EFFICACY OF THE AQUEOUS ROOT EXTRACT OF *PHYLLANTHUS MUELLERIANUS* IN ALLEVIATING ANEMIA IN RATS

By

Gershom B. Lwanga

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DECLARATION

I, Gershom B. Lwanga hereby declare that this dissertation is my own work. To the best of my knowledge, the work has not been submitted before for any degree or examination in any other university. All sources used have been acknowledged accordingly.

Date……

Signature ……

Gershom B. Lwanga

CERTIFICATE OF APPROVAL

This dissertation of Gershom B. Lwanga has been approved as fulfilling the requirements or partial fulfillment of the requirements for the award of Master of Science in Biochemistry by the University of Zambia.

EXAMINERS

Examiner 1.

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Examiner 3.

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ABSTRACT

Phyllanthus muellerianus (*P. muellerianus*) is classified under the family Phyllanthaceae consisting of approximately 1,000 species which are widely distributed in Africa, Asia, America and Australia. It is a monoecious, glabrous, straggling shrub or small tree of up to 12 meters tall. It occurs in riverine forest and wooded grasslands on deep and well-drained soils. Different parts of *P. muellerianus* are used for treatment of a number of diseases in traditional medicine context. In some parts of Zambia, it is used to treat anemia, but its potential has not been scientifically established. Therefore, this study aimed at evaluating the effect of the aqueous root extract of *P. muellerianus* on the hematological parameters of male albino rats and to determine its phytochemical profile.

Thirty-six male albino rats in six groups were used for this study. The groups comprised 100 mg/kg, 200 mg/kg, and 400 mg/kg plant extract, a group on ranferon (drug used to treat anemia), a normal (non-anemic) group and a control (anemic) group. Anemia, induced through repeated bleeding of the rats, was defined as hemoglobin (Hb) < 12 g/dL and the duration of the study was 22 days. The anti-anemic potential of the plant was determined by comparing its effect on the hematological parameters of rats on treatment to that of the control group. Blood samples were collected 3 times for hematological analysis; being at the baseline of the study, after inducing anemia and after treatment. The blood parameters studied include Hb, packed cell volume (PCV), film comment (anisocytosis, poikilocytosis and chromasia), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH) and mean cell volume (MCV). The phytochemical profile of the root decoction was determined by standard procedures. The results were analysed using SPSS followed by dunnett's test at a significance of $P<0.05$.

After medication, rats on 400 mg/kg dosage showed the greatest increase in the mean values for Hb, PCV and RBC count of 23.1 %, 23.0 % and 22.2 % respectively, when compared to the anemic control group ($P<0.05$). The preliminary phytochemical screening of the root extract of *P. muellerianus* revealed positive results for alkaloids, flavonoids, saponins, glycosides, steroids, triterpenoids and tannins.

The aqueous root extract of *P. muellerianus* was efficient against anemia in experimental groups in a dose dependent manner and the 400 mg/kg dosage was useful. The phytochemical compositions especially alkaloids, flavonoids and saponins seem to be responsible for its hematopoietic properties. Thus, the root decoction of the plant is useful in alleviating anemia and the results lend credence to its use in traditional medicine in the management of anemia. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compound/s that is/are responsible for its medicinal value.

Key words: *Anemia, Albino rats, Phyllanthus muellerianus, Phytochemicals, Traditional medicine.*

DEDICATION

This dissertation is dedicated to my loving and hard working parents, Mr. and Mrs. Lwanga, who are always doing everything possible to have their children, go to school. In addition, I dedicate this dissertation to my supportive wife Nelly Wamundila and Children, Namukoko and Silas, for their patience and understanding. With that great support, I was able to attain University Education. May the Almighty God bless you all.

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ACRONYMS / ABBREVIATIONS

B. wt- Body weight

β - Beta

DFID- Department for International Development

EDTA - Ethylene diamine tetra acetate

Etc- Et cetera

Fl - Femto liter

GBD- Global Burden of Disease

GDP- Gross Domestic Product

g/dL- Grams per deciliter

Hb- Hemoglobin

Hct- Hematocrit

HIV- Human Immunodeficiency Virus

LD- Lethal Dose

µl- Micro liter

^{o}C- Degree Celsius

MCHC- Mean Corpuscular Hemoglobin Concentration

MCV- Mean Corpuscular Volume

NFNC- National Food and Nutrition Commission

NHSP- National Health Strategic Plan

Pg- Pico gram

RBCs - Red Blood Cells

ROS - Reactive Oxygen Species

USAID- United States Agency for International Development

CHAPTER 1
INTRODUCTION

1.1 Background information

Anemia (from the Greek word, anaimia, which means 'lack of blood') is a blood condition in which there are too few red blood cells (RBCs) also called erythrocytes and are deficient in hemoglobin (Hb), the iron containing protein that carries oxygen (O_2). Anemia is a common blood disorder that affects people of all ethnicity and ages; although people at greater risk are the elderly, young women of child bearing age and infants (WHO, 2008, Johnson-Wimbley and Graham, 2011).

It is a major contributing factor to maternal morbidity and mortality (Saeed et al., 1996) and is among the top 10 causes of morbidity and mortality in Zambia (NHSP, 2011). The prevalence of anemia in Zambia is 46 %, which is a severe public health problem based on the World Health Organization (WHO) standards (WHO, 2001, MOST, 2003). Such high prevalence rate is expected on account that the diet of majority Zambians is mainly composed of cereals (maize) and starchy roots with very little micronutrient-dense foods such as animal products, fruits and vegetables (USAID, 2014, NFNC, 2014).

The causes of anemia are patho-physiologically diverse and multi-factorial. Thus, there are more than 400 types of anemia many of which are rare but in most cases, the oxygen carrying capacity of blood decreases due to reduction in the number of RBCs or the Hb concentration in RBCs (Mathew et al 2013). Some types are mild while others are severe or even life threatening if not treated. Among the common types of anemia are;

1. Pernicious anemia, a chronic ailment caused by; a genetic disorder, Crohn's disease, or surgery that removes part of the stomach leading to vitamin B_{12} deficiency.
2. Hemorrhagic anemia caused by bleeding (externally and internally due to; injuries, heavy menstrual periods, child birth, major surgical procedures, gastritis, ulcers, hemorrhoids etc.), resulting into a significant decrease in RBCs.
3. Hemolytic anemia occurs when dying RBCs outpace the bone marrow's production in an individual. It can be caused by factors that are extrinsic (destruction of RBCs by the spleen, auto-immune system, infection etc.) or intrinsic (produced RBCs are defective).

4. Sickle cell anemia is an inherited condition in which hemoglobin is abnormal and causes the RBCs to be rigid and clog (sickle-shaped RBCs). These irregularly shaped RBCs die prematurely resulting in a chronic shortage of RBCs.
5. Aplastic anemia occurs because of diseased or injured bone marrow. Bone marrow damage may result from a viral infection, cancer, radiation or exposure to toxic chemicals, including arsenic, benzene and some antibiotics and cancer medications; and
6. Iron deficiency anemia(IDA), occurs when there is an inadequate iron intake, decreased iron absorption, increased iron demand and increased iron losses (NIH, 2011, Mathew et al., 2013, Murray et al., 2003).

Although the prevalence of IDA has somehow declined globally, iron deficiency continues to be the top ranking cause of anemia worldwide. The terms anemia, iron deficiency anemia and iron deficiency are used interchangeably because anemia is the most common indicator used to screen for iron deficiency (WHO, 2008, Mathew et al., 2013).Deficiency develops in stages in humans and in rats. In the first stage, iron requirement exceeds intake, causing progressive depletion of iron stores in the bone marrow. As the stores decrease, absorption of dietary iron increases in compensation. During the later stages, deficiency impairs the synthesis of RBCs ultimately causing anemia (Queiroz and Torres, 2000, Harris, 2007).

Findings on physical examination of patients with anemia may include the following ;- pallor of the mucous membrane - a non-specific finding, koilonychias, angular stomatitis, a glossy tongue with atrophy of the lingual papillae, splenomegaly - in severe, persistent untreated cases and pseudo tumor cerebri - a rare finding in severe cases (Provan et al., 2004).In rodents, symptoms include; rapid or labored respiration, anorexia, immobility, abnormal appearance or posture periocular and nasal porphyrin discharge (National Research Council (US) Committee., 2011).In some rural communities of Zambia, Anemia is diagnosed as weakness, paleness, loss of appetite and fatigue.

Iron is an essential trace element crucial to biological functions, cell proliferation, energy production, and synthesis of molecules in the body such as Hb and deoxyribonucleic acid (DNA). It is distributed in active metabolic and storage pools. Total body iron is about 3.5 grams (g) in healthy men and 2.5 g in women; the difference relates to women's smaller body size, lower androgen levels, and dearth of stored iron because of iron loss due to menses and pregnancy (Johnson-Wimbley and Graham, 2011, Gupta, 2014). Hb synthesis requires the

coordinated production of heme and globin. Heme is the prosthetic group that mediates reversible binding of oxygen by Hb while globin is the protein that surrounds and protects the heme molecule. Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell (Figure 1.1). In the final step, the enzyme ferrochelatase (heme synthase) inserts ferrous iron (Fe^{2+}) into the ring structure of protoporphyrin IX to produce heme. Two distinct globin chains (each with its individual heme molecule) combine to form Hb. Hb is composed of four polypeptide chain, each of which contains one iron ion. The iron is the site of oxygen binding; each iron can bind one O_2 molecule, thus each Hb molecule is capable of binding a total to four O_2 molecules (Gupta, 2014, Johnson-Wimbley and Graham, 2011). The iron atom and the attached protein such as chlorophyll and cytochrome, modify the wavelength of the absorption and give Hb its characteristic colour. Deranged production of heme produces a variety of anemias. Iron deficiency impairs heme synthesis thereby producing anemia. A number of drugs and toxins directly inhibit heme production by interfering with enzymes involved in heme biosynthesis (Gupta, 2014).

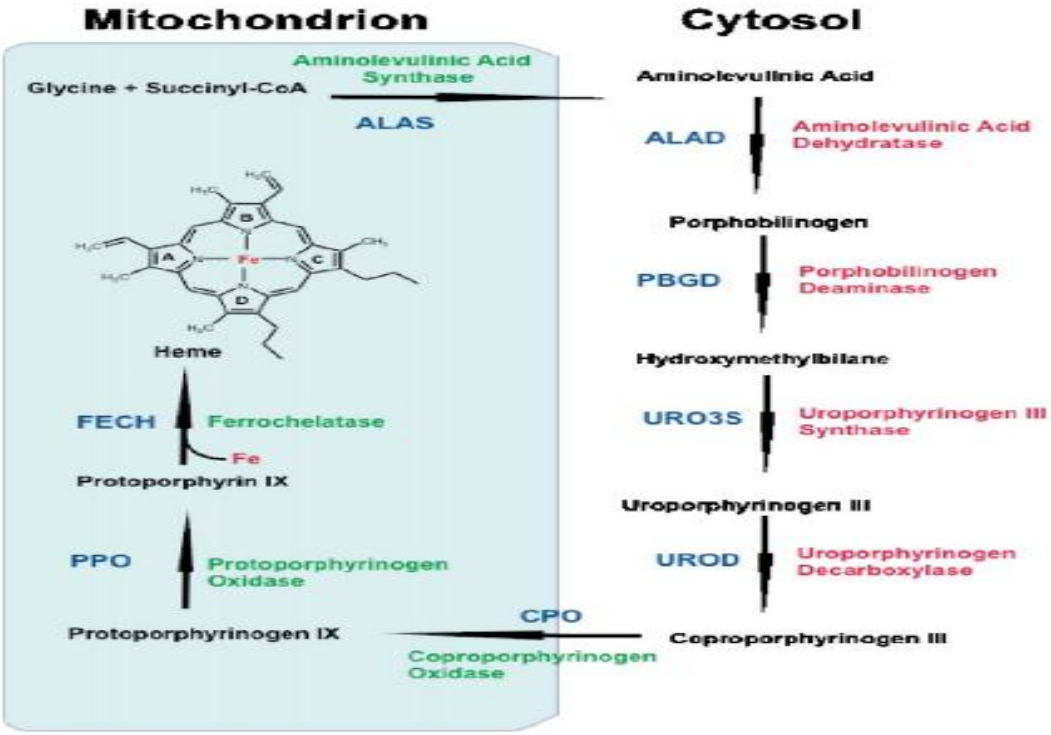

Figure 1.1Heme synthesis

Anemia associated with a serious disease is treated by treating the underlying disorder. In some cases, when symptoms persist or worsen, additional medications that boost RBCs may be necessary to avoid life threatening conditions and improve quality of life. For instance, Physicians prescribe iron pills or iron supplements and iron fortifications to treat IDA, synthetically manufactured erythropoietin that stimulates the production and growth of RBCs, blood transfusions in case of massive blood loss and removal of the spleen to prevent removal of RBCs from blood circulation or destroyed too rapidly. Nutritionists also recommend the following among others as remedies; - plant products such as palm tree fruits, legumes, groundnuts, tomatoes and spinach as well as animal products such as liver and red meat (Goddard et al., 2000, WHO, 2011, Osungbade and Oladunjoye, 2012, Anemia, 2015) .

In Africa and most Asian countries, treatment of anemia involves phytotherapy (medical treatment based on plants, taken either through self-medication or prescribed by traditional healers). These plants include; - *Khaya senegalensis, Imperata cylindrica, Hoslundia opposita, Stylosanthes erecta, Waltheria indica, Justicia secunda, Thalia geniculata, Milicia excelsa, Amaranthus spinosus, Amaranthus hybridas* and *Angelica sinensis* (Koné et al., 2012).

In this study, the potential of the root extract of *Phyllanthus muellerianus* to alleviate anemia was examined in rats.

Phyllanthus muellerianus (P. muellerianus) local names-*umupetwalupe (Ichibemba) and Mulembalemba (Kikaonde),* was formerly classified as one of the flowering trees of the spurge family (Euphorbiaceae). However, a recent re-classification has categorized Phyllanthus under the family Phyllanthaceae consisting of approximately 1,000 species which are widely distributed in Africa, Asia, America and Australia. It is a monocot, glabrous, straggling or climbing shrub or small tree of up to 12 meters tall with branches spreading or pendulous, angular, reddish tingled branchlets 15-20 cm long with several short auxiliary shoots. Leaves alternate, distichously along lateral twigs, simple, glabrous with flowers in clusters having 2-3 male flowers and 1 female flower. In each cluster (unisexual), fruits are like globoid capsules measuring 3-4 mm in diameter, usually smooth green becoming red and later black. It occurs in riverine forest and wooded grasslands on deep and well-drained soils from sea level up to 1600 meters altitude. Harvested plant parts are used whilst they are fresh or are dried, as a whole, or are powdered for future use. Young shoots

are harvested at the beginning of the rainy season; leaves, stem bark and roots can be harvested throughout the year, although it is easier to harvest the roots during the rainy season. *P. muellerianus* flowers at the end of the dry season, shortly after new leaves have formed. It has many medicinal uses especially to treat intestinal problems, body pain and as an antiseptic (Schmelzer et al., 2008).

In Zambia, the root decoction is prescribed by traditional healers to treat anemia and intestinal problems, leaves are used as antiseptics, tonic and as fodder. Woods are used as rafters and for other construction work. They are also used to make fish traps and basketry. Further, the stem and stem barks are used as a source of black and brown dyes (Simute et al 1998).

1.2 Problem Statement

Anemia is a global public health problem affecting more than 1.6 billion people worldwide. By 2010, it was responsible for 68.4 million Years of Life lived with Disability (YLD), 841,000 deaths and economic median annual loss of about $ 16.78 million or 4 % of Gross Domestic Product (GDP) of most developing countries. The most affected continents are Asia and Africa which carry 71 % of the global mortality burden besides 65 % of YLD (Stoltzfus, 2015, Ross et al., 1998, WHO, 2008, Shaw and Friedman, 2011, Stevens et al., 2013, Nicholus et al., 2013).

Anemia ranked number 9 among the 26 risk factors in the global burden of disease 2000 (Mathers et al., 2002, Queiroz and Torres, 2000, WHO, 2011). It retards physical as well as mental growth in children if it occurs early in life and it contributes to cognitive impairment (Lozoff et al., 2006, Lozoff, 2007, WHO, 2011).

Anemic mothers are at risk of giving birth to underweight babies most of whom are born prematurely and these babies have slim chances of surviving. Children with anemia: have a short attention span, exhibit greater unhappiness and fearfulness. They have lower motor scores which do not improve over time despite correction of anemia through therapy, their measure of intelligence quotient (IQ) is 1.73 points lower for each 10 g/L decrease in Hb and perform poorly in mathematics tests (Lozoff, 2007, Lozoff and Georgieff, 2006).

Anemic patients may present the following: loss of stamina, shortness of breath, dizziness, cold intolerance, reduced risk to infection, fatigue, an abnormal craving to eat substances like

dirt, paint, ice cold vegetables etc.(Haas and Brownlie, 2001, Southgate, 2002 , Lozoff, 2007, Pasricha et al., 2010)**.**

The average prevalence of anemia in Zambia is 46 % hence; anemia is a severe public health problem in Zambia going by the standards set by WHO as shown in Table 1.1. This implies that there is a great loss of man hours of healthy adults who find themselves off work to nurse or look after anemic patients (CSO, 2009, NFNC, 2014).

Table 1.1 Classification of anemia as a problem of public health significance

Prevalence of anemia (%)	Category of public health significance
<4.9	No public health problem
5.0-19.9	Mild public health problem
20.0-39.9	Moderate public health problem
>40.0	Severe public health problem

Source: WHO, 2001

Overall the prevention and successful treatment of anemia remains woefully insufficient worldwide especially among underprivileged women and children (Miller, 2013).The contributing factors to this scenario in Zambia are that;- despite efforts by the Government and other cooperating partners to make modern health services and medicines accessible, available and acceptable to all people, most of the health institutions are far from communities they serve.

Further, effective interventions are not carried out to scale in Zambia because; - the road network linking some of these communities to health facilities are mainly inaccessible especially during rain seasons and there is inadequate qualified human resource. In addition, there is poor coordination with other sectors, inadequate investment in the health sector, high disease burden and inadequate emergency facilities (DFID, 2011,NHSP, 2011).

1.3 Study Justification

In 2011, the National Food and Nutrition Commission (NFNC) estimated that, reduction of maternal anemia by one third, stunting by 1 % per year and elimination of iodine deficiency would increase Zambia's productivity by $ 1.5 billion over a period of 10 years. Further, productivity gains at a micro level from improving nutrition are estimated at 10 % of lifetime earnings and a macro level GDP gain of as high as 2-3 % (NFNC, 2014). However, there are

no significant improvements in levels of under nutrition hence nutrition related problems (such as anemia) have continued.

Correction of anemia has been found to improve many other conditions such as; chronic kidney disease, inflammatory bowel disease (IBD), cancer, athletic disturbances, aortic stenosis, idiopathic pulmonary hypertension, acute myocardial infarction etc. (Silverberg et al., 2015). That is why basic, clinical, epidemiologic and operational research is encouraged to maximize anemia reduction strategies in developing countries in a manner that encompasses short, medium and long term interventions (Pasricha et al., 2013). Furthermore, WHO encourages an integrated package of interventions to be administered proportionate to the level of prevalence of anemia in order to achieve positive results in preventing anemia (WHO, 2008).

The research was in support of the goals of WHO 2014-2023 strategic plan which are; - to harness the potential of phytotherapy to health, universal health coverage, wellness, and people centered health care. Also to promote safe, effective use of phytotherapy through regulation, research and integration of phytotherapy products, practices and practitioners into the health system (Qi, 2015).

P. muellerianus is part of the indigenous knowledge used to treat anemia in the Northern part of Zambia. However, its efficacy had not been proved scientifically. Therefore, the purpose of this study was to examine the efficacy of *P. muellerianus* in alleviating anemia in male rats.

P. muellerianus is locally available, cheap and easy to access in Zambia hence the study was biased towards finding medical solutions which are coming from our local flora (Burkill, 1994).

The study also aimed at adding wealth to the body of knowledge that already exist regarding treatment of anemia, thereby contribute to reducing the disease burden and subsequently save resources directed to health care, disease treatment and other problems associated with anemia.

1.4 Research Question

Is the aqueous root extract of *P. muellerianus* useful in alleviating anemia?

1.5 Study Objectives

1.5.1 General objective

To determine the phytochemicals and the effect of *P. muellerianus* aqueous root extract on the hematological parameters in rats induced with anemia.

1.5.2 Specific objectives

The specific objectives of this study were to;-

1. Determine the qualitative phytochemical composition of the root extract,
2. Determine the effect of the aqueous root extract of *P. muellerianus* on the hematological parameters of rats, and
3. Establish the right dosage in rats by administering various concentrations of the plant extract and the known anti-anemic drug (ranferon) to some anemic groups, and then compare their varied effects on the hematological parameters to the control group.

CHAPTER 2
LITERATURE REVIEW

2.1 Causes of Anemia

Anemia results from a wide variety of causes that could be isolated, but more often co-exist. These causes can be acquired, inherited or are sometimes unknown (Camaschella, 2015). Meanwhile, there are 3 primary causes of anemia and these are;-

1. **Reduced production of Red Blood Cells** due to; -inadequate amounts of certain micronutrients like iron, copper and riboflavin, vitamins A and B_{12}, folic acid as well as the hormone erythropoietin, strict vegetarian diets, mal-absorption and a cereal based diet which decreases iron bioavailability and chronic illnesses such as cancer, kidney disease, diabetes mellitus and IBD (Murray et al 2003).
2. **Destruction of Red Blood Cells** because of an enlarged spleen that may cause abnormal destruction of RBCs, a malfunction of the immune system in which antibodies attach to RBCs marking them for destruction, and some genetic conditions such as thalassemia, which cause defects in the structure or function of RBCs.
3. **Excessive bleeding** due to;- injury, surgery, menstruation, parasitic infections such as ascariasis, schistosomiasis and ancylostomiasis, acute and chronic diseases including malaria, cancer, tuberculosis and Acquired Immune Deficiency Syndrome (AIDS) and in some cases, excessive bleeding may occur over time, such as from bleeding ulcers or tumors of the intestinal tract (Spivak, 1994, WHO, 2008, Pasricha et al., 2010).

There are contributing causes of anemia that include inadequate knowledge about the problem of anemia, environmental factors, lack of access to services, poverty / malnutrition and blood donations (Camaschella, 2015). In addition;

1. Obesity may be associated with mild iron deficiency because of subclinical inflammation, increased serum levels of hepcidin and decreased iron absorption,
2. Some studies report a high prevalence of iron deficiency (30 %-50 %) in patients with congestive heart failure probably because of impaired iron absorption and inflammation, increased serum levels of hepcidin have been reported in the early stages of disease but not during disease progression (Goddard et al, 2000), and

3. Uses of proton pump inhibitors, anti-coagulants as well as non-steroidal anti-inflammatory drugs impair iron absorption.

In New Zealand, these additional causes resulted in anemia cases exceeding 20 % of the total anemic population (Goddard et al., 2000, Camaschella, 2015).

2.2 Treatment options for Anemia

Anemic patients receive iron supplementation. The administration of oral iron is a convenient, inexpensive and effective means of treating stable patients. Three to six months of treatment are required for the repletion of iron stores and normalization of serum ferritin levels. However, therapy with oral supplements is limited by side effects, which include nausea, diarrhea, dark colored stools, vomiting, constipation and metallic taste. These side effects are frequent and although not severe, are often worrisome to patients. In addition, a study done in Lusaka, Zambia showed that iron supplementation impaired the integrity of the small intestine mucosa of Zambian Primary School Children, while Morais and Lifschitz (2004) showed that a high iron diet increased the intestinal permeability in rats (Nchito et al., 2006, Camaschella, 2015).

Routine supplementation of iron in areas where malaria is endemic should be taken cautiously because it may reverse the potentially protective effects of iron deficiency or increase the susceptibility to co-infections. In Malawi, Calis and others found an inverse association between iron deficiency and bacteremia and reported that iron deficiency protects against infection by creating an un-favorable environment for bacteria growth (Calis et al., 2008). Results of a randomised controlled trial in Tanzanian children showed an increase in malarial episodes in iron deficient children receiving micronutrients such as zinc (Veenemans et al., 2011). Another large randomised controlled trial identified an increase in malaria and infection-related morbidity and mortality in Tanzanian Children randomised to iron and folic acid (Sazawal et al., 2006).

In vitro studies have also shown that *Plasmodium falciparum*, the malaria parasite, is less efficient in infecting iron deficient erythrocytes than in infecting iron-replete erythrocytes, a protection that is reversed with iron supplementation. Thus, some studies support the view that measurement of hepcidin levels could help to determine the best time (e.g., the end of malaria season) to provide iron supplements in these regions. The benefit of treating iron deficiency before the development of anemia remains uncertain (Camaschella, 2015, Calis et

al., 2008). Therefore, a need exists to clarify the net benefits and risks of iron supplementation.

Patients with anemia that causes cardiovascular symptoms, such as heart failure or angina, receive RBC transfusions. This approach rapidly corrects not only hypoxia but also iron deficiency, since one unit of packed red cells provides approximately 200 mg of iron. Some concern persists with regard to the long term biological effects of iron and its effects on the generation of reactive oxygen species (ROS) as well as patient susceptibility to infections. A few studies show that the administration of intravenous iron improves fatigue in women without anemia whose ferritin levels are in the iron deficient range. However, intravenous iron formulations have transient side effects including, nausea, abdominal pains, malaise, flushing, myalgia, arthralgia, back and chest pain, vomiting and headache. Thus, others recommend that, intravenous iron should be avoided in the first trimester of pregnancy because of lack of data on safety; however, it has an acceptable profile when used later in pregnancy (Lingxia et al., 2008, Silverberg et al., 2015, Camaschella, 2015)**.**

Erythropoiesis-stimulating agents are also used in selected patients with low-risk myeodysplastic syndrome and in patients with cancer who are receiving chemotherapy. In these circumstances, iron supplementation is usually limited to patients with concomitant iron deficiency or to those in whom there is no response to erythropoiesis stimulating agents; intravenous iron is preferred when high hepcidin levels create a condition that is refractory to supplementation with oral iron. The way in which iron enhances the effect of erythropoiesis stimulating agents is unclear (Johnson-Wimbley and Graham, 2011). One hypothesis suggest that increased iron in macrophages leads to the over expression of ferroportin by means of the iron responsive element-iron- regulatory protein system, which enhances the mobilization of iron for use in erythropoiesis. There are no markers that can be used to predict which patients will or will not have a response to oral iron therapy. A pilot study showed that measurement of serum hepcidin levels could help to identify patients in whom a response to oral iron is probable (those with low hepcidin levels) and those in whom it is not probable (those with elevated hepcidin levels). However, hepcidin tests are not routinely available for clinical use (Camaschella, 2015, Johnson-Wimbley and Graham, 2011).

Food facilitating factors, which include; - ascorbic acid, all kinds of meat, amino acids such as lysine, cysteine and histidine, citric and succinic acids, as well as sugars such as fructose,

determine the absorption of iron. Other determinants are food inhibitors like:- phytates found in cereals, tannins found in black tea, coffee and some soft drinks, calcium and phosphate salts found in milk protein sources, zinc and manganese supplements, peppermint and chamomile, daily foods and anti-acids which reduce stomach acid and certain antibiotics (e.g. tetracycline). Conditions such as bariatric surgery, decreased gastrin, endoscopy for evaluation of anemia and celiac disease can also inhibit iron absorption (Queiroz and Torres, 2000, U.S.DA, 2010).

To treat the diet related anemia, dietary iron intake and higher supplement doses are required primarily, while increasing iron consumption and optimizing absorption by minimizing inhibitors and maximizing enhancers through diet are encouraged for secondary prevention of iron deficiency. In this regard, the recommended iron dietary allowances (RDAs) for non-vegetarians are as shown in Table 2.1.

Table 2.1 Recommended Dietary Allowance (RDA) for iron

Age	**Male**	**Female**	**Pregnancy**	**Lactation**
Birth to 6 months	0.27 mg	0.27 mg	N/A	N/A
7–12 months	11 mg	11 mg	N/A	N/A
1–3 years	7 mg	7 mg	N/A	N/A
4–8 years	10 mg	10 mg	N/A	N/A
9–13 years	8 mg	8 mg	N/A	N/A
14–18 years	11 mg	15 mg	27 mg	10 mg
19–50 years	8 mg	18 mg	27 mg	9 mg
51+ years	8 mg	8 mg	N/A	N/A

Source: US.D.A 2010

The RDAs for vegetarians are 1.8 times higher than for people who eat meat (Queiroz and Torres, 2000, U.S.DA, 2010).

In Brazil and many other countries, treating anemia involves use of iron salts (sulfates, fumarate, gluconate, succinate and citrate) which are inexpensive and get absorbed quickly. However, they produce side effects such as nausea, vomiting, epigastric pain, intestinal obstipation, diarrhea, dark feces and in the long run, the development of dark spots on teeth (Queiroz and Torres, 2000, U.S. Department of Agriculture, 2010).

Although various treatment options for anemia such as the ones discussed previously, are available in most hospitals and clinics, most people in Latin America, Asia, Africa and Zambia in particular do not have access to them and as such, they depend on traditional medicine to treat anemia (WHO, 2003). Natural products many of which are used in traditional medicine context provide nearly one quarter of all modern medicines. In Africa, up to 80 % of the population uses traditional medicine for primary health care and consume 50,000 tons of more than 4,000 plant species annually. However, one of the problems of using plant materials as medicines is that in many cases, no definite doses are prescribed, often resulting in overdoses (WHO, 2003, Burkill, 1994).

Plants play an important role in human life, primarily, as a source of food and medicine. Importance of plants in drug discovery is growing due to vast diversity of the secondary metabolites that possess varied biological activities and act as main source of molecules leading to the discovery of new, effective, and safer drugs. For example, many drugs today are of plant origin and pharmacological history is replete with examples such as quinine, aspirin, picrotoxin, reserpine, antitumour drugs (e.g. vincristine and taxol) and asthma therapies (e.g. cromoglycate) etc., while many of the synthetic drugs are fashioned after natural plant products (Shami, and Aman, 2016., Trease and Evans, 2002, Sofowora, 1993). Other drugs derived from plants are as shown in Table 2.2.

Table 2.2 Drugs derived from plants

Drug	**Action or clinical use**	**Plant source**
Quinine	Anti-malarial	*Cinchona ledgeriana*
Pilocarpine	Parasympathomimetic	*Pilocarpus jaborandi*
Morphine	Analgesic	*Papaver somniferum*
Emetine	Emetic, amoebicide	*Cephaelis ipecacuanha*
Digitoxin	Cardiogenic	*Digitalis purpurea*
Colchicine	Anti-tumour, anti-gout agent	*Colchicum autumnale*
Atropine	Anticholinergic	*Atropa belladona*

Phytochemicals are responsible for medicinal activity of plants. Preliminary screening of phytochemicals is therefore, a valuable step in the detection of the bioactive principles present in medicinal plants. Phytochemicals are non-nutritive plant chemicals that have

protective or disease preventive properties. The plants produce these chemicals to protect themselves, but recent research demonstrates that they possess enormous physiological activities in humans and animals and can therefore, protect them against various diseases. Their known functions include cancer prevention, anti-bacterial, anti-fungal, anti-oxidative, hormonal action, enzyme stimulation, etc. They are divided into 2 groups, namely: primary and secondary metabolites, based on the function in plant metabolism. The primary metabolites are of major importance to plants, while the secondary metabolites are of medicinal value to man. The major constituents of primary phytochemicals are carbohydrates, amino acids, proteins, and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids,glycosides, flavonoids, and tannins (Trease and Evance., 2002.,Sofowora A., 1993).

2.3 Metabolites and uses of *P. muellerianus*

Plants such as *P. muellerianus* used in phytotherapy contain a wide range of substances that are used to treat different diseases and pharmaceutical industries use them as a source of bioactive agents in the preparation of synthetic medicines. A number of studies have confirmed and extended both the medicinal uses of *P. muellerianus* and different secondary metabolites that render it with medicinal properties. Screening of the leaves and stem bark showed the presence of hydrolysable tannins (ellagitannins), flavonoids, saponins, steroids, alkaloids and anthraquinones. The triterpenoids, 22β-hydroxyfriedel-ene and 1β, 22β-dihydroxyfriedelin have also been isolated from the stem bark. The dry fruits of *P. muellerianus* contained per gram: 77 mg water, 9.7 mg protein, 4.3 mg fat, 62.7 mg sugar, 21 mg fibers, 500 mg calcium, 200 mg phosphorus and 15 mg iron (Okeniyi et al., 2014, Agyare et al., 2010, Ayegba et al., 2015, Adesida et al., 1972, Schmelzer et al., 2008, Hamill et al., 2003).It is used in Africa as outlined in this report.

In West Africa, leaf sap is applied as eye drops to treat eye infections and as wound dressing. In Sierra Leone, a leaf decoction is used to treat constipation. In Côte d'Ivoire, the leaves are eaten together with young leaves of *Funtumia elastica* (Preuss), to improve male fertility. Leaves boiled with palm fruit are given to women after delivery as a general tonic. In Côte d'Ivoire and Burkina Faso, a bark decoction is taken to treat sore throats, cough, pneumonia and enlarged glands. Pulped leafy twigs are rubbed on the body to treat paralysis. In Ghana, an infusion of the young shoots is taken to treat severe dysentery. In Nigeria, a twig and root

decoction is taken to treat jaundice and urethral discharges. Leaves are sometimes cooked with food or in soup as a seasoning. In Cameroon, a maceration of the leaves and roots is used to wash the body to treat rash with fever in children. The bark is sometimes added to palm wine to render it strongly intoxicating. In Gabon, roasted powdered twigs are eaten with plant ash to treat dysmenorrhoea. In Congo, powdered roasted roots with palm oil are taken to treat stomach problems and as an anti-emetic (Schmelzer et al., 2008, Koné et al., 2012, Ziblim et al., 2013, Onocha et al., 2003).

In East Africa, the brown dye from the bark is used to dye mats and fishing lines. From the whole plant, a black dye is used to colour fibers. In Tanzania, roots are pounded in water and the liquid is drunk to treat diarrhea. Boiled roots are also applied as enema to treat stomachache. A root decoction is taken to treat hard abscesses; powdered dried roots and stem bark are sprinkled on wounds as a dressing. (Neuwinger, 2000, Radcliffe-Smith, 1987).

In the Central African Republic, fresh root bark is drunk as an aphrodisiac. In DR Congo, a leaf decoction is taken to treat anemia and as a mouthwash to treat toothache. A leaf extract is used as a bath and a vapour bath to treat venereal diseases. Dried bark powder is sniffed to treat colds and sinusitis. A root bark decoction is applied to swellings and is drunk to treat gonorrhea. Stem ash is applied to scarifications to treat rheumatism and intercostal pain (Schmelzer et al., 2008).

In Zambia, the woods are used as rafters and for other construction work. They are also used to make fish traps and basketry while the leaves are used as fodder. Further, the plant is used as a source of black and brown dyes (Schmelzer et al., 2008, Simute et al., 1998).

CHAPTER 3
MATERIALS AND METHODS

3.1 Ethical Issues/Clearance

The University of Zambia Biomedical Research Ethics Committee (UNZABREC) granted the ethical clearance for animal experimentation on REF. NO. 005-09-16.The research was anchored by the Department of Disease Control at the School of Veterinary Medicine-UNZA. All laboratory work was done according to the Guidelines for Care and Use of Laboratory animals in Biomedical Research (National Research Council (US) Committee., 2011), which included;

1. All rats were observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs,
2. The rats were maintained under standard animal house conditions (temperature: 25–30°C, photoperiod: 12-hours light and dark cycle, and relative humidity: 55–60 %) with regular access to food and distilled water,
3. The researcher and his assistants underwent appropriate training to ensure; asepsis, appropriate use of instruments, effective hemostasis etc.,
4. After recovery from anesthesia, animals were monitored for signs of behavioral changes arising from possible infections, and swelling of the eye.

In addition, the rats were euthanized in diethyl ether at the end of the study according to the American Veterinary Medicine Association guidelines (AVMA, 2013).

3.2 Plant identification

The plant was identified by Mr. Michael Nang'alelwa, a botanist /conservation ecologist (National Heritage Conservation Commission, Northwestern region in Zambia) and authenticated at Forest Research Herbarium in Kitwe.

3.3 Collection and preparation of the aqueous root extract of the plant

The roots of the plant were harvested in October 2016, for preparation of the extract from Kaunda Square area of Lusaka District in Zambia. They were thoroughly washed to remove dirt and other debris (soil). One kilogram of the roots was weighed (weight / weight) and boiled in 2 litres of distilled water until the colour of the water turned dark brown. The resulting solution was allowed to cool then sieved to remove non soluble plant matter and

finally filtered through Whatman filter paper number 4. The mixture was boiled to dryness on a heating mantle and the resulting brittle powder was weighed and kept at 4^{o}C until further use. Various Concentrations of the extract were made by dissolving the appropriate quantity of the solid extract in distilled water to make a solution on a mass by volume basis.

The percentage yield was calculated using the equation;-

$$\frac{\mathrm{M2}-\mathrm{M1}}{\mathrm{M}} X\ 100\ \%$$

Where: $M_2$is the mass of the extract and the beaker,

M_1 is the mass of the beaker alone, and

M is the mass of the initial dried sample**.**

3.4 Preliminary Qualitative Phytochemical Screening

The preliminary phytochemical screening of the plant extract was carried out at the Department of Chemistry, University of Zambia. The steps taken for the phytochemical screening of saponins, tannins, flavonoids, and other phytochemicals were in line with the procedures outlined in Trease and Evans (Trease G. E., 2002) and Sofowora (Sofowora A., 1993).

3.4.1 Test for tannins

About 2 mL of the aqueous extract was stirred with 2 mL of distilled water and filtered. 4 drops of 1 % ferric chloride solution was added to the filtrate. The formation of a blue-black, green or blue-green precipitate indicated the presence of tannins (Trease G. E., 2002).

3.4.2 Test for Saponins

Five milliliters of the extract was warmed with 5 mL of distilled water and filtered. The filtrate was shaken vigorously for about 5 minutes. The formation of a stable foam was taken as evidence for the presence of saponins (Sofowora A., 1993).

3.4.3 Test for Flavonoids

One to two fragments of metallic magnesium were mixed with 3-4 mL of the extract, then 0.5 mL of concentrated hydrochloric acid (HCl) was added ; after 5 minutes, a red colour appeared for the flavonols, orange for the flavons, red violaceous for flavanones or green for flavanols (Trease G. E., 2002).

3.4.4 Liebermann-Bourchard test for Steroids and Triterpenoids

Three to ten milliliters of hydro-alcoholic extract was evaporated into a porcelain capsule, the residue was dissolved in 0.5 mL chloroform, and then 0.5 mL of acetic anhydride was added. The resulting solution was put in the test tube followed by the addition of 1-2 mL of concentrated sulphuric acid (H_2SO_4). Colour development from violet ring, red- brown appeared after 5-10 minutes at the contact zone between the 2 liquids as well as a blue or bluish-green or violet upper layer which indicated the presence of steroids and triterpenoids (Sofowora A., 1993).

3.4.5 Test for Alkaloids

Three milliliters of the aqueous extract was stirred with 3 mL of 1 % aqueous HCl on a steam water bath. Mayer's reagent was added to the mixture; turbidity of the resulting precipitate was taken as an indication for the presence of alkaloids (Sofowora A., 1993).

3.4.6 Test for Cardiac Glycosides (Keller-kiliani test)

Two milliliters of the extract was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution. The mixture was poured into a test tube containing 1 mL of concentrated H_2SO_4. A brown ring at the interface was an indicator of the presence of a deoxy sugar, characteristic of cardenolides.

3.5 Mineral quantification of the plant extract

The iron (Fe), Zinc (Zn), and Manganese (Mn), content in the aqueous root extract of *P .muellerianus* were determined by atomic absorption spectroscopy (AAS) using Perkin-Elmer atomic analyst 400 in line with the procedure by Sharma and Tyagi (2013).The dilution factor of 25 was used for the sample and the procedure outlined here was followed;-

1. The measured volume (1000 mL) of well-mixed, preserved sample was placed into a beaker, boiled slowly and evaporated on a hot plate up to 10-20 mL,
2. At least 3 concentrations of standard solution of a particular metal to be analyzed were selected,
3. The blank solution was aspirated in a flame and the instrument adjusted to zero,
4. Each standard solution was aspirated in a flame,
5. The reading of the prepared sample solution was taken directly from the instrument, and

6. The following formula was used to determine the quantities of the elements of interest in the original sample matrix from its concentration in the sample solution;

$$Element\ (mg/L)\ =\ (C)\ (d.f)$$

Where; C - concentration of the element in the sample solution in mg/L

d. f - the dilution factor

An atomic absorption spectrophotometer is an analytical instrument based on the principle of AAS and is very useful to detect the metal ion concentration present in samples (Sharma and Tyagi, 2013).There are 5 basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest,
2. An "absorption cell" in which atoms of the sample are produced (flame, graphite furnace, MHS cell, FIAS cell, FIMS cell),
3. A monochromator for light dispersion,
4. A detector, which measures the light intensity and amplifies the signal, and
5. A display that shows the reading after it has been processed by the instrument electronics.

AAS method follows the Beer's law, which states that absorbance is directly proportional to concentration.The principle of AAS is that it makes use of the absorption of light by the element in order to measure their concentration. When a sample solution is aspirated into a flame, then the sample element is changed into atomic vapour (gaseous state) of that element. The flame contains atoms of elements. Furthermore, the flame thermally excites some atoms whereas some of them remain in ground state. The ground state atoms then absorb the radiation of specific wavelength produced by the source i.e. hollow cathode lamp of that specific metal. Now, the wavelength of radiation given off by the source or lamp is similar as that of absorbed by the atoms in the flame. Thus, the concentration of an element is determined from the amount of absorption (Sharma and Tyagi, 2013).

3.6 Study Design: Experimental/ intervention

3.7 Study Population: Male Albino rats

3.8 Sample Size: 36

3.9 Sample Size Calculation

The resource equation method in which a value, E, is calculated based on decided sample size was used (Charan and Biswas, 2013). This equation suggests that, E, should lie within 10 to 20 for optimum sample size; if the value of E is less than 10 then more animals should be included and if it is more than 20 then the sample size should be decreased. Six groups of rats having 6 rats each, were formed. Four groups were treated with different interventions. Therefore, the total number of experimental rats was; - 6x4 =24

E= [total number of experimental animals – total number of groups]

E= 24 - 6 = 18

3.10 Animals and induction of Anemia

Male albino rats weighing between 150 and 180 grams were purchased from the University of Zambia (UNZA) animal house at the Department of Biological Sciences in Lusaka. They were transported to the animal house at the School of Veterinary Medicine-UNZA, kept for 7 days to allow them to acclimatise and housed in rat cages according to the guidelines for the care and use of laboratory animals and welfare. Variations in the experimental procedure (e.g., loss of power during the period of the experiment, unexpected change in temperature in a room containing rats) were monitored (National Research Council (US) Committee, 2011).

Anemia was induced by repeated extraction of blood from the retro orbital plexus of each rat within 5 days (Brain et al., 2006, Skikne et al., 1990, Wallin et al., 2015, Dauar et al., 2015). This was done on 3 alternate days that is on days 8, 10 and 12 (Okonkwo et al., 2013). The formula used for determining the quantity of blood removed through bleeding is;-Blood volume (mL) = 0.06x body weight (g) + 0.77 as described by Lee and Blaufox (Lee and Blaufox, 1985). The bleeding was under light anaesthesia using diethyl ether as an anaesthetic.

3.11 Treatment and grouping of the rats

The methanolic and ethylacetate extracts of *P. muellerianus* have been shown by Assob et al., (2011) to have the minimum inhibition concentration (MIC) of between 0.07 and 1.25 mg/mL and a lethal dose (LD_{50}) of more than 4 g/kg body weight of male and female rats. Based on their study, they concluded that *P. muellerianus* is not toxic, taking into consideration the 5 g/kg threshold of toxic substances. Arising from their study, a dose for this study was selected (Assob et al., 2011).

The plant extract treatment started 24 hours after confirming the induction of anemia. The rats were divided into 6 groups with 6 animals in each group and various concentrations of the root extract of *P. muellerianus* were administered as shown in Table 3.1.

Table 3.1 Treatment options for the rats

Group (n=6)	**Condition of the rats**	**Treatment option for 10 days**
1	Normal (non-anemic)	distilled water
2	Control (Anemic)	distilled water
3	Anemic	200 mg ranferon
4	Anemic	100 mg/kg
5	Anemic	200 mg/kg
6	Anemic	400 mg/kg

n= number of rats

a. The 1st and 2nd groups received distilled water. The 2nd group was anemic and served as a control,
b. The 3rd group was anemic and received ranferon,
c. The 4th, 5th and 6th groups were treated with the plant extract, 100, 200 and 400 mg/kg body weight.

Ranferon, the extract and distilled water were administered by oral intubation for 10 days (Okonkwo et al., 2013, Oladiji et al., 2007).

3.12 Collection of blood samples

Rats were anesthetised in diethyl ether. When they became unconscious, blood was collected from their retro orbital plexus into heparinised ethylenediaminetetraacetate (EDTA) test tubes for hematological studies (Stone, 1954).

3.13 Hematological tests

Hematological data was collected 3 times; - at the baseline of the study, on day 12 to confirm that anemia had been induced and after treatment with the plant extract as well as ranferon. The Packed cell volume (PCV) was measured using the mean hematocrit centrifuge (Hawksley mean hematocrit centrifuge). The steps taken to determine the PCV using the

hematocrit method were as outlined here;-

1. the capillary tube was filled with blood and sealed at the end using a sealant,
2. the capillary tube was placed in the mean hematocrit centrifuge and the safety cover was screwed,
3. the lid was closed and spun at 10,062 x *g*, and
4. the spun tube was placed onto the mean-hematocrit reader, a specially designed scale, to read off the PCV as a percentage.

Hb concentration was determined from the PCV values using the formula; Hb= PCV/3. RBC count was done manually, using an Olympus microscope at the magnification of X40 and a RBC counter. The film comment was determined using a haemocytometer (Thoma haemocytometer) and an Olympus microscope at the magnification of X100.

Erythrocyte indices were determined as follows; Mean Cell Haemoglobin Concentration (MCHC) was calculated using the equation: MCHC= [(Hb x 100)/ PCV]. Mean Cell Hemoglobin (MCH) using the equation: MCH= [(Hb x10)/ RBC]. Mean Red Cell Volume (MCV) using the equation: MCV = [(PCVx10) / RBC] (Bull BS, 1958, Baker and Silverton, 1980).

3.14 Data processing and statistical analysis

The mean values and standard deviation were calculated using analysis of variance (ANOVA) followed by the Dunnett's test. Dunnett's test is a post hoc test used to compare the control group mean to the means of the entire experimental groups in an ANOVA. Results were analysed using the Statistical Package for Social Scientists (SPSS) software version 21, at a significance level of $P < 0.05$.

CHAPTER 4

RESULTS

4.1 Results for preliminary Phytochemical Screening

The medicinal value of plants lies in the bioactive phytochemical constituents of the plant that shows various physiological effects in humans. Therefore, through phytochemical screening one could detect the various important compounds, which could be used as the base for discovering modern drugs for curing various diseases including anemia (Harborne, 1973). Various phytochemical tests performed on the root extract of *P. muellerianus* showed positive results for steroids, triterpenoids, alkaloids, flavonoids, saponins, glycosides and tannins as illustrated in Table 4.1, Figures 4.1 and 4.2.

Table 4.1 Results forpreliminary phytochemical screening of the extract

Test	**Observation**	**Result**
Alkaloids	Turbidity	+++
Steroids	Green-blue colour	++
Triterpenoids	Violet upper layer	++
Flavonoids	Red colour	+++
Saponins	Stable foam	+++
Glycosides	Brown ring	+++
Tannins	Green precipitate	+++

Key: - Negative

\+ Trace,

++ Moderate, and

+++ High.

Figure 4.1 Picture for phytochemical test results

Figure 4.2 Photo of the phytochemical screening results

The color of solid extract was dark brown (Figure 4.3) and the percentage yield was 1.6 %.

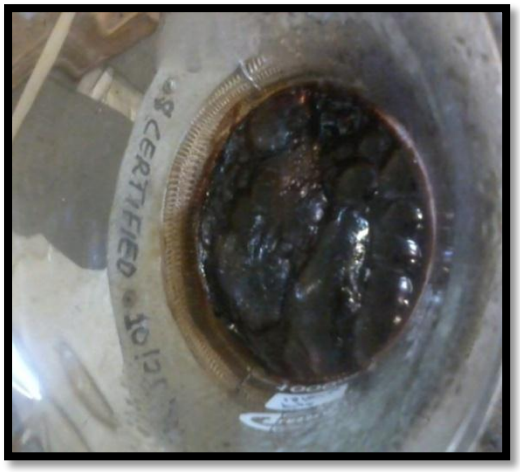

Figure 4.3 Photographs of the solid extract of *P. muellerianus*

The extractive weight of the plant extract was 16 g and it had the mineral composition of 230.5 mg Fe, 273.5 mg Mn and 138 mg Zn calculated from values in Table 4.2.

Table 4.2 Details for AAS results using Perkin-Elmer atomic analyst 400

Element	Wave-length (nm)	Slit width	Standards (mg/L)	Dilution factor	Weight (g)	Initial Volume (mL)	Extracting solution	Conc. (mg/L)
Fe	248.8	0.2	6,18,36	25	1000	2000	Distilled Water	0.922
Zn	213.9	0.7	1,3,12					0.552
Mn	279.8	0.2	2,6,12					1.094

4.2 Data analysis

A one-way analysis of variance (Table 4.3) was conducted to evaluate the research question, "**is the aqueous root extract of *P. muellerianus* useful in alleviating anemia in rats?**" The rats were divided in 6 groups based on the treatment option (Group 1: Control, Group 2:

Normal, Group 3: Ranferon, Group 4: 100 mg/kg, Group 5: 200 mg/kg and Group 6: 400 mg/ kg plant extract).

Table 4.3 Anova results

		Sum of Squares	df	Mean Square	F	Sig.
Packed Cell Volume (%)	Between Groups	371.806	5	74.361	68.641	.000
	Within Groups	32.500	30	1.083		
	Total	404.306	35			
Hemoglobin Concentration (g/ dL)	Between Groups	40.909	5	8.182	63.948	.000
	Within Groups	3.838	30	.128		
	Total	44.748	35			
Red Blood Cells (x 10^6 /µl)	Between Groups	8.688	5	1.738	2.975	.027
	Within Groups	17.522	30	.584		
	Total	26.210	35			
Mean Cell Volume (Fl)	Between Groups	599.786	5	119.957	1.185	.340
	Within Groups	3037.150	30	101.238		
	Total	3636.936	35			
Mean Cell Hemoglobin (Pg)	Between Groups	69.976	5	13.995	1.247	.312
	Within Groups	336.653	30	11.222		
	Total	406.629	35			
Mean Cell Hemoglobin Concentration (g/dL)	Between Groups	.013	5	.003	1.412	.248
	Within Groups	.057	30	.002		
	Total	.070	35			

The assumption of normality was evaluated using tests for normality (see Tables A.1 and A.2) and found tenable for Hb concentration and RBC count in all groups.

The assumption of homogeneity of variances after treatment was tested and found tenable for all the hematological parameters, $P < 0.05$ (Table 4.4).

Table 4.4 Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Packed Cell Volume (%)	4.010	5	30	.007
Hemoglobin Concentration (g/ dL)	4.141	5	30	.006
Red Blood Cells (x 10^6 /µl)	10.746	5	30	.000
Mean Cell Volume (Fl)	16.876	5	30	.000
Mean Cell Hemoglobin (Pg)	18.174	5	30	.000
Mean Cell Hemoglobin Concentration (g/dL)	4.520	5	30	.003

4.3 Effects of the plant extract on the hematological parameters.

Post hoc comparisons to evaluate the effect of the aqueous root extract of *P. muellerianus* on the hematological parameters of experimental rats compared to the control group were conducted using dunnett's test since equal variances were tenable (Table A.3).

4.3.1 Effects of the plant extract on the Packed Cell Volume and Hemoglobin

In this study, anemia was defined as Hb < 12 g/dL. Results indicated that at the baseline of the study, the mean values for PCV and Hb in the 5 comparison groups were not different ($P > 0.05$) from the control group. However, repeated bleeding decreased PCV and Hb to values less than 32.5 % and 11.5 g/dL respectively, thus leading to hypochromasia. Treatment with various concentrations of *P. muellerianus* reversed the effects of bleeding in a dose dependent manner (Table A.3, Figures 4.4 and 4.5).

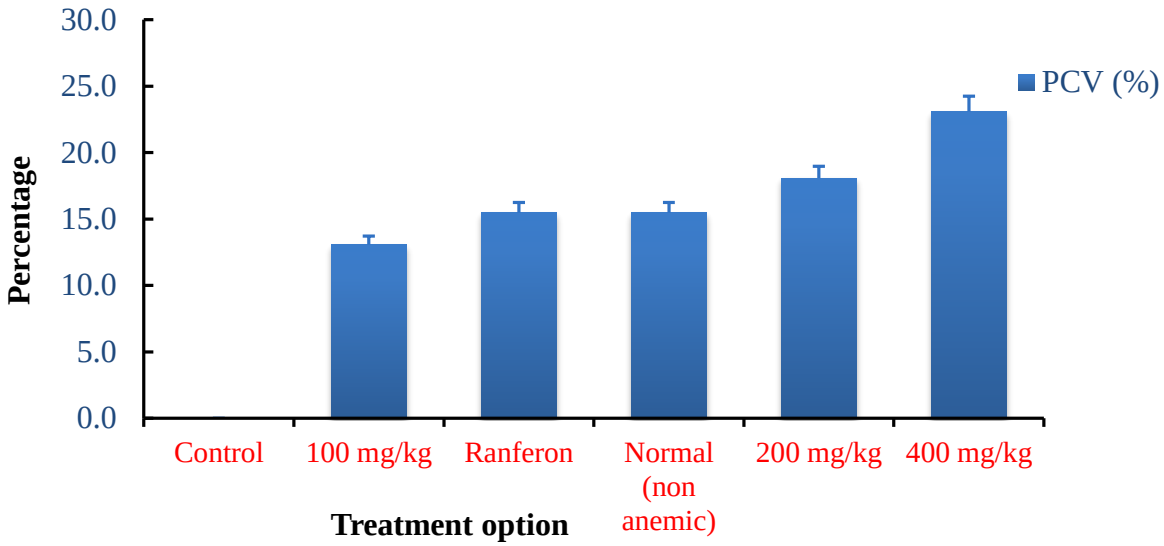

Figure 4.3 Percentage increase in PCV compared to the control.

The percentage increases in PCV and Hb for the experimental groups on plant extract from the control group were 13.1, 18.1 and 23.1 % at the dosages of 100, 200 and 400 mg/kg respectively, $P < 0.05$(Figures 4.4and 4.5).Tests of between-subjects effect (Tables A.4 and A.5) revealed the partial eta squared values of 0.914 for Hb, and 0.92 for PCV.

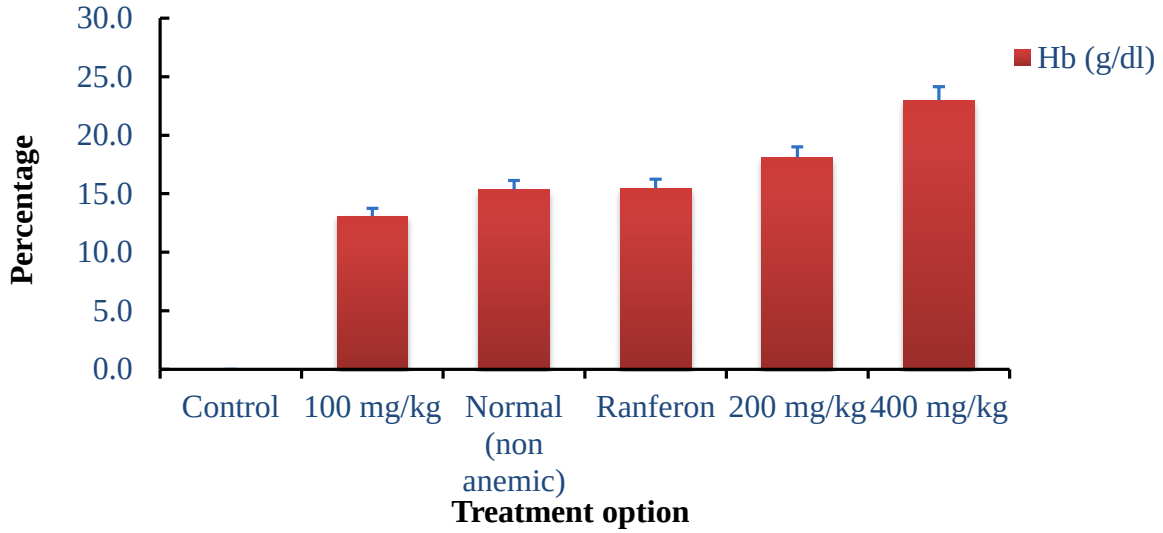

Figure 4.4 Percentage increase in Hb compared to the control.

The mean values for Hb concentration (g/dL) in this study ranged between; -14.2 and 15.3 at the baseline of the study, 9.8 and 11.4 after inducing anemia, and13.6 and 15.4 in the experimental groups after treatment.

4.3.2 Effects of the plant extract on the Red Blood Cells

The mean values of RBC count ranged from, 5.3 to 5.9x 10^6 /µl (*P > 0.05*) at the baseline of the study, 3.9 to 6.7 x 10^6 /µl after inducing anemia, and 4.3 to 6.3 x 10^6 /µl after treatment. The differences in RBC count between the control and the normal group were significant (*P < 0.05*) after inducing anemia as well as between the control and the group on 400 mg/kg dosage, after treatment. The percentage differences after treatment were that, there was an increase in RBCs in a dose dependent manner with the greatest increase (22.2 %) observed at the 400 mg/kg dosage (Figure 4.6).

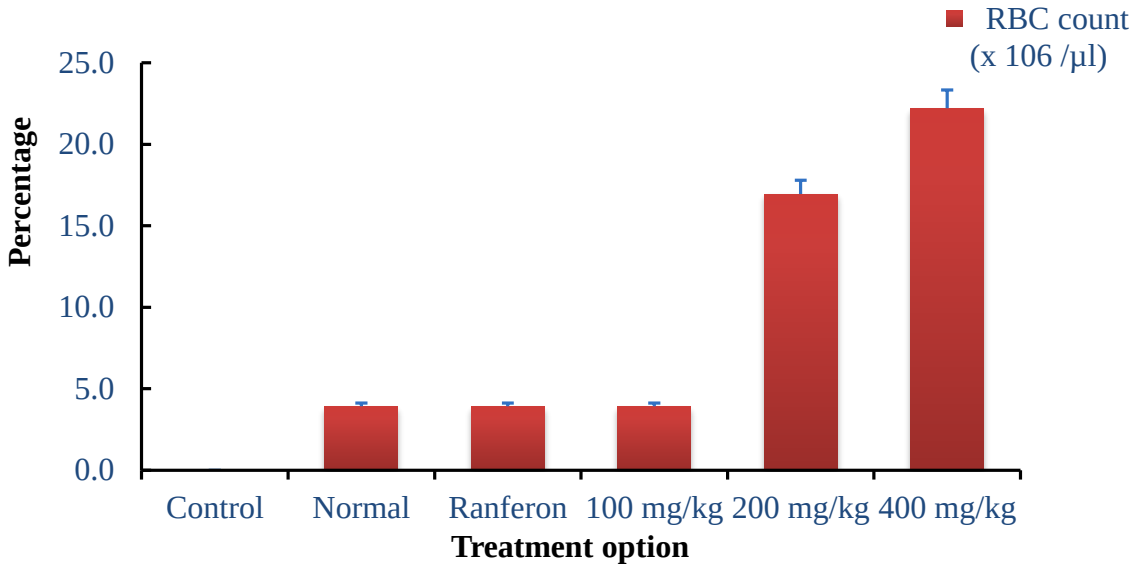

Figure 4.5 Percentage differences in RBC compared to the control.

4.3.3 Effects of the plant extract on MCV, MCH and MCHC

The plant extract at100 mg/ kg, 200 mg/kg and 400 mg/kg increased the MCV by 9.4 %, 1.3% and 1.2 % respectively, when compared to the control group. The percentage differences were neither dose dependent nor significant (*P > 0.05*). The same values were observed for MCH (Figures 4.7 and 4.8).

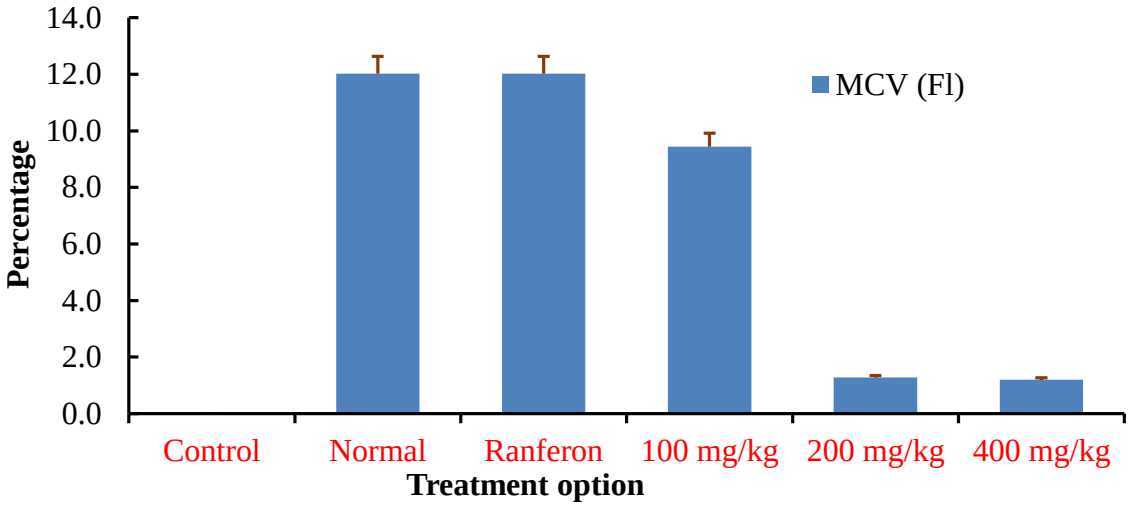

Figure 4.6 Percentage differences in MCV.

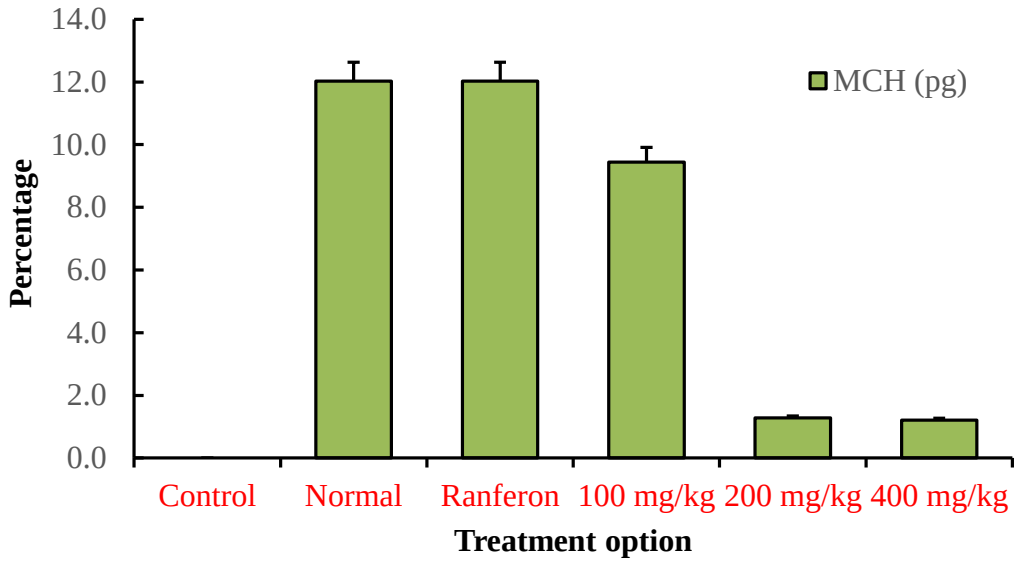

Figure 4.7 Percentage differences in MCH.
The error bars represent the standard error of the mean

As for MCHC, the mean value, 33.3 (g/dL), remained constant at all stages of the study and there were no significant differences between the experimental groups and the control.

CHAPTER 5
DISCUSSION

5.1Effect of *P. muellerianus* on the hematological parameters

Dosages of 100, 200, and 400 mg/kg of the root extract and ranferon were administered orally for 10 days to anemic male albino rats to monitor their effect compared to that of the control group that did not receive any drug but distilled water. The results demonstrated that the root extract and ranferon were able to restore the hematological indices of experimental animals to normal levels after 10 days of treatment and the significant ($P < 0.05$) effect was found to be dose-dependent when compared to the control values. After treatment, the results fell within the normal range of hematological indices of rats (Table A.7). In addition, the partial eta squared values for PCV and Hb were close to 1 suggesting that the aqueous root extract of the plant was both statistically significant and biologically/clinically efficacious against anemia. The Phytochemical and micro nutrient compositions of the root extract appear most likely to be responsible for the hematinic effect of *P. muellerianus* and their presence in the plant extract agrees with other studies (Shami et al. 2016, Katsayal, and Lamai, 2011, Yenon, et al., 2015, Yakubu, 2005).

The blood parameters, Hb, PCV, and RBCs together with the level of iron are indicative indices of anemia that could be used to indicate nutritional values of ingested diets as well. MCV, MCHand MCHC are constants for typing anemia hence they were not statistically different ($P > 0.05$) when experimental groups were compared to the control group. The MCV and MCH decreased significantly after inducing anemia, thus indicating microcytosisas their decrease reflects a release of red blood cells, which are less saturated in hemoglobin (hypochromia). This decrease was offset in the treated anemic groups of rats, and the increase reflects a release of large immature erythrocytes (macrocytes). Therefore, occurrence of anemia observed in this study is attributable to lowered values of these indices and related to the findings of Osman et al., (2013). However, the observed increase in Hb, PCV, and RBCs in experimental rats of this study might be due to the stimulating effect of *P. muellerianus* on hematopoiesis and consequently the production of RBCs into the blood stream, which in turn compensated for anemia.

Moreover, the increase in these hematological parameters appeared to be dose-dependent and could be due to high nutritional values of the plant particularly in minerals such as Fe (230.5

mg). Fe plays a significant role in erythropoiesis, synthesis of Hb and myoglobin while its deficiency causes anemia. It is also important in immune function, cognitive development, temperature regulation, and energy metabolism. However, the therapeutic potential of *P. muellerianus* could not be established based on available Fe content alone as other factors play a role in its absorption in the body. In this context, Fe is a necessity for the formation of the heme part of Hb as reported by others (Koné et al., 2012, Queiroz and Torres, 2000, Osman et al., 2013). The plant also had high concentration of Zinc and Manganese. Zn is a component of anti-oxidative enzymes, catalase, superoxide dismutase, and glutathione peroxidase. It is required for the function of over 200 enzymes and is important in growth and sexual development in man. It also lowers blood sugar. Mn is both nutritionally essential and potentially toxic. It plays an important role in a number of physiologic processes as a constituent of multiple enzymes such as manganese superoxide dismutase (MnSOD) and is an activator of other enzymes. It is important in the formation of bones, connective tissues, blood-clotting factors and sex hormones, and is involved in fat and carbohydrate metabolism, calcium absorption and blood sugar regulation. In addition, it is important for brain and nerve function (can bind with neurotransmitters and stimulate faster or more efficient transmission of electrical impulses throughout the body, in effect, speeding up cognitive function). Mn may be helpful in treating osteoporosis, arthritis, premenstrual syndrome, diabetes, and epilepsy. Fe deficiency has been shown to increase the risk of Mn accumulation in the brain. Although the specific mechanisms for Mn absorption and transport have not been determined, some evidence suggests that Fe and Mn can share common absorption and transport pathways. Absorption of Mn from a meal decreases as the meal's Fe content increases. Fe supplementation (60 mg/day for 4 months) is associated with decreased blood Mn levels and decreased activity in white blood cells, indicating a reduction in Mn nutritional status. Intestinal absorption of Mn is increased during Fe deficiency, and increased Fe stores (ferritin levels) are associated with decreased Mn absorption (Queiroz and Torres, 2000, U.S. Department of Agriculture, 2010) .

The high Fe content of the plant under investigation justifies and partly supports the traditional use of its roots in treating anemia. The results also place it under a group of plants with ant-anemic potential, which are richest in Fe content according to the related findings of Koné et al., (2012). However, Schmelzer et al., (2008) reported an insignificant Fe content of 15 mg per gram of dry fruits of *P. muellerianus.*

The methanolic and ethylacetate leaf extracts of *P. muellerianus* were shown by Assob et al., (2011) to have the minimum inhibition concentration (MIC) of between 0.07 and 1.25 mg/mL and a lethal dose (LD_{50}) of more than 4 g/kg body weight of male and female rats. Based on their study, they concluded that *P. muellerianus* is not toxic, taking into consideration the 5 g/kg threshold of toxic substances. On the other hand, Adedapo et al., (2007) showed that the leaves of *P .muellerianus* significantly ($P<0.05$) reduced the hematological parameters, had toxic potential and were therefore, poisonous to the animals.

5.2 Bleeding and induction of anemia

Repeated bleeding which was used in this study has been documented to have the ability of inducing iron deficiency anemia by decreasing the iron stores and concentrations of blood parameters such as PCV, Hb and RBCs (Brain et al., 2006, Skikneet al., 1990, Wallin et al., 2015, Dauar et al., 2015, Dina, et al., 2000,Marković, et al,. 2010). If a person or an animal losses blood in conditions like haemorrhage or severe injury, the individual is unable to absorb sufficient amount of iron from the intestines to form hemoglobin. Therefore, the RBCs formed are smaller than normal leading to microcytic, hypochromic anemia. A loss of about one-third of the total blood volume of an animal is known to precipitate anemia. Therefore, based on their body weights, one-third of the total blood volume of the rats was collected through bleeding to induce anemia. Marković, et al., (2010) reported that in the plasma and RBC of the bled rats, the concentration of reduced glutathione (GSH) was significantly high, while the concentration of oxidized glutathione (GSSG) and the ratio GSSG/2GSH, were significantly lower in the group of anemic rats. All these changes and the efficacy of the glutathione status were a consequence of the higher activity of glutathione reductase (GR) in the bled rats. Glutathione reductase catalyses the reduction of oxidized GSSG back into GSH, the latter being the co-substrate of glutathione peroxidase (GSH-Px). Higher GR activity provides conservation of GSH, which is a primary protective molecule from the oxidative stress of the RBCs. Based on their results, Marković et al concluded that the glutathione system is efficient in the anti-oxidative defence of RBCs of bleeding-induced anemic rats, but less efficient in the plasma of the bled rats considering the high levels of the thiobarbituric acid reactive substances (TBARS). Their results showed a lower level of ROS, a higher level of lipid peroxidation, and the effective anti-oxidative role of the glutathione system in the blood of bleeding rats (Marković et al., 2010). This explanation accounts for the

observed reduction in the quantity and concentration of some hematological parameters after inducing anemia through repeated bleeding.

5.3 Observed Phytochemicals and their action

Due to expanding focus on the use of traditional medicine, it has becomenecessary to document the traditional / medicinal uses, as well as expand our knowledge of the possible active principles involved in the acclaimed efficacies of plants used in this system. Plants used in the treatment of disease contain a wide range of active principles with biological activity, some of which are responsible for the characteristic odours, pungencies and colours of plants while others give a particular plant its culinary, medicinal or poisonous virtues (Katsayal, and Lamai. 2011). These chemical principles vary in distribution within the plant parts, as well as their occurrence within plant species. The presence or absence of such compounds depends largely on the extent of accumulation, the amount of plant material used, cultivation period, season of collection, plant-to-plant variability in the medicinal content and the analytical method employed (Harborne, 1973). That is whyphytochemical screening of plants must be done constantly, even on the ones whose secondary metabolites are already known.

A wide variety of phytochemical constituents from different parts of *P. muellerianus* has been reported. Some have had their pharmacological effect identified while others have not. They include;- flavonoids, alkaloids, glycosides, triterpeniods, saponins, tannins, lipids, carbohydrates, reducing sugars, resins,1 β,22 β -dihydroxyfriedelin,22 β -hydroxyfriedel-1-ene, 3-friedelanone, corilagin, furosin, and geraniin. Others are;- astragalin, isoquercitrin, quercitrin, β -sitosterol, 3,5-dicafeoylquinic acid, cafeoylmalic acid, chlorogenic acid, gallic acid, methyl gallate,bis(2-ethylicosyl)phthalate, bis(2-ethyloctyl)phthalate,phyllosea pentasaccharide, and (E)-isoelemicin (Agyare et al., 2010, Ayegba et al., 2015, Adesida et al., 1972, Schmelzer et al., 2008) .

In this study, qualitative phytochemical tests performed on the root extract of *P. muellerianus* showed positive results for steroids, triterpenoids, alkaloids, flavonoids, saponins, cardiac glycosides and tannins. The results of this study are related to the findings recorded by among others;- Schmelzer et al., (2008), Okeniyi et al., (2014),Yakubu et al.,(2005) and Yenon et al.,(2015) who reported that their presence might be responsible for the acclaimed medicinal value of plants used in traditional medicine because these secondary metabolites have many biological and therapeutic properties.

As inferred from other reports, alkaloids, the most revered of all phytochemicals are said to be pharmacologically active and their action is felt in the nervous system, blood vessels, respiratory system, and the gastrointestinal tract. They are anti-spasmodic, analgesic and have bactericidal effects. They inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase leading to accumulation of cAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins, which improves erythropoiesis (Shami et al. 2016, Katsayal, and Lamai, 2011, Yenon, et al., 2015).

Saponins are known to; inhibit platelet aggregation and thrombosis, lower the cholesterol levels, and have anti-diabetic as well as anti-carcinogenic properties. Saponins contained in herbs have been successfully used in the management of liver inflammation, as tonic sedative formulas, to promote and vitalize blood circulation, as expectorants (cough suppressants) and for hemolytic activities. Since saponins are active agents that lyse the membrane of RBCs or other walls, it is likely that the plant extract used in this study first lysed RBCs. Then the cells overcame this inhibition by producing a glycosidic enzyme that cleaves some of the terminal sugars from the saponins, which detoxified it (Shami et al. 2016, Katsayal, and Lamai, 2011, Yenon, et al., 2015). This detoxification of saponins reinforced the proper use of iron contained in the aqueous extract of *P. muellerianus* allowing it to synthesize heme/hemoglobin for new RBCs, thus leading to an observed improvement of Hb, RBCs and PCV in the plant extract treated groups. Glycosides enhance the natural resistance and have the recovery powers for the body (Shami et al. 2016, Katsayal, and Lamai, 2011, Yenon, et al., 2015).

Flavonoids are significantly recognised for their anti-oxidant, anti-carcinogenic, anti-microbial, anti-tumour properties, anti-inflammatory, anti-allergic, hepato-protective, ant-thrombotic, and anti-viral activities. They are known to possess a well-established protective effect against membrane lipoperoxidative damages. The unique chemical structures of these phenolic compounds is characterized by an aromatic ring possessing one or more hydroxyl substituents and is predictive of their anti-oxidant potential in terms of radical scavenging, hydrogen or electron-donating and metal chelating capacities. It has also been demonstrated that the anti-oxidants such as flavonoids can act:

1. Either by neutralizing ROS by directly reacting with superoxide anion, nitric oxide (NO) and peroxynitrite thereby preserving vascular function and protecting vascular injuries from ROS and perhaps from other oxidant species,

2. Or they could stimulate erythropoiesis (Shami et al. 2016, Katsayal, and Lamai, 2011, Yenon, et al., 2015).

Tannins are well known for their anti-oxidant, anti-inflammatory, diuretics, antidiarrhoeal, anti-microbial, astringent properties, anti-tumor, anti-HIV activity as well as for soothing relief, and skin regeneration. Tannins from many plants especially *Euphorbiaceae* are used to treat cells that have gone neoplastic,and to stop bleeding during circumcision. They have been used traditionally as styptic and internally for the protection of inflamed surfaces of the mouth and throat. Steroids regulate carbohydrate and protein metabolism and possesses anti-inflammatory properties.This might correspond to their ethnomedicinal significance (Shami et al, 2016).

The observed anti-anemic activity of the plant extract could therefore be due to the presence of the secondary metabolites detected especially the alkaloids, saponins, and flavonoids. Further evaluations, isolation, and characterization of these metabolites are therefore, encouraged in order to elucidate the active agents responsible for the observed anti-anemic effects of the root extract of *P. muellerianus*.

CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The results of qualitative phytochemical screening in this study showed the presence of alkaloids, saponins, flavonoids, steroids, triterpenoids, tannins and cardiac glycosides in the aqueous root extract of *P. muellerianus*. The results also showed that the aqueous root extract of *P. muellerianus* is useful in alleviating anemia as it was efficient against anemia in a dose-dependent manner and the dosage of 400 mg/kg was useful. The mechanism of action remains unknown but may involve stimulation of erythropoiesis. The rich array of trace elements and phytochemicals in the roots observed in this study (especially alkaloids, saponins and flavonoids), present *P. muellerianus* as a plant with very good potential for medicinal use in the possible treatment of anemia, as well as a myriad of other diseases.

6.2 Recommendations

From this study, it is suggested that the anti-anemic potential of the aqueous root extract of *P. muellerianus* could be explored for further research in developing a novel herbal delivery system as an alternative for the treatment of anemia that is affordable, easily accessible and cost effective.

Given the findings of this study, the anti-anemic potential of the root extract of *P. muellerianus* can be elucidated by further studies;

1. On its biological tolerance (toxicity studies),
2. To extract its specific anti-anemic active ingredient/s,
3. To understand the mechanism of action for the active ingredient, and
4. Appropriate clinical trials.

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APPENDICES

Appendix A: Statistical test results

Table A.1 Tests of normality[c] for Hb Concentration

	Treatment-option	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Hemoglobin Concentration at the baseline(g/dL)	Control	.161	6	.200*	.945	6	.697
	400 mg/kg	.220	6	.200*	.935	6	.622
	100 mg/kg	.196	6	.200*	.965	6	.860
	200 mg/kg	.268	6	.200*	.786	6	.044
	Normal	.333	6	.036	.827	6	.100
	Ranferon	.207	6	.200*	.926	6	.547
Hemoglobin Concentration after inducing anemia(g/dL)	Control	.165	6	.200*	.956	6	.789
	400 mg/kg	.283	6	.145	.863	6	.199
	100 mg/kg	.186	6	.200*	.957	6	.799
	200 mg/kg	.298	6	.103	.812	6	.075
	Normal	.167	6	.200*	.919	6	.499
	Ranferon	.184	6	.200*	.907	6	.419
Hemoglobin Concentration after medication(g/dL)	Control	.386	6	.006	.691	6	.005
	400 mg/kg	.413	6	.002	.747	6	.019
	100 mg/kg	.287	6	.133	.859	6	.184
	200 mg/kg	.227	6	.200*	.895	6	.345
	Normal	.216	6	.200*	.884	6	.290
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							
c. Hemoglobin Concentration (g/dL) after medication is constant when Treatment-option = Ranferon. It has been omitted.							

Table A.2 Tests of normality for RBC count

	Treatment option	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Red blood cell count at the baseline (x 10^6 /µl)	Control	.193	6	.200*	.890	6	.317
	400 mg/kg	.341	6	.028	.784	6	.042
	100 mg/kg	.199	6	.200*	.968	6	.878
	200 mg/kg	.281	6	.151	.852	6	.162
	Normal	.264	6	.200*	.864	6	.202
	Ranferon	.259	6	.200*	.854	6	.171
Red blood cell count after inducing anemia (x 10^6 /µl)	Control	.325	6	.047	.779	6	.037
	400 mg/kg	.258	6	.200*	.869	6	.221
	100 mg/kg	.252	6	.200*	.869	6	.221
	200 mg/kg	.215	6	.200*	.889	6	.312
	Normal	.202	6	.200*	.906	6	.411
	Ranferon	.286	6	.136	.863	6	.201
Red blood cell count after medication (x 10^6 /µl)	Control	.295	6	.111	.817	6	.083
	400 mg/kg	.285	6	.140	.711	6	.008
	100 mg/kg	.289	6	.127	.804	6	.064
	200 mg/kg	.279	6	.159	.803	6	.062
	Normal	.279	6	.159	.908	6	.421
	Ranferon	.333	6	.036	.827	6	.101

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table A.3 Dunnett's test results for Multiple Comparisons

Dunnett t (>control)[a]							
Dependent Variable:	(I) Treatment-option	(J) Treatment-option	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Packed Cell Volume (%)	Normal	Control	6.5000*	.6009	0.000	5.097	6.5000*
	Ranferon 100 mg/kg	Control	6.5000*	.6009	0.000	5.097	6.5000*
		Control	5.3333*	.6009	0.000	3.930	5.3333*
	200 mg/kg	Control	7.8333*	.6009	0.000	6.430	7.8333*
	400 mg/kg	Control	10.6667*	.6009	0.000	9.263	10.667*
Hemoglobin Concentration (g/dL)	Normal	Control	2.1500*	.2065	0.000	1.668	2.1500*
	Ranferon	Control	2.1667*	.2065	0.000	1.684	2.1667*
	100 mg/kg	Control	1.7833*	.2065	0.000	1.301	1.7833*
	200 mg/kg	Control	2.6167*	.2065	0.000	2.134	2.6167*
	400 mg/kg	Control	3.5333*	.2065	0.000	3.051	3.5333*
Red Blood Cells (x 10^6 /µl)	Normal	Control	.0500	.4412	.798	-.980	.0500
	Ranferon	Control	.0167	.4412	.822	-1.014	.0167
	100 mg/kg	Control	-.1500	.4412	.915	-1.180	-.1500
	200 mg/kg	Control	.7833	.4412	.143	-.247	.7833
	400 mg/kg	Control	1.1833*	.4412	.024	.153	1.1833*
Mean Cell Volume (Fl)	Normal	Control	7.0500	5.8091	.327	-6.515	7.0500
	Ranferon	Control	7.6167	5.8091	.289	-5.949	7.6167
	100 mg/kg	Control	8.9333	5.8091	.209	-4.632	8.9333
	200 mg/kg	Control	.4333	5.8091	.810	-13.132	.4333
	400 mg/kg	Control	-.9667	5.8091	.878	-14.532	-.9667
Mean Cell Hemoglobin (Pg)	Normal	Control	2.3833	1.9341	.320	-2.133	2.3833
	Ranferon	Control	2.6333	1.9341	.270	-1.883	2.6333
	100 mg/kg	Control	3.0333	1.9341	.200	-1.483	3.0333
	200 mg/kg	Control	.0500	1.9341	.826	-4.466	.0500

	400 mg/kg	Control	-.2667	1.9341	.871	-4.783	-.2667
Mean Cell Hemoglobin Concentration (g/dL)	Normal	Control	.0167	.0251	.573	-.042	.0167
	Ranferon	Control	.0000	.0251	.833	-.059	.0000
	100 mg/kg	Control	.0500	.0251	.098	-.009	.0500
	200 mg/kg	Control	.0333	.0251	.282	-.025	.0333
	400 mg/kg	Control	.0000	.0251	.833	-.059	.0000
*. The mean difference is significant at the 0.05 level.							
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.							

Table A.4 Tests of between-subjects effect for Hb Concentration

Source	Dependent Variable: **Hb Concentration (g/dL)**	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	at the baseline	5.096[a]	5	1.019	.437	.819	.068
	after inducing anemia	57.982[b]	5	11.596	14.912	.000	.713
	after medication	40.909[c]	5	8.182	63.948	.000	.914
Intercept	at the baseline	8055.062	1	8055.062	3457.521	.000	.991
	after inducing anemia	4619.468	1	4619.468	5940.164	.000	.995
	after medication	6930.563	1	6930.563	54168.53	.000	.999
Treatment	at the baseline	5.096	5	1.019	.437	.819	.068
	after induction	57.982	5	11.596	14.912	.000	.713
	after medication	40.909	5	8.182	63.948	.000	.914
Error	at the baseline	69.892	30	2.330			
	after induction	23.330	30	.778			
	after medication	3.838	30	.128			
Total	at the baseline	8130.050	36				
	after induction	4700.780	36				
	after medication	6975.310	36				
Corrected Total	at the baseline	74.988	35				
	after induction	81.312	35				
	after medication	44.748	35				
a. R Squared = .068 (Adjusted R Squared = -.087)							
b. R Squared = .713 (Adjusted R Squared = .665)							
c. R Squared = .914 (Adjusted R Squared = .900)							

Table A.5 Tests of between-subjects effect for PCV

Source	Dependent Variable: **Packed Cell Volume (%)**	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[d]
Corrected Model	at the baseline	44.806[a]	5	8.961	.433	.822	.067	2.163	.146
	after induction	524.667[b]	5	104.93	14.896	.000	.713	74.479	1.000
	after medication	371.806[c]	5	74.361	68.641	.000	.920	343.21	1.000
Intercept	at the baseline	72450.69	1	72450.7	3497.22	.000	.991	3497.2	1.000
	after induction	41616.00	1	41616.0	5907.63	.000	.995	5907.6	1.000
	after medication	62416.69	1	62416.7	57615.4	.000	.999	57615.	1.000
treatment	at the baseline	44.806	5	8.961	.433	.822	.067	2.163	.146
	after induction	524.667	5	104.933	14.896	.000	.713	74.479	1.000
	after medication	371.806	5	74.361	68.641	.000	.920	343.21	1.000
Error	at the baseline	621.500	30	20.717					
	after induction	211.333	30	7.044					
	after medication	32.500	30	1.083					
Total	at the baseline	73117.00	36						
	after inducing	42352.00	36						
	after medication	62821.00	36						
Corrected Total	at the baseline	666.306	35						
	after inducing	736.000	35						
	after medication	404.306	35						

a. R Squared = .067 (Adjusted R Squared = -.088)

b. R Squared = .713 (Adjusted R Squared = .665)

c. R Squared = .920 (Adjusted R Squared = .906)

d. Computed using alpha = .05

Table A.6 Tests of between-subjects effect for RBC count

Source	Dependent Variable: **Red blood cell count (x 10^6 /µl)**	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[d]
Corrected Model	at the baseline	1.158[a]	5	.232	.373	.863	.059	1.865	.131
	after inducing	31.191[b]	5	6.238	15.546	.000	.722	77.730	1.000
	after medication	8.688[c]	5	1.738	2.975	.027	.331	14.875	.785
Intercept	at the baseline	1134.57	1	1134.6	1827.5	.000	.984	1827.5	1.000
	after inducing	811.300	1	811.3	2021.8	.000	.985	2021.8	1.000
	after medication	1048.68	1	1049.	1795.5	.000	.984	1795.5	1.000
Treatment	at the baseline	1.158	5	.232	.373	.863	.059	1.865	.131
	after inducing	31.191	5	6.238	15.546	.000	.722	77.730	1.000
	after medication	8.688	5	1.738	2.975	.027	.331	14.875	.785
Error	at the baseline	18.625	30	.621					
	after inducing	12.038	30	.401					
	after medication	17.522	30	.584					
Total	at the baseline	1154.35	36						
	after inducing	854.530	36						
	after medication	1074.89	36						
Corrected Total	at the baseline	19.783	35						
	after inducing	43.230	35						
	after medication	26.210	35						

a. R Squared = .059 (Adjusted R Squared = -.098)

b. R Squared = .722 (Adjusted R Squared = .675)

c. R Squared = .331 (Adjusted R Squared = .220)

d. Computed using alpha = .05

Table A.7 Hematology data: Rats 8-16 weeks old - males

TEST	UNIT	N	MEAN	S.D	RANGE
MCH	(Pg)	181	18.7	0.8	17.1- 20.4
MCV	(fl)	181	53.5	2.4	48.9- 57.9
MCHC	(g/dL)	181	34.9	1.2	32.9- 37.5
Hematocrit	(%)	181	45.0	3.5	39.6- 52.5
Hemoglobin	(g/dL)	181	15.7	1.0	13.7- 17.6
Red blood cell	(x 10^6/µl)	181	8.39	0.67	7.27- 9.65

Source: Clinical Laboratory Parameters for Crl: WI (Han), 2008

Appendix B: Profile of *P. muellerianus*

Table A.8 Taxonomy of*P. muellerianus*

Kingdom	*Plantae*
Division	*Angiospermae*
Class	*Eudicots*
Order	*Malpighiales*
Family	*Phyllanthaceae*
Tribe	*Phyllantheae*
Genus	*Phyllanthus*
Species	*muellerianus*

Local names in Zambia: *Ichibemba - Umupetwalupe*

Kaonde - Mulembalemba

Chewa - Mkuzandola

Mambwe - Mupetwandupe

Tumbuka - Kapikanduzi

Figure B.1 Pictures of *P. muellerianus*

Figure B.2 Roots of *P. muellerianus*