NUTRITIONAL COMPOSITION OF *PARQUETINA NIGRESCENS* LEAF AND EFFECTS OF ITS AQUEOUS EXTRACT IN ALUMINIUM CHLORIDE-INDUCED ANAEMIC RATS

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ABSTRACT

Parquetina nigrescens is a plant known for its various therapeutic uses in traditional medicine. Proximate, minerals, vitamins and secondary metabolites analysis of the leaf were evaluated using standard procedure. Extraction was done using distilled water. Acute toxicity to determine the LD_{50} of the plant extract was determined, thereafter thirty (30) male albino rats were divided into 6 groups of 5 animals. Group A was given distill water, while group B to F were administered $AlCl_3$ at 2.0 mg/kg bodyweight to induce anaemia for 14 consecutive days. Animals in group A and B served as positive and negative controls respectively and therefore were not treated, while group C, D, E and F were administered 2.6 mg/kg bw of ferrous sulphate (FS), 200, 400 and 600 mg/ kg bw aqueous extract of *P.nigrescens* leaf respectively daily for another 14 days. All treatments were orally administered. Bodyweight and packed cell volume (PCV) were monitored at days 0, 14, 19, 24 and 28 to determine the bodyweight and the anaemic state of the animals respectively. Animals were anaesthetised, euthanised, and the blood samples were collected for haematological and biochemical parameters. The proximate composition showed that the leaf contains carbohydrate (46.68%), protein (21.02%), and ash (10.50%). The mineral analysis revealed the presence of Fe (13.50 mg/100g), Mn (3.95 mg/100g) and Cu (1.45 mg/100g). Vitamin C (7.35 mg/100g), vitamin E (2.25 mg/100g), vitamin B_{12} (2.42 mg/100g) and folate (2.03 mg/100g) were present. Phenols and flavonoids were significantly ($P<0.05$) high at 183.08 mg/100g and 180.24 mg/100g respectively. The extract was considered safe, since no mortality was recorded at the highest dosage of 5000 mg/kg body weight. Haematological analysis showed significant ($P<0.05$) increases in the mean Red Blood Cells (RBC), Haemogblobin (Hb), PCV, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration in the *P.nigrescens* leaf extract treatment groups. Group A had the highest PCV (41.75%) but this was not significantly ($P<0.05$) different from the PCV of 600 mg/kg extract treated group (39.75%). However, there were significant ($P<0.05$) variations with group B having the lowest Hb (4.57 g/dL) and RBC (3.79), compared with the 600 mg/kg extract treated group having Hb and RBC value of (14.57 g/dL) and (4.6) respectively. There were also significantly ($P<0.05$) increase in the serum protein, albumin, serum iron and ferritin in all the treatment groups when compared with the negative control group B. However, there was no significant ($P<0.05$) difference between the FS treatment group and the extract treatment groups. Also, White Blood Cells, serum Alanine transferase, Aspartate transferase and Alkaline phosphatase activities of the negative control group significantly ($P<0.05$) increased when compared with the extract and FS treated group. The highest anti-anaemic effect was observed in the group treated with 600 mg/kg. The result of this study suggests that aqueous extract of *P.nigrescens* improved the anaemic condition of the treated animals when compared with the induced but untreated groups. Hence, *P.nigrescens* could be beneficial in improving blood formation and in the management of anaemic related ailments.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The condition that is associated by low level of haemoglobin as a result of either decreased quality or quantity of red blood cells insufficient to meet the physiological needs of the body is known as Anaemia (Ndem *et al.,* 2013). Usually, it varies by sex, age, pregnancy status, and altitude. Oxygen is usually transported by the red blood cells (RBCs) from the lungs to various tissues and organs of the body and carbon dioxide is then removed from these body cells (George *et al.,* 2012). Often in an anaemic condition, the decreased capacity of the RBC in transporting oxygen is harmful to the body (Ashish *et al.,* 2013). However, anaemia is not considered as a disease, but a sign of an underlying illness or nutritional deficiency (Camilla and Parminder, 2019). Hence, focus is usually on identification and removal of the underlying cause in the management or treatment of anaemia (Jilani and Iqbal, 2011). Anaemia remains one of the most common public health challenges in both developed and developing countries with major effects on human health and socioeconomic development (WHO/UNICEF, 2014).

In 2016, nearly one-third of the world's populations (32.9 %) was estimated to suffer from anaemia (Sridhar *et al*., 2018). Anaemia burden in the developing countries is four times higher than in the developed countries (Osungbade and Oladunjoye, 2012). In Nigeria for example, prevalence of anaemia among reproductive age women between the ages of 15-49 as at 2016 was 49.80%, while the highest value was recorded in 1990 to be 53.70% and the lowest value was in 2014 which was 49.60% (WHO, 2019).

Although women and children continued to be at greater risk than men across all ages and all geographical regions (Omoregie and Osagie, 2010; Cusick *et al*., 2018; Camilla

and Parminder, 2019). Anaemia constitutes a serious health problem in many tropical countries including Nigeria because of the prevalence of malaria and other parasitic infections (Toma *et al*., 2015). It significantly affects human health and socioeconomic development. In 2010, anaemia accounted for about 9 % of the total global disability burden (Kassebaum *et al*., 2014; Camilla and Parminder, 2019).

Anaemia can result from deficiencies originating from poor nutrition which may include deficiencies of iron, copper, certain vitamin and protein, poverty, ignorance, high parasitic infections, chronic disease state, and drug toxicity (Allen, 2015; Cusick *et al*., 2018). Apart from physiological factors, behavioural and environmental conditions such as smoking and altitude, which affect haemoglobin concentration, exposure of red blood cells to certain chemicals and toxins has also been associated with its breakdown, resulting in haemolytic anaemia. Chemicals such as phenylhydrazine, cyclophosphamide, dapsome, divcine, hydroxylamine, and aluminium have been reported to lead to acute haemolytic anaemia on entry into the circulatory system, causing severe problems such as low birth weight in pregnant women and impaired physical and mental development in children aside other effects (Madukwe *et al*., 2013; Tata *et al.,* 2016). Aluminum for example, causes deformability of erythrocytes, and have direct effect on iron metabolism by competing with iron binding sites on transferrin, the iron transport protein (Purnima *et al.*, 2014). This led to generation of free radicals and reactive oxygen species (ROS), which result in the peroxidative damages of the erythrocytes membrane and hence, destruction of the red cells. Therefore, the depressed erythrocyte counts in animals given aluminum salt may be the consequence of the reduction in the survival time of erythrocytes and the haemolytic effect of aluminum on the erythrocytes (Renuka, 2012).

Occurrences of anaemia may be due to various red cell defect ranging from production defect, maturation defect, defects in haemoglobin synthesis, genetic defect to synthesis of abnormal haemoglobin or even physical loss of red cells (Mukherjee and Ghosh, 2012; Madukwe *et al*., 2013). Symptoms of anaemia vary with severity, but are generally associated with fatigue, headache, coldness in feet and hands, faintness, shortness of breath, and lack of power of concentration, fever, systolic murmurs, cyanosis, dyspnoea, tachycardia, nausea, vomiting and abdominal pain (Camilla and Parminder, 2019).

Anaemia is mostly diagnosed by a low haemoglobin (Hb) concentration or a low hematocrit (Schreir, 2018), it can also be diagnosed using RBC count, blood reticulocytes, mean corpuscular volume (MCV), blood film analysis, serum iron, total iron binding capacity (TIBC), serum ferritin and Hb electrophoresis (Camilla and Parminder, 2019). However, treatment and management of anaemia depends on the underlying diagnosis. Some of the examples of treatments include; medications, injections of synthetic hormones, chemotherapy, treatment of infections such as malaria, blood transfusion, and surgery such as bone marrow transplant (Moorthi *et al*., 2011). Apart from the convectional direct treatment of anaemia, several intervention programs have been implemented to combat anaemia as a public health concern. They include industrial iron fortification in foods and condiments, and administration of supplements, vitamins and/or minerals to the vulnerable populations. These approaches depend on what form of anaemia it is and the degree of the condition. The approaches may therefore be used singly or in synergy with other types of treatment (Moorthi *et al*., 2011; WHO/UNICEF, 2014).

Usually, management and treatment of anaemia are usually very expensive and not readily available to the poor, besides direct iron supplement intake when in excess may

result in iron overload, gastrointestinal microbiome, mutagenesis and other serious risk (Cusick *et al*., 2018). Also, in many developing countries, intervention programs encountered draw backs such as ineffective distribution of the supplement as a result of civil disruption, migration, poor governmental policies, war zones, illiteracy and traditional practices (WHO, 2017). However, in Africa and most part of Asia, plant products especially medicinal plants are more acceptable by the populace (Wong *et al*., 2016). In the quest to finding a lasting and stable solutions to the problem of anaemia, evaluation of phyto-medicines has been considered as an alternative to provide a safe, effective, affordable, and an available medicine for the treatment of this condition. Assessments into the phytochemistry of medicinal plants revealed the antioxidant properties of these plants to prevent and fight against diseases caused by the presence of free radicals and ROS which are capable of cell peroxidative damages (Mamta *et al*., 2013; Arvind, 2016). Generally, medicinal plants are plants that possesses rich source of bioactive compounds useful for therapeutic purposes or are precursors for drugs (Aborisade *et al*., 2017). Example of such plants used in Africa is *Parquetina nigrescens.*

Parquetina nigrescens belongs to the Perplocaceae family (Guede *et al*., 2010). It is a perennial shrub found in the secondary forest around villages in Senegal as well as in Nigeria (Ayoola *et al*., 2011, Odukoya *et al*., 2018). In some Nigerian languages, *P. nigrescens* is called ewe ogbo (Yoruba), kwankwanin (Hausa), mgbidimgbe (Igbo), Olilia or Ovieukpakoma (Etsako) (Konan *et al*., 2013). The leaves, seeds, fruits, stem, roots and the latex of the plants are commonly used in traditional medicine (Owoyele *et al*., 2011). The plant is a climber usually planted by the rural dwellers for its health benefits. The leaf is sometimes freshly crushed for its juice or as a decoction.

It has been used in traditional medicine practice for treatment of gonorrhea, gastro-intestinal disorders (GIT), menstrual disorders, wound healing, and to boost blood shortage (Imaga *et al*., 2010). Pharmacognostic studies of *P. nigrescens* has revealed that the plant is highly rich in protein, minerals such as iron and magnesium, vitamin such as tocopherol, ascorbic acid, some secondary metabolites and also possesses anti-infective, analgesic, anti-inflammatory, antioxidant, and antidiabetic activities (Adebayo *et al*., 2010; Sopeyin and Ajayi, 2016). However, despite the pharmacological importance of *P. nigrescens*, it remains one of the numerous unutilized plants in the society especially by the urban settlers (Aborisade *et al.,* 2017). Therefore, there is little or no detailed information on how the rich nutrients and some secondary metabolites properties of *P. nigrescens* may be responsible for its hematinic potential, hence the purpose for this study.

1.2 Statement of the Research Problem

Generally, anaemia poses so much negative effects on human productivity which makes it difficult to perform daily tasks (Wenger *et al*., 2017), makes women weaker during pregnancy and delivery, reducing their chances of having healthy babies and surviving blood loss during and after childbirth. Sometimes, the adverse effect of anaemia may have a lifetime impact on infants and children's health (Prieto-Patron *et al*., 2018). Anaemic children grow more slowly than non-anemic infants and children, they have increased susceptibility to infections, impaired cognitive development, anorexic, and lower physical activity (Prieto-Patron *et al*., 2018). Also, anaemia constitutes serious health problem in many tropical countries including Nigeria because of the prevalence of malaria and other parasitic infections which usually results to decrease of haemoglobin (Toma *et al*., 2015).

Furthermore, most current and available synthetic iron drugs, which although are relatively affordable to the rural dwellers who constitute the majority of the population in developing countries (Pasricha *et al*., 2013), have side effects such as constipation, stomach pain, nausea, vomiting and dark stool, and an increased risk of iron overload (Hossain *et al*., 2013). In the developing countries iron deficient anaemia continued to be a serious problem due to high prices of iron rich foods, particularly food of animal source (Shami and Aman, 2016).

In addition, most food industries in the developing countries are under developed and where they are developed, the poor people are not able to afford the fortified foods (WHO, 2017). More also, intervention programs in developing countries such as vitamin and mineral supplementation also have setbacks preventing effective and evenly distributions due to civil disruption, migration, poor governmental policies, war zones, illiteracy and traditional practices (WHO, 2017).

Not only does anaemia and iron deficiency reduce individuals' well-being, cause fatigue and lethargy, impair physical capacity and work performance thereby decreasing human productivity (Pasricha *et al*., 2014), it also results in generations of children with impaired development and learning abilities and at large a nation with impaired economic productivity and development. (Prieto-Patron *et al*., 2018).

1.3 Justification of the Study

There has been so much intervention in the management and treatment of anaemia. A major strategy is Iron supplementation or fortification (Pasricha *et al*., 2014). Fortification of staple foods with iron has been commonly recommended to prevent iron deficient anaemia, but current solutions have drawbacks such as dose limitation, food oxidation, and high cost of iron-rich foods (Muñoz *et al*., 2018). Furthermore, soluble

iron salts produce several side effects such as unpleasant taste, vomiting, pyrosis and stool darkening (Pasricha *et al*., 2013; Elisabet *et al*., 2017). Studies have also revealed that too large doses of direct iron consumption in the treatment of anaemia result in iron overload, which predisposes human to infectious diseases (Lonnerdal, 2017; Cusick *et al*., 2018). Studies have also shown that plants iron (non-heme iron) which is less well absorbed may be better source of iron than animal sources (heme iron) which is readily absorbed by the small intestine enterocytes. The heme iron is known to directly impacts many disease and physiological processes in humans (Hooda *et al*., 2014; Michael, 2017).

Attention is rapidly shifting from the use of synthetic drugs towards botanicals as an alternative in health care services due to their economic value, availability, and accessibility (Arvind, 2016; Yuan *et al*., 2016). Plant species in Nigerian vegetation is vast and diverse yet, only a little percentage of its biodiversity has been put to use for their healing potentials. For instance, there are about 250,000 species of higher plants but only about 5 to 10% of these plants have been studied thus far (Pan *et al.,* 2013). Today, approximately 80% of antimicrobial, cardiovascular, anti-cancer, immunosuppressive, and antianaemic drugs are of plant source, their sales exceeding US$ 65 billion in 2003 (Pan *et al*., 2013; Song *et al*., 2014). Therefore, the rich mineral, vitamin, antioxidants and other nutritional contents of *Parquetina nigrescens* makes it a cheap source in the management of iron-deficient anaemia.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this research study is to evaluate the nutritional composition of *Parquetina nigrescens* leaf and effects of its aqueous extract in aluminium chloride-induced anaemic rats.

1.4.2 Objectives

The objectives of this research are the determination of the:

i. proximate, mineral and vitamin composition of *Parquetina nigrescens* leaf.

ii. secondary metabolites content of *Parquetina nigrescens* leaf.

iii. *invitro* antioxidant activities of *Parquetina nigrescens* aqueous leaf extract.

iv. LD_{50} of *Parquetina nigrescens* aqueous leaf extract.

v. effects of *Parquetina nigrescens* aqueous leaf extract on the biochemical and haematological indices in aluminium chloride-induced anaemic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Anaemia: General Overview

Anaemia is a deficiency in the number of circulating red blood cells, consequently a deficiency in the haemoglobin levels (<12-13 g/dL) (Camilla and Parminder, 2019). Therefore, a reduction in the number of circulating erythrocytes is the characteristics of anaemia as shown in Figure 2.1. It also compromises the state of health of an individual by decreasing the ability of the blood to supply tissues with sufficient amounts of oxygen (Rodwell *et al*., 2015). Since haemoglobin (found inside RBCs) transports oxygen from the lungs to the tissues, the result is hypoxia (lack of oxygen) in organs. All human cells rely on oxygen for survival, varying degrees of anaemia can have a wide range of clinical consequences (Fathollahipour *et al*., 2018).

The causes of anaemia are numerous and diverse however, common etiologies in the developing countries include: nutritional deficiencies, parasitic infections, acute or chronic blood loss, malabsorption, premature hemolysis of RBCs, decreased medullary erythropoiesis, inherited genetic defects, or undefined cause (Jilani and Iqbal, 2011; Osungbade and Oladunjoye, 2012; Camilla and Parminder, 2019). Anaemia is classified into different types based on their causes. Understanding the different classifications can help to recognize the symptoms and also to avoid anaemia. There exist over 400 types of anaemia, many of which are rare, but in all cases, there is lower than a normal number of circulating RBCs. Many types of anaemia exist such as iron deficiency anaemia, pernicious anaemia, haemolytic anaemia, aplastic anaemia, megaloblastic anaemia, sickle cell anaemia, thalassemia, and sideroblastic anaemia. Iron deficiency is believed to be the most common cause of anaemia globally (Mukherjee and Ghosh, 2012; Obeagu, 2018).

There are several diagnostic tests for anaemia in which deviation from normal could be an indication for anaemic condition in an individual. They include, haemoglobin concentration, haematocrit level, red blood cell count, serum iron, ferritin, transferrin and iron-binding capacity, reticulocyte, vitamin B_{12} and folate test, certain enzymes but to mention a few (Camilla and Parminder, 2019).

Though most common cause of anaemia remain iron deficiency, however in the recent times, studies have revealed that reactive oxygen species (ROS) of erythrocytes are major causative factors of anaemia. This usually occur either by suppression of redox/ antioxidative system or by activation of ROS generation resulting in oxidative stress (Yoshihito, 2012). Punima *et al*. (2014) explained that exposure to aluminium caused impairments in glucose utilization, agonist-stimulated inositol phosphate accumulation, lipid peroxidation, free radical mediated cytotoxicity, reduced cholinergic function, impact on gene expression and altered protein phosphorylation.

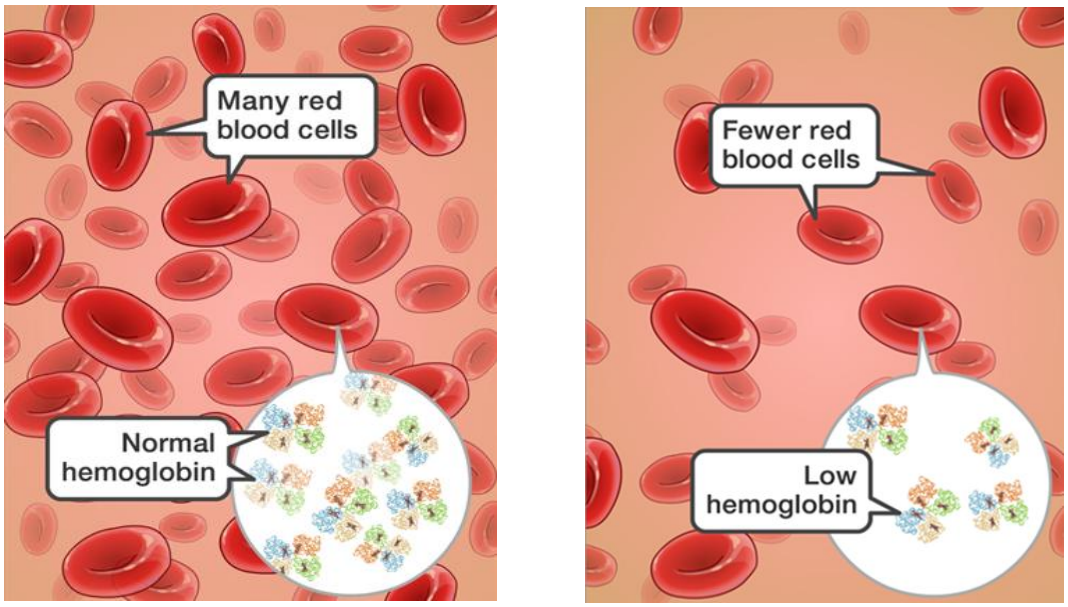


Figure 2.1: Picture of a normal and anaemic RBCs (Hariharan *et al*., 2015)

2.2 Types of Anaemia

2.2.1 Iron deficiency anaemia (IDA)

Although, there are several nutrients involved in red blood cells and haemoglobin production, iron deficiency is almost the most common cause of nutritional anaemia. This condition is a marked by a depletion of iron store and consequently, too little iron in the blood circulation (Mustapha *et al*., 2017). Microscopic examination in patient with iron-deficiency anaemia often reveals hypochromic (under pigmented) and microcytic (small sized) characteristics of the red blood cells. These deviations in the red cells occurs from decreased rates of globin synthesis as a result of low heme. Bone marrow aspiration will show no presence of iron, serum ferritin levels will be almost zero, while serum transferrin will be elevated. IDA is common in adolescents and women of child-bearing age (Rao *et al.*, 2018). Common causes include heavy menstrual flow, pregnancy, multiple births, gastrointestinal bleeding, childhood growth spurts, poor absorption of iron, iron deficient diets, bleeding from kidney, medications (aspirin, diclofenac, Ibuprofen, and Naproxen), hookworm infection, and bone marrow problems (Rodwell *et al*., 2015).

IDA may have serious debilitating effect, which include: lowered intellectual motivation, diminished capacity for work, poor performance, decreased resistance to infections (Obeagu, 2018). Other symptoms include headaches, tiredness, lethargy, feeling faint and becoming breathless easily, sore mouth, irregular heartbeats (palpitations), altered taste, and ringing in the ears (tinnitus). Low iron reserves in the body during pregnancy may also lead to anaemia in the newborn baby (Pasricha *et al*., 2010).

2.2.2 Pernicious anaemia

This is the most common type of vitamin B_{12} deficiency. This is a type of anaemia in which the bone marrow produces structurally abnormal and unusually large immature red cells. There is slow production of erythroblasts in the bone marrow leading to oversized, odd shapes and fragile membrane red blood cells due to poor absorption of folate, vitamin B12 and intrinsic factors from the stomach mucosa. This is known as megaloblast. These cells rupture easily resulting in anaemia (Shami and Aman, 2016).

2.2.3 Haemolytic anaemia

This is a condition in which red blood cell become fragile, destroyed and removed from the blood stream before their normal lifespan ended leading to serious anaemia (Shami and Aman, 2016). Haemolytic anaemia may be classified as congenital or inherited anaemia and as acquired haemolytic anaemia. Inherited haemolytic anaemia occur when the oxygen carrying capacity and life span of RBC is reduced as a result of genetic abnormalities in the synthesis of haemoglobin and increased membrane fragility (Evans, 2020). Common forms of inherited haemolytic anaemia include sickle cell anaemia, thalassaemias, hereditary elliptocytosis, hereditary spherocytosis, pyruvate kinase deficiency, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. The acquired haemolytic anaemia may occur from chemical agents, parasitic diseases, ionizing radiations, autoimmunity, and physical damage to cells (Ross and Wilson, 2006). Acquired haemolytic anaemia include immune haemolytic anaemia, autoimmune haemolytic anaemia, drug-induced haemolytic anaemia, alloimmune haemolytic anaemia, paroxysmal nocturnal haemoglobinuria, mechanical haemolytic anaemia, certain infections and substances can damage blood cells and lead to haemolytic anaemia (Jilani and Iqbal, 2011**).**

2.2.4 Sickle cell anaemia (SCA)

Sickle cell anaemia is a hereditary disorder of hemoglobin synthesis. The condition occurs as result of mutation in the same single base pair, when one single amino acid valine substitute glutamic acid at the 6th position, (GAG to GTG, Glu to Val) in the beta-globin molecule of sickle cells (HbS) (Inusa *et* al., 2019).

The body therefore makes sickle-shaped ("C"-shaped) red blood cells. These red cells contain abnormal haemoglobin (HbS) leading to a sickle shape of the red blood cells, hence the cells are not able to move easily through the blood vessels. The clusters of sickle cells block the blood flow leading to the limbs and organs, thereby causing severe pain, serious infections, and organ damage. Sickle cells are destroyed after about 10 to 20 days and the body cannot synthesize new red blood cells quick enough to substitute the ones that are being destroyed, which causes anaemia (Yoshihito, 2012). Sickle cell anaemia is a genetic, lifelong disorder common to Africans, South or Central Americans, Indians, Caribbeans, Mediterraneanians, and Saudi Arabians (Weatherall, 2011). Symptoms include fatigue, headaches, shortness of breath, coldness in the hands and feet, pale skin, dizziness, and Chest pain. Treatment includes gene therapy, nutritional supplements, bone marrow transplant, blood transfusion and hydroxycarbamide (Angastiniotis and Lobitz, 2019).

Figures 2.2.a: Normal and sickled RBC
(Hariharan *et al*., 2015)

2.2.5 Thalassaemia

This is a genetic blood disorder which cause the body to produce fewer healthy red blood cells and lower than normal level of haemoglobin. Thalassaemia could be alpha-thalassaemia or beta thalassaemia. The alpha thalassaemia major or hydrops fetalis is the most severe form of alpha thalassaemia, while thalassaemia major or Cooley's anaemia is the severe form of beta thalassaemia. Most patients with thalassaemia are diagnosed before the age of two (Sachith, 2018). Both males and females can be affected by thalassaemia and it occur most often in people of Italian, Middle Eastern, Asian Greek, and African descent (Angastiniotis and Lobitz, 2019). There are two kinds of protein chains in the haemoglobin; the alpha globin and the beta globin. When the body does not synthesize enough of these protein chains, red blood cells are not properly formed and therefore cannot carry enough oxygen. When the genes responsible for haemoglobin synthesis are absent or altered, thalassaemia occur (Soundarya and Suganthi, 2016).

Symptoms of thalassaemia are caused by a lack of oxygen in the blood. Severity of symptoms depends on the degree of the disorder. Generally, symptoms may include other health problems such as pale and listless appearance, poor appetite, and dark urine. Severe thalassaemia symptoms may include slowed growth and delayed puberty, jaundice, enlarged spleen, liver and heart, and bone problems. Three major standard treatments used in treatment of moderate and severe forms of thalassaemia are; blood transfusions, iron chelation therapy, and folic acid supplements (Soundarya and Suganthi, 2016).

2.2.6 Aplastic anaemia

This is a type of anaemia in which the bone marrow does not make enough new blood cells. It is a rare disorder characterized by suppression of bone marrow function resulting in progressive pancytopenia. It usually results in a number of health problems including an enlarged heart, heart failure, arrhythmias, infections and bleeding. Damage to the stem cells of the bone marrow result in aplastic anaemia (Scheinberg and Young, 2012).

Aplastic anaemia may be caused by several acquired factors including Toxins, such as pesticides, benzene, and arsenic, radiation and chemotherapy, medicines such as chloramphenicol, autoimmune disorders such as lupus and rheumatoid arthritis, infectious diseases such as hepatitis, HIV, Epstein-Barr virus, cytomegalovirus, and parvovirus. Inherited conditions, such as dyskeratosis, fanconi anaemia, Diamond-Blackfan anaemia and Shwachman-Diamond syndrome may also cause aplastic anaemia. Bone marrow aspiration and trephine biopsy are the most important diagnostic tools in diagnoses (Maurizio and Cario, 2015).

The symptoms of aplastic anaemia are fatigue, headaches, pale skin, gums and nail beds, dizziness, coldness in hands or feet, chest pains and shortness of breath. Treatment for aplastic anaemia includes blood transfusions, medications and blood and marrow stem cell transplants. These treatments can check or reduce complications, relieve symptoms, and enhance quality of life but bone marrow stem cell transplants may cure the disorder (Soundarya and Suganthi, 2016).

2.3 Aluminum Chloride

Aluminum metal is widely present in the earth's crust. It gained access into human body from the environment through the respiratory and the gastrointestinal tracts (Kalaiselvi *et al*, 2014). Aluminium is found as a composition of cooking utensils, spices, tea,

yellow cheese, pesticides, herbs, food additives, salts, cosmetics and medicines such as antacids, deodorants and this has permitted its easy access into the body (Davis and Littlewood, 2012). It is also added to drinking water for clarification purposes (Ozturk and Ozdemir, 2015). Aluminium has been suggested as an environmental factor that may contribute to some neurodegenerative diseases, affects several enzymes, because biochemical responses leading to unhealthy bone metabolism and learning disabilities in children (Klortz *et al*, 2017).

Salts of aluminium may bind to protein, DNA, and RNA thereby inhibiting activities of enzymes such as hexokinase, phosphodiesterase, acid and alkaline phosphatase, and phosphooxydase. Aluminium exposure can result in lipid peroxidation, impairments in glucose utilization, free radical mediated cytotoxicity, agonist-stimulated inositol, phosphate accumulation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation (Purnima *et al.*, 2014). Evidences suggested that aluminium induces alterations in hemato-biochemical parameters, increased lipid peroxidation and decreased activities of the antioxidant enzymes in plasma and in tissues of male rabbits (Kalaiselvi *et al*, 2014, Sarac *et al,* 2019). Aluminium chloride also cause enhancement of free radicals, lowers sperm quality, reproduction dysfunctions such as damage of the ovarian structure and results in alterations in antioxidant enzymes in both invivo and invitro (Wang *et al*, 2012). The mechanism of aluminium induced toxicity is that it enhances the potential of Fe^{2+} and Fe^{3+} ions to cause oxidative damage (Ige and Aiyeola, 2017).

2.3.1 Mechanism of action of aluminum chloride-induce anaemia

A number of studies have pointed to aluminum (Al) as an effective factor responsible for development of anaemia signs both in experimental animals and patients on hemodialysis (Sarac *et al,* 2019). On excess exposure of Aluminium, there is a direct

effect on haematopoiesis leading to anaemia. Daily ingestions of aluminum salt in rats produced severe anaemia within 2-3 weeks, this suggest that anaemia maybe induced by Aluminum chloride salts through decreased heme synthesis, decreased globulin synthesis and increased hemolysis (Kalaiselvi *et al*, 2014). Patients with anaemia from aluminum toxicity often have increased reticulocyte counts, decreased haemoglobin concentration, decreased hematocrit value, decreased mean corpuscular haemoglobin and decreased mean corpuscular volume. The toxic effects of aluminum on RBC's have been suggested to generate reactive oxygen species and induction of oxidative stress, which results in the oxidative deterioration of proteins, DNA, and cellular lipids through lipid peroxidation (Sarac *et al,* 2019). Recent studies show that aluminum may induce alterations in the activity of a number of anti-oxidative enzymes (xanthine oxidase, glutathione peroxidase, superoxide dismutase) (Hasona and Ahmed, 2017; Kalaiselvi *et al*, 2014). According to a study by (Newairy *et al*., 2009), Lipid peroxidation has been implicated as one of the molecular mechanisms underlying aluminum toxicity *in vitro* and *in vivo*. It was suggested that aluminum can induce morphological and functional changes in erythroid cells by a direct action on circulating erythrocytes, suggesting membrane alterations due to lipid peroxidation mechanisms. (Ameh and Aladi, 2018).

Furthermore, Aluminum may have a direct effect on iron metabolism; such that Aluminium influences absorption of iron through the intestine. Aluminium block iron transport in the serum and it displaces iron's binding to transferrin (Ige and Aiyeola, 2017). The major forms of aluminum in blood circulation have been reported to be in complex with transferrin using titration techniques, immune affinity chromatography, and spectophotometric. Transferrin is mainly known for its role in the transport and cellular uptake of iron, but it is also the major serum binding protein for aluminum. Aluminum is known to bound to serum transferrin and the complex of aluminum-

transferrin interacts with the same receptors as iron-transferrin (Ameh and Aladi, 2018). This receptor mediated cellular uptake is an important factor in the uptake of aluminum by the tissues. Studies suggest that chronic exposure to relatively high doses of aluminum can change iron metabolism in different animal species. However, the findings are not always in the same direction. Literatures have indicated a reduction in serum iron of rats exposed to high levels of aluminum, while other investigators have found no alterations or an increase in iron stores after exposure to aluminum (Kalaiselvi *et al.,* 2015; Klotz *et al*., 2017). Consequently, data concerning the mechanism of Aluminum toxicity on haematological system and iron metabolism after administration of aluminum are contradictory and seem to depend on the conditions of toxicity, which refer mainly to different doses and different routes of administration (Ige and Aiyeola, 2017).

2.4 Medicinal Plants

Medicinal plants are parts of plant or a whole plant which contain compounds that could serve for therapeutic purposes or that serves as precursors for synthesis of useful drugs (Yuan *et al*., 2016). Several drugs available today are derivatives of plant or microbial origin. From history, many novel therapeutic drugs were sourced from medicinal plants, as plant derived medicines have made large contributions to the health and wellbeing of humans (Nwachukwu *et al*., 2010). According to World Health Organization (WHO, 2014), about 65 - 80% of the global population rely on plants for their primary health care (Palhares *et al.,* 2015). The value of medicinal plants lies in the bioactive phytochemical constituents which create a physiological action on the human body (Aborisade *et al.,* 2017). Examples of these phytochemicals are phenols, alkaloids, flavonoids, tannins, essential oils, resins, and many others (Uttpal *et al*., 2019). However, some of the side effects obtained in the use of these medicinal plants as

source of medicine are mostly due to over-dosage and lack of sufficient knowledge of other harmful by-products contained in them (Ekor, 2014).

2.4.1 Medicinal plants possessing antioxidant activity and anti-anaemic potential

Plants has been said to be promising remedy for anaemia especially iron deficiency anaemia. Quite a number of plants have been reported to possess anti-anaemic activities, examples of such plant include:

2.4.1.1 *Telfairia occidentalis*

Recent studies on *T. occidentalis* described it as a nutritious vegetable in Nigeria that contains macronutrients nutrients, vitamins, minerals, and antioxidant but to mention a few. Studies has shown that high antisickling property can be attributed to the effects of *T. occidentalis* extract on the density of red blood cells and also reduced the deformability of red blood cells. Hence, the Leave extract were reported to possess significant antianaemic potentials due to the presence of various antioxidants (Kayode and Kayode, 2011; Mojisola *et al.,* 2017).

2.4.1.2 *Magnifera indica*

Magnifera indica (mango) is one of the most important tropical plants widely grown in Africa. Its leaf, stem bark and root have been reported to be efficacious in the treatment of asthma, diabetes, malaria and anaemia (Modupe and Olatunji, 2016). Studies on the plant pharmacology revealed the presence of saponnins, tannins, flavonoids, glycosides, phlobatannins and terpenoids, and that extracts of *M.indica* possesses anti-anaemic properties (Ogbe *et al.,* 2010; Ige and Aiyeola, 2017). Thus, it appears that the presence of these bioactive compounds such flavonoids, tannins and saponins, may be

responsible for the haematopoietic activity either by acting as an antioxidant thereby making iron available in the ferrous form or by aiding production of new red blood cell in the bone marrow (Abidakun *et al.,* 2018).

2.4.1.3 *Jatropha tanjorensis*

Jatropha tanjorensis plant belongs to the Euphorbiaceae family, and is commonly refers to as “hospital too far”, “lapalapa”, “catholic vegetable”, “Iyana Ipaja.” It is commonly consumed in the southwestern part of Nigeria as vegetable soup and used as herbs as remedy for several ailments such as anaemia, diabetes and cardiovascular diseases (Omoregie and Osagie, 2010). Proximate analysis revealed the presence of macronutrients in significant levels, phytochemical screening of *J.tanjorensis* leaf showed that it contains bioactive compounds such as flavonoids, alkaloids, tannins, cardiac glycoside, anthraquinones, and saponins. (Ebe and Chukwuebuka, 2019). The studies done to evaluate the anti- anaemic activity of *Jatropha tanjorensis* in Rabbits showed that the crude aqueous extract of *J.tanjorensis* leaf improved the haematological indices in anaemic-induced rats. The presence of these antioxidants in the leaf suggested to be responsible for the amelioration of damaging effects caused by the anaemic agent. (MacDonald *et al*., 2014).

2.4.1.4 *Moringa oleifera*

The leave of this plant has been reported to contain a profile of important trace elements and are good sources of proteins, beta-carotene, amino acids, minerals, vitamins, and various phenolics compounds (Galani *et al*., 2018). The leaves contain phytochemicals that include alkaloids, coumarins, anthraquinone, flavonoids, phenols, quinines and tannins (Kasolo *et al*., 2010). In the *M.oleifera* extract-treated groups, there were

significant increase in the haematological parameters when compared with the control and anaemic groups. This improvement could be explained as a result of the phytochemicals, vitamins and minerals, which have been well studied for their haemopoietic principles that have direct influence on the production of blood in bone marrow (Ameh and Aladi, 2018).

2.4.1.5 *Murraya koenigii*

Murrraya koenigii belongs to family Rutaceae. It is commonly called 'curry leaf' and is traditionally used as spice for its characteristic flavour and aroma. The leaves are considered as tonic, analgesic, antihelminthic, digestive and appetizer. The leaves are used traditionally in the treatment of piles, vomiting, inflammation, fresh cut, itching, dysentery, and dropsy. *M.koenigii* leaf has been said to contain a range of active pharmacological agents including alkaloids, carbazole, flavonoids, tannins, furanocoumarins, and terpenoids and are rich source of iron, magnesium, copper and zinc (Pushpa *et al*, 2014).

In the studies, "comparative anti anaemic activity of *Murraya koenigii* (Linn.) Spreng leaves and its combination with *Emblica officinalis* in aluminium chloride induced anaemia using rodents", it was observed that aluminium chloride was capable of causing alteration in some haematological parameters, thereby inducing anaemia. However, curry leaf in combination with amla was able to minimize these hazards by increasing iron and ferritin levels and the haematological indices in the treated groups. This suggested that the pharmacological agents in the leaves could have been responsible for the anti-anaemic activities (Purnima *et al.,* 2014; Sathassyea *et al.,* 2010)

2.5 *Parquetina nigrescens*

2.5.1 Taxonomic classification

Kingdom- Plantae

Phylum- Tracheophyta

Class- Magnoliopsida

Order- Gentianales

Family- Asclepiadaceae

Subfamily- Periplocoideae

Genus- Parquetina

Species- *Parquetina nigrescens*

(Source: Ayoola *et al,* 2011).

2.5.2 Botanical description

Parquetina nigrescens (Bullock) belongs to the family Ascelpiadaceae commonly called *ewe ogbo* in Yoruba, meaning "leaf that hears" (Ayoola *et al,* 2011). It is a perennial shrub found in low bushes in savannah areas, in equatorial West African countries including Ghana, Nigeria, Senegal, Congo basin, Cote d'Ivoire and down to South Tropical Africa (Irvine, 1961; Saba *et* al., 2010). It is a slender, glabrous long twining stems and a base tapering 10-15 cm long and 6-8 cm broad. The flowers of the plant grow from its side branches having whitish outside and inner reddish colour (Gill, 1992). The fruit also, is composed of two parts; an inner softer and an outer woody part that house the feather-like seed (Awobajo and Olatunji, 2010).

2.5.3 Ethnobotanical uses of *Parquetina nigrescens*

The leaf, root and the latex of *P. nigrescens* are commonly used in traditional medicine in treatment of all kinds of ailments and infections. Report from the traditional medical practitioners showed it use in the treatment of measles, dysentery, diarrheal, intestinal worms, rickets, gastric ulcers, skin lesions, menstrual disorders and venereal diseases

(Aderibigbe *et al*., 2011), headaches, fever, pains, and diabetes (Owoyele *et al.,* 2008). Sometimes, fresh leaves are crushed and taken as an emetic to treat severe constipation. It is also used to induce abortion (Imaga *et al*., 2010). It is given to children to treat respiratory diseases (Agbo and Odetola, 2005). The leaf juice is mixed with honey to ease fatigue, jaundice, stomach ulcer and anemia as a tonic. It is also used to treat hypotension and to ease child birth pain. The body is washed with a leave decoction to treat general fatigue. The leaf is a common ingredient in medication to treat insanity (Kayode *et al*., 2009). It has been used as dressing on wounds, carbuncles, boils, and worm infections in ethno-medicine (Kayode *et al*., 2009; Owoyele *et al*., 2011). The leaf of *Parquetina nigrescens* have been reported in literatures to contain a number of bioactive chemical compounds to which it owes its numerous pharmacological activities (Imaga *et al*., 2010; Aderibigbe *et al.,* 2011). Pharmacological activities reported in the plant include their use as analgesic, antiinfective, anti-inflammatory, antidiabetic and in the treatment of sickle cell disease (Adebayo *et al*., 2010; Saba *et al*., 2010). The antioxidant activities of the plant have been attributed to these bioactive chemical compounds and micronutrients (Akinrinmade *et al*., 2015).

2.6 Carbohydrate

Carbohydrates are one of the most important classes of biomolecules in the living system. They are organic compound that are made up of carbon, hydrogen and oxygen with the general formula of carbohydrates is $C_n (H_2O)_n$ (David *et al*., 2018). They play numerous vital roles in living organisms. They are the main source of energy depots in the body. Brain cells and erythrocytes are almost exclusively dependent on carbohydrates as the energy source, and the energy production from carbohydrates is 4k calones/g (16 k joules/g). They are structural basis for many organisms; cell wall of microorganisms, cellulose of plants, exoskeleton of arthropods (insects),

mucopolysaccharides of higher organisms, part of RNA and DNA as in ribose and deoxyribose respectively, integral features of many lipids and proteins (glycolipids and glycoproteins), in cell membrane especially where cell-cell recognition and molecular targeting is highly important (Nelms *et al*., 2011).

Based on their molecular size, they are grouped into monosaccharides, disaccharides, oligosaccharide and polysaccharides. The monosaccharides are the simple sugars and consist of a single polyhydroxy ketone or aldehyde. The most abundant monosaccharides in nature are the six-carbon sugar D-glucose, while the most abundant oligosaccharides are two unit of monosaccharides known as sucrose (cane sugar) a Disaccharides. Polysaccharides on the other hand is a sugar polymer containing 20 or more monosaccharides units (Ashley *et al*., 2018).

2.7 Proteins

Proteins are the most abundant nitrogen-containing compound in plants and animal. It is also a macromolecule aside carbohydrates and lipids. It is one of the five classes of complex biomolecules in tissues and cells, the other being DNA, RNA, lipids and polysaccharides. Amino acids are the building blocks of protein join together by peptides links according to a sequence directed by the base sequence of the DNA (Parveen *et al*., 2017). They are essential components of all living cells. They are also essential secretions such as digestive enzymes and peptides, digestive enzymes or protein hormones. Amino acids involved in the synthesis of plasma proteins, essential for transport substances through the blood, maintaining osmotic pressure, and maintaining immunity are obtained from dietary proteins. The excess protein from diets is treated as source of energy such that the ketogenic amino acids are converted to fatty acids and keto acids while the glucogenic amino acids are converted to glucose (Chatterjea and Shinde, 2012). The human body has no storage depot and thus dietary

protein must be supplied with every meal, however certain amount of the body protein undergoes a continuous breakdown process and resynthesis (Longo and Mattson, 2014). In fasting state, the breakdown of this protein is activated and the resulting amino acids are used for the production of glucose, plasma protein, synthesis of non-protein nitrogenous compounds and the essential sensory protein. In the fed state, some amino acids are utilized for energy production and as biosynthetic precursor (Andrade & Moacir, 2017). Plants are important source of dietary protein for both human beings and animals.

2.8 Lipids

Lipids are organic compounds made up of carbon skeleton with hydrogen and oxygen substitution. Some lipids contain Sulphur, phosphorus and nitrogen. Lipids are not (hydrophilic) water loving and hence are not soluble in water like the carbohydrates and proteins. This general rule is not ultimate for some lipids, since soap, short-to-medium-chain fatty acids, and some complex lipids are soluble in water (Fahy *et al.,* 2011). Lipids are classified into four, as categorized by Bloor: simple, compound (complex), derived and miscellaneous lipids. Simple lipids are esters of fatty acids with various alcohols such as cholesterol and glycerol examples include tricylglycerols, cholesteryl esters, waxes, vitamin A and D esters. Compound lipids are esters of fatty acids in combination with both alcohol and other groups, examples include phospholipids, sulpholipids, glycolipids and lipopolysaccharides.

Derived lipids are product of hydrolysis of simple and compound lipids. Examples include monoacylglycerols and diacylglycerols, sterols, straight-chain and ring-containing alcohols, and steroids. And lastly, miscellaneous lipids include some wax, vitamin E and K, carotenoids and squalene. (Lakna, 2017). Lipids have high dietary energy value, and are essential dietary constituents such as the fat-soluble vitamins and

the essential fatty acids found in natural foods. Linoleic and linolenic acids cannot be synthesized in the body so are essential fatty acids in diets. The essential fatty acids are required for maintaining integrity and functions of cellular membrane structure, metabolisms and synthesis of certain compounds. Fats is stored in adipose tissue, where it serves as an insulator and provide mechanical protection for organs (Sacks *et al.,* 2017). Evidences show that incidence of coronary heart disease, obesity and cancer are influences from dietary fats and oils. Oils are found in plant source predominantly.

2.9 Dietary Fibre

Dietary fibre are non-starch polysaccharide and lignin. They food components that cannot be broken down by human digestive enzymes (Kohn, 2016). Dietary fibres are of two types; soluble and insoluble fibre. Soluble fibres dissolves in water in the intestinal tract to form gel which helps to show down the movement of food though the intestine. Examples are gums and pectins (Dai and Chau, 2016). The insoluble fibres do not dissolve in water in the intestinal tract, but stimulate peristalsis movement, by causing a rhythmic muscular contraction of the intestines, thereby moving the digesta through the digestive tract (Gidley and Yakubov, 2019). Plants have high amounts of dietary fibre. Several health benefits of dietary fibre have been established, including reduction in chances of gastrointestinal problems such as diarrhea, slows down the rate of gastric emptying, thus the rate of nutrients absorption (Mackie *et al*., 2016). When carbohydrates food is consumed along with fibre, the rate at which carbohydrate are digested and absorbed is slowed down, thus the rise in blood sugar and insulin levels (Olive and Komarek, 2017).

2.10 Vitamins

Vitamins are organic compounds found in the diet. They are known to perform specific cellular functions and are required in small amount for reproduction, growth,

development and health. They are found in varieties of chemical forms and structures which form the basis of their classifications (Muhammad *et al.,* 2017). Vitamins have important biochemical roles in the maintenance of human health and possesses unique therapeutic role in the treatment of health disorders. The amounts sufficient to meet the body's requirement cannot be adequately synthesized by man, hence, must be supplied from the diets. Vitamins are therefore classified into two categories based on their solubility: Fat soluble vitamins which include vitamin A, D, E and K and water-soluble vitamins, which are the B complex and vitamin C. (Perlitz *et al*., 2019). Fat-soluble vitamins are related and absorbed with dietary fats, because their absorption is similar to that of fats. However, water-soluble vitamins are not related with fats and are not affected by changes in fat absorption.

2.10.1 Vitamin A

Vitamin A is a collective name for a group of lipophilic biomolecules required for vital metabolic functions. In plants, it is called provitamin A and carotenoids while it is called retinoids in animal source. The retinol, retinal, retinoic acid and retinyl esters are different forms of retinoids. α-carotene, β-carotene and β-crytoxanthin are however, the plant originated provitamin. The active forms of the vitamin are retinol, retinal, and retinoic acid, while the β-carotenes are less biologically active (Ramadhan and Ian, 2012).

Vitamin A (retinol) is an essential micro nutrient required for normal vision by contributing to the production of retinal pigments needed for vision in dim light. It is vital for cell growth and differentiation, in reproduction by supporting spermatogenesis in males and prevents fetal reabsorption in females, and enhances the immune system. It also maintains the integrity and normal functioning of glandular and epithelial tissues lining the intestine, urinary and respiratory tract, skin and eyes. It is also known to

protect against degenerative diseases because of its antioxidant activity and perform other biochemical functions (Sinbad *et al*., 2019).

Vitamin A is widely distributed in plant and animal foods. Animal foods include liver, fish, eggs, butter, meat and whole milk, while plants source include; Red palm oil, Buriti palm pulp, Carrot, Dark green leafy vegetable, Sweet potato, red, yellow, Mango, Apricot, Papaya, and Tomato. Green leafy vegetables are the cheapest source while Fish liver oils are the richest natural sources of retinol. The IU is equivalent to 0.3 µg of retinol or 0.55 µg of retinal palmitate or 0.6 µg of β-carotene (Kulkarni, 2012).

Vitamin A deficiency is a systematic disease that affects cells and organs throughout the body. Its deficiency is associated with xerophthalmia, blindness, anaemia, growth retardation, apathy, hepatosplenomegaly, keratinization of epithelia tissues including dry and scaly skin, infections and so on (Perlitz *et al*., 2019).

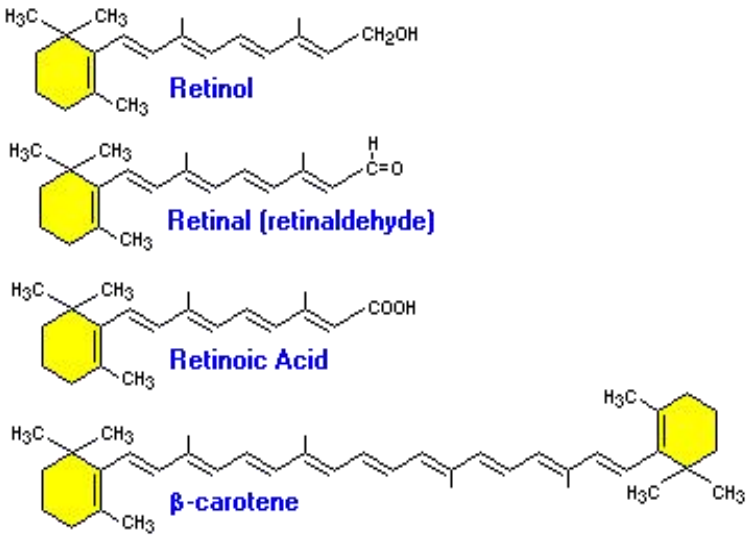


Figure 2.3: Structural formula of vitamin A (Ramadhan and Ian, 2012)

2.10.2 Vitamin D

Vitamin D is a component of diet, although it is also produced under human skin as prohormone when it is exposed to sunlight. Whether it is obtain from sunlight, diet or

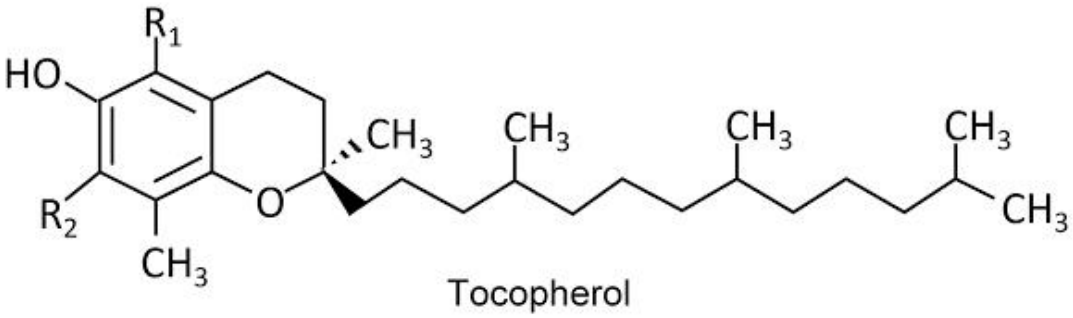
supplement, it function similarly in the body by undergoing several processes to become the physiologically active form (1, 25-dihydroxyvitamin D) (Sinbad *et al*., 2019). This active form possesses potent cell signaling abilities and is tightly regulated at the tissue level (Taylor *et al.,* 2017). It is also a key player in immunity response, as it is involved in maturation of white blood cells through calcitriol an important agent in calcium homeostasis. Vitamin D is known to act on target organs like the bones, intestinal mucosa and kidney in order to regulate calcium and phosphate metabolisms (Muhammad *et al*., 2017). Vitamin D is found in salmon, sardine, tuna, Egg yolks, mushrooms, soy milk, cow's milk, orange juice, and fortified foods (Sinbad *et al*., 2019). It deficiency is associated with numerous disorders like rickets, osteomalacia, osteoporosis, loss of balance, diabetes, rheumatoid arthritis, asthma, depression, epilepsy, and lowered immune function (Michael, 2019).

2.10.3 Vitamin E

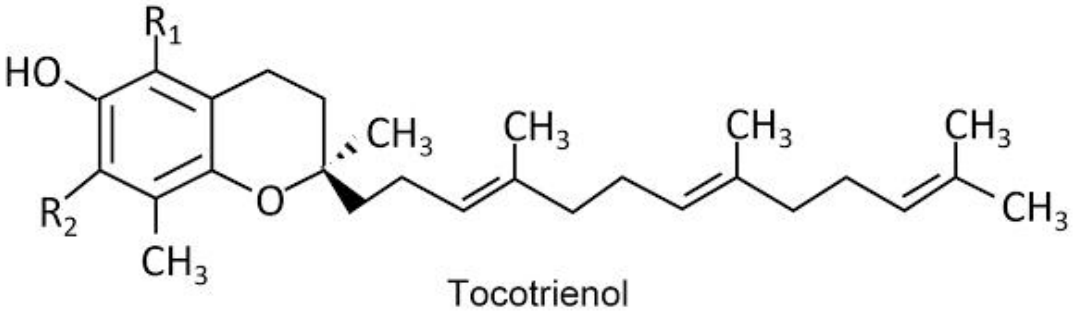
Vitamin E is a general descriptive name of two families of compounds, the tocopherols and tocotrienols. There are four forms in each family, which are alpha, beta, gamma and delta classes of tocopherol and tocotrienol. These eight classes are collectively called "tocochromanols" (Sinbad *et al*., 2019). They have different biological potency, but the alpha tocopherol is the most active in vitamin E activity (Chatterjea and Shinde, 2012). They are lipophilic in character, and due to this, they accumulate in cellular membrane, lipoproteins, and fat deposits where they act as free radical scavengers or as highly potent antioxidant and also function in the protection of polyunsaturated fatty acids from peroxidations (Jilani and Iqbal, 2011). The most important function of vitamin E is as a chain-breaking, free-radical-trapping antioxidants in the cells. This is because of the presence of phenolic-OH group on the 6th carbon of the chromane ring. (Chatterjea and Shinde, 2012).

The tocopheroxyl radical is unreactive, and so forms nonradical compounds. Usually, the topheroxyl radicals is reduced back to tocopherol by reaction with vitamin C from plasma (Rodwell *et al.,* 2015). Dietary sources of vitamin E includes, vegetable seeds and their oils, almond, fish, some leafy vegetables and fruits, nuts and some cereals (Jilani and Iqbal, 2011).

There are several signs of vitamin E deficiency in animals, most of which are related to cell membranes damage, leakage of cell contents to the external fluids, muscle and neurologic problems. Specific examples of the vitamin deficiency include, resorption of fetuses, testicular atrophy, increase concentration of creatine kinase and pyruvate kinase due to leakages of these enzymes from the muscles. In vitamin E deficiency, the erythrocyte membrane becomes abnormally fragile, which leads to increase erythrocyte haemolysis (Sinbad *et al*., 2019). Others include cancer, heart attack, stroke, Parkinson's disease, cataract, Alzheimer's disease, fibrocystic breast disease, epilepsy, and diabetes (Chatterjea and Shinde, 2012).



Tocopherol



Tocotrienol

Figure 2.4: Structural formula of Vitamin E (Chatterjea and Shinde, 2012)

2.10.4 Vitamin C

Vitamin C exist as ascorbic acids and as dehydroascorbic acid, of which both have vitamin activities. Vitamin C is a vitamin for human beings, other primates, guinea pig, passeriform birds, bats, most fishes and invertebrates because other animals synthesized it as an intermediate in the uronic acid pathway of glucose metabolism, but there is a blockage in the pathway in the species for which it is a vitamin as a result of the absence of gulonolactone oxidase (Chambal *et al*., 2013). Vitamin C serves as a cofactor for mixed function oxidases, where its act as a reducing agent in hydroxylation of proline and lysine which are required for protocollagen to crosslink adequately into collagen fibrils. Vitamin C is therefore essential for wound healing, bone formation, and for the maintenance of connective tissues (Grosso *et al.,* 2013).

In addition, ascorbic acid has a number of non-enzymatic effects due to its action as oxygen radical quencher and as a reducing agent. For instance, it aids absorption of iron in the stomach from ferric to ferrous state. It aids the conversion of folate to tetrahydrofolate and its derivative, thereby, enhancing utilization of folate. It also prevents the oxidation of vitamin A, E, and some B vitamins (Sinbad *et al*., 2019). Deficiency of vitamin C include skin changes, gum decay, tooth loss, bone fracture, fragility of blood capillaries, which lead to bruising petechiae and decrease immunocompitence. Scurvy a more severe form of deficiency is linked to decrease wound healing, hemorrhahing and anaemia (Kumar and Rizvi, 2012).

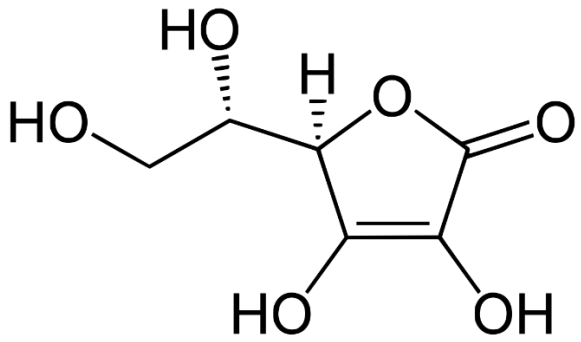


Figure 2.5: Structural Formula of Vitamin C (Ascorbic Acid)

2.10.5 Vitamin B_9 (Folic acid)

The active form of folic acid is tetrahydrofolate (pteroyl gluatamate) as shown in Figure 2.3. Folate in foods may have up to seven additional glutamate residues connected by γ-pepetide bonds. Usually, tetrahydrofolate carry one-carbon fragments attached to *N*-5 (formyl, formimino, or methyl groups), *N*-10 (formyl) or bridging *N*-5- *N*-10 (methylene or methenyl groups) (Von Fraunhofer, 2019). Due to the stability of 5-Formyl-tetrahydrofolate than folate, it is often used pharmaceutically. One carbon tetrahydrofolate derivative is required in biosynthetic reactions like synthesis of choline, glycine, serine, methionine, purines and dTMP. In protein synthesis, folate is required in conversion of methionine to S-adenosyl methionine, which is used in several methylation reactions, including DNA methylation (Soofi *et al.*, 2017).

Rapidly dividing cells are seriously affected by folate deficiency because of its large requirement for thymidine for DNA synthesis; this affects the bone marrow, resulting in megaloblastic anaemia (Mahmood, 2014). Supplements of 400 μg/ day of folate begun before conception result in a significant reduction in the incidence of spina bifida and other neural tube defects (Gorelova *et al*., 2019). Another folate deficiency is observed in elevated blood homocysteine, is a significant risk for thrombosis, artherosclerosis,

and hypertension. Studies revealed that reduced folate status led to impaired methylation of CpG islands in DNA, a factor in the development of colorectal and other cancers. Several studies suggest that folate food enrichment or supplementation may reduce the risk of developing certain cancers (Siaw-Cheok, 2016).

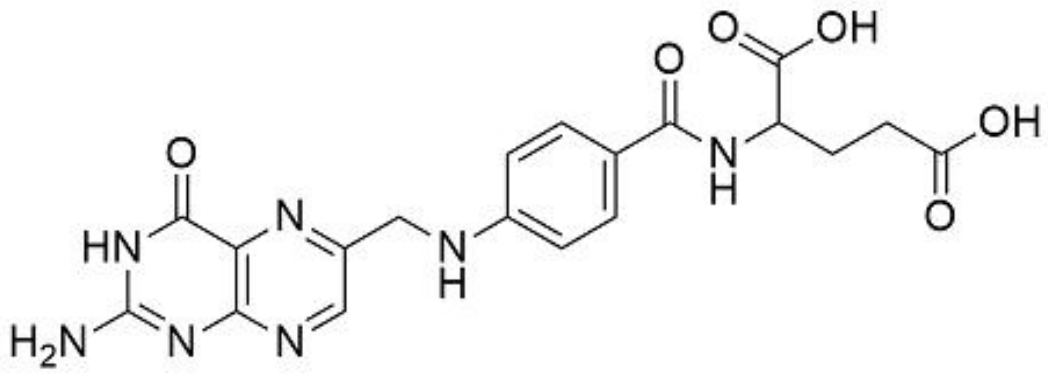


Figure 2.6: structural formulae of Folic acid (Vitamin B_9) (Soofi *et al.*, 2017).

2.10.6 Vitamin B_{12}

Vitamin B_{12} (molecular weight = 1355.4) also called 'cobalamin'- those corrinoids (meaning cobalt-containing compounds possessing the corrin ring) (Watanabe *et al*., 2014). There are different forms of this vitamin; methyl cobalamin, cyanocobalmin or hydroxycobalamin and 5-deoxycobalamin. Vitamin B_{12} take part in only two reactions in humans. The methyl derivative of vitamin B_{12} (methylcobalamin) function as a coenzyme of methionine synthase (EC 2.1.1.13), the enzyme involved in the methylation of homocysteine to methionine (methionine biosynthesis) (Sinbad *et al*., 2019). Secondly, in the catabolism of valine, isoleucine, threonine, odd fatty acid chain, thymine and the side chain of cholesterol, 5-deoxyadenosylcobalamin functions as a coenzyme for methylmalonyl CoA mutase (EC 5.4.99.2), an enzyme which convert methylmalonyl CoA to succinyl CoA. (2,3)

Vitamin B_{12} is the only vitamin found to be completely absent from plant source, therefore vegetarians are at risk of developing B_{12} deficiency (Watanabe *et al*., 2013). Although, certain bacteria are able to synthesized it, it is concentrated in the animal source foods, such as egg, milk, meat, fish, and shellfish (Watanabe *et al*., 2014). In recent times, there are several vitamin B12 enriched or fortified plant foods, such as, enriched beans and vegetables using organic fertilizers or hydrophonics (Bito *et al*., 2012) and some other plant source such as fermented Beans, edible mushrooms and edible algae (Watanabe *et al*., 2012).

The recommended daily allowance (RDA) of vitamin A in the united State of American is 2.4 μg/day (Shibata *et al*., 2013). Little amounts of the vitamin produced on the surface of fruits may be sufficient to meet requirements, but already prepared vitamin B_{12} produced by bacterial fermentation is available (Rodwell *et al.,* 2015). Vitamin B_{12} is absorbed from the distal third of the ileum through receptors that bind the intrinsic factor-vitamin B_{12} complex.

Vitamin B_{12} deficiency results in pernicious anaemia. This impairs the metabolism of folic acid, resulting in a functional folic acid deficiency that alters erythropoiesis, resulting in a megaloblastic anaemia. Vitamin B_{12} deficiency also causes neurologic problems which results from demylenation of nervous tissues (Rodwell *et al.,* 2015, Teng *et al.,* 2014). Figure 2 below shows the structural formular of vitamin B_{12}

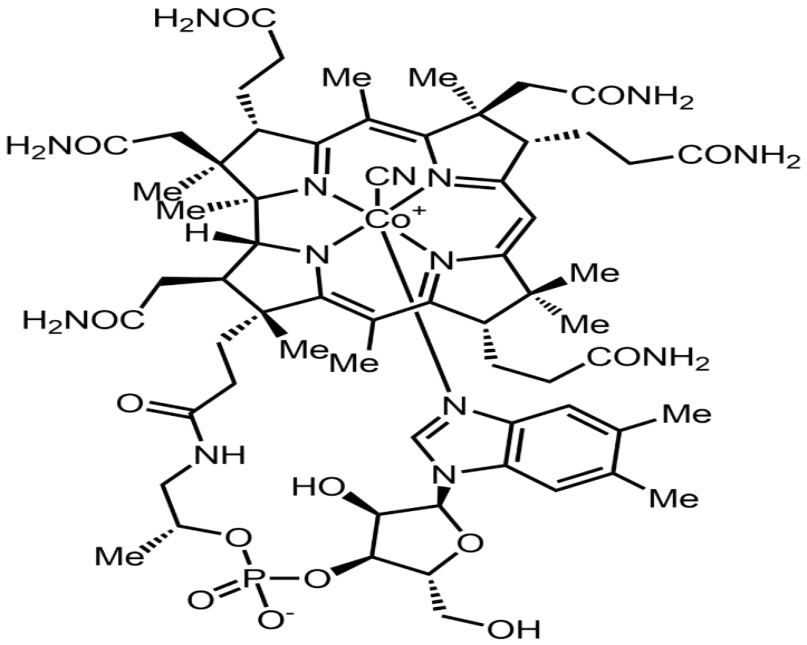


Figure 2.7: structural formulae of Vitamin B_{12} (Chatterjea and Shinde, 2012).

2.11 Minerals

Minerals are known as inorganic elements which function in a wide variety of way such as cofactors of enzymes, structural elements, acid base balance, nerve conduction, muscle stability. Mineral requirements in individuals varies from µl to mg per day. Minerals are divided into two groups namely: Microminerals and Macrominerals. Macrominerals are needed in high concentration, more than 100mg per day. They include sodium, potassium, phosphorus, calcium, magnesium, while microminerals are needed in low concentration in the body. They are known as trace elements. They include iodine, iron, zinc, copper, selenium, chromium (Chatterjea and Shinde, 2012).

2.11.1 Calcium

This is the fifth most abundant metallic element in the earth crust, it forms about 3% (Heaney *et al*., 2012). It is found in nature as granite, chalk, hard water, bone, limestone. Calcium is essential in diet; foods of vegetable origin contain some amount of calcium. Dairy products are good sources of calcium. A normal human body contains about 1200g of calcium which accounts for 1-2% of body weight. About 99% of the body's

calcium is localized in bones and teeth, if present together it is known as calcium phosphate with a small portion of calcium carbonate providing a rigid structure (Chatterjea and Shinde, 2012).

The remaining 1% is found in the muscles, extracellular fluid and other tissue. Calcium is required in the body for growth and the development of the skeleton. Ca is involved in several processes in the body such as transmission of signals across membrane, enzymatic action, contraction of muscle, coagulation of blood, and hormonal response. Inadequate intake of calcium is also associated with rickets and can result to elevated blood pressure (Cormick and Belizán, 2019).

2.11.2 Magnesium

This element is required in certain important enzymatic activity in the body, cellular reaction and in neuromuscular transmission. It is regarded as the fourth most abundant cation in the body. It helps in development of bone (60% in the bone) and chromatin condensation in gene activity. Alcoholism leads to deficiency of magnesium, which is marked in symptoms such as weakness, tremor and cardiac arrhythmia (Chatterjea and Shinde, 2012).

2.11.3 Potassium

This is an intracellular electrolyte and it exists as a water-soluble cation. There are three major electrolytes in the body which are potassium, chloride and sodium. The concentration of sodium and potassium across membrane is maintained by Na+/k+ ATPase pump is important for nerve transmission and muscle function (Vasudevan *et al*., 2011). The importance of potassium in the body affects many systems such as respiratory, cardiovascular, digestive, renal, and endocrine. Potassium is also an important cofactor for enzymes involve in energy metabolism, cell division and cell

growth. Potassium supplementation is said to have a role in treating heart failure, decrease blood pressure, improve calcium/ phosphorus balance, enhance bone formation, and maintain the activity of nerve and muscle (Chatterjea and Shinde, 2012).

2.11.4 Iron

Iron exists in the oxidation state from +2 to +6, but in the biological system the oxidation state is Fe^{2+} and Fe^{3+} interchangeably. The total store of iron in the body ranges from 2.5- 4g, about 2- 2.5g of Fe is found in the red blood cell. Iron is split from a carrier by a process aided by acid secretion before it is being absorbed into the body (Chatterjea and Shinde, 2012). Fe is stored in the liver as ferritin. Iron is required as a component of haemoglobin, myglobin for oxygen and carbon dioxide transport. It is also a component of cytochome which is involved in oxidative phosphorylation. It is also required for proper phagocytosis and killing of bacteria by the neutrophil. Deficiency of iron leads to microcytic hypochromic anemia, decrease in immunocompetence (Anand *et al*., 2014).

2.11.5 Zinc

Zinc is a component of about twenty-four metalloenzyme such as carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, zinc copper dismutase, RNA and DNA polymerase etc. Zinc (Zinc finger-zinc coordinate with four amino-acid side chains) d provides structural stability for about 300-700 proteins, it also facilitates binding DNA. Zinc is involved in protein-protein interaction and in signal transduction (Chatterjea and Shinde, 2012). Deficiency in Zinc causes poor growth development in children, poor wound healing. dermatitis, decrease taste and impaired immune function. When zinc is replaced with lead it leads to lead poisoning which causes anaemia and results to accumulation of aminolaevulinic acid (Rodwell *et* al., 2015).

2.11.6 Copper

Copper is a component of various matalloenzyme, it aids in the function of many important enzymes (Gibney *et al*., 2009). It is an important component of the following enzymes such as cytochrome C oxidase, dopamine β-hydrolase, superoxide dismutase, oxidase, tyrosinase, desaturase. In human blood copper is distributed between the erythrocytes and plasma. As a component of ceruloplasmin, it aids the transport of cells by facilitating the binding of oxidized iron to transferrin (Chatterjea and Shinde, 2012). Deficiency of copper results to nephritic syndrome, kwashiorkor, Wilson disease, vomiting and diarrhea. The symptoms of this deficiency are anemia, neutropenia (Bost *et al*., 2016).

2.11.7 Sodium

This element is makes up about 80% of dissolved matter in the water; it is the sixth abundant element in the earth. There are varieties of sodium such as sodium chloride which are used as additives in food processing. Sodium element is a cation which maintains osmotic and electrolyte balance in the body. Sodium is involved in various processes such as nerve conduction, formation of mineral for the bone, cellular transport system. Decrease in the plasma sodium is not related to diet but caused by clinical conditions such as trauma, overuse of diuretics, severe infection, liver disease, oedema, heart failure, ulcerative colitis, anorexia nervosa (Gibney *et al*., 2009). Excessive intake of salt could be involved in coronary heart disease, stroke, gastric cancer, bronchial hyperactivity, and osteoporosis.

2.11.8 Manganese

This element is widely distributed in the biosphere. It constitutes about 0.85% of the earth crust making it the 12th most abundant element on earth. There are about 25mg of

manganese in the body, 25% of it is stored in the skeleton and are not easily accessible. Research has shown that there is high concentration of manganese in the liver, pancrease, intestine and bone (Li and Yang, 2018).

Manganese is also required as a cofactor of enzymatic activities such as arginase, superoxide dismutase, pyruvate carboxylase, phosphoenol pyruvate carboxylase, glutamine synthetase, glycosyl transferase. Deficiency of manganese leads to impaired growth, of the skeletal structure, depressed function of the reproductory system, defects in lipid and carbohydrate metabolism. Manganese deficiency is reported to be more in infants due to the low concentration of manganese in the breast milk and variation in the levels of infant formulated diets (Gibney, 2009).

2.12 Secondary Metabolites

2.12.1 Flavonoids

Flavonoids are large group of secondary metabolites in plants, in which over 6000 different kinds have been reported among plant species (Scarano *et al*., 2018). They have an aromatic ring bearing at least one hydroxyl group (Aurelia *et al*., 2018; Duangjai *et al*., 2018). They are found in plant derived foods especially in skin and flesh of fruits and in the epidermis of leaves (Bubols., 2013). Based on their chemical structure and modification, Flavonoids have been classified into several subdivisions, such as flavonols, chalcones, isoflavones, flavones, anthocyanins, proanthocyanidins, and aurones (Aryal *et al*., 2019).

They have been known to perform numerous physiological roles which are of great benefits to both the plant itself and to humans. These roles include; developmental regulators of auxin transport, protection of plants against UV radiation and catabolism which are considered as the most important and ancient roles of this phytochemicals

(Mouradov and Spangenberg, 2014). Other roles include protection against phytopathogens/predators, attraction of pollinators, responsible for the colourful nature of flowers and interaction with the rhizosphere among others.

The occurrences and chemical biodiversity observed in flavonoids and in other secondary metabolites indicated their adaptation to local conditions and so their variations, genetic background, the plant developmental stage, geographical area, and external factors such as the biotic and abiotic stresses (Aurelia *et al*., 2018; Cirak and Radusiene, 2019). Food processing of varying types and degrees such as heat or temperature dependent treatment, crushing, drying, pressing, and canning have been reported to modify the organoleptic, structures and nutritional properties of the food, since many these food metabolites are volatile and easily destroyed (Cirak and Radusiene, 2019; Kårlund *et al*., 2014).

Also, food processing sometimes could cause the release of some enzymes that can promote the degradation of biocompounds. For example, flavonoids can be oxygenated and destroyed by esterases, polyphenol oxidases, glycosidases and peroxydases. For instance, the black pigmentation observed on vegetables and some fruits is often caused by the oxidation reactions of peroxidases and polyphenol oxidases, and this reduces the freshness of the vegetables (Attia-Ismail, 2015.)

Flavonoids have been identified to have several nutraceutical benefits. They include; anti-cancer activities1 hypoglycemic effects and antioxidant activities, anti-bacterial activity, anti-inflammatory and immune-modulatory activities (Qadir *et al*., 2017; Scarano *et al*., 2018).

2.12.2 Tannins

Tannins are astringent and bitter plant with a molecular weight above 500. They are polyphenolic compounds that bind or precipitate organic compounds such as proteins, amino acid and alkaloids. They decrease the quality of protein by decreasing its digestibility and palatability. Tannins also inhibit the activities of trypsin, chymotrypsin, lipase, amylase and absorption of iron from diets (Gemede and Negussie, 2014). The effect of tannin includes decrease in feed intake, decrease in growth rate, damage of intestinal tract, decrease of microbial activities, interference with the absorption of iron, carcinogenic effect. Tannins are a group of certain phytochemicals with wide range of properties such as antiviral, antiparasitic, anti-inflammatory and antiulceric (Choi and Kim, 2020). Tannin has wide usage in industries such as in the making of leathers, additives and antioxidant, and in the pharmaceuticals (Antonio, 2019).

2.12.3 Phytates

Phytates are also known phytic acid a derivate of inositol, it's found in plants. Phytic acid can form insoluble salts with calcium, iron, zinc and magnesium because of the three replaceable hydrogen atoms. Its ability to form insoluble salt makes the metal unavailable for absorption into the body (Innalegwu *et al.,* 2017). Phytate is seen as the primary storage of phosphoruss in seeds and grains. It works best at a broad pH region as a negatively ion, hence it has a negative impact on the availability of diavalent, trivalent ions. High consumption of phytate will result to deficiency of mineral (Gemede and Negussie, 2014).

2.12.4 Oxalates

Oxalate is a dicarboxylic anion which is produced in crop plants and pasture weeds. Oxalate is present in plants as soluble salts such as oxalic acid, potassium, sodium or ammonium oxalate. High oxalate in diet leads to increase in renal absorption of calcium. Oxalate binds to nutrients during digestion making them inaccessible to the body. When

food with the high amount of oxalate is taken, nutritional deficiencies are likely to occur and irritation of the lining in the gut (Troisi *et al*., 2015).

2.12.5 Saponin

Saponins are glycosides which are characterized by their bitter or astringent taste, foaming ability, hemolytic effect on the red blood cells. They occur primarily in plant kingdom, although they are widely distributed in nature (Gemede and Negussie, 2014). They have several properties such as foaming ability, bitter taste, emulsifying properties, medical properties, antimicrobial properties, haemolytic properties, insecticidal and mollusicidal activities (Augustin *et al.*, 2011). The bitter taste of this compound in food limits its intake which can cause impairment in growth (Gemede and Negussie, 2014). Saponin affects the digestion of protein by inhibiting the activities of the enzyme involved in protein digestion (Hossain, 2019).

Saponins have also been reported to have beneficial effect such as anticarcinogenic properties, immune stimulatory and hypocholesterolemic property. They act as adjuvant in viral and antibacterial activities (Yang *et al*., 2010). A high saponin diet can also be used in the treatment of lead poisoning, hypercalciuria in human and prevention of platelet aggression in human (Troisi *et al*., 2015).

2.12.6 Cyanogenic glycosides

They are derived from five amino acids (Valine, Isoleucine, Leucine, Phenyalanine and Tyrosine) and hydroxylnitrile with a sugar moiety. Consumption of diet with cyanogens results to cyanide toxicity. Cyanide interferes with aerobic respiration, it's an inhibitor of cytochrome oxidase. Hydrogen cyanides combines with sugar to form non-toxic cyanogenic glycosides, which can be degraded to form hydrogen and cyanide. The lethal dose of hydrogen cyanide is 50-60 mg/kg per body weight (Onwuka, 2005).

2.13 Haematological parameter

Haematological parameters are parameters related to blood and it's an indicator of the physiological state of the body. The analysis of the blood gives information on the physiological, nutritional, pathology of the organism. The constituent of the blood change as the physiological condition of the health of an organism change. Organism with good blood composition shows better physiological performance. Analyzing blood for their composition provides information of the diagnosis and prognosis of diseases. Red blood cells, white blood cells, mean corpuscular, and pack cell volume are examples of haematological parameters (Gibney *et al.,* 2009).

2.13.1 Red blood cells

This the commonest type of blood cells, it functions in delivering oxygen to various tissues in the body of an organism. They are about 4-6 millions/mm^3, each mature erythrocyte is about 6-8µm in diameter and 2µm in thickness. The human red blood cells are formed in the bone marrow, the red blood cell lacks nucleus, mitochondria, golgi apparatus, endoplasmic reticulum. It has a life span of 120 days, at the end of the life span it is destroyed in the spleen and the iron is used for the production of new red blood cell (Chatterjea and Shinde, 2012).

The red blood cell takes up oxygen from the lung and gill with the aid of the haemoglobin which is an iron containing biomolecule that has the ability to bind to oxygen. The haemoglobin in the red blood cell also aid in carrying carbon dioxide from the tissue to the pulmonary lung (Satheeshkumar *et al.*, 2011). When there is a reduction in the red blood cell, it leads to reduction in the amount of oxygen taken to different tissue and also the reduction in the amount of carbon dioxide been carried out from the different tissues (Elarabany, 2018). Shortage of red blood cell indicates an anaemic condition which may be as a result of malnutrition (deficiency in iron), bone marrow

suppression, damage of red blood cells. Unusual increase in red blood cells may be as a result of heart disease, lung diseases, and dehydration (Rodwell *et al*., 2015).

The haematocrit or pack cell volume which is also a haemotological parameter, measures the volume of cell as a percentage of the total volume of cells and plasma in the blood. The percentage is three times more than the haemoglobin (Stevens *et al*., 2013). The Mean cell volume is the average of the total red blood cell volume. In individuals with anaemia, the MCV provides a basis of the classification of microcytic anaemia. It is calculated as the PCV divided by red blood cell count, in femolitres. A low MCV value indicate the cells are microcytic which is as a result of iron deficiency, lead poisoning, diseases (Özkan *et al*., 2012).

The MCH which is known as the Mean corpuscular Haemoglobin is the average weight of haemoglobin per red blood cell in a sample of blood. The MCHC mean corpuscular haemoglobin concentration is a measure of the concentration of the haemoglobin in a pack cell volume. It is gotten by the amount of haemoglobin with the haemocrit. The standard reference range of blood test is 32-36 g/dl or 4.9-5.5 mmol/L (Aster, 2014). It is low in microcytic anemia and normal in macrocytic anaemia. It is the most sensitive and accurate blood test.

2.13.2 White Blood Cell

White blood cell also known as Leukocytes are responsible for the immunity of an organism. The density of WBC in the blood is said to be 5000-7000/mm^3. The WBC are divided mainly into two namely; the granulocytes and agranulocytes (Rodwell *et al*., 2015). The granulocytes have granules and are further divided into three namely: neutrophil, eosinophil and basophil, while agranulocytes, do not contain granules and are divided into two namely: lymphocytes and monocytes.

The neutrophils are first to arrive at the site of an inflammation, they have a diameter of 12-15 µl. They aid in destroying and ingesting bacteria. Neutrophils increases in conditions such as stress, inflammation, necrosis and heart attacks. The normal level of neutrophil in the blood ranges from 45-74 % (Aghara, 2014). The eosinophils are found in the skin and airways and blood streams. They are of the same size with the neutrophils and often increase in response to an allergic, inflammatory reaction, and parasitic reaction. They are rarely found in the blood stream, their normal level in the blood stream ranges from 0-7% (Özkan *et al*., 2012).

The basophils are known as mast cells, when found in other tissues but called Basophils when found in the blood stream. The basophil secretes substances such as histamine, serotonin and heparin. These substances secreted by basophils are known as anti-coagulant and vasodilators. They are said to be involved in allergic, stress reaction, prevention of blood clotting in microcirculation (Corbett, 2008). The normal blood level ranges from 0-2%. The lymphocytes are smaller than the leucocytes and have a size of 8-10µm in diameter. They are the main component of the immune system, which acts as body defense to fight against anti-bodies (Satheeshkumar *et al.*, 2011). Organisms with low lymphocytes are at a risk of infection while high lymphocytes are highly resistance to diseases.

2.14 Biochemical parameter

2.14.1 Albumin

This is a small globular protein with a molecular weight of 66.3Kda. It is the most abundant protein found in the plasma; it accounts for half of the weight of the plasma protein. Albumin is the major protein component of the urine, amniotic fluid, cerebrospinal fluid, interstitial fluid (Satheeshkumar *et al.*, 2011). It is synthesized by the hepatic parenchyma cells; its synthesis is controlled by the osmotic pressure and

protein intake. Its function is to maintain colloidal osmotic pressure in both the vascular and extravascular spaces. Albumins function in the binding and transportation of compounds such as fatty acids, phospholipids, metallic ions, amino acids, hormones, drugs (Yakubu *et al*., 2017).

Decrease in albumin concentration are seen in conditions such as inflammation, decrease in extracellular space which could be an indication of liver disease. Measurement of the albumin concentration in the plasma is use in assessing the severity of liver diseases. Low concentration of albumin in the plasma can indicate malnutrition in patients. Albumin concentration are used to monitor protein nutritional status in patients (Burtis *et al*., 2008).

2.14.2 Aspartate transaminase (AST)

This enzyme catalysis the reversible transfer of amino- acid from aspartate to a keto-acid to form glutamate and oxaloacetate. This reaction requires the coenzyme pyridoxal phosphate. Aspartate transaminase is present in high concentration in cardiac, skeletal muscle, liver, kidney and erythrocytes (Elarabany, 2018). It is released into the serum when there is an injury in the heart (myocardial infaction) and liver cell. Increase in AST concentration can also be caused by trauma or surgery (cardiac surgery) and hemolytic diseases (Chatterjea and Shinde, 2012).

2.14.3 Alanine transaminase (ALT)

This enzyme catalysis the reversible transfer of amino-group from L-alanine to Ketoglutarate, to form pyruvate and L-glutamate (Chatterjea and Shinde, 2012). ALT is present in a high concentration in the liver and low in the skeletal muscle, kidney and heart. A liver disease causes an increase in ALT, when the integrity of the liver cells is affected. ALT is more specific in the liver than AST because AST can be elevated due

to cardiac and skeletal muscle infection while ALT does not (Singh *et al*., 2011). ALT activities are rarely observed in other disease condition apart from liver diseases. In most of the liver diseases ALT activity is observed more than AST with exceptions in cirrhosis, alcoholic hepatitis, liver neoplasia (Elarabany, 2018).

2.14.4 Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) catalysis the alkaline hydrolysis of naturally occurring synthetic substrate. ALP is present in most organs of the body and is associated with membrane, cell surfaces located on mucosa of the intestine, convoluted tubule, bone, liver and placenta (Rodwell *et al*., 2015). The metabolism of ALP is not well understood, it appears to be associated with lipid and calcification process of the bone. Elevations in the serum ALP activities are as a result of bone or liver diseases such as rickets, osteolmalacia, cancer and hepatobiliary diseases (Elarabany, 2018). Trace levels of ALP may be found during healing of bone fracture. Physiological bone growth increases ALP levels, these accounts for the increase in sera concentration of ALP in growing children. The enzyme concentration is 1.5 to 1.7 times more than in a healthy adult (Adeyemi *et al*., 2015).

2.14.5 Total plasma proteins

A plasma protein contains a mixture of proteins from different origin and function. The major proteins that make up the plasma proteins are albumin, α- globulin, β- globulin (transferrin and lipoprotein), γ-globulin. The concentration of proteins in the vascular compartment is dependent on the balance between the rate of synthesis and catabolism or loss in the distribution between intravascular and extravascular compartment; the concentration depends on the amount of water in vascular containment. Abnormal concentrations do not mean abnormal protein metabolism (Özkan *et al*., 2012).

The liver cell synthesizes several plasma proteins while some plasma proteins are also synthesized by macrophages. Some plasma proteins can be taken up by pinocytosis into the endothelial cell or into the mononuclear phagocyte where they can be broken down. Some of these proteins can be lost passively through the glomerular and intestines wall some can also be reabsorbed through the renal tubule or after digestion in the intestine lumen (Rodwell *et al*., 2015).Total protein estimation has a limited clinical value. Changes in the concentration in this protein can affect the ratio of proteins in the fluid in the vascular compartment. Acute changes in the plasma concentration of the proteins can be as a result of loss or gain by the vascular containment of protein free fluid than of protein (Elarabany, 2018). Marked changes of protein constituents such as albumin, immunoglobulins are likely to affect the total protein significantly. Total protein concentrations of the plasma may be misleading, because they may be normal when there are marked changes in the constituent of the protein. For instance, a decrease in the albumin concentration of the plasma proteins can be balance by a rise in the immunoglobulin concentration (Gibney *et al*., 2009). Most individual proteins except albumin contributes very little to the total plasma protein concentration, however a large percentage change in the concentration of one of them may not cause a significant change in the total plasma protein concentration. Increase plasma concentration may be as a result of the loss of protein free fluid or an increase in one or more of the immunoglobulin. Low plasma protein can be due to hypoalbumin or deficiency of immunoglobulin (Daniel and Marshall, 1999).

A decrease in the total plasma concentration can be due to severe malnutrition or abnormal absorption. Reduction in the total protein can be an indication of liver damage, however in some liver diseases the albumin concentration is low but the immunoglobulin level is high making the total plasma concentration level normal. The

reason may be because the liver cannot produce albumin and there is an infection, which increase the concentration of immunoglobulin (Chatterjea and Shinde, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and apparatus

The equipment used for this study include; Hot- air oven, Analytical, weighing balance (Ultra V-bile P055, Rome, Italy), Crucibles (containers), Freeze dryer, Desiccators, Stop watch, Measuring cylinder, Laboratory muffle furnace, Burette, Tong, Beakers, Kjeldahl distillation apparatus, Kjeldahl digestion block, Digestion tube, Atomic Absorption Spectrophotometer -6800 Shimadzu, Pipettes, Volumetric flasks, Conical flasks, Soxhlet extraction unit Soxhlet apparatus (Sigma-Aldrich, SA64826, London,

UK), Haematological analyser, Haematocrit centrifuge (Damon IEC MB Centrifuge Micro Haematocrit, Ohai, USA), Thimble.

3.1.2 Reagents and chemicals

Chemicals used for this study were of the analytical grade and were products of Sigma-Aldric Chemical (Zayo-Sigma in Jos, Nigeria). sulphuric acid (H2SO4), hydrochloric acid (HCL), nitric acid, Sodium hydroxide (NaOH), sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), selenium oxide (SeO_2), boric acid, 2,2-dyphenyl-1-picrylhydrazyl (DPPH) trichloroacetic acid, aluminium chloride, sodium acetate, sodium carbonate (Na_2CO_3), petroleum ether, ethanol, Ferrous sulphate, methanol, and methyl red indicator.

3.1.3 Plant material collection, identification and preparation

Fresh and healthy leaf of *Parquetina nigrescens* was obtained from Omu-Aran, Irepodun Local Government Area of Kwara State, Nigeria. The obtained plant material was identified by local herbalist and authenticated by Dr Daudu, of the Plant Biology Department, Federal University of Technology, Minna, Niger State. The leaf was then rinsed with clean water and air-dried for two weeks after which were grounded into fine powder with the aid of electric blender and stored in a plastic container for further analyses using the method described by Kabiru *et al*. (2012).

Plate 2.1: *Parquetina nigrescens* Leaf (picture taken in January, 2019)

3.1.4 Experimental animals

Healthy male rats were obtained from Nigeria Institute of Trypanosomiasis Research (NITR), Kaduna, Kaduna State, Nigerian. Total of thirty-five albino male rats of the wistar stock aged within the weight of 140 – 160 g were housed in clean plastic cages and were given commercial rat pellet with water *ad libtum*. The animals were kept in the animal house of the Department of Biochemistry, Federal University of Technology. They were exposed to 12 hours of light and 12 hours of darkness and were handled according to the Departmental guidelines on animal handling.

3.2 Methods

3.2.1 Determination of proximate parameters

The crude protein, crude fibre, ash, total carbohydrate, moisture, and crude lipid matters were determined according to the method of AOAC (2016).

3.2.1.1 Moisture content

Two grams (2g) of leaf powder sample was weighed into a clean and dried crucible. The crucible with its content was transferred into an oven and dried at a temperature of 80^0C for 2 hours and 105^0C for another 4 hours until a constant weight was obtained. The sample was allowed to cool in a desiccator and the dry weight of sample plus crucible was noted. The % moisture was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3 \times 100}{W_2 - W_1}$$

Where; W_1 = Mass of crucible - Sample

W_2 = Mass of crucible + Sample

W_3 = Mass of crucible + Oven dried sample

3.2.1.2 Ash content

A clean, dried and empty crucible was placed in a Muffle furnace for 1 hour at 550ºC, which was then transferred into a desiccator for cooling and the weight was taken (W_1). Then leaf powder sample weighing two grams (2g) was placed into a pre-weighed porcelain crucible (W_2). The crucible containing the leaf powder sample was then placed in a pre-heated muffle furnace for 2 hours at 550^0C which was then allowed to char uninterruptedly until a white ash was obtained. The crucible was brought out with a tong and transferred into the desiccator to cool and was then reweighed (W_3). Weight of the ash was expressed as a percentage of the initial weight of the sample.

$$\text{Ash content (\%)} = \frac{\text{Difference in Weight of Ash} \times 100}{\text{Weight of the leaf (2 g)}}$$

$$\text{Difference in weight of ash} = \frac{W_3 - W_1}{W_2 - W_1}$$

W_1 = initial weight of crucible

W_2 = weight of crucible before ashing

W_3 = weight of crucible + Ash

3.2.1.3 Crude protein content

The protein content determination of the plant powder sample was done by micro-kjeldahl method. The powdered sample weighing zero point two five gram (0.25 g) was placed into a dry and clean kjeldahl flask of 100 ml. Six millilitre (6 ml) Concentrated sulphuric acid was added. Mixed catalyst (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in ratio (10:5:1) were added into a flask to enable digestion. It was then carefully digested on the digestion block until the mixture became clear light green for 3 hours. After it was cooled, it was transferred into 100 ml volumetric flask and distilled water was then added to make it up to the mark. Aliquot (10 ml) of the digest was then pipetted into Markam distiller and a 10 ml solution of 40 % Sodium hydroxide (NaOH) was added to the digest. The distillation process continued for at least 10 min of which ammonia (NH_3) was produced and collected as NH_4OH in a conical flask containing 5 ml of 4 % boric acid solutions and 4 drops of methyl red indicator until about 70 ml of distillate was collected. Titration of the distillate was done against a standard 0.1 N hydrochloric acid until there was a colour change was observed from green through grey to definite pink which indicated the end point of the titration. The blank solution (5 ml) containing (40 % NaOH / 4 % boric acid; ratio 1:1) also undergo the same procedure as previously described. Crude protein content (%) of the leaf was determined as follows:

$$\text{Nitrogen content (\%)} = \frac{(S-B) \times n \times 0.014 \times D \times 100}{\text{Weight of the leaf} \times V}$$

Crude Protein content (%) = 6.25 x % Nitrogen

Where;

S = Titre value of crude protein

n = Normality of HCl

B = Blank titration reading

D = Dilution factor

V = Volume of the digest used in distillation

0.014 = weight of Nitrogen

6.25 = Nitrogen to protein conversion factor

N = Nitrogen content

100 = Conversion factor to percentage

3.2.1.4 Crude fibre content

Using petroleum ether, two grams (2 g) of leaf powdered sample was defatted. The leaf sample was transferred into a 250 cm^3 Erlenmeyer flask and was boiled for 30 minutes under reflux with 200 ml of 1.25 % H_2SO_4 solution. The flask content was filtered and afterward washed with boiling water until the washed content was no longer acidic. The sample was further transferred into the flask and boiled for another 30 minutes with 200ml of 1.25% NaOH solution. This was again filtered and the residue was washed thoroughly until the residues were no longer alkaline. The sample residue was transferred to a crucible of known weight and dried in an oven at 105^0C. The crucible with its content was then incinerated at 550^0C for 30 minutes, cooled and reweighed. The change in weight was expressed as a percentage of the initial weight of the sample.

Crude fibre content (%) =

$$\frac{\text{Weight of crucible + Sample before ignited – Weight of crucible + Ash} \times 100}{\text{Initial weight of sample}}$$

3.2.1.5 Crude lipid content

Using Soxhlet apparatus the determination of crude fat was carried out. A washed and dried soxhlet extractor flask was placed in the oven and was then cooled in desiccator. Leaf powdered sample weighing two grams (2 g) was wrapped in a well labeled thimble and weighed. The labeled thimble was then placed in the soxhlet flask containing 300ml of petroleum ether boiling point at 60ºC. The extraction continued until a clear solvent

was observed. The defatted sample and thimble were removed, dried in an oven at 107 ^{0}C for 1 hour, it was then cooled in a desiccator and the weight was taken. The crude fat (%) was calculated using the following expression:

$$\text{Crude fat content (\%)} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

3.2.1.6 Carbohydrate content

The carbohydrate content determination was calculated by differences. The total value of protein, ash, moisture, fibre and lipid content was subtracted from 100%. Carbohydrate content (%) = % 100 – % (Moisture + Lipid + Ash + Protein + Fiber)

3.2.2 Minerals content

The mineral analyses of the leaf powder were carried out using method of dry ashing described by Bouba *et al*., (2012). The sample weighing one gram (1g) was transferred into a glazed, porcelain crucibles and sample was ashed in a muffle furnace at 550^0 C for 2hrs and allowed to cool. The ashed leaf sample was then transferred into a 250ml beaker, and solutions of 15ml of concentrated hydrochloric acid (HCL) and 5ml of concentrated nitric acid were added. The beaker containing this mixture was then placed on a hotplate set at 100^{0}C till the acid content evaporated completely. Distilled water (10ml) was added to the beaker containing the sample and thoroughly stirred before filtering into a 100ml volumetric flask and was then made up to the mark. The analyses were done in triplicates. Iron (Fe) content was analysed using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 710–ES series) while the content of other elements; Zn, Ca, K, Na, Mg, Mn, and Cu were determined by atomic absorption spectrophotometer (6800 Shimadzu)

3.2.3 Quantitative determination of secondary metabolites of plant leaf

3.2.3.1 Total phenol

Using the method described by Singleton *et al.* (1999), total phenol content of the leaf powder was determined. Five milliliters (5 mL) of distilled water were added to five grams (5 g) of the leaf powder in a test tube, and allow to stand for about 30 minutes for extraction purpose. Zero-point five milliliter (0.5 mL) of the extract was then oxidized with 2.5 mL of 10 % Folin- Ciocalteau's reagent (v/v) which was further neutralized by 2 mL of 7.5% sodium carbonate. Incubation of the reaction mixture was done at 45°C for 40 minutes and the absorbance was read at 765 nm using Spectrophotometer. Using gallic acid as standard, the total phenol content was subsequently calculated.

3.2.3.2 Tannin

The Folin Denis Spectrophotometric method was adopted as described by Onwuka, (2005) to determine the tannin content of sample. Weighing zero-point two gram (0.2 g) of the extract into a 50 mL beaker, 20 mL of 50 % methanol was added and was placed in a water bath at 80^0C for 1 hour covered with para film. Afterward, the mixture was thoroughly shaken and the content was subsequently transferred into a 100 mL volumetric flask. To this mixture, twenty milliliters (20 mL) of distilled water, 2.5 mL Folin-Denis reagent and 10 mL of 17% Na_2CO_3 solution were added and stirred thoroughly. For about 20 min, the mixture was allowed to stand. Bluish-green colouration was observed at the end, ranging 12.5-100 µg/mL of Tannic acid. Using a spectrophotometer, the absorbance was taken at wavelength of 760nm for tannin acid standard and the sample solution after colour development.

3.2.3.3 Saponin

The method described by Oloyede (2005), was used in the determination of saponins. Extract of known weight of zero-point five gram (0.5 g) was mixed with 1M HCl (20 mL) while for 4 hours the mixture was boiled, cooled and filtered. To the filtrate, 50 mL of petroleum ether was added and was evaporated to dryness, while to the residue, five milliliters (5mL) of acetone and ethanol was added. Ferrous sulphate reagent (6 mL) was added to the mixture followed by 2 mL of concentrated H_2SO_4. The mixture was thoroughly stirred while it was allowed to stand for 10 minutes before the absorbance was taken at 490 nm.

3.2.3.4 Flavonoid

The method of Chang *et al*. (2005) was used to determine the total flavonoid content of the leaf powder sample using the Aluminium chloride colorimetric. A mixture of solution containing 0.1mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate, 1.5 mL of methanol, and 2.8 mL of distilled water was added to a measure of zero-point five milliliter (0.5mL) and was kept for 30 minutes at room temperature. Using a double beam Shimadzu UV spectrophotometer absorbance of the reaction mixture was read at a wavelength 415 nm with. Using quercetin as standard at concentrations of 12.5 to 100 gmL^{-1} in methanol the calibration curve was prepared.

3.2.3.5 Phytic acid

Aina *et al*. (2012) method, a modified indirect colorimetric method used in the determination of phytic acid content of the leaf powder sample. The principle of the method is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample depending on an iron phosphorus in ratio of 4:6. 20ml of 3% trichloroacetic acid was added to 5g of the sample for extraction, which was then filtered. The analysis was carried out using 5ml of the filtrate. Precipitation of phytate

occurred as ferric phytate which was further converted to ferric hydroxide and soluble sodium phytate after the addition of 5ml of IM NaOH. In the presence of hot 3.2M HNO_3, the precipitate was dissolved and absorbance was taken at a wave length of 480nm. Standard curve for phytic acid was prepared as follows: standard curve of different concentrations of $Fe(NO_3)_3$ was plotted against the corresponding absorbance values of spectrophotometer to calculate the ferric iron concentration. Calculation of phytate phosphorus was from the ferric iron concentration of assuming 4:6 iron: phosphorus molar ratio.

3.2.3.6 Oxalate

Oxalate determination of a leaf powdered sample of *P.* nigrescens was evaluated by permanganate titrimetric method as stated by Onwuka (2005). Sample flour weighing 2 g added to 190 ml of distilled water was transferred to a 250 ml volumetric flask while 10 ml of 6M HCl was further added to the suspension, until the test solution turned faint yellow colour (pH 4-4.5) from a salmon pink colour.

Each portion was heated to 90^0C, which was then cooled and filtered to remove ferrous ion containing-precipitate. 10ml of 5% $CaC1_2$ solution was added to the filtrate after it has been heated to 90^0C, as it was being stirred continuously. The heated solution was then left and cooled overnight at 5^0C. However, the cooled solution was centrifuged at 2500rpm for 5mins and the supernatant was decanted while the precipitate was completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution, and the resulting filtrate from the digestion was made up to 300ml. From the filtrate, 125ml (aliquot) was heated until almost boiling and was titrated against 0.05M standardized $KMnO_4$ solution till a faint pink colour was observed which persisted for 30s. To calculate the calcium oxalate content, the formula below was used:

$$\frac{T \times (Vme)\ (Df) \times 10^5}{} \qquad (mg/100g)$$

(ME) x Mf

Where T is the titre of $KMnO_4$ (ml), Vme is the volume-mass equivalent ($1cm^3$ of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid), Df is the dilution factor V_T/A (2.5 where V_T is the total volume of titrate (300ml) and A is the aliquot used (125ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of flour used.

3.2.3.7 Cyanide

Cyanide content of the powdered leaf sample was analysed by the method described by Onwuka, (2005) using alkaline picrate. Powdered sample (5 g) was added to 50 ml of distilled water in a cooked conical flask which was allowed to stand over-night and afterward, filtered. 1ml of filtrate was added to 4ml alkaline picrate in a test tube and was corked. The mixture was incubated for 5 minutes in a water bath and colour development was observed (reddish brown) and at wavelength of 490 nm, absorbance was read. The blank (1ml distilled water and 4ml alkaline picrate solution) absorbance was also recorded. Cyanide content was inferred from the cyanide standard curve which was prepared from KCN solution of different concentration containing 5-50µg cyanide in a conical flask (500 L) followed by addition of 25ml of 1 N HCI.

3.2.4 Vitamin analysis

3.2.4.1 Vitamin A

The method described in the Njoku *et al.* (2015) vitamin A content. 10 ml of distilled water was added to leaf powder sample weighing two grams (2 g) in a flat bottom flask and an alcoholic KOH solution of twenty-five milliliters (25 ml) was also added. The mixture in the flask was heated for 1 hr on a water bath and cooled after which 30ml of distilled water was added. The obtained hydrolysate was then transferred into a

separating funnel. Using chloroform (250 ml), the solution mixture was extracted three times. To the extract, anhydrous Na_2SO_4 weighing two grams (2 g) was added to remove any available of water. This mixture was filtered into a 100 ml volumetric flask and was made up to mark with chloroform. Zero point zero zero three gram (0.003 g) of standard Vitamin A was dissolved in 100 ml of chloroform in order to prepare Vitamin A standard solution of range 0-50 µg/ml. Sample and standards absorbances of were taken at a wavelength of 328nm using Spectrophotometer (Metrohm Spectronic 21D Model) and vitamin A content was calculated.

$$\text{Vitamin A (µg/ 100g)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample}}$$

3.2.4.2 Vitamin E

Method described by Njoku *et al.* (2015) is used to analyse vitamin E**.** Leaf powdered sample weighing 1 g was added to 10 ml of methanol. The mixture was stirred thoroughly and was filtered. Into a beaker, an aliquot of the extract (0.4 ml), 7.6 ml of colour developer (containing 0.84 g sodium dihydrogen phosphate), 1.24 g of Ammonium molybdate, 8.15 ml of H_2SO_4 and 250 ml of methanol were added successively. 0.4 ml of methanol was again added to the extract mixture. The mixture was incubated for 1 hour at 90^0C and absorbance was read at wavelength of 695nm using Spectronic 21D Spectrophotometer. A standard curve was prepared and vitamin E concentration was extrapolated

3.2.4.3 Vitamin C

Onwuka (2005) method was used in determining vitamin C. Weighing five grams (5 g) of the powdered leaf sample which was mixed with 45 ml of distilled water, a suspension was formed which was filtered. While the residue was kept aside, 5 ml was measured from the filtrate into a 250 ml conical flask and glacial acetic acid (0.1 ml)

was added. Dichlorophenol indophenol was titrated against the filtrate-mixture in the conical flask until a faint pink colour was obtained from the solution. Using the obtained titre values, vitamin C was the calculated.

3.2.4.4 Vitamin B_{12}

Vitamin B_{12} content was determined according to the method of Okwu and Josiah, (2006). Leaf powder sample weighing 1 g was transferred into a 250ml volumetric flask and 100ml of distilled water was added, which was shaken for 45 minutes and was filtered into a 250 ml beaker. An aliquot of the filtrate (20 ml) was added to 5 ml of solution of 1% sodium dithionite to decolourise the yellow colour. From stock cyanocobalamin, a standard cyanocobalamin solution with concentrations ranging from 0-10 µg/ml were prepared and used to obtain gradient factor. The standard and the sample absorbance were read at 445 nm on a spectronic 21D spectrophotometer.

$$\text{Vitamin } B_{12} \text{ in } (\mu g/100g) = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample}}$$

3.3.4.5 Vitamin B_9

Folic acid determination was done according to the method described by Ibrahim and Yusuf, (2015) using High performance liquid chromatography (HPLC). Three grams (3g) of plant sample were extracted with 50ml of 0.1mol/L phosphate buffer pH 7.0 and 0.1% (V/V) of 2- mercaptoethanol was added. The solution was mixed thoroughly for 30 minutes in a vortex shaker, centrifuged at 3500rpm for 15 minute and then filtered through a Millipore filter paper before chromatography analysis. The stationary phase was immediately flushed with 5mL methanol and 5mL deionized water to activate the stationary phase, and the sample extract was passed through with a flow rate of 2-3 drops while the sample was eluted with 5mL NaOH at pH 10.0 prior to HPLC analysis. All filteration was carried out through a Millipore filter and the elute was injected into

the chromatograph. The elute was passed through the column and was monitored with a photodiode array detector at 282nm for folic acid. However, the mobile phase at pH 7.0 and KH_2PO_4: Methanol at ratio 90:10 was filtered through a 0.5nm membrane and degassed before use. The flow rate was 0.7mol/min. The column was operated at room temperature.

3.2.5 Plant extracts preparation

Six hundred grams (600 g) of the stored powdered sample was cold extracted with distill water at ratio 1:6 of sample to water respectively. The extract was dried in a freeze drier and the extract obtained was weighed, stored in a sterilized beaker covered with foil paper, and afterwards kept in the refrigerator for preservation throughout the period of the experiment. The percentage extract yield was calculated on the dry weight basis using the following formula:

$$\text{Percentage yield for dry leaf} = \frac{\text{Weight (g) of extract} \times 100}{\text{Weight (g) of pulverized leaf}}$$

3.2.6 Free radical scavenging ability

The method of Miguel *et al*. (2014) was used to determine the extract free radical scavenging ability against DPPH radical was one milliliter (1 mL) of the extract with a suitable dilution (0 - 250 µg/mL) was mixed with one milliliter (1 mL) of 0.4 mM methanol solution containing DPPH radicals. For 30 minutes, the mixture was left in the dark for incubation at room temperature and the absorbance was read at a wavelength of 516 nm in a spectrophotometer. the DPPH free radical scavenging ability was calculated with respect to the Absorbance of the blank containing the same amount of DPPH solution and methanol which was the negative control (Ao). The percentage inhibition $[(A_0\text{-}A_1/A_0) \times 100]$ was plotted against the phenol content and IC50 was determined.

3.2.7 Determination of Median lethal dose

Acute toxicity determination of *Parquetina nigrescens* leaf aqueous extract in experimental rats was done using Lorke's method (1983). The experiment was performed in two phases. In the first phase 9 rats were used, and were divided into 3 groups of 3 rats each. Groups A, B and C rats were given 100, 600 and 1000 mg/kg body weight of the extract respectively. In the second phase, the experiment similar to the first phase, the rats were administered 1600, 2900 and 5000 mg/kg body weight of the extract for group I, II, and III respectively. The animals were monitored for 24 hours, while toxicity signs and possible death were looked out for and the median lethal dose (LD_{50}) of the extract was determined and compared with values from those of the control group. The rats were further observed for 14 days for any mortality. Using the results of the two phases, the LD_{50} was calculated as the square root of the product of the highest non-lethal dose and the lowest lethal dose.

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

D_0 = highest dose that gave no mortality

D_{100} = lowest dose that produced mortality

3.2.8 Antianaemic studies

Total of thirty-five (35) albino male rats of the wistar stock aged within the weight of (140 – 160 g) were used in this study to determine the anti-anaemic effect of the selected plant. The obtained animals were kept in clean plastic cages and fed with normal commercial diet and water *ad libitum* and were allowed to acclimatize for one week to the environment before the start of the experiment**.** The animals were randomly grouped into six groups of five animals each as follows:

Group A: Normal control (Not induce, not treated)

Group B: Negative control (Induced not treated)

Group C: Induced and treated with 2.86 mg/kg body weight

Group D: Induced and treated with 200 mg/Kg bw extract

Group E: Induced and treated with 400 mg/Kg bw extract

Group F: Induced and treated with 600 mg/Kg bw extract

All induced animal were orally administered with 2.0 mg/kg body weight Aluminium Chloride ($ALCl_3$) for 14 days consecutively. This was followed by treatment which lasted for another 14 consecutive days. Animal's weight and packed cell volume (PCV) were monitored on day 0, 14, 19, 24, and 28 to determine their weight and anaemic state.

Therefore, weights were determined using the Ultra V-bile P055 electronic compact scale and the PCV was determined using microhematocrit centrifuge.

3.2.9 Collection of blood sample

At the end of the experiment, animals were weighed to obtain their final weights, while each rat was anaesthetised with mild diethylether in a covered container and euthanised according to the method of Shittu *et al*. (2014). Blood samples were collected for haematological parameters in an EDTA bottle and plain bottles were for the biochemical indices. The blood samples were collected in the plain bottles were allowed to clot for 30 minutes, afterwards centrifuged for 15 minutes at 3000rpm to obtain the serum. The serum was used for the biochemical analyses.

3.2.10 Assay for haematological parameters

Some haematological parameters of rats treated with aqueous leaf extract of *P.nigrenscens* were evaluated using an automated haematological analyzer systemex

KX-21 (Japan) as described by Dacie and Lewis (2015), which include the packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH).

3.2.10.1 Packed cell volume

i). Principle

When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

ii). Procedures

Using microhaematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until 2/3rd filled with blood. Blood filling was done for each tube. One end of each tube was sealed with plastacine and placed in the medial grooves of the haematocrit centrifuge exactly opposite each other, with the sealed end away from the center of the centrifuge. All tubes were spun at 12000 rpm for five minutes. The tubes were removed as soon as the centrifuge had stopped spinning.

iii). Calculation:

PCV was obtained by measuring the height of the RBC column and expressing this as a ratio of the height of the total blood column.

$$\text{PCV (\%)} = \frac{\text{Height of cell column x 100}}{\text{Height of total blood column}}$$

3.2.10.2 Haemoglobin, red blood cells and white blood cells count

i). Methods

Dacie and Lewis (2015) methods of haematological analyses was used to determine the haemoglobin concentration, red blood cells and white blood cells counts of the experimental animals in this study.

i). Principle

This method is based on counting and sorting of cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid pass through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume.

iii). Procedures

The blood sample were thoroughly mixed with the EDTA after which were placed in the haematological machine racks, the rack compartments were thoroughly checked to ensure the bottles were well placed. The automated haematological analyzer was made to start running, the machine processed the sample for five minutes and generated results via the output compartment.

3.2.11 Assay for biochemical parameters of the rat's blood serum

The serum obtained was analysed for AST, ALT, ALP, Albumin,Total protein, using appropriate kits, Cobas C111 Roche Autoanalyser and Shimadu UV – spectrophotometer, UV – 1800.

3.2.11.1 Total protein

i). Principle:

Compounds containing two or more peptide linkages, complex with cupric ions to give a purple coloured complex; the intensity of the colour varies with the concentration of the peptide linkages.

ii). Procedures:

Total plasma protein concentration was determined by the method of Gancheo *et al*. (2003). A plot of absorbance against bovine serum albumin concentration was used as standard. Each of the sample (. 0.1 ml) was mixed with 0.9 ml of distilled water and 4ml of Biuret's reagent were added together in a test tube. This was left for 30 minutes and the absorbance recorded. The total protein for each sample was extrapolated from the standard graph plotted.

3.2.11.2 Biochemical analysis of serum ALT activities

Determination of serum Alanine transaminase (ALT) activity was using Randox diagnostic Kit (UK) was carried out as reported by Kusiluka and Kumar (1996).

i. principle:

ALT catalyses a reaction, involving transamination of alanine and α- oxoglutatarate to form glutamate and pyruvate. 2,4-dinitrophenylhydrazone when in an alkaline medium gives a red-brown colour is formed when the produced pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH). This is the measure for monitoring ALT activity.

L – alanine + α - Oxoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + L – glutamate

ii. **proceudure:**

Serum sample of zero point one microlitre (0.1 µl) and the same measure of distilled water (blank solution) in duplicate was mixed with five hundred microlitre (500 µl) of RI buffer solution containing phosphate buffer, L-alanine and -oxoglutarate were added

into the test tubes, and was incubated at 37^0C for 30minutes at pH 7.4. Furthermore, 500 µl of R2 buffer (2,4 – dinitrophenylhydrazine) was added into test tubes. The sample mixture was homogenised and allowed to stand for 20 minutes.

Subsequently, 0.1M NaOH measuring five milliliters (5.0 ml) of was added to the sample and was thoroughly mixed. At a wavelength of 546nm, the absorbance of the sample was read against the reagent blank using Shimadu UV – spectrophotometer, UV - 1800. Using the standard curve, activity of ALT in the sample was obtained.

3.2.11.3 Determination of serum AST activities

Following the method described by Kusiluka and Kumar (1996), Serum Aspartate transaminase (AST) activity was evaluated using the Randox diagnostic Kit (United Kingdom)

i. principle:

It is based on the principle that the reversible transamination between aspartate and glutamate to form oxaloacetate and α–oxoglutarate is a reaction catalyzed by AST. Oxaloacetate formed reacts with 2,4–dinitrophenylhydrazine to form 2,4–dinitrophenylhydrazone. By monitoring the concentration of oxaloacetate hydrazone formed with 2,4 – dinitrophenylhydrazine, AST activity is being determined.

L – glutamate + L – aspartate $\xrightarrow{\text{AST}}$ α – Oxoglutarate + oxaloacetate

ii. procedure:

Serum sample of 0.1 µl and 0.1 µl of distilled water as the blank solution was mixed with 500 µl of RI buffer in a test tubes, and was incubated at 37^0C for 30minutes and 500 µl of R2 (2,4 – dinitrophenylhydrazine) was later added into test tubes. The sample mixture was well mixed and was allowed to stand for 20 minutes. Afterward, 0.1M NaOH measuring five milliliters (5.0 ml) was added to the sample and was thoroughly

mixed. At 546nm, the absorbance of the sample was read against the reagent blank. From the standard curve, activity of AST in the sample was obtained.

3.2.11.4 Determination of serum ALP activities

Serum Alkaline phosphatase (ALP) activity was evaluated by the method reported by Maundu and Tengnas (2005) using Randox diagnostic Kit (United Kingdom).

i. principle:

In the presence of magnesium ions, the substrate p – nitrophenylphosphate (colourless) was hydrolysed by alkaline phosphate to form nitrophenol (yellow colour) absorbance of which can be read at a wavelength of 450nm. Colour intensity produced is proportional to the activity of ALP.

p– nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ p– nitrophenol + Inorganic phosphate

ii. procedure:

Five microlitre (5 µl) of serum sample and distilled water of the same measure as the blank solution and was added to sample bottles, while zero-point three milliliter (0.3 ml) of substrate was added to each bottle respectively and mixed well and the initial absorbance was read at 405nm. Using a stop watch, three more absorbance of the sample and the blank was read at one minute interval using Shimadu UV – spectrophotometer, UV – 1800. Using the standard curve, activity of ALT in the sample was obtained.

3.3 Data Analyses

All values were expressed as mean ± Standard Error of Mean. Statistical analyses were carried out by one-way analysis of variance (ANOVA) and differences between the means was by assessed with Duncan's Multiple range test using Statistical Package for

Social Science (SPSS) version 20 (USA). P values < 0.05 was considered to be significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate composition

The proximate composition of dried leaf of *Parquetina nigrescens* is presented on table 4.1. On a general note, there was a significant ($p< 0.05$) difference in the proximate constituents recorded for *P.nigrescens* leaf. The carbohydrate content (46.68±0.86 %) was significantly ($p< 0.05$) higher, then was the crude protein content (21.02±0.33 %), followed by the ash (10.50±0.17 %), while the moisture content (8.00±0.46 %) and the lipid content (7.00±0.46 %) were significant ($p> 0.05$) low. However, the crude fibre content (5.80±0.58 %) was found to be significantly the lowest component in the leaf.

Table 4.1: Proximate Composition of *Parquetina nigrescens* Dried Leaf

Parameters	Compositions (%)
Moisture	8.00±0.12
Ash	10.50±0.17
Crude Protein	21.02±0.33
Crude Lipid	7.00±0.46
Crude Fibre	5.80±0.58
Carbohydrates	46.68±0.86

Values are presented in means ± SEM of three replicates.

4.1.2 Mineral composition

The result of mineral composition in (mg/100g) of *Parquetina nigrescens* leaf is represented on table 4.2. Minerals such as iron, zinc, calcium, manganese, copper, magnesium, sodium and potassium were analyzed. The result of the minerals composition revealed that magnesium (62.30±2.30) was significantly found to be the most abundant mineral component, while copper and zinc were significantly found to be the least in the sample having (1.45±0.05) and (0.07±0.00) respectively. However, iron was found to be (13.50±0.40) while sodium was (22.00±2.00) but there was no significant ($p > 0.05$) difference between the content of calcium (3.85±0.01) and that of manganese (3.95±0.30).

Table 4.2: Mineral Composition of *Parquetina nigrescens* Leaf

Elements	Composition (mg/100g)
Fe	13.50±0.40
Zn	0.07±0.00
Ca	3.85±0.01
Mn	3.95±0.30
Cu	1.45±0.05
Mg	62.30±2.30
Na	22.00±2.00
K	10.00±2.00

Values are presented in means ± SEM of three replicates.

4.1.3 Vitamin composition

The result of vitamin composition in (mg/100g) of *Parquetina nigrescens* leaf is contained on table 4.3. Vitamin C (7.35±0.01) was significantly the most abundant vitamin, while vitamin A was (1.25±0.05) and the least abundant in the plant sample. There was no significant ($p > 0.05$) difference between the value of vitamin E which was found to be (2.25±0.01), B_{12} which was (2.42±0.02) and folate which was (2.03±0.10).

Table 4.3: Vitamin Composition of *Parquetina nigrescens* Leaf

Vitamin	Compositions (mg/100g)
A	1.25±0.05
C	7.35±0.01
E	2.25±0.01
B_{12}	2.42±0.02
Folate	2.03±0.10

Values are presented in means ± SEM of three replicates.

4.1.4 Composition of secondary metabolites

Table 4.4 showed the quantitative composition of selected secondary metabolites constituents of *Parquetina nigrescens* leaf in mg/100g. The results showed that phenols and flavonoids were significantly the highest in *P.nigrescens* leaf as (183.08±2.00) and (180.24±0.39) respectively, followed by phytate (53.38±0.88), tannin (47.36±0.33), glycoside (24.80±0.01) and saponin which was (16.05±0.60). However, oxalate (0.55±0.05) and hydrogen cyanide were (0.19±0.01) significantly the least abundant.

Table 4.4: Secondary Metabolites Composition of *Parquetina nigrescens* Leaf

Phytochemicals	**Concentration (mg/100g)**
Flavonoids	180.24±0.39
Phenols	183.08±2.00
Tannins	47.36±0.33
Saponins	16.05±0.60
Glycosides	24.80±0.01
Oxalates	0.55±0.05
Phytates	53.38±0.88
Hydrogen cyanide	0.19±0.01

Values are presented in means ± SEM of three replicates.

4.1.5 Percentage yield of plant extracts

Dried leaf of *Parquetina nigrescens* had an extract yield of (15.6 %).

$$\text{Percentage yield for dry leaf} = \frac{93.6}{600} \times 100$$

Percentage yield for dry leaf = 15.6%

4.1.6 DPPH Antioxidant Activity of Extract of *P. nigrescens*

The antioxidant activity of *P.nigrescens* aqueous extract using 1,1-diphenyl-2-picrylhydroxyl (DPPH) result is detailed in Figure 4.1. This radical scavenging method revealed a dose dependent inhibition ability of *P.nigrescens* aqueous leaf extract to act as free radical scavengers. *P.nigrescens* aqueous extract gave higher inhibition with an IC_{50} of 45.53 µg/mL while Ascorbic acid (standard) was IC_{50} of 21.94 µg/mL.

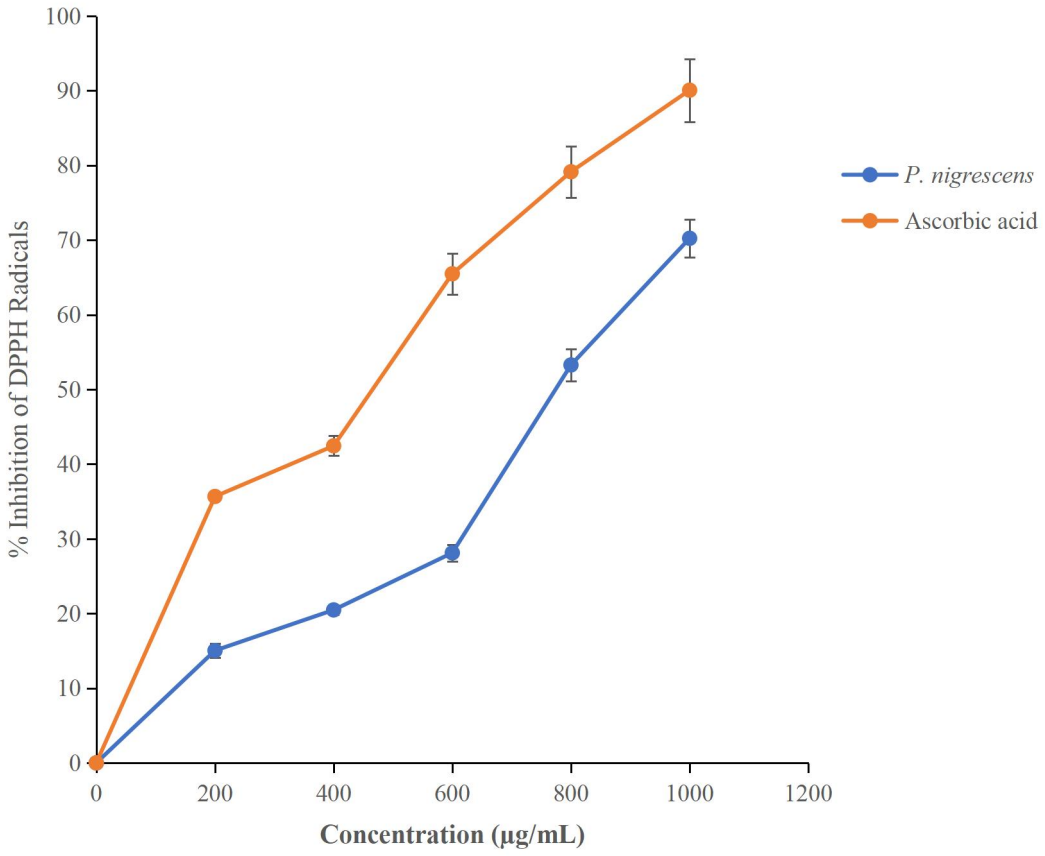


Figure 4.1: DPPH Scavenging Activity of Extract of *P.nigrescens*

4.1.7 Median lethal dose

The acute oral toxicity result of the of aqueous leaf extract *P. nigrescens* is represented on table 4.5. The acute toxicity test showed no mortality in rats during the 48 hours' experimental period in all the groups up to the dose of 5000 mg/kg body weight aqueous extract. No clinical sign or adverse effects of toxicity such as hyperactivity, ruffled fur, circling, or itching were observed.

Table 4.5: Acute Oral Toxicity of Aqueous Leaf Extract of *Parquetina nigrescens*

Treatment	Dose (mg/kg b.w)	Animals (n)	Mortality
Phase 1	10	3	0
	100	3	0
	1000	3	0
Phase 2	1600	3	0
	2900	3	0
	5000	3	0

Key: b.w – body weight

4.1.8 Effect of *P.nigrescens* aqueous extract on body weight of anaemic rats.

The results of body weights change on aluminium chloride-induced rats treated with aqueous extracts of *P.nigrescens* is represented on figure 4.2. Oral administration of aluminium chloride ($AlCl_3$) for the period of 14 days significantly ($p<0.05$) lowered the body weight of all the administered groups. Meanwhile, the positive control group which was the uninduced group had a significant ($p<0.05$) increase in their body weight throughout the study period. However, post-treatment results indicated that groups administered aqueous extract at doses of 200, 400 and 600 mg/kg body weight and the ferrous sulphate showed a progressive significant ($p<0.05$) increase in body weight during the two weeks' phase of treatment. There was also no significant ($p<0.05$) difference between the positive control group and the 200 mg/kg extract group in the post-treatment phase.

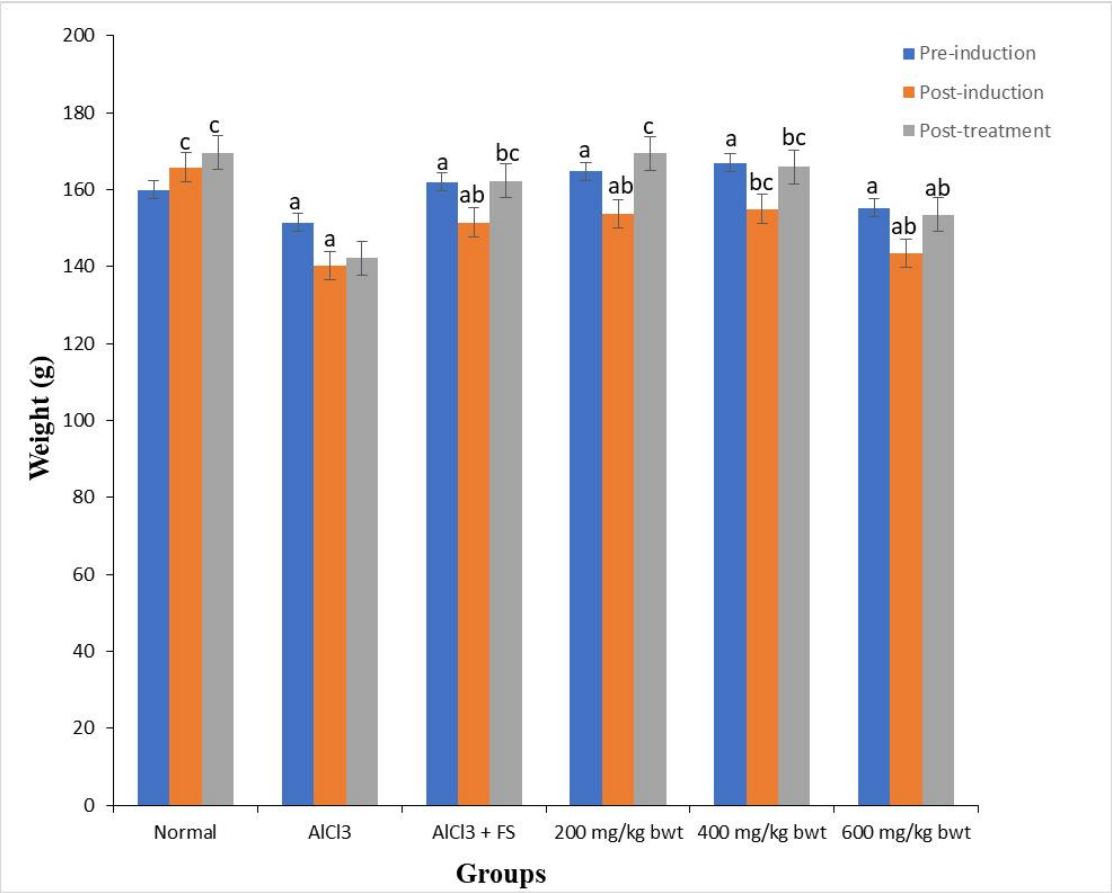


Figure 4.2: Effect of *P.nigrescens* Aqueous Leaf Extract on Body weight of Rats.

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly ($p>0.05$) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.1.9 Effect of *P.nigrescens* aqueous extract on packed cell volume of rats.

The changes in the packed cell volume (PCV) of aluminium chloride-induced rats treated with aqueous extracts of *P.nigrescens* were shown on table 4.6. Fourteen days (14 days) oral administration with aluminium chloride ($AlCl_3$) to groups, indicated significant ($p<0.05$) decrease in the Packed Cell Volume (PVC) when compared to the positive control group.

Post treatment with *P.nigrescens* aqueous leaf extract significantly ($p<0.05$) increased the PCV of the animals as it was observed with ferrous sulphate group. However, on the 28^{th} day of treatment, no significant ($p<0.05$) difference was observed between the normal control group, ferrous sulphate group and the group administered 600 mg/kg body weight aqueous leaf extract of *P.nigrescens*.

Table 4.6: Effects of Aqueous Leaf Extract of *P.nigrescens* on PCV of Rats

Treatment	Pre-treatment		Post-treatment		
Groups/Days	0	14	19	24	28
Normal	34.25±0.85^{a}	37.25±0.85^{d}	39.25±1.03^{c}	41.25±1.03 ^{d}	41.75±1.03^{d}
$AlCl_3$	33.00±0.41^{a}	23.75±0.48^{c}	22.00±0.71^{a}	19.25±0.48^{a}	19.00±3.02^{a}
$AlCl_3$ + FS	34.25±3.44^{a}	21.50±1.93^{b}	27.00±2.38^{b}	32.50±2.53^{c}	39.25±3.25^{d}
$AlCl_3$+200mg/kg bwt. extract	34.00±1.87^{a}	19.50±2.33^{a}	23.75±2.17^{ab}	26.25±2.29^{b}	31.75±1.18^{b}
$AlCl_3$+400mg/kg bwt. extract	35.00±0.82^{a}	21.00±0.71^{b}	24.00±0.41^{ab}	28.00±0.41^{b}	34.25±0.85^{c}
$AlCl_3$+600mg/kg bwt. extract	35.25±1.18^{a}	21.75±0.63^{b}	27.50±0.65^{b}	33.50±0.65^{c}	39.75±0.86^{d}

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly ($p>0.05$) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.1.10 Haematological parameters in rats treated with *P.nigrenscens*

4.1.10.1 Haemoglobin, red blood cells, and white blood cells

The results of the effect of *P.nigrescens* aqueous leaf extract on haemoglobin, red blood cells (RBC) and white blood cells (WBC) of aluminum chloride induced rats are represented on figure 4.3. The post induction result indicated a significant ($P<0.05$) decrease in the haemoglobin concentration of the negative control group (induced but not treated group) (4.57±0.27) when compared with other groups. Although there was no group significant ($P<0.05$) difference between the normal control group (14.90±0.35) and the group treated with 600 mg/kg body weight of the extract (14.57±0.10), yet they had the highest haemoglobin concentration. There was also no significant ($P>0.05$) difference between the group treated with 200 mg/kg body weight extract (11.20±0.18) and the group treated with 400 mg/kg body weight extract (12.38±0.20), and were significantly ($P>0.05$) lower than the haemoglobin concentration of the ferrous sulphate group (13.78±0.38).

Meanwhile, the RBC counts of the negative group was found to be significantly ($P>0.05$) low (3.79±0.17) and the normal control group had significantly ($P>0.05$) the highest RBC count (5.17±0.12) when compared with the standard and the treatments groups. However, there was no significant ($P>0.05$) difference between the RBC counts of the group treated with 600 mg/kg body weight extract (4.60±0.07) and the group treated with ferrous sulphate (standard drug) (4.61±0.25). There was also no significant difference ($P>0.05$) between the RBC counts of the group treated with 200 mg/kg body weight extract (4.15±0.78) and the group treated with 400 mg/kg body weight extract (4.20±0.08).

Furthermore, the negative group had a significant (P>0.05) highest count of WBC (9.14±0.12), while the normal control group had a significant lowest count of WBC (3.76±0.34) when compared to other groups. There was no significant difference (P>0.05) between the group treated with ferrous sulphate (7.05±0.13) and the groups treated with 200 mg/kg body weight extract (7.10±0.21), 400 mg/kg body weight extract (7.42±0.08), and 600 mg/kg body weight extract (7.31±0.15).

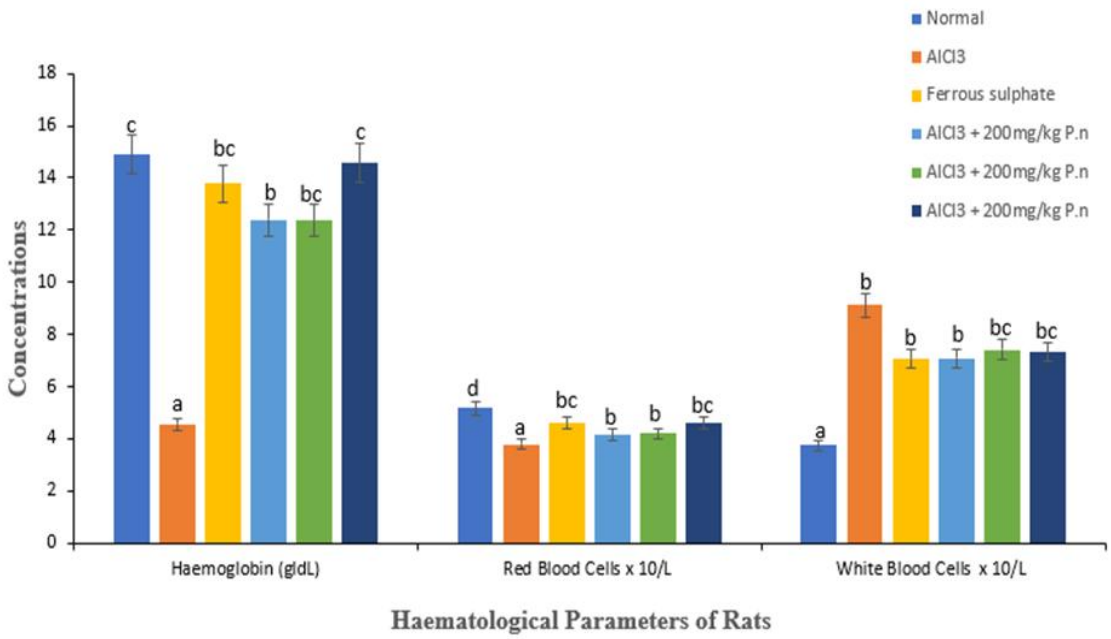


Figure 4.3: Effect of Aqueous Extract of *P.nigrescens* on Haemoglobin Parameters n Rats.

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly (p>0.05) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.1.10.2 Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC).

The effect of aqueous leaf extract of *P.nigrescens* on mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) on aluminium chloride induced rats is detailed on table 4.7. There was no significant ($p>0.05$) difference in the MCV of the ferrous sulphate group (85.14±1.47) and the 600 mg/kg body weight extract group (86.41±1.32), having significantly ($p>0.05$) the highest concentration. The negative control group was found to be significantly ($p>0.05$) the lowest (50.13±1.68), while there was no significant ($p>0.05$) difference between the groups treated with 400 mg/kg body weight extract (81.50±1.11) and the normal control group (80.75±2.00).

The results for MCH also showed that the group treated with 600 mg/kg body weight extract (31.67±0.41) was significantly ($p>0.05$) the highest, while negative control group (12.05±0.38) was significantly the lowest. There was no significant ($p>0.05$) difference between the normal control group (28.80±0.34), ferrous sulphate group (29.89±0.74), 400 mg/kg body weight extract group (29.47±0.43) and the group treated with 200 mg/kg body weight extract (26.98±0.41).

Likewise, the MCHC value showed no significant ($p>0.05$) difference between the normal control group (35.69±0.80), ferrous sulphate group (35.10±0.75) and the group treated with 200 mg/kg body weight extract (34.19±0.72). Nevertheless, the negative control group (24.05±0.65) remain the group with significantly least concentration of MCHC. Lastly, no significant ($p>0.05$) difference was also seen between the 400 mg/kg

body weight extract group (36.14±0.08) and 600 mg/kg body weight extract group (36.75±0.85).

Table 4.7: Effect of aqueous leaf extract of *P.nigrescens* on MCV, MCH, and MCHC on Rats.

Treatments	MCV (fl)	MCH (pg)	MCHC (gl/dL)
Normal	80.75±2.00^{c}	28.80±0.34^{bc}	35.69±0.80^{b}
$AlCl_3$	50.13±1.68^{a}	12.05±0.38^{a}	24.05±0.65^{a}
$AlCl_3$ + FS	85.14±1.47^{d}	29.89±0.74^{c}	35.10±0.75^{b}
$AlCl_3$+200mg/kg bwt. Extract	78.91±1.23^{b}	26.98±0.41^{b}	34.19±0.72^{b}
$AlCl_3$+400mg/kg bwt. Extract	81.50±1.11^{c}	29.47±0.43^{c}	36.15±0.08^{c}
$AlCl_3$+600mg/kg bwt. Extract	86.41±1.32^{d}	31.67±0.41^{d}	36.75±0.85^{c}

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly ($p>0.05$) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

MCV = Mean Corpuscular Volume

MCH = Mean Corpuscular Haemoglobin

MCHC = Mean Corpuscular Haemoglobin Concentration

4.1.11 Biochemical parameters of rats treated with *P.nigrenscens* aqueous extract

4.1.11.1 Serum iron and ferritin

The result of the effect of aqueous leaf extract of *P.nigrescens* on serum iron and serum ferritin in aluminium chloride induced rats is contained on figure 4.4. The serum iron level was found to be significantly ($p>0.05$) lowest in the negative control group (119.52±2.82) while no significant ($p>0.05$) difference was observed between the normal control group (193.54±0.50), 600 mg/kg body weight extract group (186.88±0.67), and the ferrous sulphate group (184.94±0.62) when compared to other groups. However, the groups treated with 200 and 400 mg/kg body weight extract were significantly ($p<0.05$) different (158.23±0.54) and (175.47±0.45) respectively.

Also, the ferritin level of the negative control group was found to be significantly ($p>0.05$) the least (47.21±0.34), while no significant difference ($p>0.05$) was observed between the standard drug/ ferrous sulphate group (154.66±0.42) and the group treated with 600 mg/kg body weight extract (155.23±0.83). However, the groups treated with 200 and 400 mg/kg body weight extract were significantly ($p<0.05$) different (131.87±0.47) and (143.96±0.48) respectively.

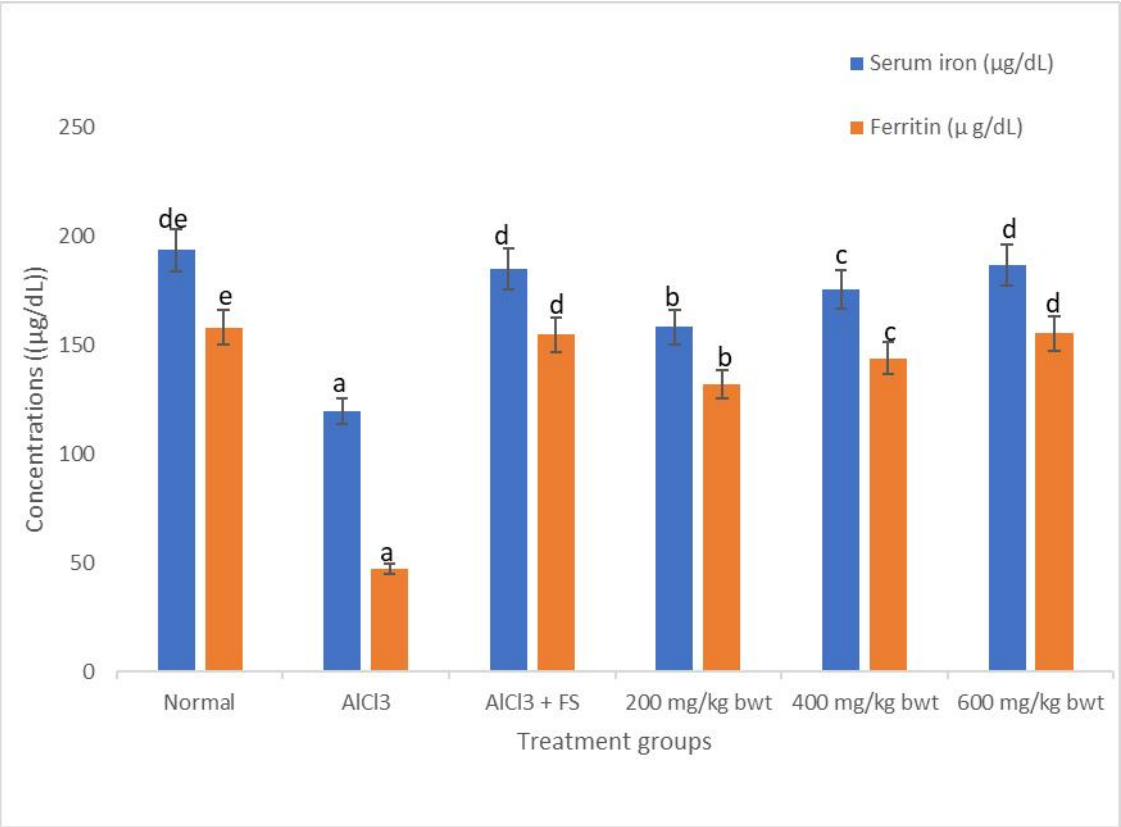


Figure 4.4: Effect of Aqueous Leaf Extract of *P.nigrescens* on Serum Iron and Ferritin on Rats.

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly ($p>0.05$) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.1.11.2 Serum albumin and total protein

The result of the effect of aqueous leaf extract of *P.nigrescens* on serum albumin and total protein in aluminium chloride induced rats is presented on table 4.8. The serum albumin level was found to be significantly ($p>0.05$) lowest in the negative control group (2.40±0.23), while positive control group was found to significantly ($p>0.05$) have the highest concentration (5.82±0.34) when compared with other groups. Also, there was no significant ($p>0.05$) difference between the group treated with the standard drug (5.05±0.22) and the group treated with 600 mg/kg body weight extract (5.18±0.11), and between the groups treated with 200 mg/kg body weight extract (4.67±0.17), and 400 mg/kg body weight extract (4.80±0.15). The serum total protein concentration level of negative control group was observed to be significantly ($p>0.05$) the lowest (3.05±0.12) and the positive control group was significantly ($p>0.05$) the highest (6.65±0.13) when compared to other groups. However, there was no significant difference ($p>0.05$) between the positive control group (6.65±0.13), and the group treated with 600 mg/kg body weight extract (6.45±0.28). Also, there was no significant difference ($p>0.05$) between the group treated with 400 mg/kg body weight extract (5.95±0.10) and the group treated with the ferrous sulphate (6.05±0.12). Nevertheless, there was a significant ($p<0.05$) difference between the group treated with 200 mg/kg body weight extract (5.30±0.23) and other groups.

Table 4.8: Effect of Aqueous Leaf Extract of *P.nigrescens* on Serum Albumin and Total Protein in Rats.

Treatments	Albumin (g/dL)	Total Protein (g/dL)
Normal	5.82 ± 0.34^{d}	6.65 ± 0.13^{d}
$AlCl_3$	2.40 ± 0.23^{a}	3.05 ± 0.12^{a}
$AlCl_3$ + ferrous sulphate	5.05 ± 0.22^{c}	6.05 ± 0.12^{c}
$AlCl_3$ + 200 mg/kg body weight extract	4.67 ± 0.17^{b}	5.30 ± 0.23^{b}
$AlCl_3$ + 400 mg/kg body weight extract	4.80 ± 0.15^{b}	5.95 ± 0.10^{c}
$AlCl_3$ + 600 mg/kg body weight extract	5.18 ± 0.11^{c}	6.45 ± 0.28^{d}

Values are expressed in as ± SEM.

Values with same superscript in the same column are not significantly ($p>0.05$) different.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.1.11.3 Serum alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP).

The result of the effects of *P.nigrescens* aqueous leaf extract on serum alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) activities in $AlCl_3$ induced rats is shown on table 4.9. The group administered $AlCl_3$ (negative control) only, had a significant ($p<0.05$) higher serum ALT (135.33±6.14) when compared to the normal control (56.13±2.27), and other treatment groups. There was no significant ($p>0.05$) difference in the ALT level of the groups treated with the standard drug (ferrous sulphate) (79.29±1.44), 400 mg/kg body weight extract (78.98±3.78) and 600 mg/kg body weight extract (70.49±2.70), while the group treated with 200 mg/kg body weight extract had a significant ($p>0.05$) concentration of (84.08±2.71).

The serum AST activities of the negative control group was found to be the highest (31.65±1.21) when compared with other groups. Although, the positive control group was the lowest (10.41±0.50), there was still no significant difference ($p>0.05$) between in the serum AST activities of the group and the normal control group (11.45±0.33). No significant difference ($p>0.05$) was observed between the ferrous sulphate group that is the standard group (12.63±0.36) and the group treated with 600 mg/kg body weight extract (12.57±0.72), and between the groups treated with 200 mg/kg body weight extract (13.57±0.72) and 400 mg/kg body weight extract (13.35±0.64).

Furthermore, the serum ALP activity for the negative control group was significantly ($p>0.05$) the highest (55.24±2.43), while the positive control group was significantly ($p>0.05$) the lowest (25.45±0.33) when compared with other groups. There was also no significant ($p>0.05$) difference in the ALP level of the groups treated with ferrous

sulphate (33.07±0.77), 400 mg/kg body weight extract (33.15±1.02) and 600 mg/kg body weight extract (32.63±1.31).

Table 4.9: Effect of Aqueous Leaf Extract of *P.nigrescens* on Serum Alkaline Phosphatase, Aspartate Transferase and Alanine Transferase Activities on Rats.

Treatments	ALT	AST	ALP
Normal	56.13±2.27[a]	11.45±0.33[a]	25.45±0.33[a]
$AlCl_3$	135.33±6.14[d]	31.65±1.21[d]	55.24±2.43[d]
$AlCl_3$ + ferrous sulphate	79.29±1.44[b]	12.63±0.36[b]	33.07±0.77[b]
$AlCl_3$ + 200 mg/kg body weight extract	84.08±2.71[c]	13.57±0.72[c]	37.35±0.86[c]
$AlCl_3$ + 400 mg/kg body weight extract	78.98±3.78[b]	13.35±0.64[c]	33.15±1.02[b]
$AlCl_3$ + 600 mg/kg body weight extract	70.49±2.70[b]	12.57±0.72[b]	32.63±1.31[b]

Values are expressed in mean ± standard error of mean of four replicates. Values with same superscript on the same column are not significantly different at $p>0.05$.

Key: $AlCl_3$= Aluminium chloride, mg/kg btw. = milligram per kilogram body weight of the animals, and FS = Ferrous sulphate

4.2 Discussion

In this study, the nutritional parameters and antianaemic activities of *Parquetina nigrescens* was evaluated in an aluminium chloride-induced anaemic rats. The proximate, minerals, secondary metabolites and antioxidants compositions of the plant were analysed, while an aqueous extraction of the leaf was carried and an animal studies. The choice of the plant was centered on the traditional medicinal (ethnomedicinal) usages in treating different kinds of diseases and infections (Aborisade *el al*., 2017; Oyagbemi *et al*., 2013; Kayode *et al*., 2009; Agbo and Odetola, 2005).

The proximate composition of *P.nigrescens* leaf shows that the plant has high carbohydrate content and protein content, moderate concentration of ash, moisture and lipid content, and a relatively low concentration of fibre content. A report by Imaga *et al*. (2010), on the phytochemical and antioxidant constituents of *Carica papaya* and *Parquetina nigrescens*, showed that *P.nigrescens* leaf has dissimilar concentration value when compared to the values obtained in this study. It is worth noting that these variations may be as a result of differences in the environmental factor, soil types and nutrients, the age of the plant at harvest, geographical locations, cultivation methods, seasonal and diurnal variations and procedure employed in preparation and extraction.

The carbohydrate content in the leaf of *P.nigrescens* was similar to that reported for *Moringa oleifera* and *Chenopodium ambroisiodes* by Madukwe *et al.* (2013), but higher than *Amarantus cruentus*, *Corchoru olitoius* as reported by Onwordi *et al.* (2009), and *P.nigrescens* by Imaga *et al.* (2010). It is however, lower than the value reported for *Telfairia occidentalis* by Omimakinde *et al.* (2018), and *Carica papaya* as reported by Imaga *et al.* (2010). Carbohydrate is one of the four macromolecules present in food. It

is a class of naturally occurring organic compounds that are essential for maintenance of life and supply of energy in plants and animals, and also serves as raw materials to industries (Aborisade *et al.,* 2017; Emebu and Anyika, 2011). The United State (U.S) Dietary Guidelines for 2015- 2020 recommends that, 45% to 65% of energy needed daily by the body should be obtained from carbohydrate (Odukoya *et al*., 2018). According to (FND, 2002), the recommended carbohydrate dietary allowance values for children, adults, pregnant and lactating mothers are 130, 130, 175 and 210 grams respectively. Carbohydrate is known to provide energy needed by the body tissues and cells such as the brain, muscle and the blood (Achi *et al.,* 2017). *P.nigrescens* leaf can therefore be said to be a carbohydrate rich food. The high carbohydrate content of *P.nigrescens* make it a suitable source of carbohydrate and may therefore be included in animal feed (Onwuka, 2005). The crude protein content of *P.nigrescens* leaf was relatively high. The value obtained in this study is comparable to the protein value in *Telfairia occidentalis* as reported by Omimakinde *et al.* (2018), *Launaea taraxacifolia* and *Solanum nigrum* as stated by Odukoya *et al*. (2018) and higher than the values stated for *Amarantus cruentus*, *Celusia argenta*, and *Corchoru olitoius* as described by Onwordi *et al*. (2009) and *Carica papaya* as reported by Imaga *et al*. (2010). Analysis of the crude protein contents of this plant showed that it is suitable for consumption as it has protein contents above the 3.3% USDA standard reference for protein (Emebu and Anyika, 2011). The protein contents also fall within the reported range of protein level found in green leafy vegetables on dry weight basis as noted by Ponka *et al*. (2005) and Hussain *et al.* (2010). Protein is a biomolecule essential in body building process and is involved in the general metabolic activities of an organism. Result from the study shows that the leaf of *P.nigrescens* may be considered as a good source of protein and may be used as protein supplement for patients with protein deficiency diseases.

The ash content of *P.nigrescens* is similar to the value in *Veronia amygdalina* and *Parquetina nigrescens* as reported by Mgbeje *et al*. (2019) and Imaga *et al.* (2010), higher than the amount reported by Mgbeje *et al*., (2019) in *Ocimum gratissimum* and lower than the amount reported by Imaga *et al.* (2010) for *Carica papaya.* Several studies have identified ash content of a sample as a reflection of the amount of mineral present in it (Aborisade *et al*., 2017; Andzouana and Monbouli, 2012; Bakare *et al*., 2010; Mgbeje *et al*., 2019; Odukoya *et al*., 2018). Therefore, the plant may contain an appreciable amount of minerals. The moisture content of *P.nigrescens* is comparable to *Telfairia occidentalis* Omimakinde *et al.* (2018). This value is relatively low when compared with certain leafy vegetable as reported by Onwordi *et al*. (2009) as in *Amarantus cruentus*, *Celusia argenta*, and *Corchoru olitoius*. It is however, higher than the moisture value of *Carica papaya* as reported by Imaga *et al.* (2010). Higher amount of moisture in plant account for low shelf life and subject plants to microbial attack. Therefore, the firmness of *P.nigrescens* leaf may be accounted for by its low moisture content and this suggest that the storage life will be higher and longer (Madukwe *et al.,* 2013). The lipid content of *P.nigrescens* leaf is significant. It is comparable to the values of *Magnifera indica* as stated by Aborisade *et al*. (2017) and *Carica papaya* as reported by Imaga *et al*. (2010). Too high fat consumption results in cardiovascular disorder such as atherosclerosis, and other chronic diseases (Raymond *et al*., 2010). Low fat content of *Parquetina nigrescens* might make the leaf useful in the diet of people living with lipid related disease since the fat content is significantly low. However, studies as shown that dietary fat increases palatability of food by absorbing and retaining of flavour (Aborisade *et al*., 2017; Antia *et al*., 2006), hence inclusion of the leaf of *P.nigrescens* in animal feed and human feed may moderartely improve flavour in the diet. The crude fibre value of *Parquetina nigrescens* in this study was

found to be relatively lower than the value in *Carica papaya* and *Parquetina nigrescens* as reported Imaga *et al*. (2010), while that of *Solanum nigrum* was relatively similar as stated by Odukoya *et al*. (2018). Plants are very good source of dietary fibre. This fibre when consumed can lowers and prevent the risk of constipation, hypertension, heart diseases, diabetes, and reduces serum cholesterol (Aborisade *et al*., 2017; Ishida *et al*., 2015). Since *P.nigrescens* contain some appreciable amount of carbohydrate, protein, lipid and ash compositions, it can be suggested that the leaf of *P.nigrescens* can be used as nutraceutical because the leaf have some nutritional value when taken as food and potent medicinal properties when used as herb.

The result of mineral analysis of *Parquetina nigrescens* revealed magnesium, sodium, iron and potassium to be significantly higher than zinc, copper, calcium and manganese. However, the value of sodium, iron, and zinc in this study were significantly lower than the reported values in *Telfaris occidentalis* and *Jatropha tanjorensis* by Madukwe *et al.* (2013). Calcium (Ca) is required for the coagulation of blood, the proper functioning of the heart and nervous system and normal contraction of muscles. Although, Ca concentration of *P.nigrescens* leaf was not significantly high, the value present may still be beneficial and readily absorbed in the gut because of the high protein and low lipid value of the leaf. According to Parveen *et al*. (2017), high protein diet favours absorption, because the presence of amino acids increase the solubility of Ca-salt thus its absorption. Its most important function is to aid in the formation of bones and teeth. The calcium content in this study is higher compared to the reported values for other higher plant sources that are already in conventional use as medicines and in human/animal diet. Iron level was found to be significantly high. The high iron level of *Parquetina nigrescens* is of significance because iron is one of the most essential trace elements in the body. It is a component of heme, invariably of haemoglobin. Deficiency

of iron is one of the most important nutritional deficiency in the developing countries. The high amount of iron in plant may be due to the soil pH, respiration and photosynthesis process and in the production of green leaf. It has been established that roots of plants readily absorb iron in a low pH soil (Rodwell *et al*., 2015). The result is in line with the study by Imaga *et al*. (2010) who reported that *P.nigrescens* is high in iron. Iron deficiency is in three stages; the iron storage depletion, iron deficiency and iron deficiency anaemia. Iron deficiency leads to derangement in cellular respiration since iron is a component of cytochrome, impaired attention, lowered memory especially in children, delayed heme synthesis (Chatterjea and Shinde, 2012). Magnesium is an abundant and important cation in human and is highly important in the protection of red blood cells. Mg is obtained in green vegetables and found as porphyrin group of chlorophyll. Dietary calcium decrease the absorption of Mg, however the calcium level in *P.nigrescens* is low, hence the absorption of Mg may not be lowered. Mg act as cofactors and an activators to wide spectrum of enzymes action. Zinc is also highly required as an integral part of many enzymes and are involved in so many cellular mechanisms. Recently in sickle cell anaemia, decreased zinc level (Hypozincaemia) with hyperzincuria have been noted (Chatterjea and Shinde, 2012).

The plant vitamin analysis revealed various vitamins that met their different recommended daily allowances (RDAs). Vitamins are essential components in diets and are required in very small amount. Several researchers have reported the variation of vitamin A, C, E, B_{12} and folate in various plants (Imaga *et al*., 2010; van Huis *et al*., 2013). These variations may be due differences in soil, species, environmental factor, time of harvest. Vitamin C was found to be the highest in the plant while there was no significant difference in the values of vitamin E, vitamin B_{12} (Cobalamin) and folate, vitamin A was found to be the lowest. This result is in agreement with the report of

Imaga *et al*., (2010) in phtochemical and antioxidant nutrient constituents of *Carica papaya* and *parquetina nigrescens* extracts, which recorded similar values for Vitamin B_{12} and folates. No value was recorded for vitamin E while vitamin C and A was higher in levels compared to the result obtained in this study. This may be due to several abiotic and biotic factor which usually cause variation in plant components. High amount of vitamin C was obtained in this study. Vitamin C is an aqueous antioxidant vitamin, essential for iron absorption. Deficiency of vitamin C or low levels of vitamin C may contribute to iron deficient anaemia by decreasing the absorption of iron in plant-based foods, therefore reducing iron metabolism. Low level of vitamin C can also result is capillaries fragility, hence haemolytic anaemia. The vitamin A level was significantly the lowest in the study. Deficiency in vitamin A is thought to cause anaemia and other diseases through multiple mechanisms, including role of retinoids in erythropoiesis. Vitamin A function in enhancing immune system is one of its significant roles in prevention or ameliorating damages caused by parasitic infections such as malaria, as well as well-established role in iron metabolism (Camilla and Parminder, 2019). Vitamin E is the most powerful natural antioxidant, and it is a chain-breaking antioxidant. It is of great importance because it helps protect lipoproteins and cellular membranes peroxidation. The red blood cells are protected from premature destruction by free radicals. According to Camilla and Parminder (2019), both folate and vitamin B_{12} (cobalamin) are highly important in Hb synthesis or iron metabolism, and their deficiency is said to result in macrocytic anaemia. Folate deficiency can result in decrease erythrocyte life span. Folate is found readily in green leafy vegetable, therefore deficiency among vegetarian is less likely. Vitamin B_{12} is significant precursors of coenzymes for enzymes of intermediary metabolism. Both vitamin B_{12} and folate are

essential for production of normal red blood cells production. Deficiency is seen in megaloblastic and pernicious anaemia.

This current study showed the presence of therapeutic secondary metabolites in the leaf of *Parquetina nigrescens* such as flavonoids, tannins, phenols, saponins, and glycosides. This is consistent with the results of Sopeyin and Ajayi, (2016) and Aborisade *et al*. (2017). Presence of these phytochemicals have been attributed to their secondary metabolic activity to ward off infections. Studies have shown that the presence of phytochemicals in plants is fundamental in protecting them from environmental hazards such as stress, pollutions, drought, pathogenic attacks and diseases. It also contributes to their colour, flavour and aroma (Mamta *et al*., 2013). Medicinal plants have therefore been used in the treatment of several diseases from the ancient times, and these abilities have been traced to the presence of bioactive plant chemicals called phytochemicals in them (Shami *et al*., 2016). Hence, the dietary values and the medicinal potentials of *P.nigrescens* may be as a results of these secondary metabolites. The quantitative phytochemical analysis of *P.nigrescens* was found to contain relatively higher amount of flavonoids, phenols, tannins and glycoside, while saponins was significantly lower. The results of the current study are in agreement with the report of Imaga *et al*. (2010) and Aborisade *et al*. (2017) who also confirm the presence of these phytochemicals in the leave extract of *P.nigrescens.* Phenols and flavonoids are weakly acidic hydroxyl-group, and are attached directly to the aromatic ring Aryal, *et al*. 2019. They have been reported to serve as an antiseptic, anti-microbial, anti-tumor, anti-inflammatory, and also as a good disinfectant (Somboro *et al*., 2011).

Flavonoids are biologically active secondary plant metabolites which have been reported to have antioxidant, anti-inflammatory, hepatoprotective, anti-allergic and anti-

carcinogenic activities (Shami and Aman, 2016; Duangjai *et al*., 2018). However, both flavonoids and phenols are water soluble, and free radical scavengers. They are used in the prevention of oxidative cell damage, allergies for free radicals and microbes. (Choi and Kim, 2020; Doughari, 2010). They are well known for effective protection against membrane lipoperoxidative damages. The strong antioxidant properties of both phenols and flavonoids can be attributed to their abilities to act as reducing agents, singlet oxygen quenchers, as metal chelators and as hydrogen/electron donators Shami and Aman, (2016). Flavonoids and phenols have been associated with several diseases such as cancer, atherosclerosis, Alzheimer's disease, anaemia, cardiovascular diseases and many others due to their health promoting abilities which have made them indispensable in medicinal, nutraceuticals, pharmaceuticals and in cosmetic applications. Saponins are class of chemical compounds found in several parts of plant species. They are amphipathic in nature, have soap-like foam when shaken in an aqueous solution (Augustin *et al.*, 2011) and have been observed to have bitter taste. It has been reported that saponins possess several biological activities such as anti-tumor, hepatoprotective, anti-ulcer, adjuvant, antimicrobial, anti-inflammatory and potential immune modulatory activities (Mamta *et al*., 2013; Troisi *et al*., (2015).

Saponins are particularly known to have strong haemolytic property because they have the ability to lyse or break the membrane of erythrocytes due to their ability to bind membrane sterols. According to findings, saponin-induced damage to lipid bilayer is irreversible. Hence, excess and continuous intake of substance containing saponin, may result to haemolytic anaemia (Hossain, 2019). Saponins are believed to have the natural role of protecting against potential pathogenic attacks, which account for their anti-microbial activity. Oral toxicity of saponins to mammals are low, yet they are extremely toxic to cold-blooded animals Troisi *et al*. (2015). As a result of this, saponins have

become highly important in several industries. For example, in the making of preservatives, detergents, agent for cholesterol removal, flavour modifiers, antibiotics, fungicidal, insecticidal and in some other pharmacological products (Yang *et al*., 2010; Chen *et al*., 2010; Hossain, 2019). Furthermore, saponin can affect nutrients absorption by inhibiting both metabolic and digestive enzymes and as well as bind with nutrients such as zinc, impair protein digestion, uptake of vitamins and minerals in the gut (Popova and Mihaylova, 2019). However, the concentration of saponin in *P. nigrescens* is significantly low and is safe for human. According to Popova and Mihaylova, (2019), tannins are said to be heat stable and exhibit antinutritional properties, by decreasing protein digestibility in humans and animals, either by inhibiting/inactivating digestive enzymes such as trypsin, chemotrypsin, lipase and amylase or by partially making protein unavailable when it forms complex with protein (tannin-protein complexes) thereby increasing fecal proteins, and it's also interfere with absorption of dietary iron. (Gemede and Negussie, 2014). Tannins has been reported to have antibacterial activity (Doughari, 2010). Tannins are a group of certain phytochemicals with wide range of properties such as antiviral, antiparasitic, anti-inflammatory and antiulceric (Choi and Kim, 2020). Tannins have wide industrial usages, which include tanning of leathers, wood adhesives, pharmaceuticals and medicinal applications, additives and antioxidant in fruit juices, beer and wine, fireproof and insulating foams, in mining of ore, flocculants and precipitation of polluting materials, as inhibitors of corrosion of Metals, mud stabilizer and drilling fluids, in horticulture and so many other applications (Antonio, 2019).

In the median lethal dose LD_{50} test of aqueous leaf extract of *P.nigrescens* among the experimental animals recorded no mortality even at a high dose concentration of 5000 mg/ kg body weight. This suggests that *P.nigrescens* was well tolerated by the rats

administered through oral route and does not cause mortality to the test animals within the test period. The result is in agreement with the result obtained by Agbo and Odetola, (2005) who stated that LD_{50} obtained was at 6.5 g/kg. The considerable high LD_{50} of 6.5 g/kg obtained for *P.nigrescens* aqueous extract suggest that oral route administration is relatively safe. Literatures have it recorded that doses for LD_{50} higher than 5 g/kg body weight are generally not considered as dose related toxicity (Lorke, 1983).

Administration of aluminium chloride for 4 weeks to some groups caused a significant decrease in their weight gain, when compared to the groups not induced. The significant weight loss may be due to the oxidative stress induced by aluminium which may result in free radical mediated cytotoxicity and decreased antioxidant enzyme (Shackley and Sohi, 2010). This result agrees with the result of Opasich *et al*. (2005) who stated that daily administration of aluminium chloride for five days led to a significant decrease in the weight of the animals. Aluminium inhibits certain enzymes required for synthesis of body fuel molecules such as hexokinase, phosphodiesterase and acid and alkaline phosphatase, which may result in tissue wasting (Scholl and Hediger, 1994). However, the administration of aqueous leaf extract of *P.nigrescens* improved the toxic effect of aluminium salt on the weight of animals. The weight of the groups treated with the extract significantly increased.

This agrees with the results of Anita *et al*. (2017), after a remarkable increase was observed in the groups of rat administered *M.oleifera* extract. The rats' weight gain could be as a result of rich nutrients present in *P.nigrescens* leaf such as protein, carbohydrate, lipids, mineral, vitamins and some phytochemicals (Toma *et al*., 2015). Aluminium chloride ($AlCl_3$) among toxic environmental metals and contaminants has a remarkable toxic potential for humans and animals (Kalaiselvi *et al*., 2015). These are known to cause oxidative stress and other deleterious effects on the morphology and

physiology of blood cells, neurological diseases and damage to organs (Kalaiselvi *et al*., 2014; Ige and Aiyeola, 2017). The haematological parameters such as the red blood cells (RBC), haemoglobin concentration (Hb), white blood cells (WBC), Packed cell volume or haematocrit values (PCV), MCV, MCH, MCHC and some biochemical parameters such as total protein, albumin and liver enzymes; ALT, AST, and ALP were monitored during the antianaemic study in rats because of their role in providing reliable information regarding contaminants or toxicants impacts in the living system and hence have become important health indicators for understanding normal and pathological processes (Aletan, 2014). Results of this study indicated that oral administration of $AlCl_3$ for 4 weeks caused a significant ($P< 0.05$) decrease in the concentration of RBC, Hb, and PCV. The reduction in red blood cell count, haemoglobin and packed cell volume in this study is in agreement with past studies of Mahieu *et al*. (2000); Kalaiselvi *et al*. (2015); Ige and Aiyeola, (2017); Ameh and Aladi, (2018) and Olorunnisola *et al*. (2012) who stated that daily administration of aluminium chloride for a period of 2 weeks induced severe anaemia in rats. Kalaiselvi *et al*. (2015) suggested that the reduction in RBC, Hb and PCV as a result of $AlCl_3$ exposure could be a reflection of disrupted hematopoietic process and an interference with stages of red cell synthesis. Another study by Ameh and Aladi, (2018), also reported that the reduction in the haematological parameters could have been due to some abnormalities due to impaired heme biosynthesis in the bone marrow. Anaemia is one of the leading public health issues in the world today with over 30% sufferers. Apart from iron deficiency which is the most common causative factors of anaemia, reactive oxygen species of the erythrocyte is another major causative factor of anaemia. Past literatures reported that Ingestion of aluminium chloride can cause oxidative damage to red cells by increasing the formation of reactive oxygen species and decreased activities of

antioxidant enzymes as a results of lipid peoxidation of the red cell membrane and therefore destruction of mature erythrocytes (Ogbonnia *et al*., 2009).

Also, transferrin is an iron transport protein (Rajangam *et al.*, 2011), and evidences from literatures had shown that aluminium obstructs iron transport in the serum by displacing iron from transferrin binding site (Mahieu *et al*., 2000; Davis and Littlewood, 2012). Literature has it that the affinity of aluminium for transferrin is much greater than transferrin-iron affinity, hence the transferrin-aluminium complex rendered serum transferrin unavailable for iron to bind. This may therefore mean the beginning of anaemia caused by deficiency of iron (Rajangam *et al.*, 2011; Ige and Aiyeola, 2017). More also, the decreased observed in the value of Hb, RBCs, PCV may be due to the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) initiated by aluminium. The ferric ion (Fe^{3+}) form may now be replaced by aluminium ion (Al^{3+}) (Omoregie and Osagie, 2010). Ferrochelatase (hemesynthetase), a mitochondrial enzyme involved in the final step of heme synthesis can be inhibited by aluminium (Rang *et al.*, 2017). However, haematological indices was significantly ($P< 0.05$) improved on administration of aqueous leaf extract of *P.nigrescens.* Owoyele *et al.* (2011), explains that ingestion of drugs or medicinal compounds can interfere with the normal range of haematological parameters. These modifications can either be positive or negative. The increased in the RBCs count, haemoglobin concentration, PCV, MCV, MCH, and MCHC in the various animal groups treated with the extract was progressive within the 14 days of treatment. This result is supported by the results of Owoyele *et al.* (2011) who reported that administration of root extract of *P.nigrescens,* restored the integrity of PCV, RBC, and Hb concentrations in albino rats.

The current result is also in agreement with the findings of Agbo & Odetola, 2005), who checked the effect of *P.nigrescens* on erythrocytes indices of rats following acute blood

loss in rats. They found out that the administration *P.nigrescens* extract reversed the decreased haematological parameters after the period of treatment. The improved haematological indices is an indication of erythropoiesis. This may be as a result of various processes activated by the presence of bioactive principles in the plant which has been earlier discussed, such as protein, vitamin B_{12}, folate, vitamin E, iron, zinc, flavonoids, phenols and tannins which serve as haematopoietic factors. The involved processes may include activation of erythropoietin in kidney and bone marrow and protection of red cells membrane from peroxidation by the activities of antioxidant. In this study, activation of antioxidant enzymes such as superoxide dismutase, glutathione peroxide and catalase which are important in checking the reactive oxidants, may be the reason for the increase in the haemoglobin, RBC count, PCV within the groups of rats treated with the extract when compared to groups not treated (Beltowski *et al*., 2010). Flavonoids and phenols for example are known to have a well-established protective effect against membrane lipoperoxidative damages (Shami and Aman, 2016). Their antioxidants activities could be attributed to their ability to act as a reducing agents, electron or hydrogen donor, singlet oxygen quenchers and metal chelaters. (Shami and Aman, 2016). Oxidation of aluminium which generate reactive oxygen species and complex radicals are degraded in the presence of flavonoids and phenols (Beltowski *et al*., 2010). The white blood cells count was elevated in all the groups administered $AlCl_3$ and significantly highest in the negative control group and was significantly low in the positive control group. The higher value of WBC is in agreement with the reports by (Mbaegbu, 2012); Kalaiselvi *et al*., 2015). White blood cells are regarded as the regulators of immune system, while an elevation in its value may be as a result of immune responses or a protective toxicant stress response. Usually, stimulated release of lymphocytes from lymphomyeloid tissues under stress may result in an increase

number of WBC. The elevated WBC count may also indicate low-grade inflammation (Silitonga and Silitonga, 2017). Therefore, the presence of $AlCl_3$ might have been regarded as a foreign substance in the body and might have been due to a normal cell-mediated immune response or an activation of the immune system in the animals (Ameh and Aladi, 2018), this is also applies to the extract treated groups.

In the present study, administration of aluminium chloride in rats given no treatment resulted in a significant decreased in the serum iron and ferritin concentration. The obtained result was consistent with the findings of Purnima *et al.* (2014). Aluminium chloride is known to cause oxidative damage to the red cells leading to the release of free haemoglobin and heme in the plasma and hence free iron, which could be excreted through the kidney. The presence of certain proteins in the plasma such as transferrin and ferritin helps in scavenging mechanism (Rodwell *et al*., 2015). Administration of P.nigrescens aqueous leaf extract improved the serum iron and ferritin concentration of the treated groups. The improvement in the serum iron and ferritin concentration in the groups administered treatment may be as a result of some available haematinic nutrients such as iron, vitamin C, protein, vitamin B_{12}, and folate in the extract. The obtained result was in agreement with the findings of Purnima *et al*. (2014) in the comparative anti anaemic activity of *Murraya koenigii* Spreng leaf and its combination with *Emblica officinalis* in aluminium chloride induced anaemia using rodents, who reported a substantial increase in the levels of ferritin by the tested sample than the synthetic iron.

Serum iron and ferritin is one of the important markers in determining the iron level in the plasma. Serum iron measures the amount of circulating iron bounded to transferrin. Serum ferritin on the other hand is a complex formed by apoferritin and an iron core (Fe^{3+}) having a high capacity to bind and store iron to maintain the relative balance of

iron supply and haemoglobin content (Robinson *et al*., 2016). Too low ferritin indicates iron deficiency while too high values is an indication for iron overload.

The albumin and total protein values of groups induced with anaemia was significantly ($p<0.05$) reduced when compared to the normal group and the extract control group which were not induced with anaemia. The administration of aluminium chloride may lead to formation of reactive oxygen species (ROS) which is capable of causing protein and DNA damage (Ozturk and Ozdemir, 2015). Clinical measurement of albumin and total protein are important markers of secretory, synthetic, and excretory functions of kidney and liver (Yakubu and Musa, 2012). Albumin is the most abundant protein in the plasma with about half of the weight of the plasma protein (Leonard et al., 2003). They are involved in the binding and transport of compounds in the plasma (Yakubu *et al*., 2017). Synthesis of albumin and many other plasma proteins is done by the hepatic parenchyma cells. Little quantity may be filtered through the glomeruli and majority reabsorbed by proximal tubule cells (Pervaiz and Holme, 2009). Malnutrition may also occur when the liver function is defective (Hussain and Dera, 2016). Decreased albumin and total protein levels observed in the groups of rats administered aluminium chloride may be an indication of a deficiency in the synthesis of albumin in the liver of a defect in liver function (Butis *et al*., 2008). This result is in alignment with the findings of Newairy *et al*. (2009) and Ameh and Aladi (2018) who had reported that administration of $AlCl_3$ to rats can cause necrosis with subsequent release of AST and ALT as a result of injury of the hepatocytes.

The decreased concentration of albumin and total protein may be as a result of higher concentration of aluminium in the intracellular fluid in the liver which could lead to reduced enzymes of protein synthesis (Tripathi, 2009). Treatment with aqueous leaf extract of *P.nigrescens* following anaemia induction with $AlCl_3$ significantly improved

the reduction in the serum albumin and total protein concentrations possibly by enhancing the antioxidant status thereby scavenging the released reactive oxygen species (ROS) and free radicals. *P.nigrescens* has a high concentration of phenols, flavonoids and moderate concentration of tannin, phytochemicals known for their antioxidant and healing properties. The high protein content of *P.nigrescens* which may also be contributing factor in the improvement of plasma albumin and total protein concentration. Imaga *et al* (2010) reported that protein is crucial in body building and maintenance of wellbeing of an organism.

There was significant increase in the activities of serum liver enzymes in the groups induced anaemia with $AlCl_3$ as compared with non-induced rats. Elevated plasma activities of liver enzymes are a marker; hence these findings suggest possible indication for liver damage or injury. Alanine aminotransferase (ALT) and aspartaste aminotransferase (AST) are cytosolic marker enzymes, are involved in amino acid metabolism and are used as markers in hepatic diseases (Ebuehi and Mbara, 2011). ALT is considered to be more specific to liver damage and disease than AST, since elevation of AST is observed in the condition of muscle and skeletal injury while ALT is not. Elevated level of ALT and AST is observed in severe haemolytic condition, skeletal muscle disease, cirrhosis, hepatitis, cholestatic jaundice and various drugs. Serum alkaline phosphatase (ALP) is increased in obstructive jaundice and in bone disease, therefore the increase observed in the ALP activity in the anaemic rats may be explained as severity in bome demineralization and disease (Ebuehi and Mbara, 2011; Onakpa *et al*., 2011; Rodwell *et al*., 2015). The treatment with aqueous leaf extract of *P.nigrescens* in the $AlCl_3$ induced anaemic rats showed a reverse in the level of elevated AST, ALT and ALP when compared to the non-induced groups. The notable lowering activities observed in the extract-treated groups indicate its hepatoprotective and

anaemia curative effect. The result is in line with the report of Ameh and Aladi (2018) who stated that reduction in serum liver enzymes following administration of ethanol leave extract of *Moringa oleifera* to aluminium chloride-induced anaemic rats reflect recovery from oxidative damage. This is an indication of the variation of antioxidant and healing properties of medicinal plants. Oral administration of aqueous leaf extract of *P.nigrescens* seemed to have either neutralize the effect of reactive oxygen species, or the extract possess the required antioxidants to clear up the reactive oxides generated by the presence of aluminium chloride.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The leaf of *Parquetina nigrescens* was found to be good source of carbohydrate, protein, vitamins and minerals but was low in lipids and fibre. *Parquetina nigrescens* leaf also was found to contain secondary metabolites such as phenols, flavonoids, tannins, saponins, phytate, and hydrogen cyanide below the permissible limits. It was also found to possess vitamins and antioxidant activity. The acute toxicity test reveals that the plant leaf was safe for consumption. The decrease in weight and packed cell volume was as a result of erythrocytes breakdown. There was a significant improvement in the weight, haematological and biochemical parameters of the rats such as the haemoglobin concentration, packed cell volume (PCV), red blood cells count (RBC), MCV, MCH, MCHC, total protein, albumin, serum iron, ferritin and in the concentration of certain liver enzymes such as ALT, AST and ALP. Consequently, leaf of *Parquetina nigrescens* could be useful as nutraceutical when added to animal feed or as herbs.

The aqueous leaf extract of *Parquetina nigrescens* possessed erythropoietic potentials because it was able to ameliorate the deleterious effects of aluminium chloride on

animal body weight, haematological and biochemical parameters. The normal concentration of liver enzymes checked for indicated that consumption of the plant as herb will not result in hepatic damage. Conclusively, the study has demonstrated that the aqueous leaf extract of *P. nigrescens* exerts potential dose dependent antianaemic effect in animal model.

5.2 Recommendation

i. Experimental animals such as guinea pig or rabbits should be used for further studies on anaemia-related experiment since the volume of blood in albino rats was too low to carry out detailed analysis.

ii. Induction of anaemia may be done using various methods, in order to check the effectiveness of the extracts in the treatment of anaemia of various types.

iii. Domestic cultivation and consumption of *Parquetina nigrescens* should be encouraged since its relatively safe and it could be used as preventive herbs for other ailments.

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APPENDICES

Appendix A

Groups	Before induction	After induction	After treatment
200 mg/kg bwt	164.75±2.75^{a}	153.75±2.95^{abc}	169.50±3.80^{c}
400 mg/kg bwt	167.00±2.41^{a}	155.00±2.74^{bc}	166.00±3.03^{bc}
600 mg/kg bwt	155.25±6.73^{a}	143.50±5.51^{ab}	153.50±6.06^{ab}
Negative control	151.50±3.93^{a}	140.25±4.87^{a}	142.25±5.72^{a}
Ferroglobin (2.5mg/kg bwt)	162.00±2.48^{a}	151.50±2.40^{ab}	162.25±2.66^{bc}
Normal	160.00±3.34^{a}	165.84±4.43^{c}	169.62±3.40^{c}

Values are expressed in mean ± standard error of mean of quadruplet determination. Values with same superscript on the same column have no significance difference at $p<0.05$

Key: NITE = Not induced but treated with 200 mg/kg bwt of the extract

Table 4:7: Effect of Aqueous Extract of *P.nigrescens* on Haemoglobin, Red Blood Cells, and White Blood Cells on Rats.

Treatments	Hb (g/dL)	RBC (10^6)	WBC (10^6)
Normal	14.90 ± 0.35^{bc}	5.17 ± 0.12^{d}	3.76 ± 0.34^{a}
$AlCl_3$	4.57 ± 0.27^{a}	3.79 ± 0.17^{a}	9.14 ± 0.12^{d}
$AlCl_3$ + FS	13.78 ± 0.38^{bc}	4.61 ± 0.25^{c}	7.05 ± 0.13^{c}
$AlCl_3$+200mg/kg bwt. Extract	11.20 ± 0.18^{b}	4.15 ± 0.07^{b}	7.10 ± 0.21^{c}
$AlCl_3$+400mg/kg bwt. Extract	12.38 ± 0.20^{bc}	4.20 ± 0.08^{b}	7.42 ± 0.08^{c}
$AlCl_3$+600mg/kg bwt. Extract	14.57 ± 0.10^{d}	4.6 ± 0.07^{c}	7.31 ± 0.15^{c}

Values are expressed in mean ± standard error of mean of four replicates. Values with same superscript on the same column are not significantly different at $p>0.05$.

Table 4.9: Effect of Aqueous Leaf Extract of *P.nigrescens* on Serum Iron and Ferritin on Rats.

Treatments	Serum iron (µg/dL)	Ferritin (µ g/dL)
Normal	193.54±0.50 [de]	158.14±0.14 [de]
$AlCl_3$	119.52±2.82 [a]	47.21±0.34 [a]
$AlCl_3$ + ferrous sulphate	184.94±0.62 [d]	154.66±0.42 [d]
$AlCl_3$ + 200 mg/kg body weight extract	158.23±0.54 [b]	131.87±0.47 [b]
$AlCl_3$ + 400 mg/kg body weight extract	175.47±0.45 [c]	143.96±0.48 [c]
$AlCl_3$ + 600 mg/kg body weight extract	186.88±0.67 [d]	155.23±0.83 [d]

Values are expressed in mean ± standard error of mean of four replicates. Values with same superscript on the same column are not significantly different at $p>0.05$.

Key: NITE = Not induced but treated with 200 mg/kg bwt of the extract