a short time has claimed millions of lives across Africa and beyond (WHO 2016a; WHO 2016b). The complexity of structure and the released toxins in host are capable of damaging vital organs in host, thereby causing death.

Every drug in use has a primary function to eliminate the parasite strain and neutralize its toxins. Therefore, many of these available drugs have setbacks which limit effectiveness against the parasite and toxins. Isolated phytochemicals in some medicinal plants have great potentials to

alleviate these limitations and successfully eliminate the pathogens in host. Traditional herbalist use to combine these plants for antitrypanosomal without scientific evidence. Hence, a comprehensive study and scientific evidence of the mechanism of action by the phytochemicals are imperative.

1.3 Justification for the Study

Caesalpinia bonduc, Anthocleista nobilis and *Kigelia africana* combined extracts have a promising approach to alleviate Trypanosoma infection and the limitations of drugs. In traditional medicine, the combination was effective against the parasite without relapse. The observation become limited as no scientific evidence to show the pharmacological activities of used plants. Therefore, this study becomes important as it focuses on removing some of these challenges by screening for antitrypanosomal and biochemical activities of combined crude extracts and fractions in rats.

1.4 Aim and Objectives of the Study

This study aimed at determining the antitrypanosomal and biochemical activities of *Caesalpinia bonduc, Anthocleista nobilis* and *Kigelia africana* combined crude extracts and fractions in rats.

The objectives are to:

i. extract the three plant materials using aqueous solvent and partition with solvents of different polarities.
ii. determine qualitative and quantitative phytochemical contents of the crude extracts and fractions.
iii. screen for antitrypanosoma activity of combined crude extracts and fractions.
iv. determine some biochemical parameters and packed cell volume of treated animals.
v. determine the effect of treatment on percentage organ body-weight ratio.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Trypanosomiasis (sleeping sickness)

Trypanosomiasis is a sleeping sickness caused by protozoan, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense.* This parasite has two identical subspecies which inhabits fluid of blood, lymph, and interstitial fluids of man hosts. *Trypanosoma brucei* has digenetic life cycle that is develop in vertebrate and invertebrate hosts. *Trypanosoma brucei* are unicellular eukaryotes with a single flagellum and a visible DNA. The parasite gets transmitted via the anterior part (proboscis) of tsetse fly via biting of host.

2.2 Trypanosome Classifications

Trypanosomes have four subgenera; *Duttonella (D), Nannomonas (N), Trypanozoon (T) and Pycnomonas (P).* The subgenera have different genome, an important factor responsible for the diverse structure and function. Some subgenera can adapt, proliferate and infect human while some are animal pathogens (Kreier and Baker, 1987). All trypanosome is transmitted by tsetse flies.

2.3 Trypanosome Life Cycle

Trypanosoma brucei is a unicellular parasite with is a spindle-shaped structure. It has flagellum at the posterior end that extends beyond the anterior end of the cell. *Trypanosoma brucei*

undergoes asexual reproduction by binary fission. The life cycle of *Trypanosoma brucei* is shown in Figure 2.1. Its mode of transmission into host is through tsetse fly. A bite by tsetse fly injects the parasite into host blood stream. This injection enhances proliferation and multiplication of trypanosomes locally at the site within few days. Hence, higher number of the parasites enter the circulatory system, and into other tissues and organs.

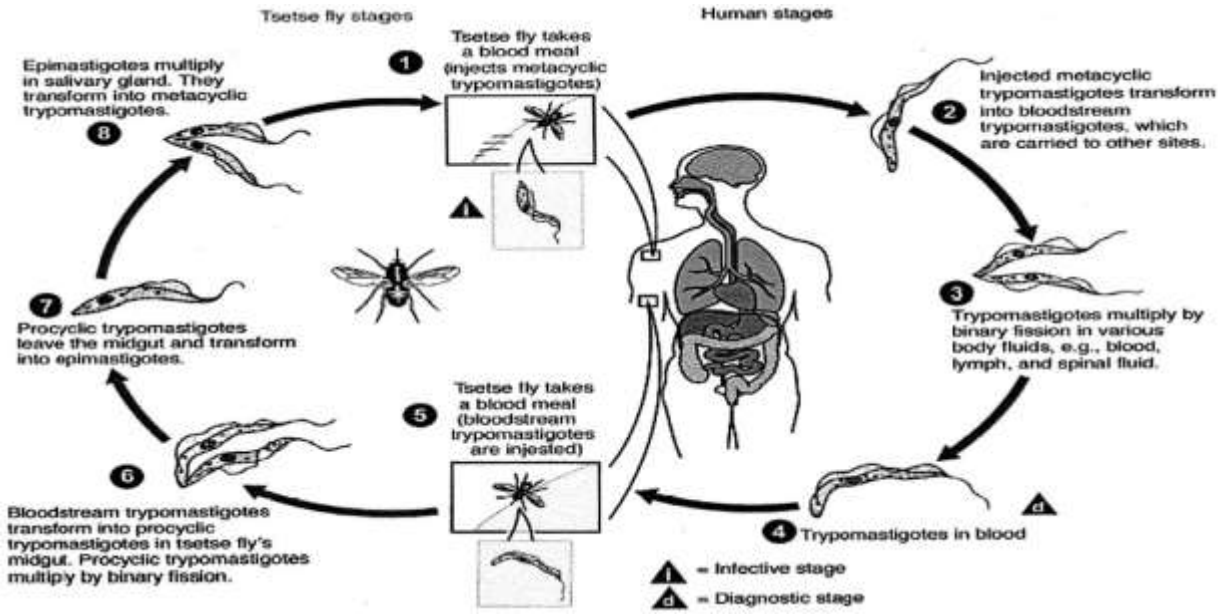

Figure 2.1: Diagrammatic Representation of Trypanosome Life Cycle in Man.

The parasite cell possessed a special protein (glycoprotein) that prevent recognition and destruction by host cells (Borst and Fairlamb, 1998). This special protein can only be identified and eliminated by host specific antibodies. This unique surface glycoproteins were encoded by genes in parasite genome (Borst, 2002; Vanhamme *et al.*, 2001). A small amount of the parasite can evade humoral immune system, proliferate and infect host. Only a new specific antibody produced by host can target and destroy them.

2.4 Trypanosome Evading Mechanism of Immune System

Trypanosome can hide from the immune system activity throughout its life span. The parasite has genes that encoded a unique protein called Variable Surface Glycoprotein (VSG). The protein is produced by a systemic DNA rearrangement. The parasite antigen surface change is facilitated by polyclonal activation of B and T lymphocytes (Vincendeau and Bouteille, 2006). VSG can prevent access to specific and unspecific immune systems and ensure safety of the parasites in host (Field and Carrington, 2009).

2.4.1 Innate immune system evasion

Innate immune system is the unspecific defense response against trypanosomes. This parasite can be eliminated by a special cell called trypanosome lytic factor (TLF) (Raper *et al.*, 2001), also recognized as apolipoprotein L1 (APOL1) (Locordier *et al.*, 2014). Parasites can secrete proteins that can help avoid APOL1 (Pays *et al.*, 2014). These proteins form a complex that inhibits APOL1 activity. Thus, the parasite remains in host blood stream. If the protein complex is removed, the parasite is easily destroyed by host immune cells (Capewell *et al.*, 2013).

2.4.2 Adaptive immune system evasion response

(1) Antigenic variation

Parasite has the ability to produce special protein (VSG) and form protein complex in host. These attributes prolong its life span in host circulatory system. The parasite does utilize such advantage to attack host humoral immune system (Morrison *et al.*, 2009). The special surface protein and protein complex regularly produced by parasite can be cleared by host specific antibodies. The host specific antibodies production can be initiated by the layer of VSG. This leads to parasite ingestion and elimination by host cells.

(2) Humoral response

At early stage of parasite infection, there is a decrease humoral response of B cells which cause its cell activation, loss and apoptosis (Bockstal *et al.*, 2011, Radwanska *et al.*, 2008).

(3) Antibody clearance

Parasite can rearrange its surface protein to form a pocket, a structure that prevent lysis by host. The host cell via endocytosis can eliminate the surface protein and complex formed from host (Pal *et al.*, 2003).

(4) Immunosuppression

Parasite infection does stimulate new antibody secretion which could cause immunosuppression of immune system. This is a pathological disorder attributed to trypanosome. This occurrence can be suppressed by macrophages and T cells (Tabel *et al.*, 2008). Parasite does inhibit antigen MHC class II activity and T cell activation (Namangala *et al.*, 2000). Macrophage and T cell activities can suppress parasite antigen expression (Ding *et al.*, 1993).

2.5 Trypanosomes Energy Metabolism

Host infected bloodstream does undergo rapid glycolysis and enzymes concerned are found in a glycosome. In aerobic condition, some intermediate is produced and the enzyme involved shuttle from host glycosome to parasite mitochondrion. This product re-enters the glycosome and undergoes further metabolism. In anaerobic condition, some intermediate can be converted to another for use. In the glycolytic pathway, nine enzymes are involved in the conversion of glucose into phosphoglycerate.

Amino acid required by trypanosome parasites is mainly obtained from host (Gutteridge and Coombs, 1977). Proline and Threonine are required by parasite for respiration and fatty acid elongation. Ornithine is a main component of polyamine, and important chemical for parasite multiplication in host (Pegg and McCann, 1988).

2.6 Clinical Symptoms of Trypanosome

The molecular biology of trypanosomes showed its diversity. Some species affects human, while others affect animal with severe symptoms (Vanhamme *et al.*, 2001). The *T. b. gambiense* causes chronic infection with about 4 years life-span and *T. b. rhodesiense* causes acute infection with about 9 months in host before mortality.

Trypanosome infection phase in host can be distinctly separated into three phases (Vanhamme *et al.*, 2001).

(i) INFECTION PHASE 1

This infection phase occurs after a bite by tsetse flies. A deep black spot developed as trypanosomes proliferate within a short time at this site. Then, inflammation of the subdermal infected tissue emerged with a tender, erythema, and heat.

(ii) INFECTION PHASE 2

The proliferated parasite circulates round through blood and lymphatic vessels, and further multiply in the respective cells. The symptoms can be vastly related to parasitaemia waves. The symptoms include joint pain, fever and headache. The depth of symptoms varies with the nature of infection, chronic or acute.

(iii) INFECTION PHASE 3

The untreated infection can cause brain disorders accompany by aggressiveness and sleepiness. This infection can cause organ damage like heart, nervous system, liver and spleen.

2.7 Biological Parameters Affected by Trypanosome

High or low packed cell volume of host infected blood can reflect chronic or acute inflammation. It is essential to evaluate packed cell volume and body organ-weight of infected animals through blood, lymph and other fluids.

(i) Antibody Detection:

Infected host antibody can be detected through some specific antibody in the blood plasma and intestinal fluid. The parasites have a complex antigenic structure in the host that stimulate the proliferation of a large variant of antibodies. IgG and IgM antibodies concentrations are high and directed against parasite surface protein antigens. The presence of these antigens complex can help determine their specificity and sensitivity (Vanhamme *et al.*, 2001). A positive analysis and interpretation of test result will require previous information on patient antibodies as infection can relapsed after cure (Paquet *et al.*, 1992).

(ii) Trypanosome Detection:

Infected blood and CSF via microscopic examination can help in diagnosis of parasite. This provides direct evidence and allows a definite diagnosis. A daily examination of the blood over a period of time can improve test sensitivity (Herbert and Lumsden, 1976). Diagnostic method that enhances the use of large blood facilitate parasite visualization and improve test sensitivity. Adequate monitoring of the time between sample collection and examination is important to avoid parasite immobilization and lysis. Ultra-violet rays can rapidly kill parasite in few hours of exposure.

(iii) Blood film examination

A wet and thick blood film require 5 to 10µl of infected blood placed on a slide and examined via microscope at ×40 magnification (Herbert and Lumsdey, 1976). This enables to visualize a moving parasite between the erythrocytes. Although, the sensitivity of this method might be low, but a high detection limit of about 10,000 count per ml can be seen. This method become more relevant because it is cheap and simple. The disadvantages include time wasting and required expertise to recognize the parasite.

2.8 Chemotherapy Treatment of Trypanosome

The use of drug has been successful in the treatment of trypanosome parasites. However, early detection and treatment is very important. The use of drug to induce mortality and morbidity of parasite is more successful at early stage than late stage. Therefore, early detection and treatment enhance the control of the symptoms.

One of effective drug in use against the parasite is Suramin, usually administered to early-stage patients. This drug does not cross the blood brain barrier. Suramin, a poly-sulfonated naphthylamine derived from trypan red is antitrypanosomal. Its toxicity can cause nephrotoxicity, optic atrophy, blindness, and inadequate adrenal hormone (Pepin and Milord, 1994). The mode of action involves inhibition of several enzymes of the parasite such as glycolytic enzymes and mitochondrial glycerol phosphate oxidase.

Pentamidine isethionate and some aromatic diamidines were previously discovered to be antitrypanosomal. The mode of action of pentamidine isethionate and suramin are similar. Pentamidine isethionate can clear parasite in host blood before the use of other complementary drug melarsoprol. Pentamidine isethionate toxicity includes nephrotoxicity and hepatotoxicity (Sands *et al*., 1985; WHO 1986; Pepin and Milord, 1994). Pentamidine isethionate can strongly bind basic divalent molecule, inhibits parasite enzymes and binds DNA in the minor groove. Pentamidine isethionate can be metabolized by host cytochrome P_{450} (Berger *et al*., 1990).

There exist another drug (Melarsoprol) that cross the blood–brain barrier. This drug is successful against the late stage of infection. Some disadvantages associated with Melarsoprol are drug metabolism and effect of drug resistance parasites. Melarsoprol inhibits a several enzymes of parasite with toxicity ranging from fever, encephalopathy, headache, gastrointestinal pain and

hypertension (Pepin and Milord, 1994; WHO 1986). The three drugs discussed are antitrypanosoma.

2.9 *Caesalpinia bonduc*

Caesalpinia bonduc (family: *Caesalpiniaceae*, genus *Fabaceae*) is commonly known as gray nicker nut in *English* and Ayóo in *Yoruba* (Sonibare *et al.*, 2009). The tree is shrub having grey, hard, globular seeds with shining surface (Nadkarni, 1954) (Plate I). This is a medicinal plant found in most tropical and sub-tropical regions of Africa and Asia (Gupta *et al.*, 2003).

Plate I: Leaf of *Caesalpinia bonduc*. Photograph taken by Adesuyi Temitope in a Farmland, Aisegba-Ekiti.

2.9.1 Medicinal application of *Caesalpinia bonduc*

Caesalpinia bonduc is a medicinal plant with seed use to produce tonic effective against several infections (Kapoor, 2010). A soaked seed in water showed no antivenom against snake bite (Kirtikar and Basu, 1975). The seed kernel is very effective against irregular fevers and liver disorder (Tummin, 1930). Roasted kernel extract is effective against asthma and acute orchitis,

ovaritis and scrofula. The root-bark helped relinquish the placenta after child birth, intestinal worms, amenorrhea, cough, anthelmintic and tumor (Kirtikar and Basu, 1975).

2.9.2 Pharmacological activity

Caesalpinia bonduc species exhibit wide characteristics against fungi, bacteria, viral, inflammation and free radicals (Carvalho *et al*., 1996; Hemalatha *et al.*, 2007; Shukla *et al.*, 2010; Devi *et al.*, 2008). Some related species have antinociceptive effects.

(i) **Antioxidant activity**

The ethanol extract of seed of *C. bonduc* has phenolic elements which inhibit free radicals by terminating the radical chain reaction (Shruti *et al.*, 2009). The leaf extract by ethanol has antioxidant activity. Chloroform extraction of seeds displayed antioxidant activity (Nikhil *et al*., 2010). The leaf and twigs have phenolic compounds which are antioxidant and possess catalase and peroxidase enzymes.

(ii) **Anti-hepatotoxic activity**

Caesalpinia bonduc possess anti-hepatotoxic activity (ArshadAli *et al.*, 2010). The mechanism of action is unknown but it is found to mediate glutathione detoxification and free radical suppression (ArshadAli *et al.*, 2010). The liver and blood concentration of glutathione is elevated by plant extract. The liver ionic molecules movement improved and serum enzymes are regulated. Thus, the liver functional integrity is protected and maintained (Rajesh, 2011).

(iii) **Antibacterial and antimicrobial activity**

Several solvents for extraction showed the plant is antibacterial and antimicrobial (Billah *et al.*, 2013). The antimicrobial activities of *Caesalpinia bonduc* extracts implied it contained several bioactive phytochemicals effective against a wide range of antibiotics (Stephano *et al.*, 2017).

(iv) **Antimalarial Activity**

Solvent extraction of *Caesalpinia bonduc* inhibits growth of *plasmodium falciparum* (Pudhom *et al.*, 2007). The antimalarial activity is dose dependent

2.9.3 Phytochemicals in *Caesalpinia bonduc*

Caesalpinia bonduc possess several phytochemicals such as steroidal, saponin, alkaloids, glycosides, terpenoids, phytosterols, isoflavones, and phenolics. These phytochemicals are abundant in its many parts. The various parts possess a great number of enzymes like amylase, peroxidase, protease, urease, catalase and oxidase.

2.10 *Anthocleista nobilis*

Anthocleista nobilis has 14 species widely distributed in Africa (Leeuwenberg, 1992; De Wilde, 2011). Young and adult plant is unbranched in stem with branched top (Figure 2.3) (Keay, 1989). Across Nigeria, several ascribed names are ‘Apa oro’ or ‘sapo’ in Yoruba (Keay *et al*., 1964), ‘Sapo sapo’ in Ijebu land (Richards, 1939; Ross, 1954), ‘Kwari’ in Hausa, and ‘Mpoto’ in Ibo (Anyanwu *et al.*, 2013). *Anthocleista nobilis* can be 6–20m high and in trunk diameter 15–55 cm, Plate II. The twigs spines are opposite and the leaf is very large (Hyde *et al.*, 2015).

(a) (b)

Plate II: Trees of *Anthocleista nobilis*. (a) A growing *Anthocleista nobilis* plant and, (b) A young growing *Anthocleista nobilis* plant.

Anthocleista nobilis is a source of food for forest animals (Basabose, 2002; Babalola *et al.*, 2012). Tree stem is rough and coarse, thus enable hosting some epiphytes (Addo Fordjour *et al.*, 2009). In traditional medicine, *Anthocleista nobilis* is potent against some diseases (Dalziel, 1955; Ateufack *et al.*, 2014).

2.10.1 Pharmacological activities

(i) Antidiabetic activity

Some parts are macerated in water and the extract is orally administered to treat diabetes (Olowokudejo *et al*., 2008; Soladoye *et al.*, 2012). The *A. nobilis* and its other species are antidiabetic (Soladoye *et al*., 2012). The various phytochemicals present have been responsible

for the antidiabetic activity. The mechanisms exhibited for the antidiabetic activity is uncleared but is found to lowers the blood glucose level by stimulating insulin secretion and decreasing glucose absorption rate.

(ii) Antioxidant activity

Anthocleista species produce several antioxidants for self-protection and usefulness. The antioxidant chemicals include vitamin E, vitamin C, carotenoids and flavonoids. Antioxidants prevent the formation of free radicals, reactive oxygen and nitrogen species capable of damaging DNA, proteins, lipids, and nucleic acids. Antioxidants can be taken as dietary supplements to prevent diseases (Baillie *et al.*, 2009).

(iv) Antitrypanosomal activity

Anthocleista species and its related species extracted by several solvents are not antitrypanosomal (Atindehou *et al*., 2004; Madubunyi and Asuzu, 1996). In contrast, Abu *et al.* (2009) studied aqueous and solvent extracts of *A. vogelii* root which exhibited antitrypanosomal in-vitro. The varied result indicated difference in phytochemicals components of the parts used.

(v) Toxicity studies

Solvent extract of various plants showed a LD_{50} of 200mg/kg (Madubunyi and Asuzu, 1996). *Anthocleista nobilis* is safe and non-toxic at a significant high dose. The study of plant toxicity showed its antioxidant activity in liver and against free radical. Therefore, various plant extract is safe and acceptable over all parameters of health and usage.

2.10.2 Phytochemicals in *Anthocleista nobilis*

Anthocleista nobilis possessed several phytochemicals (Irvine, 1961; Chapelle, 1976). Solvent extraction of several parts showed they contain various bioactive phytochemicals like saponins,

tannins, flavonoids, terpenoids, alkaloids, anthraquinones and steroids (Anyanwu *et al.*, 2013; Gboeloh *et al.*, 2014; Jegede *et al.*, 2011).

2.11 *Kigelia africana*

Kigelia africana has a huge cucumber-like fruit. It is fondly called cucumber tree. It is ascribed multiple names in Nigeria languages: Bechi (Nupe); Uturubein (Igbo); Pandoro (Yoruba); Rawuya (Hausa) and Ebie (Igala); (Mann *et al*., 2003; Otimenyin and Uzochukwu, 2012). *Kigelia africana* is vastly distributed in riverine and wet-savannah of tropical Africa, Plate III. The tree grows tall during rainfall and deciduous in dry season. The flowers and fruits are suspended downwards along the stems.

(a) (b)

Plate III: *Kigelia africana* in riverine area. (a) A tree of *Kigelia africana* and*; (b)* A plugged leaf of *Kigelia africana.*

Kigelia africana can grow to become a huge tree with big fruit. Matured fruit has many seeds eaten by wild animals (Olatunyi and Atolani, 2009). Several parts have capacity against fungal infections, eczema boils, dysentery, tapeworm, malaria and diabetes (Jackson and Beckett, 2012). The phytochemicals have activities against protozoa, fungal and bacteria.

2.11.1 Medicinal use of *Kigelia africana*

(i) Anti-Protozoal Activity

Solvent extraction of *Kigelia africana* is effective against malaria and trypanosome (Gessler *et al.*, 1994; Weiss *et al.*, 2000; Moideen *et al.*, 1999). Some of the phytochemicals isolated from *Kigelia africana* showed activities against trypanosome at IC_{50}<5μM. Solvent extraction of stem back and root bark exhibited anti-trypanosome activity as it contained naphtho-furan-4,9-quinone and naphtho-quinoids (Moideen *et al.*, 1999).

(ii) Anti-Diabetic and Antioxidant Activities

Kigelia africana exhibited antioxidant and anti-diabetic activities. Infected animal treated with extract of *Kigelia africana* improved blood biochemical activities. The extract helped to stabilize some hormone level by improving its activities. Insulin played an important role in glucose absorption and metabolism. The insulin level increased in treated animal and decreased in untreated diabetic animal. The antioxidant and anti-diabetic activities worked is a dose-dependent.

(iii) *Kigelia africana* Toxicity

Aqueous extract of some part exhibits diuretic activity. The extract acute toxicity is tolerance with no side effects after oral administration. A significantly high dose toxicity caused death with LD_{50} estimated as 3,981.07 mg/kg (Azu *et al.*, 2010; Zofou *et al.*, 2011).

Extract of *Kigelia africana* was administered daily at 500mg/kg body weight for 30 days to infected rats with no side effect on hematological and biochemical activities. Likewise, liver enzymes were not affected (Nyarko *et al.*, 2005). However, no significant change in organ weight was observed when sacrificed.

2.11.2 Phytochemicals in *Kigelia africana*

Kigelia africana parts contained various bioactive phytochemicals which serve several medicinal uses. Some of the profound phytochemicals include naphthaquinones, flavonoids, sterols, coumarins, and alkaloids with well-established structures (Owolabi and Omogbai, 2007).

The stem bark consists of phytochemicals that are antimicrobial and antitrypanosomal in action. Some prominent phytochemicals are iridoids (Neelam *et al.*, 2006), naphthoquinones (Moideen *et al.*, 1999), balaphonin and kigelinol (Akah 1998; Akunyili and Houghton, 1993).

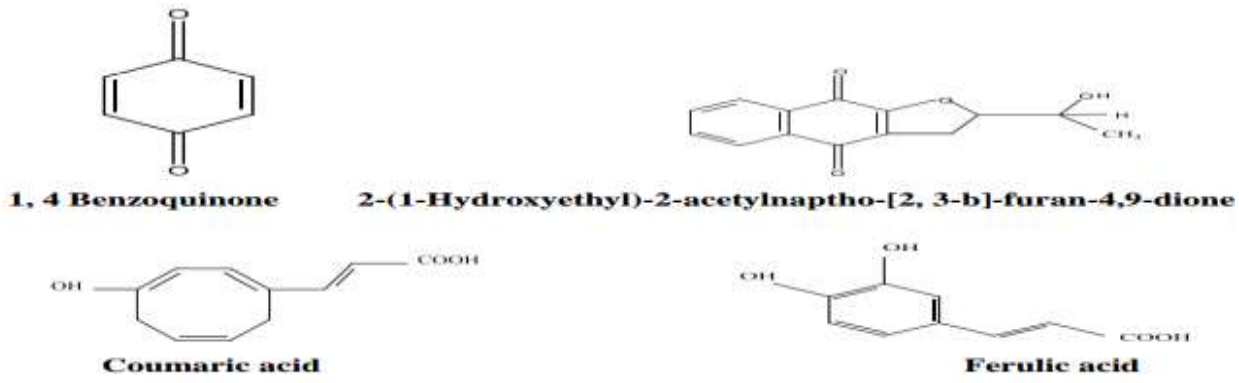

Figure 2.2: Basic Structure of Some Phytochemicals in Stem.

The root of *Kigelia Africana* contained several phytochemicals similar to those in stem, figure 2.3 (Neelam *et al.*, 2006). Coumarins, steroids and iridoids, Figure 2:2 (Akunyili and Houghton, 1993), iso-coumarins (Folashade and Olufunsho, 2012), stigmasterol and lapachol, Figure 2.6 (Houghton *et al.*, 1994), naphthoquinones (Kolodziej, 1997; Houghton, 2007) and non-quinonoid aldehydes (Olatunyi and Atolani, 2009; Houghton *et al.*, 1994) have all been established.

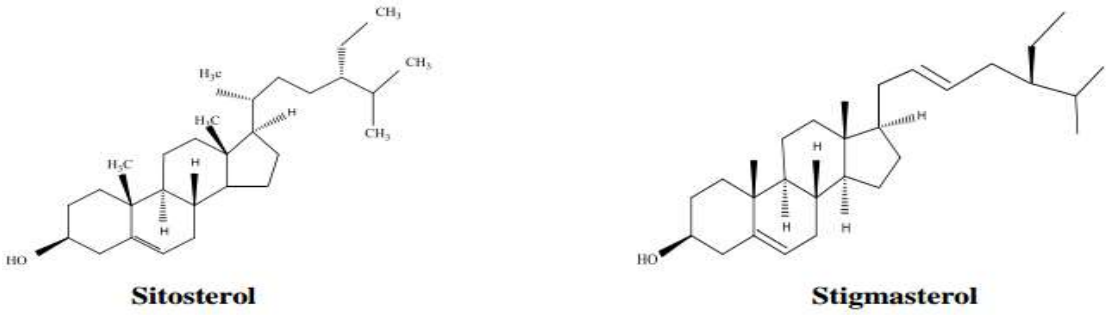

Figure 2.3: The Structure of Some Phytochemicals in Root.

The leaf extract possessed high hydrocarbons and flammable compounds. A comprehensive analysis of the leaf extract revealed to possess valuable phytochemicals (Khan and Mlungwana, 1999; Atawodi and Olowoniyi, 2015).

2.12 PHYTOCHEMICALS

Content of the phytochemical in the medicinal plants consist of compounds and components of unique biological activity. Plants can be identified by their phytochemicals properties. Most parts of the plants used for the analysis of the phytochemical properties were leaves, roots, stem barks, and fruits. Medicinal plants can be investigated for phytochemical constituents of ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water) extraction of different phytochemicals. The abundance of the phytochemicals in medicinal plants can demonstrate high efficiency to restore the damage caused by parasites.

(i) Alkaloids

Alkaloids are one of the main and largest components produced by plants, and they are metabolic byproducts that are derived from the amino acids (Naseem, 2014). Alkaloids can be extracted from the different parts of the plants using different solvents such as ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water). These types of

solvents extraction of phytochemical components can be applied on medicinal plant parts like leaves, roots, stem bark, and fruits. Alkaloids involves mostly nitrogen base compounds, which naturally occur in plants effective against bacteria, animals and fungi. Alkaloids exhibit antimalarial (e.g., quinine), antibacterial (Cushnie *et al.*, 2014), anticancer (Kittakoop *et al.*, 2014) and antihyperglycemic activities (Qiu *et al.*, 2014).

(ii) Flavonoids

Flavonoids consist of a large group of polyphenol compounds having a benzoyl-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by the phenylpropanoid pathway. Flavonoids are secondary metabolites of a phenolic nature that are responsible for the variety of pharmacological activities (Pandey, 2007). Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). Flavonoids have antioxidant effects and inhibit the initiation, progression and promotion of tumors (Kim *et al.*, 1994). It can significantly reduce coronary heart disease (Hertog *et al.*, 1993), protect against platelet aggregation, tumors, ulcers, allergies, free radicals and inflammation (Barakat *et al*., 1993).

(iii) Tannins

Tannin is a complex large biomolecule of polyphenol nature having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Navarrete, 2013). Tannins were detected in most plant parts like leaves, roots, stem bark, and fruits. Tannins are generally used in the tanning process and used as healing agents in inflammation, burn, piles, and gonorrhea (Boroushaki *et al.*, 2016). Tannin is an active ingredient of some medicines (Haslam, 1996). Tannins are antioxidants in beverages (Falbe and

Regitz, 1996) which exhibit antiviral, antibacterial, and antitumor (Haslam, 1996; Khanbabaee and Van, 2001).

(iv) Saponins

Saponins are an important group of plant secondary metabolites that are widespread throughout the plant kingdom. Saponins are phytochemicals that are found in most vegetables, beans, and herbs (Francis *et al.*, 2002). Saponins can be detected in most medicinal plant parts like leaves, roots, stem bark, and fruits. Saponins is antimicrobial effective against yeast and fungal infections (Sheikh *et al*., 2013). Saponins can bind cholesterol and block uptake by the intestines.

(v) Steroids

The word steroid was derived from sterol, a natural or synthetic chemically active hormone-like element. A steroid is one of a large group of chemical substances classified by a specific carbon structure. Steroids include drugs used to relieve swelling and inflammation, such as prednisone and cortisone; vitamin D; and some sex hormones, such as testosterone and estradiol (Hill *et al.*, 2007). Steroids were detected in most plant parts like leaves, roots, stem bark, and fruits.

(vi) Phenolic

Phenolic compounds are secondary metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization (Derong Lin *et al.*, 2016). Phenolic can be detected in most the medicinal plant parts like leaves, roots, stem bark, and fruits.

Phenols is an antioxidant found in plants and human (Dillard and German, 2000). Phenol is known to improve dietary intake of nutrients rich in antioxidant properties (Haslam, 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical reagents

The laboratory reagents were analytical grade, product of Japanese drug house. The reagents were n-hexane, ethyl acetate and methanol, ammonia hydroxide, butanol, sodium chloride, olive oil, ferric chloride, hydrochloric acid, acetic anhydride, chloroform, Fehling's solution (A and B), ammonia solution, tetraoxosulphate (vi) acid, berenil, Dragendorff's reagent, ethanoate, ethanol, folin-denis reagent, glucose test kit (Randox product), sodium tri-oxo-carbonate, diethyl ether, total protein and albumin test kit (Randox product) and capillary tubes.

3.1.2 Apparatus and equipment

Electronic weighing balance (AR223CN), water bath (BJL-458-010S), UV-Spectrophotometer (UV-1800 Shimadzu), Soxhlet extractor, electric microscope, litmus papers and Whatman filter paper.

3.1.3 Collection of plant samples

The leaf of *Caesalpinia bonduc*, root back of *Anthocleista nobilis* and stem of *Kigelia africana* were harvested from a farm settlement in Aisegba-Ekiti, Ekiti-State.

3.1.4 Experimental animals

Albino rats were bought from Olatunji farm, Ilorin. The animals were fed with chicken feeds and tap water for the entire period.

3.1.5 Parasite strain and libitum

The parasite (*T. b. brucei*) was obtained from the Nigeria Institute for Trypanosomiasis Research (NITR) Kaduna, Kaduna-state, Nigeria, and maintained in the laboratory by serial blood passage to uninfected rat.

3.2 Methods

3.2.1 Preparation of plant sample

Fresh samples were rinsed with tap water, oven dried at 27^0C for four days at the research laboratory of the Department of Biochemistry Federal University of Technology, Minna, Niger-state. Dried samples were grinded to powder via mortar and pestle.

3.2.2 Extraction of plant materials

Exactly 150 g of each powdered sample was transferred into 750 ml of distilled water and extraction carried out in Soxhlet. The aqueous crude extract of *Caesalpinia bonduc* leaf, *Anthocleista nobilis* root back were concentrated separately using water bath set at 45^{o}C. The percentage yield of each crude extract was calculated using the equation 3.1.

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{Weight of original sample}} \times 100 \qquad (3.1)$$

3.2.3 Partitioning of crude extracts

The crude extracts were partitioned using three different solvents of different increasing polarity such as n- hexane, ethyl acetate, and methanol respectively. Crude extract (20 g) of different plants were dissolved in 20 ml distilled water. Thereafter, the solution obtained was vigorously shaken and separately for two minutes with 800 ml n-hexane, ethyl acetate and methanol in order of polarity in a separating funnel. The mixture was allowed to stand and decanted. Each supernatant was collected and concentrated in water bath as fractions.

3.2.4 Phytochemical content of plant extracts

3.2.4.1 Qualitative phytochemical content of plant extracts

i. Tannins:

A solution of 1.5 g extract dissolved in 10 ml distilled water was filtered via Whatman filter paper. Add two drops of 0.1% ferric chloride to 2 ml filtrate. A brownish green or a blue black colour indicated a positive test (Ejikeme *et al.*, 2014).

ii Flavonoids:

A solution of 1.5 g extract dissolved in 5 ml distilled water was filtered. Add 5 ml filtrate to 2 ml 1.0 M dilute ammonia solution and 2 ml concentrated tetraoxosulphate (VI) acid. Yellow colour appeared and disappeared on standing which indicated a positive result (Sofowara, 1993).

iii Alkaloids:

A solution of 1.5 g extract dissolved in 5 ml distilled water was mixed with 2 ml chloroform and 1.0 M dilute tetraoxosulphate (VI) acid. Add 0.5 ml Dragendorff's reagent to 2 ml mixture and a precipitate of orange colour indicated a positive result (Hikino *et al*., 1984).

iv Phlobatannins:

A solution of 1.5 g extract dissolved in 10 ml distilled water was mixed with 2 ml of 1% dilute hydrochloric acid and boiled for 10 minutes. A deposit of red precipitate showed a positive test (Ejikeme *et al*., 2014).

v Saponin:

A solution of 1.5 g extract dissolved in 10 ml distilled water was boiled for 10 minutes and filtered. Add 5 ml of distilled water to 2 ml filtrate and shake vigorously for a stable froth. Formation of emulsion on addition of three drops of olive oil gave positive result (Ejikeme *et al.*, 2014).

vi Steroid:

A solution of 1.5 g extract dissolved in 5 ml distilled water was mixed with 2 ml acetic anhydride and 2 ml concentrated tetraoxosulphate (VI) acid. A violet, blue or green colour indicates the presence of steroids (Ejikeme *et al.*, 2014).

vii Terpenoids:

A solution of 1.5 g extract dissolved in 5 ml distilled water was mixed with 2 ml chloroform and 3 ml concentrated tetraoxosulphate (VI) acid. A reddish brown colour output indicates positive results (Ejikeme *et al.*, 2014).

viii Glycoside:

A solution of 1.5 g extract dissolved in 5 ml distilled water was filtered. The filtrate can be tested as follows:

(a) add 0.2 ml Fehling's solutions A and B to 2 ml filtrate and heat, a brick-red colour appeared to show a positive result.

(b) add 10 ml 1.0 M sulphuric acid to the mixture above. Precipitation indicates presence or absence of glycoside (Hikino *et al*., 1984).

3.2.4.2 Quantitative phytochemical content of plant extracts

i Tannins:

A solution of 1.5 g extract dissolved in 5 ml distilled water was filtered. Mix 2 ml filtrate with 2 ml Folin-Denis reagent and 2 ml of sodium trioxocarbonate (iv). Take the absorbance of the mixture at 395 nm wavelength within 10 minutes. The concentration of sample was extrapolated from the spectrum curve (Ejikeme *et al*., 2014)

ii **Phenols:**

A solution of 1.5 g extract dissolved in 5 ml distilled water. Add 2 ml ammonium hydroxide solution and 1 ml concentrated phenol to the mixture. Allow the mixture stand for 30 minutes to

develop colour. Take absorbance at 505 nm wavelength using spectrophotometer. The concentration is extrapolated from the spectrum curve (Keay *et al.,* 1964).

iii Alkaloids:

A solution of 1.5 g extract dissolved in 5 ml 10% ethanoate. The mixture was allowed to stand for I hour, filtered and concentrated. Add concentrated ammonium hydroxide to concentrate until its precipitate completely. Allow the solution to settle, wash the precipitate with dilute ammonium hydroxide and filter. The residue was dried and weighed (Ejikeme *et al.,* 2014).

$$\% \text{ alkaloid} = \frac{\text{weight of alkaloid}}{\text{weight of extract}} \times \frac{100}{1} \quad (3.2)$$

iv Saponins:

A solution of 1.5 g extract dissolved in 10 ml diethyl ether was vigorously shaken in a separating funnel. Recover the supernatant, add 10 ml butanol to the mixture and wash twice with 10 ml 5% sodium chloride. Heat the left-over solution and concentrate to a constant weight (Ejikeme *et al.*, 2014).

$$\% \text{ Saponins} = \frac{\text{weight of saponins}}{\text{weight of extract}} \times \frac{100}{1} \quad (3.3)$$

v Flavonoids:

A solution of 1.5 g extract was repeatedly extracted with 10 ml of 80% aqueous methanol at room temperature. Filter the solution and concentrate to dryness in a water bath (Ejikeme *et al.*, 2014). The percentage flavonoid is determined using equation 3.4.

$$\% \text{ Flavonoids} = \frac{\text{weight of flavonoids}}{\text{Weight of extract}} \times \frac{100}{1} \quad (3.4)$$

3.2.5 Combination of extracts and fractions

A solution of 0.32 g extract from each plant was combined at ratio 1:1:1 and dissolved in 20 ml distilled water to form combined crude extract and stored in sample bottles before use. Similarly,

fractions from different plants were also separately combined in 1:1:1 for hexane, ethyl acetate and methanol in order of polarity.

3.2.6 Inoculation of animals

Infected blood was collected from affected rats by cutting the tail end and diluted in physiological saline. Rats from each group was inoculated with 0.05 ml of diluted blood samples as inoculums. The number of parasites per ml was obtained as described by Herbert and Lumsden, (1976).

3.2.7 Screening for antitrypanosomal activities

The experimental design required thirty-five rats (35) grouped into seven, five (5) each. The infected rats in group A-D were orally administered 200 mg/kg body weight extract for 10 days. The details of animals grouping were as shown below:

Group A: infected and treated with crude extracts

Group B: infected and treated with n-hexane fractions

Group C: infected and treated with ethyl acetate fractions

Group D: infected and treated with methanol fractions

Group E: infected and treated with berenil

Group F: infected and untreated

Group G: uninfected and untreated

Two animals were sacrificed to obtain blood sample after day six (6) of post treatment from each group for the determination of some biochemical parameters and packed cell volume, while the remaining animals were used for monitoring the parasite until death or permanent cure. The biochemical parameters analysed include glucose, total protein, albumin and globulin.

3.2.8 Monitoring of parasitaemia

The animals were screened for parasitaemia at two days interval by wet blood film obtained from tail blood at x40 magnification of electric microscope. The parasite count per field was as described by Herbert and Lumsden, (1976).

3.2.9 Biochemical analysis of blood samples

i. Determination of blood glucose.

Blood glucose were analyzed as enzyme glucose oxidase reacted with glucose, water and oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide was then used to oxidize a chromogen or the consumption of oxygen that was measured to estimate the amount of glucose present. The hydrogen peroxide formed reacted with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator with absorbance taken at 578 nm. Glucose concentration is the product of glucose standard concentration and ratio of sample absorbance to standard absorbance. Glucose test kits, a product of Randox laboratories, U.K. were used.

ii. Determination of albumin

Serum albumin was measured based on its quantitative binding to the bromocresol green, BCG indicator in a buffered medium to form a green coloured complex. The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance is directly proportional to the concentration of albumin in the sample. Test kits products of Randox laboratories, U.K. were used.

iii. Determination of total protein

Serum total protein was determined as copper II ions in an alkaline medium interacted with protein peptide bonds resulting in the formation of blue-violet complex. Protein in the sample combined with the reagent to produce alkaline copper-protein chelate. The colour formed is proportional to the protein concentration and absorbance is monitored at 545 nm. Test kits products of Randox laboratories, U.K. were used.

The difference between total protein and albumin is globulin.

3.2.10 Determination of packed cell volume (PCV)

The Packed cell volume (PCV) was measured as the ratio of the occupied by the red blood cells to the volume of the whole blood. The blood sample was drawn into a capillary and centrifuged for five minutes at 1200 gm revolution. PCV value was read via micro hematocrit reader (Hawsley, England) and expressed in percentage.

3.2.11 Determination of percentage organ and body-weight ratio

The percentage organ and body-weight ratio of two animals from each group were weighed and slaughtered. Organs such as liver, spleen, kidney, heart and lungs were harvested after sacrificing the animals, washed in normal saline and weighed separately after day six (6) of post treatment. The percentage organ ratio is as determined by equation 3.5.

$$\% \text{ Organ weight} = \frac{\text{weight of organ}}{\text{Body-weight}} \times 100 \qquad (3.5)$$

3.2.12 Data analysis

Data was analyzed using the analysis of variance (ANOVA) and SPPS package (version 25) . The data were presented as mean standard error (SEM). A mean value at 5% confidence level ($p<0.05$) was significantly considered (Mahajam, 1997).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percentage yield of plant extracts

The percentage yield of aqueous extract of the plant samples was presented in Table 4.1. The *Anthocleista nobilis* extract had the highest percentage yield, followed by *Kigelia africana* and the least is *Caesalpinia bonduc*.

Table 4.1: Percentage Yield of Crude Extracts

Plant sample	Extraction solvent	Sample weight (g)	Weight of extract (g)	Yield (%)
Caesalpinia bonduc	Aqueous	150	105.29	70.19
Kigelia africana	Aqueous	150	141.87	94.58
Anthocleista nobilis	Aqueous	150	143.69	95.79

4.1.2 Percentage yield of partition extracts

The percentage yield of partition extracts is shown in Table 4.2. Ethyl acetate fractions had the highest percentage yield, while all n-hexane factions had relatively lower yields.

Table 4.2: Percentage Yield of Partition Extracts

Sample	Sample weight	Solvent	Solvent volume (ml)	Extract weight (g)	Yield (%).
Caesalpinia bonduc	20.00	n-Hexane	800	3.60	18.00
		Ethyl acetate	800	6.68	34.40
		Methanol	800	4.44	22.20
Kigelia Africana	20.00	n-Hexane	800	4.20	21.00
		Ethyl acetate	800	6.52	32.60
		Methanol	800	4.62	23.10
Anthocleista nobilis	20.00	n-Hexane	800	4.04	20.20
		Ethyl acetate	800	6.80	34.00
		Methanol	800	4.80	24.00

4.1.3.1 Qualitative phytochemical constituents in crude extracts and fractions

The phytochemical constituents isolated from the crude extracts and fractions were presented in Table 4.3. Tannins, saponin, steroid, flavonoid, terpernoids and alkaloid were readily present in all extracts, while glycoside was relatively present in some groups and absent in some groups.

Table 4.3: Qualitative Phytochemicals in Crude Extracts and Fractions

Plant extract	Tannins	Saponin	Steroid	Flavonoid	Terpenoids	Alkaloid	Glycoside
A	+	+	+	+	+	+	-
B	+	+	+	+	+	+	-
C	+	+	+	+	+	+	+
D	+	+	+	+	+	+	-
E	+	+	+	+	+	+	-
F	+	+	+	+	+	+	-
G	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+
I	+	+	+	+	+	+	+
J	+	+	+	+	+	+	+
K	+	+	+	+	+	+	+
L	+	+	+	+	+	+	+

Key: Present: +; Absent: −

4.1.3.2 Quantitative phytochemical constituents in crude extracts and fractions

The phytochemicals constituents in crude extracts and fractions were presented in Table 4.4. In total, 20.6% saponin, 11.9% flavonoid, 26.9% alkaloid, 0.031% phenol and 6.46% tannins were present in extracts and fractions.

Table 4.4: Quantitative Phytochemicals Contents in Crude Extracts and Fractions

Plant extract	Saponin (%)	Flavonoids (%)	Alkaloids (%)	Phenol(%)	Tannins (%)
A	4.0	1.2	2.4	0.002	1.195
B	3.5	2.4	7.9	0.001	1.240
C	3.2	2.1	8.0	0.002	1.350
D	0.9	0.2	0.5	0.009	0.345
E	1.0	0.3	0.2	0.010	0.017
F	0.6	0.5	0.1	0.001	0.077
G	1.3	0.9	1.0	0.001	0.192
H	1.9	1.2	0.9	0.001	0.175
I	1.5	1.0	0.5	0.001	0.185
J	0.8	0.6	1.9	0.001	0.543
K	0.9	0.7	2.2	0.001	0.735
L	1.0	0.8	2.3	0.001	0.410

Key:

A*: Caesalpinia bonduc* (crude)

B: *Anthocleista nobilis* (crude)

C*: Kigelia africana* (crude)

D: *Caesalpinia bonduc*(n-hexane) fraction

E: *Anthocleista nobilis* (n-hexane) fraction

F: *Kigelia africana* (n-hexane) fraction

G: *Caesalpinia bonduc* (ethyl acetate) fraction

H: *Anthocleista nobilis* (ethyl acetate) fraction

I: *Kigelia africana* (ethyl acetate) fraction

J: *Caesalpinia bonduc* (methanol) fraction

K: *Anthocleista nobilis* (methanol) fraction

L: *Kigelia africana* (methanol) fraction

4.1.4 Antitrypanosomal activities of crude extracts and fractions

The diagnosis of parasites in the blood stream of the infected rats was confirmed after forty-eight hours (2 days). The group E treated with berenil cleared the parasite within forty- eight hours post treatment. The group B-D treated with fractions cleared the parasite within 10 days post administration. Only crude extract cleared the parasite within 6 days of post-treatment. However, the infected untreated recorded increase parasitemia and mortality within 10 days.

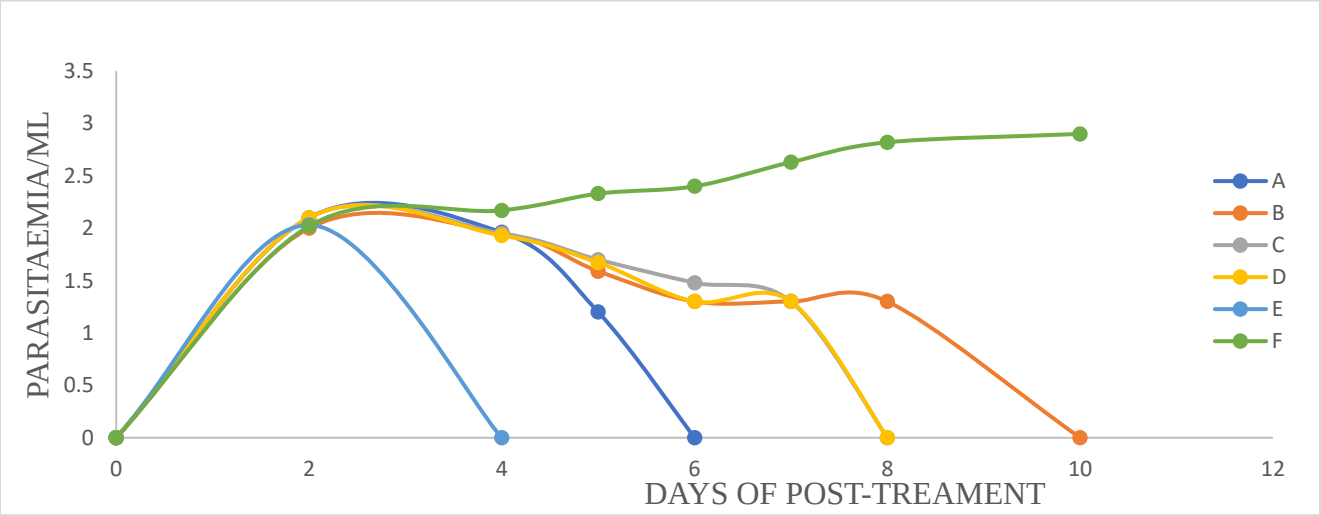

Figure 4.1: Parasite Count in Infected animals.

4.1.5 Summary of screened animals

It is important to note that animals treated with crude and fractions have elongated life span after infection than the high rate of mortality in the untreated infected. The life span of treated rats greatly improved ($p<0.05$) from day zero to day ten of treatment. High mortality rate in untreated infected rats from day zero to day seven of treatment showed significant decrease ($p<0.05$) in life span.

Table 4.5: A Summary of Screened Animals

Group	Range	Days of parasite clearance	Minimum	Maximum
A	2-30	6	0	20
B	2-30	10	0	20
C	2-30	8	0	20
D	2-30	8	0	20
E	2-30	4	0	20
F	2-7	D	0	7
G	2-30	0	0	20

Key: D- Died.

A: crude extract *B: n-hexane fractions*

C: ethyl acetate fractions *D: methanol fractions*

E: standard berenil *F: infected untreated*

G: uninfected untreated

4.1.6 The packed cell volume in infected rats

Packed cell volume (PCV) of infected rats is represented in Figure 4.2. On day six of post-treatment, the PCV of all infected treated rats increase greatly ($p<0.05$) compared to a significant reduction ($p<0.05$) in infected untreated groups. The extracts and fractions improved the PCV of all infected treated groups remarkably compared to the depreciation experienced in infected untreated group.

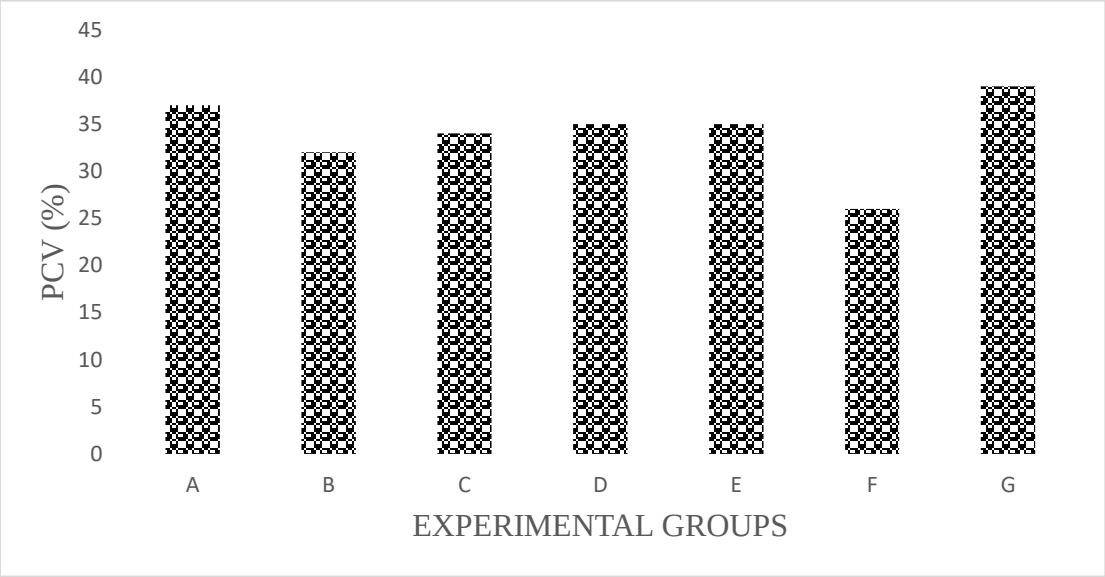

Figure 4.2: Packed Cell Volume in Infected Rats.

4.1.7 The glucose level in infected animals

The glucose level in infected and treated rats is represented in Figure 4.3. On sixth day of post-treatment, glucose level increased significantly ($p<0.05$) compared to uninfected untreated. The extracts and fractions improved the glucose level of all infected treated groups compared to the decrease experienced in infected untreated group.

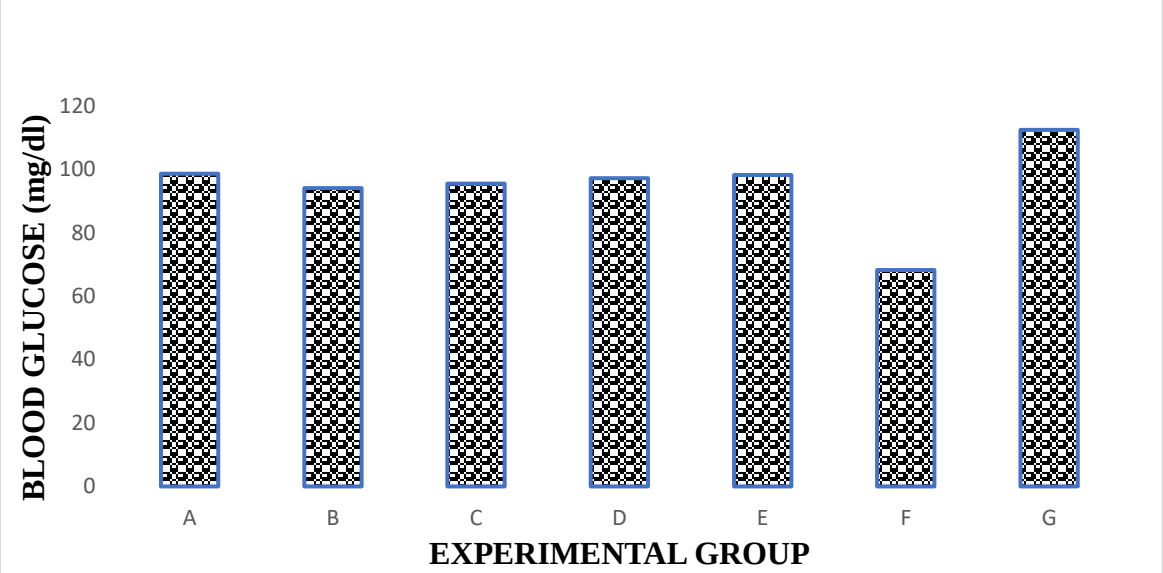

Figure 4.3: Glucose Level in Infected Rats

4.1.8 Total protein in infected and treated animals

The total protein of infected and treated rats is represented in Figure 4.4. On day six of post-treatment, total protein increased significantly ($p<0.05$) compared to significant reduction ($p<0.05$) in infected untreated group. The extracts and fractions improved the total protein concentration of all infected treated groups compared to the reduction experienced in infected untreated group.

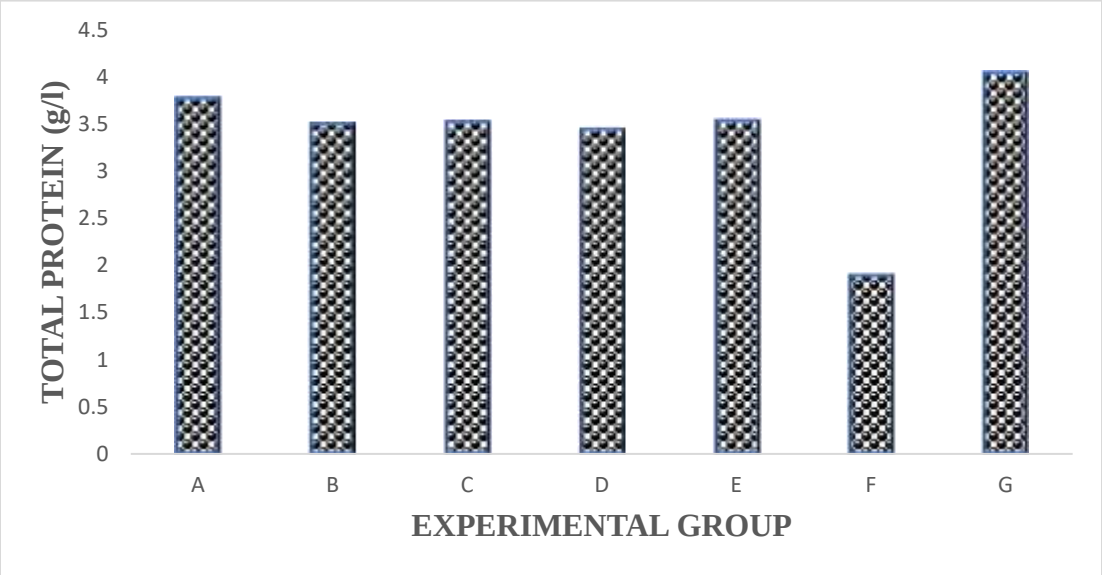

Figure 4.4: Total Protein in Infected and Treated Rats

4.1.9 Albumin in infected and treated animals

Serum albumin of infected and treated rats is represented in Figure 4.5. On day six of post-treatment, albumin increased significantly ($p<0.05$) compared to significant reduction ($p<0.05$) in infected untreated group. The extracts and fractions improved the albumin concentration of all infected treated groups compared to the depreciation experienced in infected untreated group.

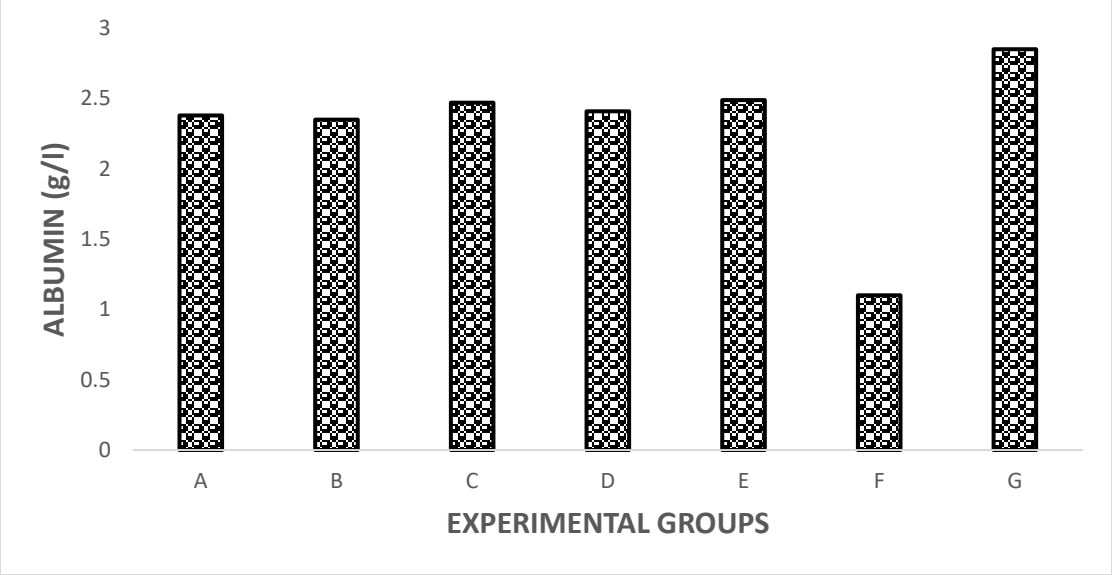

Figure 4.5: Albumin in Infected and Treated Rats

4.1.10 Globulin in infected and treated animals

Serum globulin of infected and treated rats is represented in Figure 4.6. On day six of post-treatment, globulin increased significantly ($p<0.05$) compared to significant reduction ($p<0.05$) in infected untreated group. The extracts and fractions improved the globulin concentration of all infected treated groups compared to a depreciation experienced in infected untreated group.

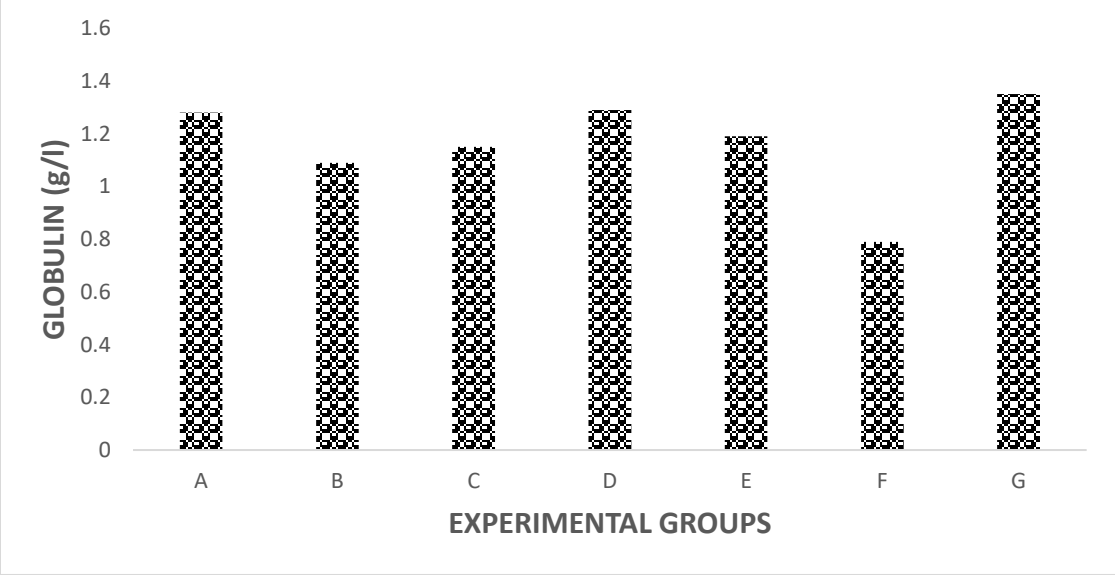

Figure 4.6: Globulin in Infected and Treated Rats

4.1.11 Percentage organ body-weight ratio in infected animals

Infected animals treated with crude extracts and fractions first experienced a decline body and organ-weight at day zero post treatment, but appreciated after the parasite was cleared. The body weight improved significantly ($p<0.05$) at day six of post treatment for group A-E, while group F decreased significantly ($p<0.05$) at the same day of post treatment. Likewise, the organ weight improved significantly ($p<0.05$) at day six of post treatment compared to a significant reduction ($p<0.05$) in infected untreated group at day six. The abundance of saponin, flavonoid and alkaloid could have improved the percentage body and organ-weight ratio of infected treated groups compared to the weight-loss experienced in untreated group.

Table 4.6: Percentage Body and Organ-weight Ratio in Infected Animals

Group	Body-weight (%)	Lungs (%)	Heart (%)	Kidney (%)	Liver (%)	Spleen (%)
A	99.14	1.28	0.44	1.09	5.67	0.53
B	98.97	1.25	0.42	0.78	5.34	0.52
C	99.95	1.26	0.41	0.83	5.60	0.56
D	99.95	1.39	0.56	0.93	7.03	0.57
E	99.95	1.12	0.54	1.21	8.01	0.77
F	69.94	0.65	0.27	0.56	4.01	0.30
G	100.14	1.76	0.64	1.10	7.08	0.69

4.2 Discussion

The use of drug for the treatment of trypanosomal infection is associated with drug toxicity, parasite resistant strains and financial issues. The use of medicinal plants has added advantage to clear the infection with less side effects. Therefore, associated setback to conventional medicine can be eliminated.

This study showed a difference in percentage yield of aqueous plant extracts, *Anthocleista nobilis* had 95.79 %, *Kigelia africana* had 94.58 % and *Caesalpinia bonduc* had 70.19 % extracts. This suggested a higher aqueous-soluble components were found in the *A. nobilis* compared to other plant extracts (Table 4.1). Water has the highest polarity, a property that make polar substance more soluble in it (Mohd *et al.*, 2012).

This study exploited several solvents to examine a difference in the percentage yield of fractions and crude extracts. The n-hexane fractions had a percentage of 20 %, ethyl acetate fractions had 33.3 % and methanol had 23.0 %. These disparity in result was attributed to variation in the solvent polarity. Ethyl acetate fractions was the highest compare to n-hexane and methanol fractions. Therefore, more polar compounds could be found in ethyl acetate fractions (Table 4.2).

The qualitative and quantitative phytochemical constituents of extracts showed they contained some bioactive chemicals which might be effective against trypanosome (Table 4.3 and Table 4.4). These phytochemicals include 26.6 % alkaloids, 20.6 % saponins, 0.031 % phenol, 6.46 % tannins, and 11.9 % flavonoids. These phytochemicals might have worked in synergy to ameliorate the parasite activity and toxicant. Also, a known naphthoquinones isolated in *Kigelia africana* extract was antitrypanosomal (Moideen *et al*., 1999; Abu *et al.*, 2009). Although, other isolated phytochemicals were not known to exhibit antitrypanosoma activity (Houghton, 2007; Anyanwu *et al*., 2013). Most phytochemicals could serve as natural antibiotics and antimicrobial

agents (Sodipo *et al*., 2000). Phytochemicals play many significant pharmacological roles in the body mostly on vital organs. Glycoside for instance is anti-congestive to heart failure (Braunwald *et al*., 1961).

The abundance of phytochemicals in groups treated with fractions of *Caesalpinia bonduc*, *Kigelia Africana* and *Anthocleista nobilis* (Figure 4.1) might have enhanced anti-trypanosome. In addition, Madubunyi and Asuzu (1996) reported that crude extract of *Anthocleista nobilis* was not antitrypanosomal, but the root bark of *A. vogelii* extract was (Abu *et al*., 2009). Also, the aqueous extracts of *Anthocleista nobilis* and *Caesalpinia bonduc* were successful antimalaria and not anti-trypanosoma (Irshad *et al*., 2011; Malan *et al*., 2015). The n-hexane, ethyl acetate and methanol fractions exhibited antitrypanosomal, a possible reason could be the presence phenolic compound (naphthoquinone) found in *Kigelia africana* with known antitrypanosomal activity (Moideen *et al.*, 1999). In contrast, ethanol extract of *Caesalpinia bonduc* and *Anthocleista nobilis* were not antitrypanosomal but was effective against malaria (Irshad *et al*., 2011; Malan *et al*., 2015). Therefore, the combination of crude extracts and fractions of the plant samples demonstrated antitrypanosomal and proved to elongate lifespan of the infected treated rats after the extracts were orally administered.

The life span of treated animals varies, nevertheless increase proliferation of the parasite speed up the high rate of mortality in infected untreated rats (Table 4.5). However, crude extracts and fractions were trypanosomal within the ten days of post-treatment. The biochemical parameters, nutritional level and physiological functions of the treated rats were restored such that no case of the parasite relapsed until their eventual death after thirty days of post treatment. This evidence agrees with Moideen *et al*. (1999) and Abu *et al*. (2009) assertions that the extracts elongate the life span of infected animals. In contrast, the untreated infected animals experienced high

mortality, a condition attributed to increased differentiation and proliferation of the parasite in the hosts. Increase evasion of organs with subsequent damage and ill-functions could accelerate mortality (Uche and Jones, 1992).

Crude extracts and fractions exhibited antitrypanosomal activity accompanied by correspondent increase in PCV (Figure 4.2). A unique clinical symptom of trypanosomiasis is anaemia (Suliman and Fieldman, 1989). Anaemia caused reduced blood volume, a primary reason for mortality (Loso and Ikede, 1972). Anaemia gets worsen with increased proliferation of the parasite. The prolonged lives of the treated animals could be associated to the clearance of parasite and its toxin. This fact agreed with Moideen *et al*. (1999) and Abu *et al*. (2009) who had previously worked on *A. vogelii and Kigelia africana* respectively. The low PCV in untreated group showed the severity of the infection. This assertion agreed with Akanji *et al*. (2009) which stated that parasite caused hemolysis.

The blood glucose level of animals treated with crude extracts and fractions improved with better physical state than those in the untreated infected group (Figure 4.3). This corroborated the appreciated weight sustenance by the treated animals and a consequent weight lost by the untreated infected, a condition enhanced by rapidly glucose consumption by multiplied parasites (Chaudhuri *et al.,* 2006).

Variation in Total protein, albumin and globulin of treated and untreated animals was caused by trypanosoma activity (Figure 4.4). The total protein, albumin and globulin of crude extracts and fractions treated animals could restore the attack initiated by trypanosome. Increase total protein is associated with increase organ enzymes and some intracellular proteins (Orhue and Nwanze, 2004) produced reduce parasitaemia activity on infected cell. Blood albumin increased in the treated animals, on the contrary a reduction is observed in the untreated. Animals treated with

crude extracts and fractions exhibited a sustained balance in serum albumin level compare to the untreated infected. Albumin level reduced due to decrease protein synthesis in the liver or increase protein loss through kidney (Cheesbrough, 1998). Globulin level showed an appreciable increase in the animals treated with crude extracts and fractions as oppose to the reduction in untreated infected. Sustained level of total protein, globulin and albumin by the rats indicated the extracts were antitrypanosoma (Igbokwe and Mohamed, 1992).

The body weight of infected rats (Table 4.6) showed a difference in physical condition of the broad categories. The treated animals could resist weight loss associated with trypanomiasis. Moideen *et al*. (1999) and Abu *et al*. (2009) shared a similar result on extract of *Kigelia africana* and *A. vogelii.* Weight loss in untreated animals complied with Mann *et al*. (2008)

The body and organ-weight of treated animals improved significantly (Table 4.6). Previously, the extracts had shown ability to sustain life treated animals as the active components were delivered to the target site (Moideen *et al*., 1999). A significant depreciation in the body and organ-weight of the untreated infected animals was attributed to trypanosomal infection. This agrees with the Damayanti (1993) and Sadun *et al*. (1973) earlier report that trypanosome causes impairment of body and organs weight. These change in body and organ weights of infected animals was associated with cellular damage caused by parasite toxicants or immunological reactions. Irreversible change accompanied cells and tissues decay, a result of the failure of cell-repair mechanisms to promptly reverse the damaged part. The destructive and irreversible damages in the organs of untreated rats increase rate of mortality. Failure of each organ causes death, and all damaged tissues caused by parasitemia increase mortality (Debjani *et al*., 2019). The formation of host-parasite complex exhibits extreme association of biochemical, nutritional and physiological adaptations. Trypanosome displays a broad mechanism to evade immune

system and with prior memory ahead of host immune response (Cox, 1993). Liver is the most essential organ affected by this infection. Liver congestion, hemorrhage, cellular infiltration and fatty deterioration were known pathological changes (Damayanti, 1993). The pathological changes in the liver shows that the infection induces irreversible transformation in animals. Spleen is a vital lymphatic tissue and mediate a first line of defense against parasite invasion. Suppression of T lymphocyte and B cell is an important symptom of parasite infection. Kidney organ is sensitive to blood diseases as parasite toxins and immune complex accumulation can impair its structure and function. Known kidney effects are glomerulus shrinkage, renal cortex and medulla fibrosis, and tubular epithelium disintegration (Uche and Jones, 1992). Lungs of infected rats exhibit changes in the progress of the disease. Extensive damage in host lungs deprived a protective defense which cause a destructive change that is death of animals. Heart failure during infection is caused by myocarditis. Myocardial collapse and focal hemorrhages are important heart lesions that increase mortality in infected rats.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This research work had provided evidence that combined crude extracts and fractions are antitrypanosomal and mediated some biochemical activities in *T. brucei* infected animals. The combination of crude extracts and fractions elongated life span, improve anaemia and packed cell volume (PCV), weight loss and biochemical changes associated with trypanosome infection in experimented animals. All phytochemicals analyzed were present in appreciable concentration. Therefore, combination of various parts of sampled plants; *Caesalpinia bonduc, Kigelia africana* and *Anthocleista nobilis in* ratio 1:1:1 has proved to be antitrypanosomal as used in traditional medicine and the abundant phytochemicals could be responsible for these activities.

5.2 Recommendation

Further work is recommended on:

i. screening of each crude extract for anti-trypanosoma activity.

ii. structural elucidation of active phytochemicals in each extract.

iii. toxicity test for the combined extracts.

iv. histopathological test of *T. brucei* infected organs

5.3 Contribution of Research to Knowledge

The experimental study proved that *A. nobilis* had 95.79 %, *K. africana* had 94.58 % and *C. bonduc* had 70.19 % aqueous extracts. The fractionated crude extracts had 20 % n-hexane, 33.3 % ethyl acetate and 23.0 % methanol. The phytochemical constituents of extracts and fractions had 20.6 % saponin, 11.9 % flavonoid, 26.9 % alkaloid, 0.031 % phenol and 6.46 % tannins. The

crude extract cleared the parasites within six days post-treatment, all fractions cleared the parasites within 10 days post-treatment, while the untreated group died within seven days. The PCV and biochemical parameters of treated groups improved significantly compared to the rapid depreciation and eventual death experienced by untreated group. The body weight and percentage organ weight ratio of treated rats improved to 98 % of original body weight while there was 25 % loss in body weight of untreated group. The abundant phytochemical constituents present in extracts might have been responsible for the antitrypanosomal activity.

REFERENCE

Abu, A. H., Uchendu, C. N. & Ofukwu, R. A. (2009). In vitro anti-trypanosoma activity of crude extracts of some Nigerian medicinal plants. *Journal of Applied Bioscience,* 21, 1277–1282.

Addo-Fordjour, P., Anning, A. K., Addo, M. G. & Osei, M. F. (2009). Composition and distribution of vascular epiphytesina tropical semideciduous forest, Ghana. *Africa Journal Ecology,* 47, 767-773.

Akah, P. A. (1998). Antidiarrheal activity of *Kigelia africana* in experimental animals. *Journal of Herbs, Spices and Medicinal Plants,* 31-38.

Akanji, M. A., Adeyemi, O. S., Oguntoye, S. O., & Sulyman, F. (2010). Psiduim guajava extract reduce trypanosomiasis associated lipid peroxidation and raise glutathione concentration in infected animals. *Experimental and clinical science, international online journal for advance in science*, 8, 148-54.

Akunyili, D. N. & Houghton, P. J. (1993). Monoterpenoids and naphthoquinones from *Kigelia pinnata* bark, *Phytochemical,* 1015-1018.

Anyanwu, G. O., Onyeneke, E. C., Usunobun, U. & Adegbegi, A. J. (2013). Impact of *Anthocleista vogelii* root bark ethanolic extract on weight reduction in high carbohydrate diet induced obesity in male wistar rats. *African Journal of Biochemical Resources*, 7, 225–232.

ArshadAli, N., Khushboo, B., Karan, A. G. & Kale, M. K. (2010). Protective Effect of Methanolic Extract of *Caesalpinia bonduc* (L.) on Artesunate -Induced Hepatotoxicity in Rats. *Pharmacology*, 3: 401-408.

Atawodi & Olowoniyi (2015). Pharmacological and Therapeutic activities of *Kigelia africana*. *Annual research and review in biology,* 5(1), 1-17.

Ateufack, G., Nguelefack, T. B., Wabo, H. K., Tane, P. & Kamanyi, A. G. (2014). Anti-ulcerogenic Activity of 1-hydroxy-3,7, 8-trimethoxyxanthone isolated from the methanol extract of *Anthocleista vogelii* planch in rats. *Ulcers*, 1–6.

Atindehou, K. K., Schmid, C., Brun, R., Koné, M. W. & Traore, D., (2004). Anti- trypanosomal and anti-plasmodial activity of medicinal plants from Côted'I-voire. *Journal of Ethnopharmacology,* 90, 221–227.

Azu, O. O., Duru F. I. O., Osinubi, A. A., Noronha, C. C., Elesha, S. O. & Okanlawon, A. O (2010). Protective agent, *Kigelia africana* fruit extract, against cisplatin-induced kidney injury in Sprague- Dawley rats. *Asian Journal Pharmacology of Clinical Resources,* 3(2):84-8.

Babalola, F. D., Amusa, T. O., Wala, Z. J., Ivande, S. T., Ihuma, J. O., Borokini, T. I., Jegede, O. O. & Tanko, D., (2012). Spatial distribution of turaco-preferred food plants in Ngel Nyaki Forest Reserve, Mambilla Plateau, Taraba-State, Nigeria. *Biodiversity*, 13, 100-107.

Baillie, J. K., Thompson, A. A. R., Irving, J. B., Bates, M. G. D., Sutherland, A. I., Macnee, W., Maxwell, S. R. J. & Webb, D. J., (2009). Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized place bio-controlled trial. *Journal of Medicine*, 1–8.

Barakat M. Z, Shahab, S. K., Darwin, N & Zahemy, E. I. (1993). "Determination of ascorbic acid from plants," *Analytical Biochemistry*, 53, 225–245. pg 15, 42-45.

Basabose, A. K., (2002). Diet composition of chimpanzees inhabiting the montane forest of work. *African Botanicals*, 1-10. pg 15, 42-45.

Berger, B. J., Lombardy, R. J., *et al*. (1990). Metabolic N-hydroxylation of pendamidine in vitro. *Antimicrobial Agents Chemotherapy*, 34, 1678–85.

Billah, M. M, Islam, R., Khatun, H., Parvin, S., Islam, E., Islam S. M. A. & Mia, A. A (2013). Antibacterial, anti-diarrhea and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonduc*ella (L.) Roxb leaves. *The official journal of the International Society for Complementary Medicine Research*, 13, 101.

Bockstal, V., Guirnalda, P., Caljon, G., *et al*. (2011). *T. brucei* infection reduces B lymphopoiesis in bone marrow and truncates compensatory splenic lymphopoiesis through transitional B-cell apoptosis. *Pathogen*, 7, 100-2089.

Boroushaki, M. T., Mollazadeh, H. & Afshari, A. R. (2016). Pomegranate seed oil: a comprehensive review on its therapeutic effects. *International Journal of Pharmacological Science Resource*, 7(2):430. pg 15, 42-45.

Borst, P. (2002). Antigenic variation and allelic exclusion. *Cell*, 109, 5–8.

Borst, P. & Fairlamb, A. H. (1998). Surface receptors and transporters of *Trypanosoma brucei*. *Annual Review of Microbiology*, 52, 745–778.

Braunwald, E., Bloodwell, R. D., Goldberg, R. I. & Morrow, A. G. (1961). "Studies on digitals IV observations in man on the effects of digitalis preservations on the contractility of the nonfailing in female *Clarias gariepinus*. *Agriculture and Biology Journal of North America*, 1(5), 791-95.

Burkill, H. M. (2000). The useful plants of West Tropical Africa. Families J-L. Kew; Royal Botanic Gardens, 3. pg 15, 42-45.

Capewell, P., Clucas, C., DeJesus, E., *et al* (2013). The TgsGP gene is essential for resistance to human serum in *Trypanosoma brucei gambiense*. *Pathogens*, 9, 100-3686.

Carvalho, J. C. T., Teixeira, J. R. M., Souza, P. J. C., Bastos, J. K., Filho, D. S. & Sarti, S. J. (1996). Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. *Journal of Ethnopharmacology*, *53*, 175–178.

Chapelle, J. P., (1976). Vogeloside and secologanic acid, secoiridoid glucosides from *Anthocleista vogelii* (author's translation). *Plant Medicine*, 29, 268–274.

Chaudhuri, M., Ott, R. D. & Hill, G. C. (2006). Trypanosome alternative oxidase: from molecule to function. *Trends parasitology*, 22, 484-91.

Cheesbrough, M. (1998). District laboratory practice in tropical countries, part 1. Cambridge university press, Cambridge, 355-358.

Cox, F. E. G. (1993). "Modern Parasitology: A Textbook of Parasitology," 2nd edition Blackwell, Oxford.

Cragg, G. M.& Newman, D. J (2013). Natural products: A continuing source of novel drug leads. *Biochemical Biophysics Acta,* 1830, 3670–3695.

Cushnie, T. P., Cushnie, T. B. & Lamb, A. J. (2014). "Alkaloids: an overview of their antibacterial, antibiotic-enhancing and anti-virulence activities," *International Journal of Antimicrobial Agents*, 44(5), 377–386.

Dalziel, J. M., (1955). The Useful Plants of West Tropical Africa. Crown Agents for the Colonies, London, UK, 46.

Damayanti, R. (1993). The pathology of natural *T. evansi* infection in the buffalo (*Bubalus bubalis*). *Penyakit Hewan,* 25**,** 34–39.

De Wilde, J. J. (2011). A new species of *Anthocleista (Gentianaceae)* from Gabon. *Blue measure*, 56, 1-3.

Debjani, B., Choudhury, A. & Musa, K. (2019). Histopathology of trypanosoma (Trypanozoon) evansi infection in Bandicoot rat visceral organs. *Experimental parasitology*, 99, 148-159.

Derong, L., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type diabetes. *Molecules,* 21:1374.

Devi, R. A., Tandan, S. K.; Kumar, D., Dudhgaonkar S. P. & Lal, J. (2008). Analgesic activity of *Caesalpinia bonducella* flower extract. *Pharmaceutical Biology,* 46, 668–672.

Dillard, C. J & German, J. B. (2000). "Phytochemicals: nutraceuticals and human health," *Journal of the Science of Food and Agriculture*, l (80), 1744–1756.

Ding, L., Linsley, P., Huang, L., *et al* (1993). IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *Journal of Immunology*, 151, 1224-34.

Dixon, R. A., Dey, P. M. & Lamb, C. J. (1983). Phytoalexins: enzymology and molecular biology. *Advance Enzymology Related Areas and Molecular Biology,* 55:1–136.

Ejikeme, C. M., Ezeonu, C. S. & Eboatu, A. N. (2014). "Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria," *European Scientific Journal*, 10 (18), 247–270.

Falbe, J. & Regitz, M. (1996). *CD RO¨MPP Chemie Lexikon.* Georgia Thieme, Stuttgart, Germany, 1.0.

Field, M. & Carrington, M. (2009). The trypanosome flagellar pocket. *Natural Revolution Microbiology,* 7, 775-86.

Folashade, O. O. & Olufunsho, S. B. O. (2012). Quantitative evaluation of the anti-psoriatic activity of sausage tree (*Kigelia africana*). *African Journal of Pure and Applied Chemistry,* 6 (13), 214-218.

Francis, G., Zohar, K., Harinder, P. S. & Klaus, B. (2002). The biological action of saponins in animal systems. *Journal on Nutrition*, 88:587–605.

Gboeloh, L. B., Okon, O. E. & Udoh, S. E. (2014). Anti-plasmodial effect of *Anthocleista vogelii* on albino mice experimentally infected with *Plasmodium bergheiberghei* (NK 65). *Journal of Parasitology Resources,* 6.

Gessler, M., Msuya, D., Nkunya, M., Mwasumbi, L., Schar, A., Heinrich, M. & Tanner, M. (1994). Traditional healers in Tanzania: The treatment of malaria with plant remedies. *Journal of Ethnopharmacology*, 48, 131-144.

Gill, L. S. (1992). Ethnomedical uses of plants in Nigeria, Uniben Press, Benin City, 143.

Gupta, M., Mazumder, U. K., Kumar, R. S. & Kumar, T. S. (2003). Studies on anti-inflammatory, analgesic and antipyretic properties of methanol extract of *Caesalpinia bonducella* leaves. *Iranian Journal of Pharmacology and Therapeutics*, 176 (3), 1724-32.

Gutteridge, W. E. & Coombs, G. H. (1977). Biochemistry of parasitic protozoa. Baltimore: Baltimore University Park Press, 89–107.

Haslam, E. (1998). *Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action*, Cambridge University Press, Cambridge, UK, 4, 60-65.

Haslam, E. (1996). "Natural polyphenols (vegetable tannins) as drugs: possible modes of action," *Journal of Natural Products*, 59 (2), 205–215.

Hemalatha, K., Kiran, A. S., Bannappa, U. & Satyanarayana, D. (2007). Analgesic activity of *Caesalpinia sappan* heartwood. *Pharmaceutical Biology*, 45, 360–362.

Herbert, W. J. & Lumsden, W. H. R. (1976). *Trypanosome brucei*. A rapid matching method for estimating the hosts Parasitaemia. *Experimental Parasitology*, 40, 423-431.

Hertog M. G. L, Feskens, E. J. M., Hollman, P. C. H., Katan, J. B. & Kromhout, D. (1993). "Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study," The Lancet, heart and on total vascular resistance." *Journal of Clinical Investigation*, 40 (1), 52–59.

Hikino, H., Kiso, Y., Wagner, H. & Fiebig, M. (1984). "Antihepatotoxic actions of flavonolignans from Silybum marianum fruits," *Plant Medical*, 50 (3), 248–250.

Hill, J. A., Suker, J. R., Sachs, K. & Brigham, C. (2007). The athletic po1ydrug abuse phenomenon. *America Journal on Sports Medicine,* 8(4):269–271.

Hong, J. Y. (2011). Natural product diversity and its role in chemical biology and drug discovery. *Current Opinion on Chemical Biology*, 15, 350–354.

Houghton, P. J. (2007). The sausage tree (*Kigelia pinnata*), Ethnobotany and recent scientific test. *Tropical Medicine and International Health,* 7, 685–692.

Houghton, P. J., Photiou, A., Uddin, S., Shah, P., Browning, M., Jackson, S. J. & Retsas, S. (1994). Activity of extracts of *Kigelia pinnata* against melanoma and renal carcinoma cell lines. *Plant Medicine,* 60(5):430-433.

Hyde, M. A., Wursten, B. T., Ballings, P. & Coates, P. M. (2015). *Anthocleista,* Flora of Zimbabwe: Genus page: ⟨http://www.zimbabweflora.co.zw/speciesdata/ genus.php?genus_id¼1089⟩.

Igbokwe, I. O. & Mohammed, A. (1992). Some plasma biochemical changes in experimental trypanosoma brucei infection in sokoto red goats. *Review of Elevated Medical Pays Tropicals,* 45(3-40), 287-290.

Irshad, S., Mannan, A., Mirza, B. (2011). Antimalarial activity of three Pakistani medicinal plants. *Pakistan Journal of Pharmaceutical Sciences*, 24(4), 589-591. pg 43.

Irvine, F. R. (1961). Woody Plants of Ghana. Oxford University Press, London, United Kingdom.

Iwu, M. M. (2014). Handbook of African Medicinal Plants. 2nd Edition. Boca Raton, FL; CRC Press.

Jackson, S., Beckett, K. (2012). Sausage Tree Kigelia pinnta: An Ethnobotanical and Scientific Review, Herbal Gram; *America Botanical Council*, 94, 48-59.

Jegede, I. A., Ibrahim, J. A. & Kunle, O. F. (2011). Phytochemical and pharmacognostic studies of the leaf and stem-bark of *Anthocleista vogelii* (Planch). *Journal of Medicinal Plant Resources,* 5, 6136–6139.

Kapoor, L. D. (2010). Hand of Ayurvedic Medicinal Plants, CRC Press, 88.

Keay, R. W. J. (1989). Nigerian Trees. Clarendon Press, Oxford and New-York.

Keay, R. W. J., Onochie, C. F. A. & Stanfield, D. P. (1964). Nigerian trees: Federal Department of Forest Reserve, Ibadan Nigeria, 2, 368–370.

Khan, M. R. & Mlungwana, S. M. (1999). γ-Sitosterol, A cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. *Fitoterapia*, 70 (1), 96-97.

Khanbabaee, K & van, R. T. (2001). "Tannins: classification and definition," *Natural Product Reports*, 18 (6), 641–649.

Kim, S Y, Kim, J. H., Kim, S. K., Oh, M. J. & Jung, M. Y. (1994). "Antioxidant activities of selected oriental herb extracts," *Journal of the American Oil Chemists' Society,* 71 (6), 53.

Kirtikar, K. R. & Basu, B. D. (1975). Indian Medicinal Plants, 2nd edition, New Delhi.

Kittakoop, P., Mahidol, C. & Ruchirawat, S. (2014). "Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation," *Current Topics in Medicinal Chemistry*, 14 (2), 239–252.

Kolodziej, H. (1997). Protective role of *Kigelia africana* fruits against benzo(a) pyrene-induced fore-stomach tumourigenesis in mice and against albumen-induced inflammation in rats. *Pharmaceutical Pharmacology Letter*, (7) 2/3, 67-70.

Kreier, J. P. & Baker, J. R. (1987). Parasitic protozoa. Boston, Massachussetts: Allen and Unwin, 53.

Lecordier, L., Uzureau, P. & Tebabi, P. (2014). Identification of *Trypanosoma brucei* components involved in trypanolysis by normal human serum. *Molecular Microbiology*, 94, 625-36.

Leeuwenberg, A. J. M. (1992). *Anthocleista grandiflora*. *Flower Plant of Africa*, 52, 2080.

Loso, G. J. & Ikede, B. O. (1972). Review of the pathology of domestic and laboratory animals caused by *T. congolense, T. vivax, T. brucei, T. rhodesiense, and T. gambiense. Veterinary pathology*, 9, 1-71.

Madubunyi, I. I. & Asuzu, I. U. (1996). Pharmacological screening of *Anthocleista nobilis* root bark. *Pharmacological Biology*, 34, 28–33.

Mahajan, B. K. (1997). Significance of difference in means. In: Methods in Biostatistics for medical and research workers. 6th edition New JAYEET Brothers medical publishers, 130-155.

Malan, D. F., Neuba, D. F. R. & Kouakou, K. L. (2015). Medicinal plants and traditional healing practices in ehotile people, around the lagoon (eastern littoral of Côte d'Ivoire). *Journal of Ethnobiological Ethnomedicine,* 11, 21.

Mann, A., Gbate, M. & Umar, A. N. (2003). Medicinal and Economic Plants of Nupe land, Jube-Evans Books and Publications, Bida. 1st Edition, 377.

Mohd, F. B., Abdul-Razak, P. Y., Zamree, M. D., Shah, L. A., Soh, S. Y. & Ir, T., Y. (2012). The effect of varying solvent polarity on extraction yield of orthosiphon stamineus leaves. *Journal of Applied Sciences*, 12, 1207-1210.

Moideen, S. V. K., Houghton, P. J., Rock, P., Croft, S. L. & Aboagye-Nyame, F. (1999). Activity of extracts and naphthoquinones from *Kigelia pinnata* against *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense. Plant Medicine,* 65(6), 536-540.

Morrison, L., Marcello, L. & McCulloch, R. (2009). Antigenic variation in the African trypanosome: molecular mechanisms and phenotypic complexity. *Cell Microbiology*, 11, 1724-34.

Muschietti, L., Vila, R., Filho, V. C. & Setzer, W. (2013). Tropical Protozoan Diseases: Natural Product Drug Discovery and Development. *Evidence Based Complementary Alternate Medicine of Immunology (Baltimore Medicine), 1*:*950*.

Nadkarni, A. K. (1954). *Indian Materia Medica*, 13th edition, Dhootapapeshwar Prakashan Limited.

Namangala, B., Brys, L., Magez, S., *et al* (2000). *Trypanosoma brucei brucei* infection impairs MHC class II antigen presentation capacity of macrophages. *Parasite Immunology*, 22, 361-70.

Naseem, U., Muhammad, Z., Farhat Ali, K. & Shazeb, K. (2014). A review on general introduction to medicinal plants, its phytochemicals and role of heavy metal and inorganic constituents. *Life Science*, 11(7s):520–527.

Navarrete, P., Pizzi, A., Pasch, H. & Rode, K. (2013). Delmotte characterization of two maritime pine tannins as wood adhesives. *Journal on Adhesive Science Technology*, 27(22), 2462–2479.

Neelam, B., Shailendra, S., Fermida, N. & Amir, A. (2006). Isolation and in vitro anti amoebic activity of iridoids isolated from *Kigelia pinnata*. *General papers Arkivoc*, 69-76.

Nikhil, Kumar Sachan, Shikhar Verma, Anupam K. Sachan & Hussain Arshad (2010). An investigation to antioxidant activity of *Caesalpinia bonduc*ella seeds. *Annual pharmacy and pharmaceutical sciences*, 1 (2), 88-91.

Nyarko, A. K., Okine, L. K. N., Wedzi, R. K., Addo, P. A. & Ofosuhene, M. (2005). Sub-chronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat: Absence of organ toxicity and modulation of cytochrome P450. *Journal of Ethnopharmacology*, 97(2), 319-25.

Olatunji, A. G. & Atolani, O. (2009). Comprehensive scientific demystification of *Kigelia africana*: A review. *Africa Journal of Pure and Applied Chemistry*, 3(9):158-64.

Olowokudejo, J. D., Kadiri, A. B. & Travih, V. A., (2008). Anethno-botanical survey of herbal markets and medicinal plants in Lagos-State of Nigeria. *Ethnobotanical Leaflet*, 12,851–865.

Orhue, E. A. J. & Nwanze, E. A. C. (2004). Effect of scoparia dulcis on trypanosoma brucei induced alterations in serum transaminase alkaline phosphatase and bilirubin in the rabbit. *Journal of medical science*, 4(3), 194-197.

Otimenyin, S. O. & Uzochukwu, D. C. (2012). Spasmolytic and Anti-diarrhea effects of the bark of *Erythrina senegalensis* and root of *Kigelia africana*. *Journal on Pharmacology and Clinical Resources*, 3(4):11-14.

Owolabi, O. J. & Omogbai, E. K. I. (2007). Analgesic and anti-inflammatory activities of the ethanolic stem bark extract of *Kigelia africana* (*Bignoniaceae*), *Africa Journal of Biotechnology*, 6(5), 582-585.

Pal, A., Hall, B., Jeffries, T., *et al.* (2003). Rab_5 and Rab_{11} mediate transferrin and anti-variant surface glycoprotein antibody recyclic in *Trypanosoma brucei*. *Biochemistry Journal*, 374, 443-51.

Pandey, A. K. (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Parihenium histerophorus: an in vitro study. *Natural Academic Science Letter*, 30:383–386.

Paquet, C., Ancelle, T., Gastellu-Etchegorry, M., Castilla, J. & Harndt, I. (1992). Persistence of antibodies to *Trypanosoma brucei gambiense* after treatment of human trypanosomiasis in Uganda. *Lancet,* 340, 250.

Pays, E., Vanhollebeke, B., Uzureau, P., *et al* (2014). The molecular arms race between African trypanosomes and humans. *National Review of Microbiology*, 12, 575-84.

Pegg, A. E. & McCann, P. P. (1988). Polyamine metabolism and function in mammalian cells and protozoans. In ISI Atlas of Science: Biochemistry. Philadelphia: Institute for Science Information. *Biotechnology,* 6(15), 1677-80.

Pepin, J. & Milord, F. (1994). The treatment of human African trypanosomiasis. *Advanced Parasitology*, 33, 2–47.

Pudhom, K., Sommit, D., Suwankitti, N. & Petsom, A. (2007). Cassane Furanoditerpenoids from The Seed Kernels of *Caesalpinia bonduc* from Thailand. *Journal of Natural Product,* 70(9), 1542-1544.

Qiu, S. H., Sun, A. H., Zhang *et al.* (2014). "Natural alkaloids: basic aspects, biological roles, and future perspectives," *Chinese Journal of Natural Medicines*, 12 (6), 401–406.

Radwanska, M., Guirnalda, P., De Trez, C., *et al* (2008). Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. *Pathogen,* 4, 100-78.

Rajesh, J. M. (2011). Hepatoprotective Activity of Ethanolic Extract of *Caesalpinia bonduc* (L.) in Paracetamol Intoxicated Albino Rats. *International Journal of Pharmaceutical technology Research*, 3(1), 431-434.

Raper, J., Portela, M., Lugli, E., *et al* (2001). Trypanosome lytic factors: novel mediators of human innate immunity. *Current Opinion on Microbiology*, 4, 402-8.

Richards, P. W. (1939). Ecological Studies on the Rain-Forest of Southern Nigeria. The structure and floristic composition of the primary forest. *Journal of Ecology*, 27 (1), 1–61.

Ross, R. (1954). Ecological Studies on the Rain-Forest of Southern Nigeria. Secondary succession in the Shasha forest reserve. *Journal of Ecology*, 42, 259–282.

Sadun, E. H., Johnson, A. J., Nagle, R. B. & Duxbury, R. E. (1973). Experimental infections with African trypanosome. V. Preliminary parasitological, clinical, haematological observations in monkeys infected with *T. rhodesiense. American Journal of Tropical Medicine and Hygiene*, 22**,** 323–330.

Sands, M., Kron, M. A. & Brown, R. B. (1985). Pentamidine. A review. *Review of Infectious Disease*, 7, 625–34.

Sheikh, N., Kumar, Y., Misra, A. K. & Pfoze, L. (2013). "Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India," and a Public-Health Policy Failure." *Public Health*, 359, 2188–94.

Shruti Shukla, Archana Mehta, Jinu John, Siddharth Singh, Pradeep Mehta & Suresh Prasad Vyas (2009). Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonduc*ella seeds; Elsevier Limited. All rights reserved, 4, 40.

Shukla, S., Mehta, A., Mehta, P., Vyas, S.P., Shukla, S. & Bajpai, V. K. (2010). Studies on anti-inflammatory, antipyretic and analgesic properties of *Caesalpinia bonducella* F. seed oil in experimental animal models. *Food Chemical Toxicology,* 48, 61–64.

Singh, V, Raghav, P. K. (2012) Review on pharmacological properties of Caesalpinia bonduc. *London International Journal of Medicine of Aromatic Plants,* 2, 514-530.

Sodipo, O. A, Akiniyi, J. A. & Ogunbano, U, S. (2000). "Studies on certain characteristics of extracts of bark of *Pausinystalia johimbe* and *Pausinystalia macroceras* (K.Schum.) Pierre ex beille," *Global Journal of Pure and Applied Sciences,* 6 (1), 83–87.

Sofowara, A. (1993). Medicinal Plants and Traditional Medicine in Africa, Spectrum Books, Ibadan, Nigeria.

Soladoye, M. O., Chukwuma, E. C. & Owa, F. P. (2012). An "Avalanche" of plant species for the traditional cure of diabetes mellitus in South-Western Nigeria. *Journal of Natural Product and Plant Resources,* 2, 60–72.

Sonibare, M. A., Moody, J. O. & Adesanya, E. O. (2009). Use of medicinal plants for the treatment of measles in Nigeria. *Journal of Ethnopharmacology,* 122, 268–272.

Stephano, H., Mlozi1 Musa Chacha & Regina Peter (2017). Anti-mycobaterial and Antibacterial Activities of Extracts from *Caesalpinia bonduc* (L.). *American Journal of Research Communication,* 5(2), 27-36.

Suliman, T. Y. & Fieldman, B. F. (1989). Pathogenesis and etiology of anaemia in trypanosomiasis with special reference to *T. brucei and T. evansi. Protozoology Abstracts*, 13, 37-45.

Tabel, H., Wei, G. & Shi, M. (2008). T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunology Revolution,* 225, 128-39.

Tummin, K. M. C. (1930). Chemical Examination of Seeds of *Caesalpinia bonduc*ella. *Journal of Indian Chemical Society,* 7:207.

Uche, U. E., & Jones, T. W. (1992). Pathology of experimental *Trypanosoma evansi* infection in rabbits. *Journal of Comparative Pathology,* 106**,** 299–309.

Valiathan, M. (1998). Healing plants. *Current science,* 75: 1122-1127.

Vanhamme, L., Pays, E., McCulloch, R. & Barry, J. D. (2001). An update on antigenic variation in African trypanosomes. *Trends Parasitology*, 17:338–343.

Vincendeau, P. & Bouteille, B. (2006). Immunology and immunopathology of African trypanosomiasis. *An Academic Branch of science,* 78:645-65.

Weiss, C. R., Moideen, S. V. K., Croft, S. L. & Houghton, P. J. (2000). Activity of extracts and isolated naphthoquinones from Kigelia pinnata against Plasmodium falciparum. *Journal of Natural Product,* 63, 1306-1309.

World Health Organization (1986). Epidemiology and Control of African Trypanosomiasis. Technical Report Series No. 739., Geneva.

World Health Organization (2015). "Neglected Tropical Diseases." http://www.who.int/neglected_diseases/diseases/en/.

World Health Organization (2016a). "Leishmaniasis." http://www.who.int/mediacentre/factsheets/fs375/en/.

World Health Organization (2016b). "Trypanosomiasis, Human African (Sleeping Sickness)." Media Center, WHO Fact Sheet. http://www.who.int/mediacentre/factsheets/fs259/en/Trypanosomiasis.

Zofou, D., Kengne, A. B., Tene, M., Ngemenya, M. N., Tane, P. & Titanji, V. P. (2011). In vitro anti-plasmodial activity and cytotoxicity of crude extracts and compounds from the stem bark of *Kigelia africana* (Lam.) Benth (*Bignoniaceae*). *Parasitology Resources*, 108(6), 1383-90.