ANTI-ANAEMIC EFFECT OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *Jatrophatanjorensis* ON PHENYLHYDRAZINE INDUCED HAEMOLYTIC ANAEMIC RATS

BY

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DECLARATION

I declare that, this dissertation entitled "ANTI-ANAEMIC EFFECT OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *JATROPHA TANJORENSIS* ON PHENYLHYDRAZINE INDUCEDHAEMOLYTIC ANAEMIC RATS" has been performed by me in the Department of Biochemistry Ahmadu Bello University, Zaria under the supervision of Prof. D.A. Ameh and Prof. S. Ibrahim.

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

Ameloko, Ugbede Joy.		
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "ANTI-ANAEMIC EFFECT OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *JATROPHA TANJORENSIS* ON PHENYLHYDRAZINE INDUCEDHAEMOLYTIC ANAEMIC RATS" by Ameloko, Ugbede Joy, meets the regulations governing the award of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God almighty, the creator of heavens and earth.

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ABSTRACT.

Antianaemic effect of aqueous and 70% methanolic extract (ME) of *Jatrophatanjorensis* leaves on phenylhydrazine (PHZ) induced haemolyticanaemic albino rats was investigated. Phytochemical quantification (mg/g) carried out on the methanolic extract (ME) sample revealed that flavonoids (3.13±0.03) was the most abundant followed by phenol (2.40±0.02), saponins (1.2600±0.00), tannin (0.967±0.013) respectively. Quantitative phytochemical analysis (mg/g) carried out on the aqueous extract sample revealed glycosides (0.95 ± 0.07) was the most abundant, followed by saponins (0.71 ± 0.03), alkaloids (0.54 ± 0.07), tannins (0.03 ± 0.01), flavanoids (0.10 ± 0.01) respectively. Preliminary investigation carried out to determine the extract with potent haematopoietic potential by administration of both aqueous and 70% methanolic extract at 200mg/kg body weight to PHZ- induced haemolyticanaemic rats for 7 days revealed methanolic extract as the more potent extract because it increased the packed cell volume (PCV) by 14%. 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity indicated that the methanolic leaf extract of *J. tanjorensis*exhibited a low percentage inhibition than vitamin C. The percentage change in body weight recorded a non significant ($p>0.05$) increase in the anaemic control group, anaemic treated groups and non anaemic groups treated with200mg/kg of methanolic leaf extract of *Jatropha. tanjorensis*. Induction of anaemia significantly ($p<0.05$) decreased Pack cell volume (PCV), haemoglobin concentration (Hb), red blood cells (RBC), and significant ($p<0.05$) increase in white blood cell count (WBC) , mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) in all the induced groups when compared with the normal control group. After treatment for 21 days in the groups treated with the methanolic extract at doses of (50,100, and 200mg/kg), the induced rats remainedanaemic because Hb,

PCV, RBC, platelet values were significantly ($p<0.05$) decreased while a significant ($p<0.05$) increase in the values of WBC, MCV, MCH, MCHC of the anaemic rats treated with the ME was observed when compared with the normal control group.Malondialdehyde (MDA), recorded a significant ($p<0.05$) increase in the anaemic group treated with 200mg/kg compared with the normal control group. This increased MDA level is an important indicator of lipid peroxidation. Catalase (CAT) recorded a significant ($p<0.05$) decrease in the anaemic control group, anaemic treated groups with ME and non anaemic group treated with 200mg/kg bw of ME. A reduction in catalase lead to the accumulation of hydrogen peroxide .There was a significant ($p<0.05$) increase in alanine aminotransferase (ALT), alkaline phosphatase (ALP) in the anaemic control group, anaemic treated groups with ME of *J. tanjorensis* leaves and a significant ($p<0.05$) increase in the total bilirubin, conjucated bilirubin and aspartateaminotransferase (AST) in the anaemic group treated with 200mg/kg of ME compared with the normal control group. An elevation in AST, ALP, and ALT is a sensitive marker for liver cell damage. Urea and creatinine recorded a significant ($p<0.05$) increase in theanaemic animals treated with 100mg/kg body weight of ME of J *tanjorensis* leaves when compared with the normal control group. Increase in urea levels is associated with urinary tract obstruction. Evaluation of the effect of extract on relative organ weights demonstrated a significant ($p<0.05$) increase in relative spleen weight in the anaemic control group, anaemic groups treated with ME of *J. tanjorensis* leaves when compared with the normal control group, therefore,from this present study, methanolic leaf extract could not ameliorate the haemolyticanaemia induced at the various doses administered, but the extract is not toxic to non anaemic rats that was administered 200mg/kg body weight of the extract.

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LIST OF ABBREVIATIONS

%: Percentage

ALB: Albumin

ALP: Alkaline Phosphatase

ALT: Alanine Amino Transferase

AST: Aspartate Amino Transferase

BW : Body weight

CAT: Catalase

C.BIL Conjugated Bilirubin

Creat: Creatinine

DPPH: 1,1 diphenyl-2-picrylhydrazyl

J. tanjorensis:Jatrophatanjorensis

Hb: Haemoglobin

IC_{50}: Median Inhibitory Concentration

LD_{50}: Median Lethal Dose

ME Methanolic Extract

MCH: Mean Corpuscular Haemoglobin

MCHC: Mean Corpuscular Haemoglobin Concentration

MCV: Mean Corpuscular Volume

PCV: Packed Cell Volume

PHZ: Phenylhydrazine

RBCs: Red Blood Cells

ROS: Reactive Oxygen Specie

SOD: Superoxide Dismutase

TBARS: Thiobarbituric Acid Reactive Substances

T. Bil.: Total Bilirubin

T.P: Total Protein

Vit B_{12}Vitamin B_{12}

WBCs: White Blood Cells

WHO: World Health Organisatiion

CHAPTER ONE

1.0 INTRODUCTION

Anaemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. Aneamia is characterized by a decrease in Haemoglobin Concentration (Hb), Red Blood Cells Count (RBC) and Packed Cell Volume (PCV) (Okochi *et al.,* 2003; Diallo *et al.,* 2008). The anaemia prevalence remains high in Africa, with an overall incidence of 64.6% in children, 55.8% among pregnant women and 44.4% among young girls (WHO, 2006).

This condition is not a disease but could develop as a result of various diseases. There are many types of anaemia (Ogbe *et al*., 2010), many of which are rare but in all cases there is lower than normal number of circulating red blood cells. Presently, more than half of the world's population experience some form of anaemia in their life time (Duff, 2008). The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites like, plasmodium, trypanosomes and helminthes infestation. It is also known that women are susceptible to anaemia during pregnancy due to high demand from the developing foetus (Ong, 1973).

Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs (UNESCO, 1998). The increasing discovery of more medicinal plants demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them.

Green plants are most significant in their role as producers of food. From them directly or indirectly come animal foods. Proteins, carbohydrates and fats as well as accessory nutrients such as vitamins and minerals are all made available to man and other animals through green plants. Plants are also used as medicine. Medicinal plants are the sources of many important scientific drugs of the modern world (Oduola *et al*., 2007).

Jatropha tanjorensis (J. tanjorensis) belongs to the family, 'Euphorbiacea'. Other species are *Jatropha curcas, J. glandulifera, J. gossypifolia, J. chevalieri, J. elliptica J. multifida, J. podagrica, and J. intergerrima* (Oduola *et al*., 2007). *J. tanjorensis* is a natural hybrid between *J. curcas* and *J. gossypifolia* (Parabakaran and Sujatha, 1999). *Jatropha tanjorensis* is a native of Central America and has become naturalized in some tropical and subtropical countries, like India, Nigeria and Canada (Parabakaran and Sujatha, 1999). Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetable and as medicine (O'Hara *et al*., 1998). *J. tanjorensis* leaf is commonly consumed as vegetable in many parts of Southern Nigeria. It is commonly called hospital too far, catholic vegetable, lapalapa (Iwalewa *et al*., 2005).

J. tanjorensis is popular as a natural remedy against diabetes in Southern Nigeria (Olayiwola *et al.,* 2004).The leaf is used as heart tonic and remedy for hypertension in some parts of Nigeria (Iwalewa *et al*., 2005). The leaf extract has hypoglycemic activity. The aqueous leaf extract of *J. tangorensis* exhibit antibacterial activity against bacteria, Staphylococcus aureus and Escherichia coli (Oboh and Masodje, 2009).



Figure 1.1: *Jatropha tanjorensis* plant (www.svele.com.)

1.1 STATEMENT OF RESEARCH PROBLEM

The incidence of anaemia is higher in the third world than in developed countries due to the incidence of many aggravating factors such as poor nutrition, high prevalence of blood parasitic infections for example, plasmodium, trypanosomes and helminthes infestation.

Although there are various drugs for the treatment of anaemia, they are not affordable to many poor people especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anaemia, so they depend heavily on plants and herbal products for the treatment of diseases. As a result of the fact that anaemia is very common and the incidence is likely to increase in future (Duff, 2008), there is need to prevent it or

seek for more cost-effective and better treatment strategies. Since there are many complications associated with the use of many drugs in the treatment of anaemia, there is a need for an alternative plant that can be used with fewer side effects.

1.2 JUSTIFICATION.

Blood transfusion is the fast and reliable technique of saving lives in severe cases of haemolytic anaemia, but some religious and cultural beliefs are strongly opposed to receiving or donating of blood for anothers' survival. Disease conditions such as hepatitis, ebola and AIDS have remained a great threat to blood transfusion as blood screening techniques are poorly handled especially in rural dwelling, mainly due to absence of necessary expertise or equipment. Psychological trauma, blood incompatibility and clinical complications (such as iron overload) at times pose challenge for this procedure. It is therefore pertinent to opt for a more affordable, convenient, acceptable and less traumatic strategy for treatment or prevention.

Although a lot of work has been done (Oluwale and Akingbala, 2011) on this plant like nutritional and antimicrobial properties of *Jatropha tanjorensis* leaves, but studies on the potency of this plant as a remedy to haemolytic anaemia have not been reported.

The result from this study can give some indication if this plant can ameliorate haemolytic anaemia, and can be used as an alternative for the prevention of haemolytic anaemia.

1.3 AIM AND OBJECTIVES OF THE STUDY

1.3.1 Aim

The aim of the study is to investigate the antianaemic effect of aqueous and methanolic leaf extracts of *Jatropha tanjorensis* on phenylhydrazine induced haemolytic anaemic rats.

1.3.2 Specific Objectives

i. To assess the qualitative and quantitative phytochemical content of both aqueous and methanolic leaf extract of *J. tangorensis*

ii. To investigate the anti-aneamic potentials of the plant extracts in phenylhydrazine induced haemolytic anaemic rats, by periodic monitoring of haematological parameters

iii. To determine the effect of the methanolic leaf extract on some biochemical parameters of phenylhydrazine induced anaemic rats

iv. To investigate the *in vitro* and *in vivo* antioxidant potential of the extract.

v. To analyze the effect of the extract on relative organ weight and on the body weight of the experimental animals.

1.4 RESEARCH HYPOTHESIS

1.4.1: Null hypothesis

The aqueous and methanolic leaf extracts of *J. tanjorensis* do not have anti-anaemic effect on phenylhydrazine induced haemolytic anaemic rats.

1.4.2: Alternate hypothesis

The aqueous and methanolic leaf extracts of *J. tanjorensis* have anti-anaemic effect on phenylhydrazine induced haemolytic anaemic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 *Jatropha tanjorensis*

Jatropha tangorensis (*J. tangorensis*) belongs to the family "Euphorbiacea". Other species are *Jatropha curcas, Jatropha glandulifera, Jatropha gossypifolia, Jatropha multifida, Jatropha podagrica and Jatropha intergerrima*. It is a bushy, gregarious shrub of about 1.8meters in height. The leaves are 3-5 lobed, palmately, 20cm glandular hair. *J. tanjorensis* is a perenual shrub, up to about 15ft (5m) tall having a stem with thick green glabrous. It is mostly herbaceous or somewhat succulent, becoming woody at the base. The leaves are alternate with long petiole, palmate veined, the leaves margin are irregular or with 3.5 shallow lobes. The flower is point which is acute to obtuse, small, yellow, unisexual that clusters in leaf axils, mostly hidden in foliage. The fruits are ovoid with 3-locular capsule which are initially green and fleshy, which turns brown or almost black and dry at maturity (Little *et al*., 1974)

2.1.1 Taxonomy and common names

Kingdom: Plantea

Division: Magnoliophyta

Class: Magnoliopsida

Oder: Malpighiales

Family: Euphobiaceae

Subfamily: Crotonoideae

Tribe: Jatropheae

Genus: Jatropha species (Oudhia, 2001).

It is commonly called hospital too far, catholic vegetable (Iwalewa *et al*., 2005).

In Hausa *J. tanjorensis* is called Bindazugu

In Yoruba, it is called Lapalapa

In Igbo, it is called Ncheogbo

In Igbirra, it is called Ochiga

2.1.2 Functional uses of *Jatropha tanjorensis*

Jatropha tanjorensis is a native of Central America and has become naturalized in many tropical and subtropical countries, including India, Africa and North America (Prabakaran and Sujatha, 1999). Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetable and as medicine (Iwalewa *et al.*, 2005). The plant leaves were initially and popularly consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume. It is grown in the premises of the catholic churches as ornamentals (Iwalewa *et al.*, 2005).

2.1.3 Medicinal uses of *Jatropha tanjorensis*

The leaf is used as heart tonic and remedy for hypertension in some parts of Nigeria (Iwalewa *et al*., 2005), however there is no sufficient scientific validation of these claims. Toxicity and histopathological studies of the leaf extract on rats revealed no significant abnormalities in the tissues except for mild effects on the lungs and liver (Omobuwajo *et al.*, 2011). The ethanol/ water (1:1) extract was reported to have a significant glucose lowering activity in glucose loaded rats (Olayiwola *et al*., 2004).

The leaves are also employed traditionally in the treatment of anaemia (as a haematinic agent), diabetes and cardiovascular diseases (Iwalewa *et al*., 2005; Orhue *et al*., 2008; Omoregie and Osagie, 2011; Oyewole *et al*., 2012).

The leave extract has hypoglycemic activities and it is taken as medicine for diabetes symptoms (Olayiwola *et al*., 2004).

The antioxidant potential of the leaf extract has been investigated and was discovered to have anti-oxidative potential against reactive oxygen species produced in protein energy malnutrition (Omoregie and Osagi, 2011).

2.1.4 Mineral Content of *Jatropha tanjorensis* leaves

The dominant constituent of *J. tanjorensis* leaves is water, which accounted for 78.77% of their weight. Fresh leaves have a protein content of 2.01% and an ash content of 0.51%. The leaves have moisture content similar to published values of cassava 80.0%, cabbage 79.0% and cowpea 85.0% and sweet potatoes leaves. Protein content of the leaves was lower than published values for cabbage, cassava, cowpea and sweet potato leaves (Van and Wijingaart, 1997; Madambu *et al*., 2006 and Oboh and Masodje, 2009).

Phosphorus constituent of fresh *J. tanjorensis* leaves is 77.0µg /g (i.e 0.59% of the RDA of phosphorous is present in 100g of leaves).Selenium constituent in *J. tanjorensis* leaves is 0.0054µg/g. Thus about 0.1% of the RDA for selenium 0.54µg is present in 100g of leaves. Zinc constitute 6.1µg/g of fresh leaves (i.e 4.1% of the RDA for zinc is present in 100g of leaves) (Oboh and Masodje, 2009). Iron content in *J.tanjorensis* is 9.4µg/g i.e 53-9.5% of RDA of iron is present in 100g of leaves. Hence, *J.tanjorensis* contain a modest amount of

phosphorous and also appear to be a modest dietary source of trace element selenium and a good source of iron and zinc (Oboh and Masodje, 2009).

2.1.5 Phytochemicals

The medicinal value of a plant depends on its bioactive phytochemical constituents that produce definite physiological action in the body. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins (Krishnaiah *et al*., 2009) Several authors have evaluated the phytochemical composition of *J. tanjorensis.* (Oluwole and Akingbala, 2011; Ilondu and Enwa, 2013) revealed the presence of saponins, cardiac glycosides, flavonoids, terpenoids and tannins in the leaf extract.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Venkatalakshmi *et al*., 2012). This plant based traditional medicinal system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Omonkhelin *et al.*, 2007). It is well known that these plants generally contain those secondary metabolites (flavanoids, saponins, alkaloids, and tannins) which have been associated with numerous physiological activities in mammalian cells in various studies (Mishra *et al*., 2009).

Flavonoids are water soluble polyphenolic molecules with antioxidant activity which have many beneficial effects on the cardiovascular system (Evans, 1989). Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake (Brown, 1996). Flavonoids prevent the oxidation of low-density lipoproteins, lower the blood levels

of cholesterol and triglycerides thereby reducing the risk for the development of atherosclerosis (Subramani and Casimir, 2002). Red wine contains high levels of flavonoids. Many studies have confirmed that one or two glasses of red wine daily can protect against heart disease (Brown, 1996). Flavonoids have also been reported to have vaso-dilatory and inhibitory effects on platelet aggregation thereby preventing coronary heart diseases (Okwu, 2001)

Saponins have been reported to have beneficial effects on blood cholesterol levels (Oyewole and Akingbala, 2011). They bind with bile salt and cholesterol in the intestinal tract. This binding causes a reduction of blood cholesterol by preventing its re-absorption. The non-sugar part of saponins also has antioxidant activity which may help to reduce risk of heart diseases (Oakenfull and Sidhu, 1990).

Cardiac glycosides have been variously used as diuretics and heart tonics due to their beneficial effects on the heart. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation. They increase the force of contraction of the heart and are very useful for heart failure patients. They act by affecting the availability of intracellular Ca^{2+} for myocardial contraction or increasing the sensitivity of myocardial contractile proteins (Walker *et al*., 2002).

Tannins are group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution. It has been suggested that consumption of tannin containing beverages, especially green teas and red wines can cure or prevent a variety of illnesses including heart related diseases (Van-Burden and Robinson, 1981).

Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Terpenoids occur in nearly every natural food and have been associated with protection from and treatment of heart disease due to their antioxidant properties (Wagner and Elmadfa, 2003).

2.2 ANAEMIA.

Anaemia is from Ancient Greek, meaning *lack of blood*; it is a decrease in number of red blood cells (RBCs) or less than the normal quantity of haemoglobin in the blood. However, it can include decreased oxygen-binding ability of each haemoglobin molecule due to deformity or lack in numerical development as in some other types of haemoglobin deficiency (Halterman *et al*., 2001).

Haemoglobin which is found inside red blood cells normally carries oxygen from the lungs to the capillaries, anaemia leads to hypoxia (lack of oxygen) in organs. Since all human cells depend on oxygen for survival, varying degrees of anaemia can have a wide range of clinical consequences (Halterman *et al*., 2001).

Anaemia is the most common disorder of the blood. Several kinds of anaemia are produced by a variety of underlying causes. It can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discernible clinical spectra, etc. The three main classes include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (haemolysis) or deficient red blood cell production (ineffective hematopoiesis) (Halterman *et al*., 2001).

2.2.1 Signs and Symptoms of Anaemia.

The clinical manifestation and severity of anaemia vary considerably among individual patients. Moderate anaemia can typically cause signs and symptoms such as headache, palpitations, tachycardia and shortness of breath. Chronic anaemia can result in severe organ damage affecting the cardiovascular system, immune system, lungs, kidneys, and the central nervous system (Ludwig and strasser, 2001). A common anaemia-related problem is fatigue, which impairs the patient's ability to perform normal daily activities. As fatigue is a multifactorial syndrome not only caused by anaemia, trying to correlate the relative contribution of anaemia to fatigue is complex. Even when anaemia is improved, the full symptoms of fatigue might not be relieved, because fatigue can be present independent of anaemia (Ahlberg, 2003).

Many individuals with mild anaemia will have no complaints and be unaware that they are anaemic. Others may complain of symptoms such as shortness of breath or fatigue, especially with exercise. Patients with severe anaemia are often symptomatic at rest. When signs and symptoms of anaemia occur, the most commonly observed include pallor of the skin and mucous membranes, soft systolic murmurs, palpitations of the heart, dyspnea (shortness of breath), lethargy, and fatigability (Bunn, 2012).

2.2.2 Haemolytic anaemia

Haemolysis is the destruction or removal of red blood cells from the circulation before their normal life span of 120 days. While haemolysis can be a lifelong asymptomatic condition, it most often presents as anaemia when erythrocytosis cannot match the pace of red cell destruction. Haemolysis also can manifest as jaundice, cholelithiasis, or isolated reticulocytosis (Ucar, 2002).

There are two mechanisms of haemolysis. Intravascular haemolysis is the destruction of red blood cells in the circulation with the release of cell contents into the plasma. Mechanical trauma from a damaged endothelium, complement fixation and activation on the cell surface, and infectious agents may cause direct membrane degradation and cell destruction .The more common extravascular haemolysis is the removal and destruction of red blood cells with membrane alterations by the macrophages of the spleen and liver. Circulating blood is filtered continuously through thin walled splenic cords into the splenic sinusoids (with fenestrated basement membranes), a sponge like labyrinth of macrophages with long dendritic processes. A normal 8-micron red blood cell can deform itself and pass through the 3-micron openings in the splenic cords. Red blood cells with structural alterations of the membrane surface (including antibodies) are unable to traverse this network and are phagocytosed and destroyed by macrophages (Ucar, 2002).

2.2.3 Diagnosis of anaemia

Anaemia is the most common hematologic disorder. Anaemia is best defined in relation to H&H (haemoglobin (Hb) and haematocrit (HCT) levels below the normal reference range, because a patient's symptoms and physiologic consequences are the result of decreased

oxygen-carrying capacity of the blood (Barros *et al.,* 2010) . According to World Health Organization (WHO) criteria, anaemia is diagnosed in males when Hb is <130 g/L (13 g/dL) and HCT is <0.39 (39%); and in females, when Hb is <120 g/L (12 g/dL) and HCT is <0.36 (36%).Other terms that indicate the presence of anaemia are;

- Haemoglobin concentration (Hb) is an estimate of the oxygen-carrying capacity of the blood
- Haematocrit value or ratio (HCT) also called packed cell volume (PCV). A measure of the relative volume occupied by RBCs in capillary or venous whole blood samples (Barros *et al.,* 2010). The HCT reflects, therefore, the body's red cell mass divided by the total blood volume. The HCT is used to detect the presence or absence of anaemia and polycythemia. The HCT can be determined mechanically, by spinning the cells in a microcentrifuge, and electronically, using an automated cell counter. The spun microhaematocrit suffers from an inherent problem of plasma trapping and is often slightly higher (0.01–0.03 or 1–3%) than the automated haematocrit (Dacie and Lewis, 1991). This phenomenon causes an erroneously high manual HCT in samples with deformed RBCs such as sickle cells. In normal samples, the microhaematocrit and the electronic HCT should agree within ±3% (HCT units). Although the spun microhaematocrit has limitations, it is the reference method for HCT measurements. The haematocrit on Cell-Dyn hematology analyzers is a calculated parameter, expressed as percent or L/L (SI units) derived from the following equation: HCT (L/L) = RBC (1012/L) _ MCV (fL)/1000 (Groner and Simson, 1995).

- Mean cell volume (MCV); a measure of cell volume (in fL) that is more reproducible than the RBC size information obtained from the blood film. Clinicians arbitrarily use a range of 80–100 fL as normal. MCV is very useful in determining the anaemia type, because only a few pathologies cause abnormal MCV results. In conditions of rapid blood loss or chronic disease, the MCV will be normal with a low HCT. Iron deficiency and thalassemia show a decreased MCV. Folate or vitamin B_{12} deficiency, alcoholism, and myelodysplastic syndrome result in an increased MCV (Groner and Simson, 1995).

Lactate dehydrogenase: When red cells undergo haemolysis, they release their contents, which are mostly comprised of haemoglobin but also include lactate dehydrogenase (LDH), an enzyme found in high concentration in red cells. Most patients with haemolysis will have an elevated LDH level, making this a sensitive test. However, because many other processes, including liver disease and pneumonia, also raise the serum LDH level, this finding is not specific for haemolysis (Seve *et al*.,2008).

Serum bilirubin. Haemoglobin is salvaged by haptoglobin, and the heme moiety is broken down first to bilirubin and then to urobilinogen, which is excreted in the urine. Bilirubin produced from the breakdown of heme is not conjugated, but rather is delivered to the liver, where it is conjugated and excreted into the bile. In haemolysis, the concentration of *unconjugated* bilirubin (indirect bilirubin) is increased, while in liver disease (such as cholestasis) the level of *conjugated* bilirubin (direct bilirubin) is increased. However, if the patient has concomitant liver disease with an increased direct bilirubin level, the serum bilirubin test is not reliable (Barros *et al.,* 2010).

Serum haptoglobin. Haptoglobin binds free serum haemoglobin and is taken up by the liver. Haptoglobin usually falls to very low levels in haemolysis. A confounder is that haptoglobin is an acute phase reactant and can rise with systemic disease or inflammation. However, patients with advanced liver disease will have low haptoglobin levels due to lack of synthesis (Pinner *et al*., 2010).

Serum haemoglobin. If the haemolysis is very rapid, the amount of free haemoglobin released will overwhelm the binding capacity of haptoglobin and lead to free haemoglobin in the plasma. This can be crudely quantified by examining the plasma color. Even minute amounts of free haemoglobin will turn the plasma pink. In fulminant haemolysis, the plasma will be cola-colored (Garratty, 2005).

Reticulocyte count. In most patients with haemolysis, the destruction of red cells is accompanied by an increase in the reticulocyte count. Reticulocytes are red cells that still contain RNA and are a marker of red cells that are approximately 24 hours old or less. Traditionally, reticulocytes were measured manually by staining the blood smear with vital blue and counting the percentage of cells that absorb the stain; this percentage needs to be adjusted for the haematocrit. Usually a percentage above 1.5% is considered indicative of an elevated reticulocyte count. Recently, automated complete blood count machines have taken advantage of the fact that reticulocytes will absorb certain stains; these machines can directly measure the reticulocyte count via flow cytometry, which results in an "absolute" reticulocyte count. The reticulocyte count obtained using this method does not have to be corrected for haematocrit, and levels of approximately 90,000/μL are considered raised. However, the reticulocyte count can also be raised in blood loss or in patients who have other causes of anaemia (e.g, iron deficiency) under treatment (Michel, 2011).

Urinary haemoglobin. One other sign of haemolysis is the presence of haemoglobin in the urine. A quick way to demonstrate haemoglobinuria is to check the urine with a dipstick followed by a microscopic examination. In haemolysis, the dipstick will detect "blood," while the microscopic examination will be negative for red cells (Blackall, 2011).

2.2.4 Treatment of anaemia and their short comings.

Erythropoietin (also known as EPO) is a growth factor that stimulates the production of red blood cells. Most of the cells in the blood are red blood cells, whose main function is to carry oxygen throughout the body (Nikolsky *et al*., 2004).

Erythropoietin is used to treat anaemia resulting from kidney failure or cancer treatment. It is considered to be an alternative to blood transfusions.

There are several different types of erythropoietins. Procrit and Epogen are two brands of epoetin alfa. Aranesp (darbepoetin alfa) is another type of erythropoietin. The major difference between Procrit/Epogen and Aranesp is that Aranesp is given less often than Procrit. Otherwise, all three agents work equally well in the treatment of anaemia (Nikolsky *et al*., 2004)

Erythropoietic agents are generally well tolerated. However, studies have shown that these drugs have been associated with an increased risk of blood clots (Nikolsky *et al*., 2004)

2.3 Red blood cells

Red blood cells, 5 to 6 million/mm3 are **biconcave disks** which function in transporting oxygen and carbon dioxide to and from tissues. Their shape facilitates both volume and surface area. Their structure is that of a flexible membrane sack (Coin and Olson, 1979).

This feature allows erythrocytes, which have a 7µm diameter, to squeeze through capillaries as small as 3 µm wide (Branemark and Lindstrom, 1963). Erythrocytes contain tremendous amounts of haemoglobin, the protein that binds oxygen. In order to make room for more haemoglobin to carry more oxygen, erythrocytes lose their nucleus and other organelles as they develop in the bone marrow. Because they lack a nucleus and other cellular machinery, erythrocytes cannot repair themselves when damaged; consequently they have a limited life span of about 120 days (Williams and Morris, 1980). The removal of old and dying erythrocytes is carried out by the spleen. Erythrocyte production must equal erythrocyte death or the cell population would decline.

2.3.1 Red Blood Cell Counts

The typical red blood cell count is 4,600,000-6, 2000,000 cells per mm^3 for males and 4,500,000-5,100,000 cells per mm^3 for females. The number of red blood cells is a measure of the blood's oxygen-carrying capacity (Coin and Olson, 1979).

2.3.2 Destruction of Red Blood Cells

With age, red blood cells become increasingly fragile and are damaged by passing through narrow capillaries. Macrophages in the liver and spleen phagocytize damaged red blood cells. Haemoglobin from the decomposed red blood cells is converted into heme and

globin. Heme is decomposed into iron and biliverdin. Iron is recycled into new haemoglobin or stored in the liver. Some biliverdin is converted into bilirubin. Biliverdin and bilirubin are excreted in bile as bile pigments (Coin and Olson, 1979).

2.3.3 Red Blood Cell Production

In the embryo and fetus, red blood cell production occurs in the yolk sac, liver, and spleen; in the adult it occurs in the red bone marrow. The average life span of a red blood cell is 120 days. The total number of red blood cells remains relatively constant due to a negative feedback mechanism utilizing the hormone erythropoietin, which is released in response to low oxygen levels detected in the kidneys and liver (Coin and Olson, 1979).

2.3.4 Dietary Factors Affecting Red Blood Cell Production.

Vitamins B_{12} and folic acid are needed for DNA synthesis, so they are necessary for the reproduction of all body cells, especially in hematopoietic tissue. Iron is needed for haemoglobin synthesis. A deficiency in red blood cells or quantity of haemoglobin results in anaemia (Coin and olson, 1979).

2.4 Phenylhydrazine

Phenylhydrazine ($C_6H_8N_2$; molecular weight 108) exists as yellow to pale brown crystals or as a yellowish oily liquid, with a freezing point of 19.6 °C, a boiling point of 243.4 °C, and a vapour pressure of 133 Pa at 72 °C. It is soluble in water (values ranging from 145 to 837 g/litre at 24 °C have been reported by WHO, 2000) and is miscible with alcohol, ether, chloroform, benzene, and acetone.

Phenylhydrazine is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. Some companies use phenylhydrazine to produce a chemical intermediate for use in the photographic industry while other manufacturers use the chemical in the synthesis of organic chemicals (Datta *et al.*, 1989).

Phenylhydrazine reacts readily with the carbonyl group, –C=O, which is common among biological molecules. It is therefore expected that direct binding to biological molecules would occur. Evidence from toxicokinetic and toxicity studies and from human experience indicates that phenylhydrazine is well absorbed by the inhalation, oral, and dermal routes in animals and humans. Once absorbed, some phenylhydrazine appears to be rapidly taken up by red blood cells, where destructive intracellular reactions may occur (Datta *et al.*, 1989).

Evidence from a number of studies *in vitro* and *in vivo* suggests that phenylhydrazine interacts with haemoglobin and cytochrome P-450 in an oxidation reaction resulting in the generation of destructive free radicals, which are responsible for subsequent haemolysis (Falconer 1933; Itano *et al*., 1975). Phenylhydrazine is toxic by single exposure via the oral route (LD50 80–188 mg/kg body weight) and is expected to be toxic by the inhalation and dermal routes (data from these routes of exposure are less clear). Phenylhydrazine solution was severely irritating to rabbit eyes; hence, it is reasonable to predict that it would have significant eye irritation potential in humans (Itano *et al.*, 1975).

Phenylhydrazine also has skin irritation potential, and there is evidence from human case reports that it has skin sensitizing properties. Exposure to phenylhydrazine may cause

damage to red blood cells, potentially resulting in anaemia and consequential secondary involvement of other tissues, such as the spleen and liver. The dose (exposure)–response characteristics for the induction of damage to the red blood cells is poorly defined, and a no-effect level has not been identified. Where phenylhydrazine has been used therapeutically in humans, via the oral route, for the treatment of blood disorders, daily doses of the order of 1.5–4 mg/kg body weight per day have caused a reduction in the numbers of red blood cells; given the health status of the individuals concerned, however, these data are of limited use. Phenylhydrazine is mutagenic *in vitro*, and, although not conclusive, there is some evidence to indicate that it may express genotoxic activity *in vivo*. The substance is clearly carcinogenic in mice following oral dosing, inducing tumours of the vascular system. The mechanism for tumour formation is unclear, and, given the genotoxic profile of phenylhydrazine, a genotoxic component cannot be excluded. Carcinogenic potential in humans cannot be excluded given the profile of genotoxicity and animal carcinogenicity, particularly as other expressions of phenylhydrazine toxicity are common to a number of species, including humans (Latunde-Dada *et al.*, 2006).

The spleen plays important roles in processes other than blood storage and immune competence, and in PHZ-induced hemolytic anaemia in rodents and rabbits, it acts as the main erythrophagocytic organ (Latunde-Dada *et al*., 2006). This implies that damaged cells are removed intact by the spleen, on the one hand, and that intravascular lysis is minor with respect to extravascular lysis, on the other. In humans, the spleen plays no significant role in erythropoiesis, and adaptation to anaemia is not as effectively accomplished as in rodents (Mattson *et al.*, 1984).

2.4.1 Mechanism of Action of Phenylhrdrazine

A number of hemolytic agents are known that induce nonindigenous redox processes in erythrocytes. These drugs, by reducing the life span of red blood cells below that deemed normal (about 120 days), can lower the circulating red blood cell population in susceptible individuals. Presumably, drug-mediated processes cause a large enough change in certain properties of a significant number of red blood cells, e.g., membrane deformability (Clark *et al*., 1983), that they are removed from circulation by the spleen and liver and then hemolyzed. Persons with genetic deficiences in key enzymes involved in red cell metabolism (e.g., glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase) are particularly susceptible to induction of hemolytic anemia by these redox drugs. Such enzymes are important components of biochemical pathways that maintain hemoglobin in its functional reduced state and help avoid or repair the effects of redox processes that challenge the integrity of the erythrocyte. Phenylhydrazine (PHZ), a particularly potent redoxactive drug, can induce hemolytic anemia, even in individuals without erthrocytic enzyme deficiencies (Beutler, 1969). Indeed, the ability of PHZ to cause removal of erythrocytes from circulation. Observations in the last century indicated that PHZ could induce dramatic changes in erythrocytes in vitro, as well as in vivo. Rabbits treated with PHZ was brown in color and that addition of PHZ to suspended erythrocytes gave them also a brown coloration; spectroscopic studies indicated that oxyhemoglobin (HbO2) had disappeared (Beaven and White, 1954).

Hemoglobin, whether free in solution or within erythrocytes, reacts with PHZ to yield"green hemoglobin", a form in which the heme group is modified (Beaven and White, 1954). Processes induced by PHZ also cause destabilization of the globin portion of

hemoglobin, leading to denaturation and precipitation (Beaven and White, 1954). A number of small molecules have been experimentally detected as products of the reaction of hemoglobin with PHZ, some of them presumably acting as intermediates in inducing the irreversible products. This group of compounds includes benzene, nitrogen, hydrogen peroxide, superoxide anion, and the phenyl radical (Beaven and White, 1954). Other species are suspected of being involved in the reaction of hemoglobin with PHZ, including phenyldiazene, phenylhydrazyl, and phenyldiazenyl radicals, and the benzenediazonium ion. These metabolites react with plasma membrane to cause lipid peroxidation and protein oxidation resulting in the destruction of RBCs and hemolytic anemia (Beaven and White, 1954).

2.5 Vitamin B_{12} and Haemolytic Anaemia

Vitamin B_{12} or cobalamin deficiency is common in elderly population (Stabler, 2013). The prevalence in general population is around 20% in industrialized nations, although it can range from 5% to 80% depending on definition and study population (Dali-Youcef and Andrès, 2009). The main source of vitamin B_{12} is animal product such as meat, milk, egg, fish, and shellfish. Certain plant such as blue-green algae contains large amount of vitamin B_{12}, but the compound was found to be inactive in mammals. Hence, strict vegetarians have a greater risk of developing vitamin B_{12} deficiency (Stabler, 2013).

Vitamin B_{12} functions as a cofactor or coenzyme that participates in various biochemical reactions, including DNA synthesis (Dali-Youcef and Andrès, 2009), which promotes normal maturation of blood cells. It also contributes to the myelination of central nervous system as well as maintenance of its function (Stabler, 2013). Intramedullary destruction or

haemolysis of fragile and abnormal red blood cell precursors is the result of ineffective erythropoiesis secondary to defective DNA and cell maturation. The haemolytic picture may resemble microangiopathic haemolytic anaemia. If such condition persisted, it may deplete iron storage and lead to concomitant iron deficiency anaemia (Stabler, 2013).

Concurrent haemolysis in patients with vitamin B_{12} deficiency has been attributed to intramedullary destruction of red blood cells (ineffective erythropoiesis) (Antony, 2005). However, studies revealed that homocysteine accumulation due to vitamin B_{12} and folate deficiency increased haemolysis in vitro. The role of homocysteine in increasing the risk of haemolysis in vitamin B_{12} and folate deficiency has been demonstrated in vitro. However, this phenomenon has not been well appreciated as a possible cause of haemolysis in the clinical setting (Antony, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Chemicals and Reagents:

All chemicals and reagents used were of analytical grade. PHZ (CAS Number : 59-88-1 Product Number : P0184) was purchased from TCI, America.

3.1.2 Plant Collection and Identification

The leaves of the plant were harvested in october from Ochaja in Dekina local government area of kogi state. The plant was authenticated at the Herbarium of the Department of Biological Science, ABU Zaria with voucher number of 24015. The leaves were washed and air dried at room temperature. Dry samples were ground and the powdered sample stored for further use.

3.1.3 Experimental Animals

A total of fifty (50) wister albino male and female rats weighing between 100-150g were obtained and kept in well aerated laboratory cages in the Animal house, Department of Pharmacology, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The animals were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed with water and grower mash from Vital Feed *ad libitum.*

3.2 Methodology

3.2.1 Preparation of extracts.

Two extracts; aqueous, 70 % methanol were prepared. One litre of the various solvents (distilled water, methanol) was used to dissolve 100g of the powder of the leaves. The prepared solutions were allowed to stand for 48 hours. Each extracted solution was filtered off using a Whatman® filtered paper (Cat no 1001 125) of pore size 125mm. Each extracted analyte was concentrated by distilling off the solvent and then evaporated to dryness on a water bath set at 45^{o}C. The extract obtained without the solvent was weighed. The percentage yield was calculated in terms of air dried weight of the plant material as: Percentage yield = (Amount of extract obtained / Amount of initial sample) x 100

3.2.2 Determination of LD_{50}

The LD_{50} was determined by the method of Lorke (1983) using 13 rats. In the first phase the rats were divided into 3 groups of 3 rats each and were treated with the methanolic extract of the plant at doses of 10,100 and1000mg/kg body weight orally. They were observed for 24h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were treated with both extracts at doses of 1000, 1600, 2900, and 5000mg/kg body weight orally. From the results of the two phases, the LD50 was calculated as the square root of the product of the highest non-lethal dose and the lowest lethal dose.

3.2.3 Induction of Anaemia

Induction was done by modified the method of Iwalewa *et al.* (2005). Rats were injected twice intraperitoneally for two days with 40 mg/kg of phenyl hydrazine hydrochloride. Anaemia was allowed to establish in 24 hours after the second induction. Packed Cell volume < 35% was considered as an index for anaemia.

3.2.4 Experimental design

Animal grouping: The experimental rats were randomly divided into seven (7) groups, with five animals per group and treated for a period of twenty-one (21) days. They were fed with commercial rat feed and water *ad libitum* daily. Except for group one and group seven, all other groups were anaemia-induced.

Group 1: non-anaemic control (normal control)

Group 2: Anaemic rats without treatment

Group 3: Anaemic rats treated with 50mg/kg body weight of methanolic extract of *Jatropha tanjorensis* leaves orally.

Group 4: Anaemic rats treated with 100mg/kg body weight of methanolic extract of *Jatropha tanjorensis* leaves orally.

Group 5: Anaemic rats treated with 200mg/kg body weight of methanolic extract of *Jatropha tanjorensis* leaves orally

Group 6: Anaemic rats treated with vitamin B_{12} (100µ mils/Kg of Vitamin B_{12}) orally.

Group 7: Non anaemic rats treated with 200mg/kg body weight of methanolic extract of *Jatropha tanjorensis* leaves orally.

3.2.5. Phytochemical Screening of Aquoeus and Methanolic leaf extract of *Jatropha tanjorensis*

3.2.5.1. Qualitative Phytochemical Screening.

This chemical test was carried out on the extract using a standard procedure for identification of phytochemical constituents as described by Harborne, (1973); Trease and Evans, (1983) and Sofowora, (1993). Extract solution was prepared by dissolving 10g of extract in distilled water to make up 100ml solution. The extract solution was then used for phytochemical screening.

3.2.5.1.1. Test for Glycosides

Concentrated sulfuric acid (5ml) was added to the 2g of the extracts each and boiled for 15.mins. This was then cooled and neutralized with 20% KOH and was divided into two portions. Another part of the extract was dissolved in distilled water, this was used as a control; no acid hydrolysis.

Fehlings Solution Test: Fehlings solution A and B (5ml) were added to this portion and boiled for few minutes, red precipitate indicates the glycone portion as a result of hydrolysis.

Ferric Chloride Test: Ferric chloride solution (3 drops) was added to this portion. Green to black precipitate indicates phenolic aglycone as a result of hydrolysis of glycoside (Trease and Evans 1983)

3.2.5.1.2 Test for Anthraquinones Derivatives

Test for free Anthraquinones (Bornirager's Test): To extracts (2g) each, were shaken with 10ml of benzene and filtered. 5ml of 10% of ammonia solution was added to each extract filtrate and stirred. The production of a pink- red or violet colour indicate the presence of anthraquinones

3.2.5.1.3 Test for Saponins

Frothing test: Extracts (1g) each were dissolved each in 10ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb formed for more than 30 minutes indicates saponin.

3.2.5.1.4 Test for Flavanoids

Shinoda Test**:** Extracts (0.5g) were dissolved each in 1-2ml of 50% methanol. Metallic magnesium and four to five drops of concentrated HCl were added. A red or orange colour indicates the presence of flavanoid aglycones.

Sodium Hydroxide Test: Three drops of aqueous NaOH were added to 5ml of each of the extracts, a yellow colouration shows the presence of flavanoids (Trease and Evan 1983).

3.2.5.1.5 Test for Tannins

Lead acetate solution (3 drops) was added to the solution of each extract. A red precipitate indicates tannins.

Ferric Chloride Test. 0.5ml of the extract was dissolved in 10ml of distilled water, and then filtered. Ferric chloride (3drops) solution were added to each filtrate. Formation of a blue

black precipitate indicates hydrolysable tannins and green precipitate indicates the presence of condensed tannin

3.2.5.1.6 Test for Alkaloids

Meyer Test: Reagent (3 drops) was added to 1g of the extracts in each test tube. Green precipitate indicates alkaloids.

Dragendoff's Test: Reagent (4 drops) was added to 0.5g of each extract. Rose- red precipitate indicates the presence of Alkaloids

3.2.5.2 Quantitative Analysis of Phytochemical

3.2.5.2 .1 Determination of the Polyphenol Content

The total polyphenol content of the plant extracts was estimated using Folin-Ciocalteu Phenol Reagent according to the method described by Amin *et al.,* (2004).

Procedure: An aliquot of 0.1 ml of 0.5 mg of extract dissolved in 1 ml methanol was transferred into test-tubes and the volumes made up to 0.5 ml with distilled water. Then, 0.25 ml of Folin-Ciocalteu phenol reagent (10% v/v in distilled water) and 1.25 ml of 20% aqueous sodium carbonate solution were added; the tubes were vortexed and allowed to stand for 40 minutes before taking absorbance at 725 nm against a blank solution containing 0.1 ml of the solvent instead of the extract. The determination was done in triplicate.

The standard curve was prepared using 0.1-0.5 mg/ml solutions of Gallic acid in methanol and the total phenolic content was expressed as Gallic Acid Equivalent (GAE) in milligrams per gram of dry matter of sample.

3.2.5.2 .2 Alkaloids Determination

The alkaloid content of the plant sample was determined according to the method of Harborne, (1980).

Procedure: Five (5) g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was then added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3.2.5.2 .3 Tannins Determination

Concentration of tannins was determined using the standard method decribed by AOAC (1980), 2g of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non absorbent cotton wool. A volume of 25ml of the infusion was measured into a 2 litre porcelain dish and tritrated with 0.1 N potassium permanganate (0.1 N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green. The few drops of 0.1 N potassium permanganate was added at a time until the solution turns golden yellow.

The tannin content in the sample was calculated by multiplying the volume of 0.1N potassium permanganate used (titre value) by 0.0066235g. Using the equation below;

1ml of 0.1N potassium permanganate (titre value) = 0.0066235g Tannins

Therefore, Tannins content in the sample= titre value × 0.0066235g.

3.2.5.2 .4 Saponins Determination

Principle: A gravimetric method of AOAC (1984) employing the use of a soxhlet extractor and two different organic solvents were used. The first solvent (aacetone) extracts lipids and interfering pigments while the second solvent (methanol) extracts saponins.

Method: Two grams of *Jatropha tanjorensis* leaves sample was weighed into a thimble and put in a soxhlet extractor with a condenser fitted on top. Extraction was done with acetone in a 250ml round bottom flask for 3hrs, after which another weighed 250ml round bottom flask containing methanol was fitted to the same extractor and extraction continued for another 3hrs. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven dried to remove the remaining solvent in the flask. The flask was allowed to cool in a dessicator and weighed.

Calculation:

% Saponins= A- B/ W× 100

Where A= Weight of flask and extract (saponins), B= weight of empty flask

W= weight of sample.

3.2.5.2 .5 Flavonoids Determination

The flavonoids concentration was determined according to the method of (Boham and Kocipai-Abyazan, 1974).

Procedure:

Exactly 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

3.2.6 *In Vitro* Antioxidant

1,1- Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging activity of the plant extracts were assayed by the DPPH radical scavenging method of (Chan *et al*., 2007) with slight modification.

Principle:

1,1- Diphenyl- 2-picrylhydrazyl (DPPH) contains an odd electron in its structure. Its purple colour is reduced to yellow- coloured diphenylpicrylhydrazine when it reacts with an antioxidant, which can donate a hydrogen atom or an electron to it. The change in colour was measured spectrophotometrically at 520 nm using a UV/Visible light spectrophotometer.

Procedure: A DPPH solution was prepared by dissolving 0.6% of DPPH in 100 ml of methanol. To 1ml of various concentrations of the extract (0.020, 0.040, 0.060, 0.080, 0.100 mg/ml), 2 ml of DPPH solution (0.1Mm) was added. DPPH (0.6%) in 100 ml of methanol served as control. The mixture was shaken vigorously and was left to stand in dark for 30min. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm. The experiments were performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated using the following formula:

Scavenging activity (%) = ((1- Absorbance of the sample)/ (Absorbance of the control) ×100

DPPH radical scavenging activities of the extracts were expressed as IC50 values. IC50, the effective concentration of the extract required for 50% scavenging of DPPH radical was calculated from the graph of scavenging activity plotted against sample concentration using Microsoft Excel software (Appendix 1).

3.2.7 Determination of Effect of Extract on Anti-anaemic Parameters

Hematologic parameters were determined by standard methods. Blood sample was collected through occular puncture and used for hematological analysis.

- Packed cell volume was determined by microhaematocrit method as described by Dacie and Lewis, (1995) using microhaematocrit centrifuge. PCV was therefore done by obtaining blood through ocular puncture and allowing the blood to flow through a capillary tube to more than two-third of the tube. The end with blood was then sealed off using plasticine. The capillary tubes were placed in the centrifuge and centrifuged for 15 min at 3,000 rpm. The capillary tubes were then placed on the microhemotocrit to determine the packed cell volume.

- Haemoglobin concentration was determined by the Cyanomethaemoglobin method (Jain, 1986).

Principle: Blood mixed with dilute hydrochloric acid. This process haemolyses the red cells disrupting the intergrity of the red cell's membrane and causing the release of haemoglobin, which in turn, is converted to a brownish coloured solution of acid heamatin.The acid haematin solution is then compared with the a colour standard.

Procedure:

Sample solution and standard solutions were prepared as follows.

- Random sample: 500µl of Drabikin reagent was mixed with 20µl of distilled water.
- Standard: 500µl of Drabikin reagent was mixed with 20µl of sample solution of haemoglobin.
- Target sample: 500µl of Drabikin reagent + 20µl of blood. The concentration of haemoglobin was marked with Drabikin's method with the use of a spectrophotometer, at 540nm wavelength. Once Drabikin reagent was mixed with the blood, the solution was incubated at room temperature for the duration of 5mins and absorbance was measured. The spectrophotometer was set to zero using distilled water.

Measurement formula: Haemoglobin and its derivatives affected by drabicin reagent transform into cyanmethomoglobin, who optical density may be measured photometrically.

$$\text{Haemoglobin concentration (g/dl)} = \frac{\text{Absorbance of tested sample}}{\text{Abssorbance of standard}} \times \text{Concentration of the standard in g/dl}$$

- Determination of White Cells and Red Blood Cells Count

Improved Neubeur haemocytometer (counting chamber) was used to count both the WBCs and RBCs under the light microscope (Dacie and Lewis, 1995).

Principle:

Blood is diluted with a fluid containing glacial acetic acid that lyses the cells the red cells but not the white cells to facilitate counting.

Procedure:

The white blood cell and red blood pipettes were used to draw blood and fill to 0.5 marks of both WBC and RBC pipettes. WBC dilution fluid (2% acetic acid and 1% gentian violet in distilled water) and RBC diluting fluid (Hayem's fluid made up of 1g of Nacl, 5g of Na_2SO_4, 0.5g of mercuric chloride per 200ml solution). Were drown to 11 and 101 marks respectively. The fluid and the blood were then mixed gently and transferred into the counting chamber. It was allowed to settle for 2mins and the chamber placed on the stage of microscope for counting. The white blood cells count was done using the ×10 objective while the red blood cells counts were done using the ×40 objective.

Calculation: Number of cells counted divided by volume of one square ($0.1mm^3$) multiplying number of squares counted and multiply by dilution factor 20 for WBC count and 200 for RBC.

- MVC, MCH and MCHC were calculated by the method of (Dacie and Lewis, 2001)

- ***Mean corpuscular volume*** **(MCV):**
- MCV (millimicron) = PCV% x 10 / RBC count (x million per mm3)

- Mean corpuscular haemoglobin (MCH):

MCH (picogram) =Hb g/dl x 10 / RBC count (x million per mm3)

- Mean corpuscular haemoglobin concentration (MCHC)**:**

(MCHC picogram) = Hb g/dl x 100 / PCV %

3.2.8 Determination of Anti-oxidant Potential of the Extract

Superoxide Dismutase (SOD) activity

Superoxide Dismutase (SOD) activity was measured using the method of Martin *et al.* (1987).

Principle;

Auto-oxidation of heamatoxylin (with increase in absorbance at 560nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of the amount of SOD present within a specific range. SOD activity in the sample is determined by measuring the amount of heamatin present.

The basic principle of the assay is shown schematically by the following equation:

$O_2 + HTH_2 \xleftarrow{SOD} H_2O_2 + HT$ (Abs560↑)

Procedure:

Tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.20ml of the diluted microsome was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM

adrenaline. The reference mixture contains 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.20ml of distilled water. Absorbance was measured over 30s up to 150s at 480nm.

Calculations

Increase in absorbance per minute $= \frac{(A_5 - A_1)}{2.5}$

% inhibition = 100 - $\frac{\uparrow A_s}{\uparrow A_x} x100$

Where $\uparrow A_s$ is increase in absorbance of substrate and $\uparrow A_b$ is increase in absorbance of the blank

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

$$\text{SOD activity} = \frac{\text{Percentage Inhibition}}{\text{50 X weight of organ}}$$

Catalase Activity

Catalase activity was assayed using the method described by Aebi, (1986).

Test principle:

Catalase scavenges hydrogen peroxide and converts it to water and molecular oxygen. The activity of catalase in the sample is determined by following the rate of decrease in absorbance at 240nm and by monitoring the consumption of H_2O_2 substrate at 240 nm spectrophotometrically.

$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + 2O_2$

Procedure;

Tissue homogenate (10µL) (100-150µg of protein) was added to 2.8ml of 50mM potassium phosphate buffer (pH 7.0) in 3ml cuvette. The reaction was initiated by adding 0.1ml of fresh 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes in a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to calculate catalase activity which was in mole H_2O_2 decreased/min/mg/protein.

Malondialdehyde.

MDA activity was determined using the method Ohkawa *et al.* (1979).

Test principle:

The MDA assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.

Procedure;

After incubation for 24 hours, the treated blood cultures were centrifuged at 3000 g for 20 min, and subsequently the supernatants were collected. In a microcentrifuge tube 1300 µL of R1 was taken. 1ml of supernatant was diluted 10 times with Tris HCl and 200 µL of this diluted supernatant from each culture was added to 200 µL of distilled water and vortexed. 300 µL of R2 to each tube, which was then vortexed and incubated at 45°C for 40 min. After incubation, the tubes were cooled in ice and centrifuged at 15000 g for 10 min at 4°C. All samples were read in a spectrophotometer at 586 nm.

3.2.9 Biochemical analysis

Determination of Serum Aspartate Aminotransferase (AST)

The serum aspartate aminotransferase was determined as described by Reitman and Frankel end-point technique (1957).

Assay Principle:

α-Oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + Oxaloacetate

Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with of 2, 4-dinitrophenylhydrazine at 540 nm and 37^{o}C.

Procedure:

Exactly, 0.5µl of reagent 1, made up of phosphate buffer, L-aspartate and α-oxoglutarate were added to a test tube containing 0.1 ml of serum, mixed and incubated for exactly 30 minutes at 37^{o}C. 0.5 ml of reagent 2, made up of 2,4-dinitrophenylhydrazine was added, mixed and allowed to stand for exactly 20 minutes at 20-25^{O}C. Then 0.5ml of sodium hydroxide solution was added, mixed and absorbance was read against the blank (also prepared) at 540nm after 5 minutes. The AST concentration (U/L) was determined from the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

Determination of Serum Alkaline Phosphatase (ALP)

The procedure described by Bassey *et al*. (1946) as modified by Wright *et al*. (1972) using Randox kits was used for the assay.

The substrate p-nitrophenyl phosphate is hydrolyzed by alkaline phosphatase from the sample in the presence of magnesium ions, to form nitrophenol which is yellow and can be

read at 405 nm. The intensity of color produced is proportional to the activity of alkaline phosphatase.

Principle:

Para-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + para-nitrophenol

Alkaline phosphatase is measured by monitoring the concentration of phosphate hydrazone formed with 2,4-dinitrophenylhydrazine at 405 nm and 37^{o}C.

Procedure:

In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm, and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min. Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

Determination of Serum Alanine Aminotransferase (ALT)

The serum alanine aminotransferase was determined as described by Reitman and frankel end-point technique (1957)

Assay principle:

α-Oxoglutarate + L-alanine $\xrightarrow{ALT}$ L-glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 540 nm and 37^{o}C.

Procedure:

Exactly, 0.5µl of reagent 1 which is made up of phosphate buffer, L-alanine and α-oxoglutarate was added in a clean test tube containing 0.1 ml of serum, mixed and

incubated for exactly 30 minutes at 37^{O}C. 0.5 ml of reagent 2 which is made up of 2,4-dinitrophenylhydrazine was added, mixed and allowed to stand for exactly 20 minutes at 20-25^{O}C. Then 0.5ml of sodium hydroxide solution was added, mixed and absorbance was read against the blank (also prepared) at 540nm after 5 minutes. The ALT concentration (U/L) was determined from the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

Determination of Creatinine

The colourimetric method used to determine serum creatinine concentration is according to Bertels and Bohmer (1973) using Randox assay kits.

Assay principle:

Creatinine present in the serum or urine reacts with alkaline picrate to form a colored complex. The rate of formation of colored complex is directly proportional to creatinine concentration. This rate of reaction (intensity of color produced) is measured photometrically at 510nm and is compared with that of the standard.

Procedure:

Working reagent (1ml) containing picric acid and sodium hydroxide was added separately into two test tubes, containing 0.1ml of sample and 0.1ml of standard solution each. The content in each test tube was mixed and after 30 seconds, the absorbance A1 of the standard and sample was read at 490nm in a spectrophotometer.

Calculation

The concentration of creatinine in serum was calculated using the formular below;

$$\triangle \text{A Sample} \times \frac{\text{Standard conc. (mg/dl)}}{} = \text{mg/dl}$$

△ A standard

$A_2 - A_{1=}$ △A standard or △A Sample

Determination of Serum Total and Unconjugated Bilirubin

Total bilirubin (TB) was estimated by acid diazo method as described by Doumas *et al.* (1973), using assay kits (Randox Laboratories Ltd).

Principle:

Sulphanilic acid is diazotized by the nitrous acid produced from the reaction between sodium nitrite and hydrochloric acid. Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid (diazo reagent) to form azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotised sulphanilic acid. Unconjugated bilirubin is obtained by the difference.

Procedure:

For total bilirubin; exactly, 0.05ml of reagent 2 made up of sodium nitrate was added into a clean test tube containing 0.2ml of reagent 1 which is made up of sulphanilic acid and hydrochloric acid and 1.0ml of reagent 3 made up of caffeine and sodium benzoate was added followed by 0.2ml of serum, mixed and allowed to stand for 10 minutes at room temperature. Reagent 4 (1ml) made up of tartarate, and sodium hydroxide was also added, mixed and allowed to stand for 10 minutes at room temperature. The absorbance of the sample against sample blank (ATB) was read at 560 nm in a spectophotometer.

Calculations

Total bilirubin (mg/dl) = 10.8 × ATB

Unconjugated bilirubin (mg/dl) = 10.8 × ATB - 14.4 × ADB

ATB = Absorbance of total bilirubin, ADB = Absorbance of direct bilirubin

Determination of the Serum Urea Concentration

Principle: The serum urea concentration was determined using urea Randox assay kit. Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured photometrically by Berthelot's reaction (Fawcett and Scout, 1960).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenols (blue compound)}$$

Procedure:

Reagent 1 (100µl) containing 10µl sample, 10µl standard reagent and 10µl distilled water (blank), the content in each of the test tube was mixed and incubated at 37^{o}C for 10min, 2.5ml of reagent 2 containing dilute phenol and reagent 3 containing dilute sodium hypochlorite and sodium hydroxide were added to each of the test tube, and the content of the test tube was mixed immediately and incubated for 15min. The absorbance of the test sample (A sample) and standard (A standard) were read against blank at 500nm in a spectrophotometer.

Calculation

The serum urea concentration was calculated using the formular below;

$$\text{Urea Concentration} = \frac{\triangle \text{ A sample} \times \text{Standard Conc. (mg/dl)}}{\triangle \text{ A Standard}}$$

$\triangle$ A sample= Change in absorbance for sample

$\triangle$ A standard = Change in absorbance for standard

Determination of Albumin

The serum albumin was determined by the method of Doumas *et al. (*1971) using randox assay kit.

Principle:

In an acidic medium, albumin binds with bromocresol green (BCG) causing a shift in the absorption spectra of the yellow BCG dye. The blue green colour formed is directly proportional to the albumin present when measured at 630 nm.(600-650nm or with Red filter).

Procedures:

Bromocresol green reagent (R1) (1ml)was added to each test tubes containing 0.02ml of serum sample, 0.02ml of standard and 0.02ml of distilled water (reagent blank). The mixture in each of the test tube was incubated for 10min under room temperature. The absorbance of the serum sample (A Smple) and of the standard (A standard) was read at 630nm against the reagent blank in a spectrophotometer.

Calculation

The serum albumin concentration was calculated using the formular below

$$\text{Albumin Concentration} = \frac{\triangle \text{ A sample} \times \text{Standard Conc. (mg/dl)}}{\triangle \quad \text{A Standard}}$$

$\triangle$ A sample= Change in absorbance for sample

$\triangle$ A standard = Change in absorbance for standard

Determination of total protein

Total protein was determined colorimetrically using Randox assay kit as described by Lowry *et al*, (1951).

Principle;

In an alkaline medium, protein reacts with the copper in the Biuret reagent causing an increase in absorbance. The increase in the absorbance, at 540nm (530-570nm or with Green/Yellow filter) due to formation of the coloured complex, is directly proportional to the concentration of protein.

Procedures:

Buiret reagent (1ml) was added to each test tube containing 0.02ml of serum, 0.02ml of standard and 0.02ml of distilled water (blank), the content in each of the test tube was mixed, incubated for 10min under room temperature. The absorbance was read at 540nm in a spectrophotometer. Total protein concentration was calculated using the formular below

$$\text{Total prot. Concentration} = \frac{\triangle \text{ A sample} \times \text{Standard Conc. (mg/dl)}}{\triangle \text{ A Standard}}$$

$\triangle$ A sample= Change in absorbance for sample

$\triangle$ A standard = Change in absorbance for standard

3.2.10 Determination of Body and Relative Organ Weights

The weight of the rats was determined using My Weigh 7001DX Multi-Purpose Digital Scale on day 0 (before induction of anaemia) and at the end of every week for three weeks. The net change in body weight (difference between final body weight and initial body

weight) was determined for all the animals. On day 22, the organisms were sacrificed after an overnight fast. The liver and kidneys, heart, spleen were removed and their weights determined. The relative liver, kidney, heart and spleen weights were derermined using the formular

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \text{X}\,100$$

3.2.11. STATISTICAL ANALYSIS

Results are presented as mean ± standard error of mean (SEM). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS 17.0 for windows Computer Software Package). Significant differences were compared by Duncan's new Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant (Duncan, 1955).

CHAPTER FOUR

4.0 RESULTS

4.1: PERCENTAGE YIELD OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *Jatropha tanjorensis*.

The percentage yield of aqueous and 70% methanol, extracts of *Jatropha tanjorensis leaves* is represented in Table 4.1. The percentage yield of aqueous extract (13.1±0.06) was significantly ($P<0.05$) higher than yield of methanolic extract (8.7±0.61).

Table 4.1: Percentage Yield of Aqueous, and Methanolic Leaf Extracts of *Jatropha tanjorensis*

Plant extract	Weight of dry samples (g)	Weight of extracts (g)	Percentage (%) yield
Aqueous	10.00	1.31 ± 0.06^{c}	13.10 ± 0.61^{c}
Methanoilc	10.00	0.87 ± 0.06^{a}	8.70 ± 0.61^{a}

Values are means ± SD of three extractions. Values with different superscript down the column are significantly ($P<0.05$) different

4.2: QUALITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *Jatropha tanjorensis* .

Table 4.2. Shows the absence of anthraquinones, the presence of cardiac glycosides saponins, steroids, tannins and alkaloids in aqueous leaf extract of *J. tanjorensis.*

Methanolic leaf extract of *J. tanjorensis* revealed the absence of anthraquinones, but the presence of cardiac glycosides, saponins, steroids, tannins and alkaloids.

Table 4.2; Qualitative Phytochemical Determination of Aqueous and Methanolic Leaf Extracts of *Jatropha tanjorensis*.

Parmeters	Aqueous Extract	Metanolic Extract
Anthraquinones	-	-
Cardiac glycosides	+	+
Saponins	+	+
Steroid	+	+
Tannins	+	+
Alkaloids	+	+

+ = present, - = not detected,

4.3: QUANTITATIVE PHYTOCHEMICAL SCREENING OF METHANOLIC AND AQUEOUS LEAF EXTRACTS OF *Jatropha tanjorensis*

Table 4.3 shows the quantitative phytochemical analysis of both methanolic and aqueous leaf extract of *J. tanjorensis*. The table shows that flavonoids (3.13±0.03mg/100g) was the most abundant, followed by alkaloids (2.40±0.02mg/100g), phenol (1.2600±0.015mg/100g), saponins (1.2600±0.06mg/100g), and tannins (0.967±0.013mg/100g) respectively in the methanolic leaf extract.

The quantitative phytochemical analysis of aqueous leaf extract of *J. tanjorensis* showed that glycosides (0.95 ± 0.07mg/100g) was the most abundant, followed by saponins (0.71 ± 0.03mg/100g), alkaloids (0.54 ± 0.07mg/100g), tanins (0.03 ± 0.01mg/100g), flavanoids (0.10 ± 0.01mg/100g) respectively.

Table 4.3: Quantitative Phytochemical Analysis of Aqueous and Methanolic Leaf Extracts of *Jatropha tanjorensis*

Parameters	Aqueous Extract(mg/100g)	Methanolic Extract(mg/100g)
Saponins	0.7±0.0	1.3 ±0.0
Flavonoid	0.1±0.0	3.1±0.0
Tannins	0.0±0.0	0.1±0.0
Alkaloid	0.5±0.1	2.4±0.0
Phenol	1.0±0.1	1.5±0.0

Values are mean±standard deviation.

4.4: EFFECT OF AQUEOUS AND METHANOLIC LEAF EXTRACT OF *J. tangorensis* ON PHZ INDUCED HEAMOLYTIC ANAEMIC RATS

Preliminary studies revealed that administration of 80mg/kg body weight of phenyl-hydrazine hydrochloride in one day led to the death of the animals, however this dose was spread into two days i.e the administration of 40mg/kg body weight of PHZ on the first and second day, proved a successful induction of anaemia, hence the first day induction was confirmed becomes day 1.

The effects of aqueous and methanolic extracts of *Jatropha tanjorensis* leaves on Packed Cell Volume (PCV) of phenylhydrazine (PHZ)-induced haemolytic anaemic rats after one week of administration of 200mg/Kg of each extract is summarised on Table 4.6. This experiment was carried out to determine the extract with potent antianaemic potential. The fall in PCV values of PHZ-induced animals on day 1 compared with PCV values of day 0 and with PCV values of the normal control group, confirmed induction of haemolytic anaemia using PHZ. Increase in PCV values of the two PHZ-induced treated groups on day 4 indicates antianaemic potential of the two extracts. The antianaemic potential of the aqueous extract was lower than the antianaemic potential of the methanolic extract. PCV values on Day 7 generated some concern about the safety of aqueous extract as deaths were recorded in the group, though the antianaemic potential of the group seemed unaffected. At the end of the experiment, methanolic extract which had substantial haematopoietic potential generated 14% increament in PCV and was relatively safe for administration compared to the aqueous extract. Methanolic extract was therefore used for the studies.

Table 4.4: Effects of Aqueous and Methanolic Leaf Extracts of *J. tanjorensis* on Packed Cell Volume of Phenylhydrazine-Induced Anaemic Rats

Group	Initial PCV (%) (Day 0)	PCV(%) after induction (Day 1)	PCV(%) on Day 4	PCV(%) on Day 7	Net increment in PCV (%) (Day7-Day1)	Mean Net increase PCV (%)
NC	35.5±0.8[a]	34.5±0.7[b]	38.5±2.1[b]	38.0±0.0[b]	+4	+2
AC	43.5±4.9[a]	21.0±15.6[a]	14.0±19.8[a]	16.00±2.2[ab]	-5	-2.5
AQ	42.5±0.7[a]	26.0±2.8[a]	15.5±22.0[a]	0.00±0.00[a]	-26	-13
AM	44.5±2.1[a]	20.5±0.7[a]	30.5±0.7[a]	48.5±7.8[b]	+28	+14

Values are means ± SD. Values with different superscripts down the columns are significantly ($P<0.05$) different.

NC: Normal control animals; AC: Anaemic control animals; AM: Anaemic rats +200mg/kg of methanol extract; AQ: Anaemic rats + 200mg/kg of aqueous extract.

4.5: LETHAL DOSE DETERMINATION

Table 4.7 shows the lethal dose determination of methanolic leaf extract of *Jatropha tanjorensis* in albino rats for 48 hours. There was no mortality within 48 hours after oral administration of 10, 100 and 1000 mg/kg body weight of extracts to the rats in the first phase and also oral administration of 1000, 1600, 2900 and 5000 mg/kg body weight of the extract in the second phase. There was no mortality in all the groups administered with 1000, 1600, 2900, 5000 mg/kg body weight in 48 hours.

Table 4.5 Lethal Dose Determination of Methanolic Extract of *Jatropha tanjorensis* Leaves on Abino Rats for 48hours

First phase	Dose (mg/kg)	Number of animals	Number of death after 48hours	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group1	1000	1	0	0
Group 2	1600	1	0	0
Group 3	2900	1	0	0
Group4	5000	1	0	0

LD_{50} > 5000mg/kg body weight

4. 6: RADICAL SCAVENGING ACTIVITY OF METHANOLIC LEAF EXTRACT OF *Jatropha tanjorensis.*

Figure 4.1 presents the percentage (%) inhibition of different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml) of methanolic leaf extract of *Jatropha tanjorensis* as well as vitamin C, a standard antioxidant on 1,1-diphenyl 2-picryl hydrazyl (DPPH). This plot depicts the antioxidant activity of the extracts of *J. tanjorensis* based on their scavenging ability on DPPH which is a stable purple-coloured radical and in the process reducing it into yellow-coloured diphenylhyrazine. From the result, the % inhibition of the extract is lower than that of vitamin C at all concentrations.

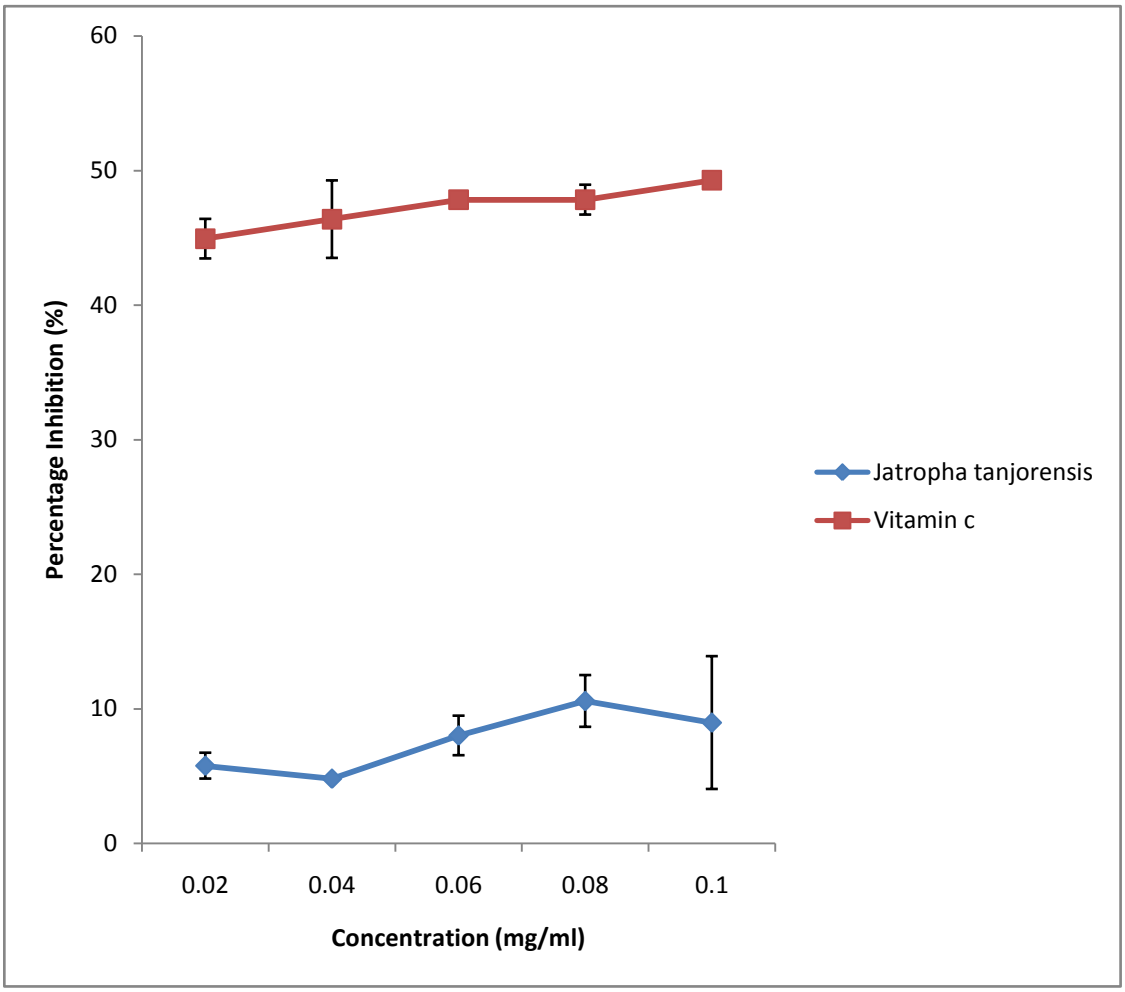


Figure 4.1: Percentage Inhibition of Different Concentrations of Methanolic Leaf Extract of *Jatropha tanjorensis* and Vitamin C on DPPH Scavenging Activity.

4.7: EFFECT OF METHANOLIC LEAF EXTRACT OF *J. tanjorensis* ON BODY WEIGHT OF PHENYLHYDRAZINE INDUCED HEAMOLYTIC ANAEMIC RATS.

Table 4.6, shows the body weight change of rats treated with methanolic leaf extract of *J. tanjorensis*. The percentage change in body weight recorded a non significant ($p>0.05$) increase in the anaemic control group , anaemic treated groups and non anaemic group administered with 200mg/kg body weight of the ME compared with the normal control group.

Table 4.6: Body Weight Change of Animals Treated with Methanolic Extract of *Jatropha tanjorensis* Leaves and Vitamin B_{12} for 21 days

Group(n=5)	Mean initial body weight(g)	Mean final body weight(g)	Percentage change in body weight
NC	115.8±2.8[a]	127.0±14.0[ab]	7.1±9.7[ab]
AC	119.0±2.4[a]	146.6±5.4[b]	18.7±3.4[b]
A+50mg/kg	126.4±3.4[a]	128.2±14.9[ab]	3.0±7.8[ab]
A+100mg/kg	120.2±10.7[a]	119.6±13.9[a]	0.2±5.4[a]
A+200mg/kg	121.6±12.1[a]	135.8±39.0[ab]	12.6±18.6[ab]
A+ Vit B_{12}	123.2±12.4[a]	112.0±14.9[a]	6.5±16.7[ab]
N+200mg/kg	119.2±7.7[a]	122.6±8.7[ab]	3.3±3.8[ab]

Values are means ± SD. Values with different superscripts down the columns are significantly ($P<0.05$) different.

NC=Normal control, AC=Anaemic control, A+50mg/kg=Anaemic rats treated with 50mg/kg of the methanolic extract, A+100mg/kg=Anaemic rats treated with 100mg/kg of methanolic extract, A+200mg/kg=Anaemic rats+200mg/kg, A+ Vit B_{12} =Anaemic rats+ standard drug (Vit B_{12}), N+200mg/kg= Non anaemic rats+200mg/kg of methanolic extract.

4.8: EFFECT OF METHANOLIC LEAF EXTRACT OF *J. tanjorensis* ON THE PACKED CELL VOLUME (PCV) AND HEAMOGLOBIN CONCENTRATION (Hb) OF PHENYLHYDRAZINE INDUCED HAEMOLYTIC ANAEMIC RATS

Tables 4.9 and 4.10 show the PCV and Hb concentration values of anaemic and non anaemic albino rats treated with methanolic leaf extract of *Jatropha tanjorensis* and vitamin B_{12} for the period of 21 days. In these tables, on day 0, the animals selected for the study were all non anaemic (i.e Hb concentration was ≥ 13g/dl and PCV≥45%).

On day 1, that is, after induction, all the animals, except the animals in the normal control group and non anaemic group administered 200mg/kg of the extract were all anaemic i.e their Hb concentration <14g/dl and PCV <45%. On day 7 and 14, 21, all the animals induced with PHZ remained anaemic, except for the group treated with vitamin B_{12}.

Table 4.7: Effect of Methanolic Extract of *Jatropha tanjorensis* Leaves and Vitamin B_{12} on the Packed Cell Volume (PCV (%)) of PHZ Induced Haemolytic Anaemic rats and Non Anaemic Rat for 21 Days

Group (n=5)	Day 0	Day 1	Day 7	Day 14	Day 21
NC	49.0 ± 2.7^{ab}	48.6 ± 2.6^{b}	48.8 ± 2.9^{c}	48.8 ± 3.0^{c}	43.8 ± 1.9^{bc}
AC	55.0 ± 1.6^{c}	30.4 ± 2.2^{a}	31.0 ± 1.0^{a}	38.0 ± 5.2^{a}	33.8 ± 1.6^{a}
A+50mg/kg	51.2 ± 4.0^{abc}	28.6 ± 2.6^{a}	33.8 ± 2.9^{a}	39.0 ± 3.8^{a}	34.4 ± 5.0^{a}
A+100mg/kg	49.4 ± 2.7^{ab}	26.0 ± 3.4^{a}	33.2 ± 2.0^{a}	40.6 ± 4.6^{ab}	32.3 ± 4.5^{a}
A+200mg/kg	53.8 ± 4.5^{bc}	29.6 ± 2.7^{a}	32.8 ± 2.3^{a}	37.0 ± 3.9^{a}	33.0 ± 6.5^{a}
A+ Vit B_{12}	49.6 ± 3.6^{abc}	27.8 ± 0.8^{a}	42.2 ± 7.6^{b}	45.6 ± 2.3^{bc}	45.6 ± 2.0^{bc}
N+200mg/kg	47.2 ± 6.3^{a}	41.8 ± 2.4^{b}	50.2 ± 1.5^{a}	50.2 ± 1.5^{c}	42.8 ± 2.4^{bc}

Data are expressed as mean± SD.Values with different superscripts down the columns are significantly ($p<0.05$) different.

NC=Normal control, AC=Anaemic control, A+50mg/kg=Anaemic rats treated with 50mg/kg of the methanolic extract, A+100mg/kg=Anaemic rats treated with 100mg/kg of methanolic extract, A+200mg/kg=Anaemic rats+200mg/kg, A+ vit B_{12} =Anaemic rats+standard drug (vit B_{12}).

N+200mg/kg = Non anaemic rats +200mg/kg of methanolic extract.

Table 4.8: Effect of Methanolic Extract of *Jatropha tanjorensis* Leaves and Vitamin B_{12} on the Hb concentation (g/dl) of PHZ Induced Haemolytic Anamic Rats and non Anaemic Rat for 21 Days

Group (n=5)	Day 0	Day 1	Day 7	Day 14	Day 21
NC	16.3 ± 0.9^{ab}	14.7 ± 2.0^{b}	16.4 ± 1.1^{c}	16.3 ± 1.0^{c}	14.3 ± 0.7^{b}
AC	18.3 ± 0.5^{c}	10.7 ± 0.6^{a}	10.3 ± 0.3^{a}	12.7 ± 1.7^{a}	8.7 ± 0.5^{a}
A+50mg/kg	17.1 ± 1.3^{abc}	9.9 ± 0.8^{a}	11.3 ± 1.0^{a}	13.0 ± 1.3^{a}	7.8 ± 0.6^{a}
A+100mg/kg	16.5 ± 0.9^{ab}	9.3 ± 1.3^{a}	10.8 ± 1.1^{a}	13.5 ± 1.5^{ab}	7.3 ± 0.6^{a}
A+200mg/kg	17.9 ± 1.5^{bc}	10.2 ± 0.7^{a}	10.1 ± 0.8^{a}	12.3 ± 1.3^{a}	7.4 ± 0.7^{a}
A+ Vit B_{12}	16.5 ± 1.2^{abc}	9.8 ± 0.4^{a}	14.1 ± 2.5^{b}	15.2 ± 2.0^{bc}	15.2 ± 1.0^{b}
N+200mg/kg	15.7 ± 2.1^{a}	14.5 ± 0.7^{b}	16.7 ± 0.5^{c}	16.6 ± 0.6^{c}	14.1 ± 2.9^{b}

Data are expressed as mean± SD.Values with different superscripts down the columns are significantly ($p<0.05$) different.

NC=Normal control, AC=Anaemic control, A+50mg/kg=Anaemic rats treated with 50mg/kg of the methanolic extract, A+100mg/kg=Anaemic rats treated with 100mg/kg of methanolic extract, A+200mg/kg=Anaemic rats+200mg/kg, A+SD=Anaemic rats+ standard drug (vit B_{12}),

N+200mg/kg = Non anaemic rats +200mg/kg of methanolic extract.

4.9: RESULT OF SOME HAEMATOLOGICAL PARAMETERS AFTER INDUCTION OF HAEMOLYTIC ANAEMIA

Table 4.9 shows the result of some hematological parameters of anaemic induced treated groups, anaemic control group and non aneamic group admistered with 200mg/kg bw of ME of *J. tanjorensis* leaves. In this, there was a significant increase ($p<0.05$) in the values of WBC, MCH, MCHC and platelet in the anaemic control group and anaemic treated groups compared with the normal control group. There was also a significant ($p<0.05$) decrease in the RBCs values in the anaemic control group, anaemic treated groups and non anaemic administered with 200mg/kg of the ME.

Table 4.9: Result of Some Hematological Parameters after Induction of Anaemia on Day1

Group(n=5)	WBC(10^3/µl	RBC(10^6/ µL)	MCV(fl)	MCH(pg)	MCHC(g/dl)	PLATELET(10^3/ml)
NC	14.5±2.9^{ab}	6.7±1.2^{c}	59.3±2.3^{a}	18.9±0.9^{a}	31.9±0.5^{a}	791.6±98.2^{ab}
AC	30.1±4.5^{c}	5.1±0.4^{b}	56.7±2.3^{a}	20.0±0.8^{ab}	35.3±1.0^{b}	969.4±106.5^{c}
A+50mg/kg	21.0±9.1^{b}	5.1±0.4^{b}	57.0±2.6^{a}	19.8±0.1^{ab}	34.7±1.7^{b}	757.4±187.1^{a}
A+100mg/kg	20.8±7.7^{b}	4.2±0.6^{a}	62.2±3.0^{b}	22.4±1.3^{c}	36.0±0.9^{b}	852.2±159.5^{ab}
A+200mg/kg	22.4±4.9^{bc}	5.2±0.5^{b}	57.1±2.1^{a}	19.8±1.2^{ab}	34.6±1.2^{b}	940.4±75.3^{ab}
A+Vit B_{12}	29.4±5.9^{c}	4.8±0.2^{ab}	58.0±0.7^{a}	20.6±0.8^{b}	35.6±1.0^{b}	899.2±132.0^{ab}
N+200mg/kg	10.9±2.0^{a}	7.2±0.4^{c}	57.5±1.5^{a}	18.7±0.4^{a}	32.4±0.5^{a}	821.2±87.7^{ab}

Data are expressed as mean±SD.Values with different superscripts down the columns are significantly ($p<0.05$) different.

NC;NC=Normal control, AC=Anaemic control, A+50mg/kg=Anaemic rats treated with 50mg/kg of the methanolic extract, A+100mg/kg=Anaemic rats treated with 100mg/kg of methanolic extract, A+200mg/kg=Anaemic rats+200mg/kg, A+ Vit B_{12}=Anaemic rats+standard drug(vit B_{12}), N+200mg/kg = Non anaemic rats +200mg/kg of methanolic extract.

WBC = White blood cell count, RBC = Red blood cell count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration

4.10: RESULT OF SOME HAEMATOGICAL PARAMETERS AFTER TREATMENT FOR 21 DAYS

Table 4.10 shows the result of some hematological parameters of anaemic induced treated group, anaemia control group and non anaemia group of albino rat after 21 days of treatment.

After treatment for 21 days, there was a significant increase ($p<0.05$) in the values of WBC,MCV,MCH in the anaemic treated compared with the normal control except in the value of WBC for the group treated with vitamin B_{12} that was significantly different ($p<0.05$) compared with the normal control. Also, after 21 days of treatment, there was a decrease ($p<0.05$) in the values of RBC, MCHC and platelet in the anaemic control group and aaemic treated groups compared to the normal control.

Table 4.10: Result of some Hematogical Parameters after Treatment for 21 Days

Group(n=5)	WBC(103/µl)	RBC(106µl)	MCV(fl)	MCH(pg)	MCHC(g/d)	PLATELET(10^3/ml)
NC	12.6±3.6[a]	6.9±0.7[b]	63.5±5.5[a]	20.9±1.9[a]	32.8±0.3[b]	950.2±44.4b[c]
AC	147.3±9.3[d]	3.2±0.2[a]	119.7±5.5[bc]	27.4±0.5[bc]	22.9±1.0[a]	748.2±56.2[a]
A+50mg/kg	128.3±12.3[cd]	2.8±0.7[a]	125.0±12.2[6c]	28.3±1.8[c]	22.5±0.9[a]	774.2±129.0[a]
A+100mg/kg	112.1±40.9[bc]	2.7±0.5[a]	123.0±10.8[bc]	27.7±1.0[bc]	22.6±1.2[a]	853.6±148.4[ab]
A+200mg/kg	91.4±45.4[b]	2.7±0.3[a]	124.6±8.6[bc]	28.0±2.1[bc]	22.4±0.6[a]	866.2±9620.0[ab]
A+ Vit B_{12}	24.3±9.4[a]	6.2±1.0[b]	111.9±13.9[b]	26.3±1.0[b]	23.6±2.9[a]	82.0±76.7[ab]
N+200mg/kg	8.6±1.9[a]	7.2±0.4[b]	59.8±1.9a	19.8±0.7[a]	33.0±0.5[b]	1017.8±136.8[c]

Data are expressed as mean±SD. Values with different superscript down the column are significantly ($p<0.05$) different.

NC=Normal control, AC=Anaemic control, A+50mg/kg=Anaemic rats treated with 50mg/kg of the methanolic extract, A+100mg/kg=Anaemic rats treated with 100mg/kg of methanolic extract, A+200mg/kg=Anaemic rats+200mg/kg, A+vit B_{12}=Anaemic rats+standard drug (vit B_{12}), N+200mg/kg = Non anaemic rats +200mg/kg of methanolic extract.

WBC=White blood cell count, RBC = Red blood cell count, MCV=Mean Corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration

4.11: EFFECT OF METHANOLIC EXTRACT OF *Jatropha tanjorensis* LEAVES ON BIOCHEMICAL PARAMETERS

Table 4.11 shows the effect of methanolic leaf extract of *Jatropha tanjorensis* on biochemical parameters of PHZ anaemic rats. ALT and ALP recorded a significant increase ($p<0.05$) in the anaemic control and anaemic treated groups compared with the normal control group. Also, serum AST showed a significant increase ($p<0.05$) in the anaemic group treated with 200mg/kg of ME compared with the normal control group. Albumin concentration was significantly decreased ($p<0.05$) in the anaemic control group and anaemic treated group compared with the normal control group.

There was a significant increase ($p<0.05$) in the concentration of urea and creatinine in the anaemic control group, anaemic treated groups compared with the normal control group. The mean value of conjucated bilirubin was significantly ($p<0.05$) higher in the anaemic groups treated with 200mg/kg b/w when compared with the normal control group. Also, there was a significant ($p <0.05$) increase in the value of total bilirubin in the anaemic group treated with 200mg/kg b/w of methanolic leaf extract when compared with the normal control group

Table 4.11: Effect of Methanolic Extract of *Jatropha tanjorensis* Leaves on Some Biochemical Parameters of Phenylhydrazine Induced Haemolytic Anaemic Rats.

Group(n=5)	ALT (µ/l)	AST(µ/l)	ALP (µ/l)	T.P (gm/dl)	ALB (g/dl)	UREA (mg/dl)	CREAT (mg/dl)	T.BIL (mg/dl)	C.BIL (mg/dl)
NC	33.8±5.3^{a}	25.4±5.5^{a}	58.8±8.3^{a}	68.4±3.1^{a}	39.4±4.2^{c}	3.2±0.8^{a}	3.2±0.8^{a}	11.3±1.3^{a}	6.7±1.0^{a}
AC	46.8±6.6^{bc}	27.6±5.5^{a}	75.6±8.0^{b}	67.4±5.8^{a}	38.2±6.1^{bc}	3.7±0.6^{ab}	3.7±0.6^{ab}	12.4±2.9^{ab}	6.2±2.2^{a}
A+50mg/kg	50.6±7.0^{c}	30.2±3.0^{a}	81.2±8.0^{b}	69.0±6.4^{a}	38.6±5.1^{bc}	3.8±0.4^{ab}	3.8±0.4^{ab}	13.3±1.6^{ab}	5.3±1.6^{a}
A+100mg/kg	43.4±3.2^{bc}	31.0±3.9^{a}	70.8±6.0^{b}	67.0±4.9^{a}	38.8±3.9^{bc}	4.1±0.4^{b}	4.1±0.4^{b}	14.4±1.1^{a}	6.8±1.4^{a}
A+200mg/kg	95.2±8.7^{d}	56.6±11.2^{b}	101.4±10.1^{c}	65.0±3.7^{a}	32.4±1.7^{a}	3.5±0.5^{ab}	3.5±0.5^{ab}	23.2±2.9^{c}	10.4±1.3^{b}
A+ Vit B_{12}	37.8±4.0^{ab}	32.6±4.0^{a}	60.0±8.5^{a}	65.6±3.0^{a}	35.6±2.6^{abc}	3.6±0.6^{ab}	3.6±0.6^{ab}	10.2±0.9^{a}	7.0±1.9^{a}
N+200mg/kg	38.4±4.3^{ab}	28.2±5.1^{a}	75.8±7.9^{b}	71.0±2.6^{a}	42.6±2.4^{c}	3.3±0.4^{a}	3.3±0.4^{a}	10.8±1.3^{a}	5.4±0.8^{a}

Data are expressed as mean±SD.values with different superscript down the columns are significantly ($p<0.05$) different.

AC=Anaemic control, NC=Normal control, A+50mg/kg=Anaemic+50mg/kg, A+100mg/kg=Anaemic + 100mg/kg, A+200mg/kg=Anaemic treated with 200mg/kg, A+ Vit B_{12}=Anaemic treated with vitamin B_{12}, N+200mg/kg=Normal rats given 200mg/kg of the methanolic leaf extract.

ALT=Alanineamino transferase, AST=Aspartateamino transferase, ALP=Alanin phosphatase, T.P=Total protein, ALB=Albumin

T.BIL=Total bilirubin, C.BIL=Conjucated bilirubin, Creat =creatinine

4.12: EFFECT OF METHANOLIC EXTRACT OF *Jatropha tanjorensis* LEAVES ON CATALASE (CAT), SUPEROXIDE DISMUTASE (SOD) AND MALONDIALDEHYDE (MDA).

Table 4.12 shows the values of CAT , SOD and tissue MDA in the anaemic control group, anaemic treated groups and the non anaemic rats administered 200mg/kg bw of the methanolic leaf extract of *Jatropha tanjorensis*. In this table, the values of tissue MDA was significantly higher ($p<0.05$) in the anaemic treated groups compared with the normal control group, also, there was a significant decrease ($p<0.05$) in the values of catalase in the anaemic treated group compared with the normal control.

Table 4.12: Effect of Methanolic Extract of *Jatropha tanjorensis* Leaves on Catalase (CAT), Superoxide dismutase (SOD) and Malondialdehyde (MDA) of Phenylhydrazine Induced Haemolytic Anaemic Rats.

Group(n=5)	MDA (mmol/g of tissue)	SOD (mmol/min/g of tissue)	CAT (moles of H_2O_2/min/g of tissue)
NC	1.3 ± 0.3^{a}	2.2 ± 0.2^{a}	66.6 ± 3.5^{d}
AC	1.5 ± 0.2^{ab}	1.9 ± 0.2^{a}	56.2 ± 4.0^{c}
A+50mg/kg	1.4 ± 1.9^{ab}	4.9 ± 7.3^{a}	54.8 ± 6.2^{bc}
A+100mg/kg	1.4 ± 0.2^{ab}	1.4 ± 0.2^{a}	49.4 ± 4.8^{b}
A+200mg/kg	2.4 ± 0.3^{c}	2.4 ± 0.3^{a}	42.2 ± 2.9^{a}
A+Vit B_{12}	1.3 ± 0.2^{a}	2.3 ± 0.2^{a}	60.0 ± 3.9^{d}
N+200mg/kg	1.7 ± 0.2^{b}	1.7 ± 1.6^{a}	51.0 ± 3.2^{bc}

Data are expressed as mean±SD.values with different superscript down the columns are significantly ($p<0.05$) different.
AC=Anaemic control, NC=Normal control, A+50mg/kg=Anaemic+50mg/kg, A+100mg/kg=Anaemic + 100mg/kg, A+200mg/kg=Anamic treated with 200mg/kg, A+VitB_{12}=Anaemic treated with vitamin B12, N+200mg/kg=Normal rats given 200mg/kg of the extract.
MDA= Malondialdehyde, SOD=Superoxide dismutase, CAT=Catalase

4.13: EFFECT OF METHANOLIC EXTRACT OF *Jatropha tanjorensis* LEAVES ON THE RELATIVE ORGAN WEIGHT

Table 4.13; shows the relative organ weight of anaemic treated, anaemic control and non anaemic group administered 200mg/kg body weight of methanolic leaf extract of *Jatropha tanjorensis.* In this table, there was a significant increase ($p<0.05$) in the weight of spleen in the anaemic control group and anaemic treated group compared with the normal control group.

Table 4.13: The Effect of Methanolic Extract of *Jatropha tanjorensis* Leaves on the Relative Organ Weight of Phenylhydrazine Induced Haemolytic Anaemic Rats After Treatment for 21 Days.

Group(n=5)	LIVER	KIDNEY	LUNGS	SPLEEN	HEART
NC	3.6±0.5^{a}	0.7±0.6^{ab}	1.0±0.1^{abc}	0.3±0.5^{a}	0.4±0.1^{a}
AC	3.16±0.9^{a}	0.6±0.5^{ab}	0.7±0.5^{a}	1.7±0.1^{b}	0.4±0.4^{a}
A+50mg/kg	3.6±0.7^{a}	0.7±0.2^{ab}	0.8±0.3^{ab}	1.8±0.4^{b}	0.5±0.1^{a}
A+100mg/kg	3.5±0.34^{a}	0.6±0.1^{ab}	0.9±0.1^{abc}	1.8±0.4^{b}	0.5±0.6^{a}
A+200mg/kg	3.4±1.2^{a}	0.8±0.3^{b}	1.2±0.2^{c}	1.9±0.7^{b}	0.5±0.2^{a}
A+ VitB$_{12}$	3.0±0.2^{a}	0.7±0.5^{ab}	1.0±0.2^{abc}	0.5±0.3^{ab}	0.5±0.0^{a}
N+200mg/kg	3.0±0.4^{a}	0.5±0.3^{a}	1.0±0.3^{abc}	0.2±0.6^{a}	0.4±0.0^{a}

Data are expressed as mean±SD.values with different superscript down the columns are significantly ($p<0.05$) different.

AC=Anaemic control, NC=Normal control, A+50mg/kg=Anaemic+50mg/kg, A+100mg/kg=Anaemic + 100mg/kg, A+200mg/kg=Anamic treated with 200mg/kg, A+VitB$_{12}$=Anaemic treated with vitamin B12, N+200mg/kg=Normal rats given 200mg/kg of the extract.

CHAPTER FIVE

5.0 DISCUSSION

The qualitative phytochemical study of aqueous and methanolic leaf extract of *J. tanjorensis* (Table4.1) revealed the absence of anthraquinones, the presence of cardiac glycosides, saponins, steroids, tannins and alkaloids. Table 4.2 showed the quantitative phytochemical analysis of methanolic and aqueous leaf extracts of *J. tanjorensis.* It was observed from the table that the quantitative phytochemical analysis of methanolic leaf extract of *J. tanjorensis* revealed that flavonoids (3.13±0.03mg/100g) was the most abundant, followed by alkaloids (2.40±0.02mg/100g), phenol (1.2600±0.015mg/100g), saponins (1.2600±0.06mg/100g), and tannins (0.967±0.013mg/100g) respectively. Also, the quantitative phytochemical analysis of aqueous leaf extract of *J. tanjorensis* showed that glycosides (0.95 ± 0.07mg/100g) was the most abundant, followed by saponins (0.71 ± 0.03mg/100g), alkaloids (0.54 ± 0.07mg/100g), tannins (0.03 ± 0.01mg/100g), flavanoids (0.10 ± 0.01mg/100g) respectively

Alkaloids are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, respiratory system, and gastrointestinal tract. In addition, alkaloids are antispasmodic, analgesic and also have bactericidal effects (Okwu, 2001). Tannins are well known for their anti-oxidant and anti-microbial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretic properties. Saponins lower cholesterol level, have anti-diabetic and anti-carcinogenic properties. In addition, saponins are expectorants, cough suppressant and for haemolytic activities (Van-Burden and Robinson, 1981). Flavonoids are significantly recognised for their anti-oxidant, anti-carcinogenic and anti-microbial and anti-tumour properties (Okwu, 2001).

Figure 4.1 presents the percentage (%) inhibition of different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml) of methanolic leaf extract of *Jatropha tanjorensis* as well as vitamin C, a standard antioxidant on 1, 1-diphenyl 2-picryl hydrazyl (DPPH). From the result, the % inhibition of the extract is lower than that of vitamin C at all concentrations. This shows that the antioxidant potential of methanolic leaf extract as a radical scavenger is lower than that of vitamin C.

Antioxidant potential is inversely proportional to Inhibitory concentration (IC_{50}) value, which was calculated from the linear regression of the % inhibition versus extract concentrations. The IC_{50} of methanolic leaf extract of *J. tanjorensis* value was found to be 0.756mg/ml and that of vitamin C value was 0.077mg/ml (Appendix 1), this means that, the extract has a lower antioxidant potential than vitamin C.

1,1-diphenyl 2-picryl hydrazyl (DPPH) radical scavenging activity is considered to be primary antioxidant activity (Norshazila *et al.,* 2010), because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials actively by a chain-breaking mechanism (Maisuthisakul *et al*., 2005) and is a model for a lipophilic radical. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The ME of *J. tanjorensis* leaves required a high concentration to give positive DPPH test suggests that the sample was relatively not a free radical scavenger. A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value (Pattanayak *et al.*, 2012).

Table 4.6, shows the bodyweight change of rats treated with methanolic extract of *J. tanjorensis* leaves. The mean final weight and the percentage change in body weight showed a non significant ($p>0.05$) increase in the anaemic control group, anaemic treated groups and non anaemic group administered 200mg/kg body weight of the extract, compared with the normal control group.

The non significant ($P>0.05$) change of percentage body weights and in the mean final body weight of non anaemic animals treated with 200mg/kg of ME of *J. tanjorensis* leaves compared to the normal control group may be because the dose has not accumulated significantly to manifest any significant change (Peters and Boyd, 1966). The non significant ($P>0.05$) increase in the percentage change in body weight of the anaemic control group, anaemic treated groups compared to the normal control group possibly indicates the body's ability to reverse the effects of haemolytic anaemia on the body weight.

Body weight is normally investigated as a sensitive indicator of chemically induced changes to organs. The comparison between the ratiometric differences of organ to body weight of the control group and the treated groups has been used to evaluate the toxic effect of the test substance (Peters and Boyd, 1966), from this result, it shows that the extract is not toxic.

A pilot study was conducted to know the effect of both aqueous and methanolic leaf extracts of *J. tanjorensis* on PHZ induced haemolytic anaemic rats (table 4.4) . The significant ($p<0.05$) decrease in PCV in the haemolytic anaemic induced groups on day 1, shows a

successful induction of haemolytic anaemia by PHZ. However, on day 7, deaths were recorded in the anaemic group treated with 200mg/kg of aqueous extract which generated a concern on the safety of the aqueous extract in the management of haemolytic anaemia. The group treated with 200mg/kg body weight of methanolic extract shows a 14% mean net increase in PCV, hence it was used for the *in vivo* study.

Tables 4.7, 4.8, 4.9, 4.10 show the PCV, Hb concentration values and some heamatological results after induction and after treatment for 21 days respectively, of anaemic and non anaemic albino rats treated with methanolic leaf extract of *Jatropha tanjorensis* and vitamin B_{12}. Intraperitoneal administration of PHZ on day1 generated a significant ($P<0.05$) decrease in PCV, Hb and RBC, This is in line with the findings of Turaskar *et al.,(*2013), who carried out a research in which PHZ altered the function of RBC by haemolysis characterized by 74.06% decrease in RBC, 48.17% decrease in Hb concentration and 41.68% decrease in PCV. It has been demonstrated that intraperitoneal administration of phenylhydrazine decreased Hb concentration, Red Blood Cells number and haematocrit (O'Riordan *et al*., 1995; Criswell *et al*., 2000). Also Agbor *et al*. (2005) demonstrated that oral administration of 10 mg/kg phenylhydrazine for 8 days reduced haematological indices by 50%. This significant ($P<0.05$) reduction in PCV, Hb and RBC confirms induction of haemolytic anaemia. Reinduction of animals (on day 7 and 14) resulted in significant ($P<0.05$) decrease of PCV, Hb and RBC of the anaemic treated rats, except with the anaemic group treated with vitamin B_{12} that had a non significant ($p>0.05$) increase in PCV, Hb and RBC when compared to the normal control group. After 21 days administration of the extract, non significant ($p>0.05$) increases in PCV, Hb, RBC, Platelet, MCHC were recorded in the non

anaemic group given 200mg/kg body weight and in the anaemic group treated with Vit B_{12}. Significant decreases ($p<0.05$) in PCV, Hb, RBC, Platelet, MCHC were observed in the anaemic control group, anaemic groups treated with the extract compared to the normal control group. A significant ($p<0.05$) increase was recorded in the WBC, MCV, MCH in the anaemic treated groups and anaemic control group compared with the normal control group. Phenylhydrazine was reported to induce the development of Heinz bodies on RBC membranes after six days of exposure (Bowman and Rand, 1980; Gordon-Smith, 1980). It has been also reported that phenylhydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species (Clemens *et al.,* 1984; Hill and Thornalley, 1982). However, alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species. Phytochemical screening of the methanolic extract revealed the presence of saponins, flavonoids and alkaloids. Thus, it appears that the presence of these antioxidants in the plant extract was not so much to reverse the damaging affect of phenylhydrazine.

In haemolytic anaemia, there is a destruction of circulating red blood cells before the normal life span (120 days), thereby leading to a decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in females (Okochi *et al*., 2004). PHZ decreases haemoglobin level, red blood cell concentration, and packed cell volume, and impairs erythrocyte deformability. It induces reticulocytosis, alters osmotic resistance, free plasma haemoglobin, MCH, MCHC, and erythropoietin levels, and extramedular haematopoiesis in the spleen and liver (Berger, 2007). The determination of haematological indices provides physiological information on a proper blood assessment. According to

Okonkwo *et al. (*2004), accurate laboratory determination of blood parameters remains the only sensitive and reliable foundation for ethical and rational research, diagnosis, treatment and prevention of haemolytic anaemia. Researchers have shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters (Ajagbonna *et al*., 2001). The major concern of the scientific communities with regard to medicinal plants and haematological studies focuses on the measures that can maintain a normal haematological state of being and reverse any negative haematological status associated with various anaemic conditions (Alexander and Griffiths, 1993).

From table 4.11, it was observed that the values of ALT and ALP was significantly ($p<0.05$) increased in the anaemic control group, anaemic treated groups with the extract compared with the normal control group. The value of AST was significantly ($p<0.05$) increased in the anaemic group treated with 200mg/kg body weight of the extract. A significant ($p<0.05$) decrease was recorded in the value of albumin in the anaemic group treated with 100mg/kg bodyweight of the extract when compared with the normal control group.

Elevation in ALT and ALP in the anaemic control group and anaemic groups treated with ME of *J. tanjorensis* leaves compared to the normal control group is in line with the findings of Oluwole *et al***. (**2012) that carried out a research on methanolic leaf extract of *J. tanjorensis* and discovered that the ALT and AST, ALP of the animals given the extract of *J. tanjorensis* were elevated.

Measurements of the activities of various enzymes in body fluids play a significant role in disease investigation and diagnosis and detection of tissue damage (Malomo, 2000). Marker

enzymes located in some cells can leak into the serum or other parts of the living system as a result of injury to the cell where they are located (Oluwole *et al*.**,** 2012). ALT is produced within the cells of the liver and is the most sensitive marker for liver cell damage (Meyer and Kulkarni, 2001). Any form of hepatic cell damage can result in leaking of ALT into the bloodstream leading to a rise in the serum levels (Meyer and Kulkarni, 2001). Therefore, the observed elevation of serum ALT in the anaemic control group and anaemic groups treated with ME of *J. tanjorensis* leaves and also the elevation in AST in the anaemic group treated with 200mg/kg bodyweight of ME of *J. tanjorensis* leaves are indications of their leakage into the bloodstream due to liver damage (Adeyemi *et al*., 2015).

The significant ($P<0.05$) decrease in the level of albumin in the anaemic group treated with 200mg/kg bw of ME of *J. tanjorensis* leaves compared to the normal control group may indicate that the synthetic function of the liver has been significantly affected, since albumin is synthesized only in the liver. Albumin which is produced only in the liver is the major plasma protein that circulates in the blood stream. It helps move many small molecules through the blood, including bilirubin, calcium, progesterone, and medications. ALB also plays an important role in keeping the fluid from the blood from leaking out into the tissues (Vasudevan and Sreekumari, 2007)

Urea and creatinin show a significant ($P<0.05$) increase in the anaemic group treated with 100mg/kg body weight of ME of *J. tanjorensis* leaves when compared with the normal control group. Also a significant ($p<0.05$) increase in the values of total billirubin and conjugated billuribin in the anaemic group treated with 200mg/kg body weight of the ME of

J. tanjorensis leaves when compared with the normal control group was observed. This is in line with the findings of (Kori-Siakpere and Ogoruvwe, 2012) who observed an elevated leavel in the concentrations of urea, uric acid and creatinine in the tissue/organ of fish exposed to crude extract of *J. tanjorensis* after 96 hours.

Urea and creatinine (Table 4.11) are non electrolytes found in the body. Urea is produced from metabolism of amino acid and creatinine is formed from the metabolism of muscle creatinine and creatine phosphate. They are both excreted by the kidneys (Guyton and Hall, 2006). The excretion of urea and creatinine is used to ascertain renal function. This process is actively carried out by the kidneys. All these processes may also be responsible for the increases in the urea and creatinine concentration in the anaemic treated group with 100mg/kg body weight of the extract as they are excreted by the kidneys (Omigie and Agoreyo, 2014). Creatinine is usually produced at a fairly constant rate by the body and filtered out of the blood by the kidneys. If the filtering capacity of the kidney is deficient, creatinine blood levels rise (Guyton and Hall, 2001). In general, increased urea levels are associated with nephritis, renal ischemia and urinary tract obstruction (Oluwole *et al*., 2012). Urea is the major end product of protein catabolism in mammals and is the primary vehicle for removal of toxic ammonia from the body. It is primarily produced in the liver and secreted by the kidneys (Oluwole *et al***.,** 2012).

Table 4.12, shows the effect of methanolic extract of *Jatropha tanjorensis* leaves on SOD, tissue catalase and MDA of phenylhydrazine induced haemolytic anaemic rats. There was a significant ($p<0.05$) increase in the levels of MDA in the anaemic group treated with

200mg/kg and a significant ($p<0.05$) reduction in the values of catalase in the anaemic control group, anaemic groups treated with the ME *of J. tanjorensis* leaves when compared with the normal control group.

Catalase is an enzymic antioxidant widely distributed in all animal tissues and the highest activity is found in the red blood cells and in the liver. Catalase decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance *et al*., 1952). Therefore, a reduction in the activity of catalase may result in a number of deleterious effects due to the accumulation of hydrogen peroxide. MDA is the major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA level is an important indicator of lipid peroxidation (Omoregie and Osagie, 2011).

Table 4.13 shows the effect of the ME of *Jatropha tanjorensis* leaves on relative organ weight of PHZ induced anaemic rats. The significant ($p<0.05$) increase in the relative spleen weight in the anaemic control group and in the aneamic treated groups was seen as a result of direct toxicity of PHZ indicating that, it is caused by the uptake of erythrocyte by the microphages in the spleen (Berger, 2007).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDTIONS

6.1 SUMMARY

a. From the present study, quantitative phytochemical analysis of methanolic leaf extract of *J. tanjorensis* revealed that flavonoids (3.13±0.03mg/100g) were the most abundant, followed by alkaloids (2.40±0.02mg/100g), phenol (1.2600±0.015mg/100g), saponins (1.2600±0.06mg/100g), and tannins (0.967±0.013mg/100g) respectively. Quantitative phytochemical analysis of aqueous leaf extract of *J. tanjorensis* also revealed phenol (0.95 ± 0.07mg/100g) was the most abundant, followed by saponins (0.71 ± 0.03mg/100g), alkaloids (0.54 ± 0.07mg/100g), tannins (0.03 ± 0.01mg/100g), flavanoids (0.10 ± 0.01mg/100g) respectively.

b. Comparative study of the aqueous and methanol extracts revealed that, methanolic extract recorded mean 14% increament in PCV within 7 days after administration of 200ml/Kg body weight with no death of rats recorded. This extract was used for the *in vivo* assays.

c. Percentage (%) inhibition of different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml) of methanolic leaf extract of *Jatropha tanjorensis* as well as Vitamin C, a standard antioxidant on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) showed that the IC_{50} of methanolic leaf extract of *J. tanjorensis* value was found to be 0.756mg/ml and that of vitamin C value was 0.077mg/ml.

d. The percentage change in body weight recorded a significant ($p<0.05$) increase in the anaemic control group and a significant ($p<0.05$) decrease in the anaemic group treated with

vitamin B_{12} and the non anaemic group administered with 100mg/kg body weight of the methanolic leaf extract of *J. tanjorensis*.

e. The preliminary study of induction showed that the interperitonial injection of 80mg/kg body weight of PHZ according to Iwelewa *et al.*, 2005, led to the death of the animals but when this dose was spread into two days i.e 40mg/kg bodyweight of PHZ for two days, induction became successful. Hence this method is called the modified method of Iwelewa *et al.*, 2005.

f. After treating the anaemic animals for 21 days with ME, the extract could not improve heamatological indices of the anaemic rats as a significant ($p<0.05$) reduction was observed in the Hb concentration, PCV, RBC and platelete in the animals treated with methanolic leaf extract of *J. tanjorensis* .

g. Levels of ALT and ALP in the anaemic control group and anaemic groups treated with ME of *J. tanjorensis* leaves was significantly ($p<0.05$) higher compared to the normal control group. There was a significant ($P<0.05$) decrease in the level of albumin in the anaemic group treated with 200mg/kg bw of ME compared to the normal control group

h. A significant ($P<0.05$) increase was recorded in the values of urea and creatinine in the anaemic group treated with 100mg/kg body weight of ME of *J. tanjorensis* leaves when compared with the normal control group. Also a significant ($p<0.05$) increase was observed in the values of total billirubin and conjugated billuribin in the anaemic group treated with 200mg/kg body weight of the ME of *J. tanjorensis* leaves when compared with the normal control group.

i. A significant ($p<0.05$) increase was observed in the levels of tissue malonaldehyde (MDA) in the anaemic group treated with 200mg/kg and a significant ($p<0.05$) reduction in the values of catalase in the anaemic control group, anaemic groups treated with the ME of *J. tanjorensis* leaves when compared with the normal control group.

j. There was a significant ($p<0.05$) increase in the relative spleen weight in the anaemic control group and in the aneamic treated groups when compared with the normal control group.

6.2 CONCLUSION

The 70% methanol extract of *J. tanjorensis* leaves appeared to have a non stimulatory effect on haematopoiesis . The extract (especially dose 200mg/kg) significantly ($P<0.05$) altered biochemical parameters as well as the level of tissue MDA and catalase in the liver of haemolytic anaemic albino rats and a non significant ($p>0.05$) effect in the non haemolytic anaemic group administered with 200mg/kg. The extract is therefore relatively not safe for the treatment of haemolytic anaemia at the doses administered, since it has relative negative effects on haematinic parameters.

6.3 RECOMMENDATION

1. From the present study, the methanolic leaf extract of *J. tanjorensis* is not toxic to non anaemic rats since it has LD50 >5000mg/kg body weight.

2. Although the plant leaves of *J. tanjorensis* were initially and popularly consumed in Nigeria as soups, the leaves are also employed traditionally as heamatinic agent. The result of this study showed that the beneficial effect of consuming the leaves of this plant to manage haemolytic anaemia may not be up to what is being claimed and therefore, consumption should be with caution.

3. Further study should be carried out to investigate the chemopreventive effect of *Jatropha tanjorensis* leaf supplementation on phenylhydrazine induced haemolytic anaemic rats.

4. Further study should also be carried out on the plant to investigate the heamoglobin regenerating potential of this plant on iron deficiency anaemia.

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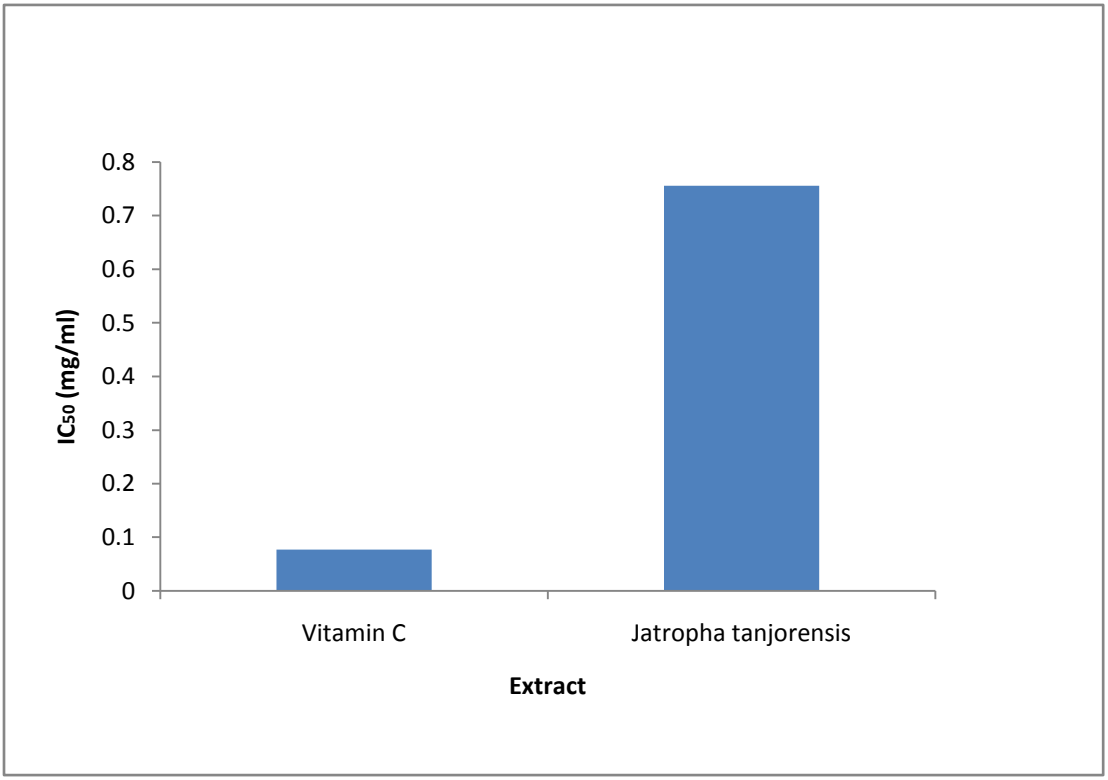
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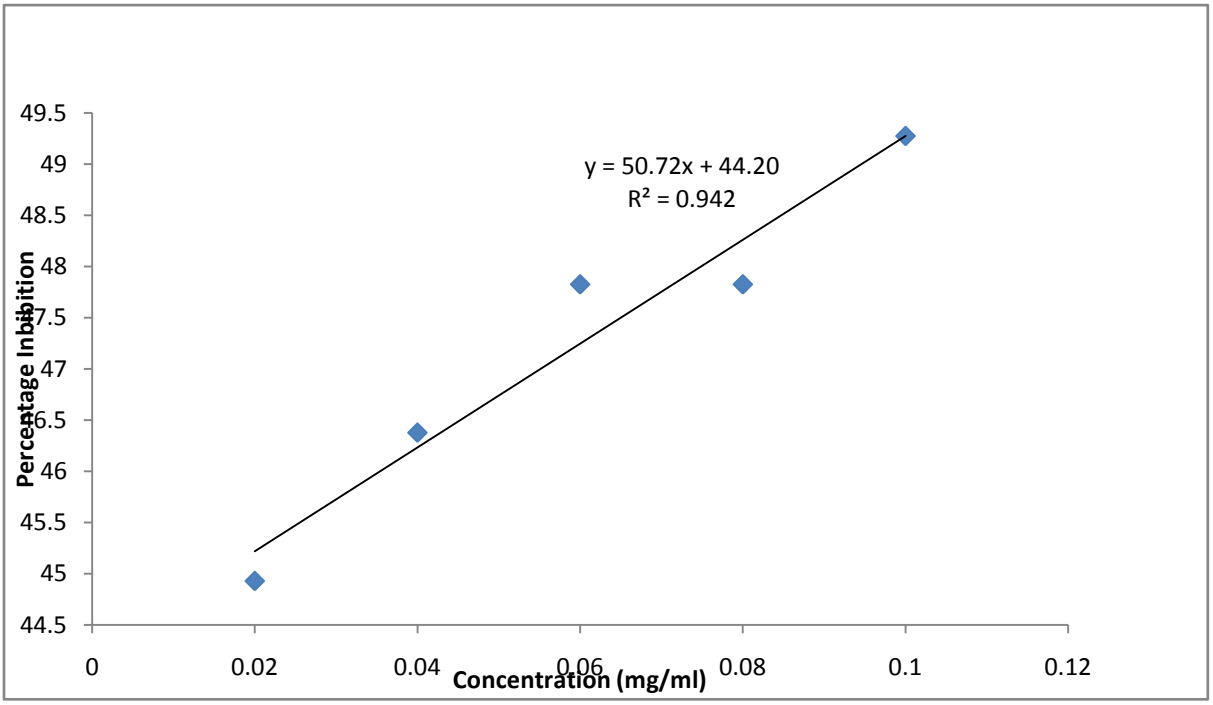
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APPENDIX 1



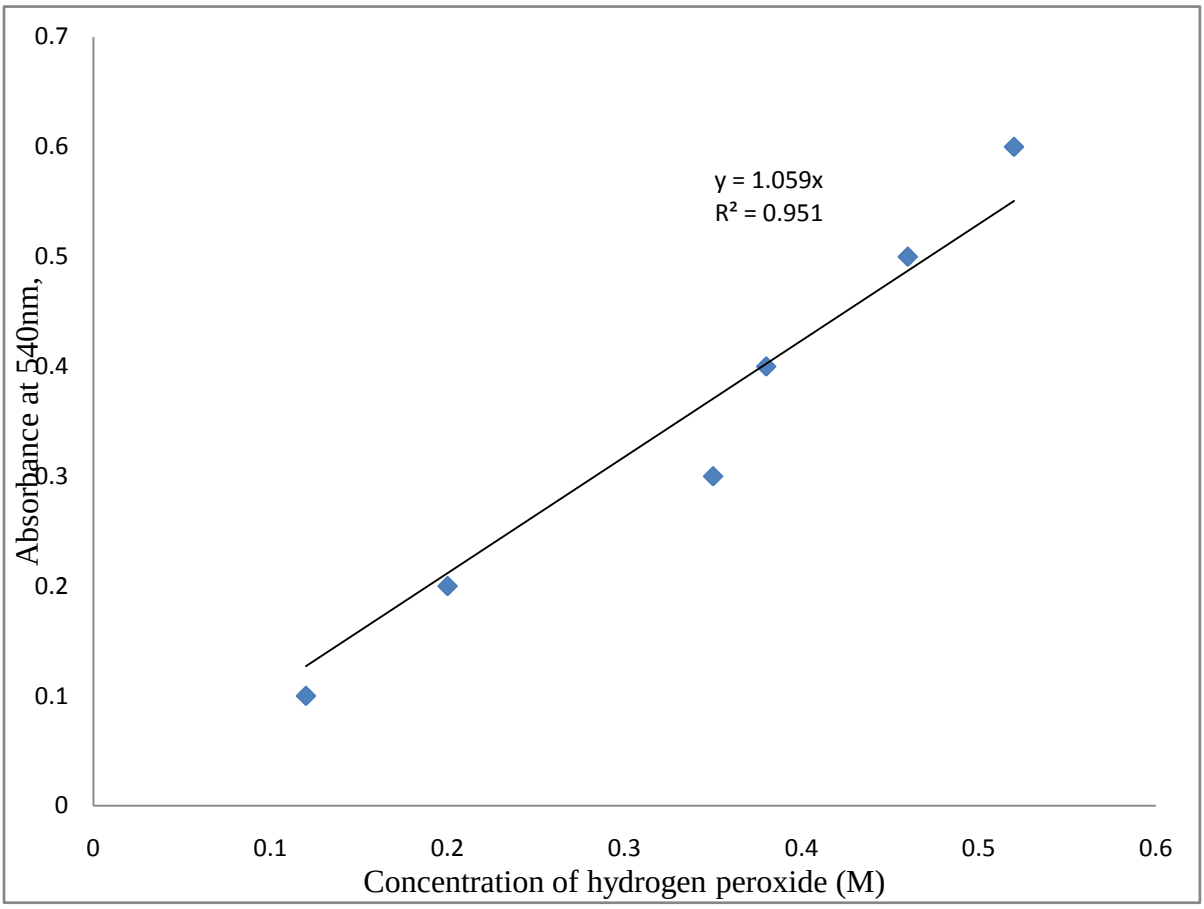
IC_{50} of methanolic leaf extract of *Jatropha tanjorensis*

APPENDIX 2



Plot of Percentage Inhibition of Free Radicals and Concentrations of Vitamin C

APPENDIX 3



Standard Curve for Hydrogen Peroxide

APPENDIX 4

Calculations and Conversions Used in Some Analyses

A. Conversions and calculations Required in the Preparation of Varying Concentration of Extract for DPPH Radical Scavenging Activity of the Extract

An electronic balance was used to weigh 100µg (0.1mg) of extract. The extract was dissolved in distilled water to produce 10ml stock solution of concentration 0.1mg/ml. Stock solution was further diluted in distilled water to obtain desired concentrations of extract as demonstrated in the table below. Each dilution process was done in triplicate.

Volume of extract solution (ml)	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Volume of distilled water (ml)	0.8	0.6	0.4	0.2	0.0
Final concentration of solution (mg/ml)	0.02	0.04	0.06	0.08	0.1