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Research Article

Hematinic effect of locust bean (*Parkia biglobosa*) seeds on phenylhydrazine-induced anaemia in rats

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Keywords:
Parkia biglobosa, phenyhydrazine, hematology, rats

ABSTRACT

Background: Hematinic effect of the extract of locust bean seeds was investigated following Phenylhydrazine (primarily used as antipyretics) administration in wistar rats. **Methods**: The phytochemical, chemical and mineral constituents of the locust bean seeds were evaluated. 25 adult male wistar rats were randomly divided into 5 groups: Group 1 rats were untreated control; Group 2 received only phenylhydrazine: (negative controls); Group 3 received phenylhydrazine +100 mg/kg of extract; Group 4 received phenylhydrazine +1000 mg/kg of extract; Group 5 received phenylhydrazine + 0.23 ml/kg of Bioferon®: phenylhydrazine (40mg/kg body weight) was administered via intraperitoneal route on day 0, with two additional doses given at 9am and 6pm, on day 1 of the experiment; the seed extracts of *Parkia biglobosa* and Bioferon were both administered orally for 14 days. On day 15, the rats were anesthetized by chloroform and blood was collected by direct cardiac puncture. Packed cell volume (PCV), hemoglobin (Hb), Red blood cell counts (RBC), Mean corpuscular volume (MCV), Mean concentration of hemoglobin (MCH), Reticulocytes and Mean corpuscular hemoglobin concentration (MCHC) were determined using automated machine (Sysmex apparatus of the type 8999). **Results**: The phytochemical analysis of the extract revealed the presence of tannis, alkaloids, flavonoid, saponin, steroid and glycosides. The chemical analysis indicated the presence of moisture, protein, crude fat, crude fibre, ash, carbohydrate, and nitrogen. The mineral contents of the extract revealed the presence of: Sodium, Potassium, Calcium, Magnesium, Zinc, Iron, Manganese, Copper and Phosphorus. Phenylhydrazine significantly reduced all erythrocyte parameters in group 2 rats when compared with group 1 rats ($p<0.05$). However, administration of the extract and Bioferon significantly increased values of all erythrocyte parameters in groups 4 and 5 rats respectively compared with group 2 rats. **Conclusion**: The results suggest a possible hematinic effect of the extract of locust bean seeds on the parameters of erythrocyte following phenylhydrazine administration. The observed effects of the extract could possibly be from the rich nutritional content of the *P. biglobosa* seeds.

INTRODUCTION

Various plants from different botanical sources have been used by many Traditional Medical Practitioners in Nigeria for the treatment and cure of many diseases that are locally endemic (Builders *et al*., 2007; Asase *et al*., 2005; Jullian *et al*., 2006). *Parkia biglobosa* (*P. biglobossa*) is one of such plants. *P. biglobosa* seed which is also known as dawadawa (Hausa), African locust bean (English) and Igba/Iyere (Yoruba), is native to Africa and its tree is an important multipurpose tree of West African Savannah land which has been used to treat variety of diseases (Abbiw, 1990). African locust bean (*Parkia biglobosa)* belongs to the family of P*arkia* and has species that are grown in the sub-Saharan Africa (N.A.S, 1979). It is a perennial

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leguminous crop with edible seeds, pods, fruit pulp and leaves that are used as cooking or drinking ingredients. The tree is majorly valued for its seeds which are fermented to make a condiment called "soumbala", "dawadawa", "netetu" or "afinti"; it also has the strong purgent smell of French cheese. This condiment is used for sauce, soup seasoning and constitutes part of the most important commercial products traded in Western Africa (Heuzé *et al.*,2019)

P. biglobosa is a major source of protein, oil, carbohydrates, vitamins, and minerals and is composed of 30-40% protein, 31-40% oil, 11.7- 15.4% carbohydrate, 8.82 – 94% crude fibre, 4.40-5.38% Ash NFE of 37% and 93.7% content of dry matter (Campbell,1980). The incidence of anaemia, globally, 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 example, 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 (Asrie, 2017; Victor *et al.,* 2020)

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by traditional medicine practitioners (Ogbe *et al*., 2010).

It is the most prevalent nutritional deficiency disorder in the world. WHO defines it as the condition in which the hempglobin content of blood is lower than normal as a result of deficiency of one or more essential nutrients (WHO,2000).

It affects people of all ages with the elderly, young women of childbearing ages and infants at higher risks.

The occurrence of anaemia can also be because of exposure to chemicals and administration of some drugs which have been found to alter the lifespan of red blood cells (RBCs) in the body e.g phenylhydrazine (PHZ) (Berger,2007; Kale *et al*.,2019)

PHZ ($C_6H_8N_2$) derivatives are primarily used as antipyretics but due to their toxic actions on red blood cells; these make their use dangerous. It is worthy of note that they are commonly used for experimental induction of anaemia in animals (Keerti *et al.*,2014) as well as for studying mechanisms in anaemia (Stevens *et al*.,2015). Furthermore, they may cause oxidative stress by increasing lipid peroxidation metabolites (Malondialdehyde, MDA) and decreasing antioxidant status, respectively (Luangaram *et al*., 2007; McMillan *et al*.,2005). This study examined the hematinic effect of the extract of locust bean seeds following phenylhydrazine administration in wistar rats.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh seeds of *P. biglobosa were* collected from Ajibode village in Ibadan, Oyo State, Nigeria. The seeds after ripening were removed from the pods, dried and ground into powder.

Preparation of plant extract

The seeds of *P. biglobosa* were air dried, pulverized and subjected to cold aqueous extraction as described by Njar *et al.* (1993) and Raji (1995). The pulverized seeds (1000g) were poured into a muslin bag and soaked in 4.5 L of distilled water, for 72 h. The extract was obtained after filtration of the supernatant with Whatman No.1 filter paper. Subsequently, complete removal of the solvent from the supernatant was achieved with a water bath placed in a fume cupboard because of the pungent smell emanating from it. A sticky, dark brown coloured extract weighing 154.61 g (a yield of 15.46%) was obtained after boiling in the water bath for about 12 h.

Induction of experimental anaemia

Phenylhydrazine was dissolved in normal saline and injected intraperitoneally, at 40mg/kg body weight on day 0; two additional injections were given at 9am and 6pm, on day 1 as described previously (Ashour,2014).

Anaemia was considered to have been induced when red blood cell (RBC) level as well as hempglobin concentration of the blood reduced by about 30%. (Anupam *et al*., 2014)

Treatment and animal groupings

Twenty-five male Wistar rats were used for this study. The rats were aged 8-10 weeks and weighed between 175 and 210g. They were divided into five Groups: 1 to 5, consisting of 5 rats per group. Rats in each group were placed in separate cages in the Animal House of Madonna University, Nigeria under natural day and night cycles. The rats had free access to normal rat chow and tap water ad libitum. They were allowed two weeks of acclimatization to their environment and the study was conducted in accordance with the guidelines for the care and use of laboratory animals by the US Institute for Laboratory and Animal Research (Derrell Clark *et al.*,1997). After acclimatization, anaemia was induced (via intra-peritoneal injection of 40mg/kg of PHZ) in all rats except group 1 rats, on day 0 and two additional injections at 9am and 6pm on day 1 of the experiment as previously described by Ashour, 2014. Anaemia was confirmed in the rats by estimating RBC and hempglobin levels. The rats with RBC and

hempglobin below 30% were used for the study. The rats were subsequently treated as follows:

Group 1: Control group. Rats in this group received only 2 ml/kg body weight of extract vehicle.
Group 2: PHZ only. – This is the negative control group. Rats in this group were left untreated after receiving 40 mg/kg doses of PHZ.
Group 3: PHZ + Low dose extract group. Rats in this group were treated with 100 mg/kg body weight of the extract of the locust beans after receiving 40 mg/kg dose of PHZ.
Group 4: PHZ + High dose extract group. Rats in this group were treated with 1000 mg/kg body weight of the extract of locust beans after receiving 40 mg/kg doses of PHZ.
Group 5: PHZ + 0.23ml/kg body weight of Bioferon. Rats in this group were treated with a known blood tonic after receiving 40mg/kg doses of PHZ. All treatments were orally administered daily to the rats for 14 days using an oral cannula.

Qualitative phytochemical analysis
Test for Tannin
1ml of extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed green or a blue-black coloration which confirms the presence of tannin.

Test for saponin
About 5ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirms a positive presence of saponin.

Test of flavonoids
3ml of Aluminium chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of diliute ammonia solution was added to the above mixture followed by addition of concentrated H_2SO_4.A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicates a positive test for flavonoids.

Test for steroids
2ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H_2SO_4.The colour changed from violet to blue or green indicate the presence of steroids.

Test for cardiac glycosides and cardenolides (Keller-Killani test):
5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glycoside.

Test for alkaloids
1ml of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide -solution) or Wagner's reagent (solution of iodine in potassium iodide) or Dragendorff's reagent (solution of Potassium bismuth iodide). The formation of a cream colour with Mayer's reagent and reddish-brown precipitate with Wagner's and Dragendorff's reagent give a positive test for alkaloids.

Determination of Chemical Analysis and Mineral Contents
The chemical analysis of the air-dried seeds was determined by standard methods (AOAC, 1999). These include the determination of crude protein, crude fat, crude fibre, ash, moisture content, carbohydrate, and nitrogen. The minerals analysed include sodium, potassium, calcium, phosphorus, magnesium, iron, manganese, copper and zinc. Sodium and potassium contents were determined by flame photometry (Jenway Limited, Donmow Essex, UK) and phosphorus was determined by the vanado-molybdate method (AOAC, 1995). Calcium, magnesium, iron, manganese, copper and zinc were determined after wet digestion with a mixture of nitric, sulphuric and perchloric acid using atomic absorption spectrophotometer (Buck Scientific, East Norwalk, CT, USA). (Anna *et al*., 2019)

Haematological Analysis of blood samples.
Haematological Measurements
Blood samples were collected through cardiac puncture from the anaesthetized rats into sample bottles tubes coated with Ethylene Diamine Tetra-Acetic Acid (EDTA). The samples were immediately analysed for haematological parameters using automated Sysmex apparatus of the type 8999. The parameters included: Hempglobin (Hb), Mean Cell Volume (MCV), Red

Blood Cells Count (RBCs), White Blood Cell Count (WBCs), Mean Corpuscular Hempglobin Concentration (MCHC). However, MCHC, MCV and MCH values were calculated as follow:

$$MCHC = \frac{\text{Haemoglobin in grams per 100ml blood}}{\text{Volume of red cells in ml per 100ml blood}} \times 100\%$$

$$MCV = \frac{\text{Volume of red cells in ml per 100ml blood}}{\text{Number of red cells per 100ml blood}}$$

$$MCH = \frac{\text{Haemoglobin in grams per 100ml blood}}{\text{Number of red cells per 100ml blood}}$$

Statistical analysis

Significant differences were determined using the One-way Analysis of Variance (ANOVA) followed by the LSD post hoc test. A p value<0.05 was considered statistically significant. Results obtained are presented in table 1-4 as Mean ± SEM).

RESULTS

Table 1 shows the phytochemical constituents of the seeds of *P. biglobosa* which include tannin, alkaloid, flavonoid, saponin, steroid and glycosides.

Table 2 showc the chemical composition of the seeds of *P. Biglobosa* : moisture, protein, crude fat, crude fibre, ash, carbohydrate, and nitrogen.

Table 1: Phytochemical constituents of the seed of *P. biglobosa*

S/N	Composition	Abundance
1.	Tannins	++
2.	Alkaloids	+
3.	Flavonoids	+
4.	Saponin	++
5.	Steroid	++
6.	Glycoside	+++

$^{+}$minute, $^{++}$less abundant, $^{+++}$more abundant

Table 2: Chemical composition of the seed of *P. biglobosa*

S/N	Composition	Value
1.	Moisture	13.49± 0.001
2.	Protein	25.57± 0.00
3.	Crude fat	6.63± 0.01
4.	Crude fibre	8.83± 0.01
5.	Ash	4.82± 0.01
6.	Carbohydrate	40.66± 8.01
7.	Nitrogen	4.09 ± 0.01

Table 3 :Mineral compositions of the seed of *P. biglobosa*

S/N	Mineral Composition	Value
1.	Sodium	1.67
2.	Potassium	121.31
3.	Calcium	54.86
4.	Magnesium	62.71
5.	Zinc	16.32
6.	Iron	7.85
7.	Manganese	0.98
8.	Phosphorus	54.40
9.	Copper	6.99

Table 3 shows the :mineral contents of the seeds of *P. biglobosa*: Sodium, Potassium, Calcium, Magnesium, Zinc, Iron, Manganese, Copper and Phosphorus.

The results of blood parameters in rats are shown in Table 4.

The results showed that there was significant increase ($p<0.05$) in PCV, Hb, MCV, MCH, reticulocytes and MCHC in group 1 rats compared with group 2 (Negative Control Group) rats.

Significant increases $p<0.05$ were observed in PCV, Hb, RBC, MCV, MCH, while a significant decrease was observed in reticulocytes in group 3 rats compared with group 2 rats ($p<0.05$).

PCV, Hb, RBC, MCV, MCH and Reticulocytes were significantly increased in group 4 rats compared to group 2 rats ($p<0.05$).

There was significant increase ($p<0.05$) in PCV, Hb, RBC, MCV , MCH, reticulocytes and MCHC in group 5 rats when compared with group 2 rats.

DISCUSSION

The phytochemical analysis of the extract of the locust bean seeds showed that it is very rich in some phytochemicals such as Tannin, Alkaloids, Flavonoids, Saponin, Steroids, Glycosides.

The chemical analysis revealed that the seeds of *P. biglobosa* contained higher protein which is in accordance with previous reports of (Okpala, 1990; Alabi,1993;Obizoba, 1998;Alabi *et al*, 2004). African locust bean has been shown to be rich in protein and may thus be used to add protein to a protein-deficient diet (Odunfa, 1983; Ikenebomeh and Kok, 1984; Dike and Odunfa, 2003).

The mineral analysis showed that the seeds of *P. biglobosa* contain higher levels of minerals. These values compare favourably with the report of (Okpala,

Table 4: Effect of extract on some erythrocyte parameters following administration of Phenylhydrazine.

PARAMETERS	GROUP 1 (Control)	GROUP 2 (Negative control)	GROUP 3(PHZ+100mg/kg extract)	GROUP 4 PHZ +1000mg/kg extract	GROUP 5 PHZ+0.23ml/kg Bioferon
PCV (%)	0.35 ± 0.01*	0.27 ± 0.01	0.42 ± 0.01*	0.54± 0.02*	0.35 ± 0.01*
Hb (g/l)	12.52 ± 0.69 *	9.78 ± 0.48	14.42± 0.74*	19.32 ± 0.44*	13.94 ± 0.34*
RBC (10^6µl-1)	4.65 ± 0.20	4.97 ± 0.12	5.27 ± 0.24*	7.00± 0.26*	5.82 ± 0.04*
MCV (fl)	78.2 ± 3.18*	48.6 ± 2.15	74.2± 5.89*	79.8 ± 0.37*	69.4 ± 9.04*
MCH (g/l)	27.2 ± 0.37*	19.4 ± 2.29	29.0 ± 0.77*	27.2 ± 1.15*	29.6 ± 1.81*
Reticulocytes (%)	0.4 ± 0.04*	0.04± 0.03	0.08 ± 0.04*	0.6± 0.04*	0.10 ± 0.03*
MCHC (g/l)	356.80 ± 12.7*	304.80 ± 14.73	312.00 ± 7.51	337.20± 9.10*	328.20± 14.91*

*All values=Mean SEM*indicates significant difference compared to group 2 rats at p<0.05*

1990). These minerals include Ca, Mg, K, Na, Mn, Fe, Cu and Zn.

Calcium is a particularly important mineral that is required for formation of bone and neurological function of the body. Sodium is an essential mineral that helps in the regulation of body fluid and maintenance of electric potential present in the body tissue. Zinc is an important micronutrient that has been associated with several numbers of enzymes, especially those related with synthesis of ribonucleic acid (Guil-Guerrero *et al*, 1998). Iron is essentially required for formation of blood and is essential for normal functioning of the central nervous system (Adeyeye and Fagbohun, 2005). Oxidation of carbohydrate, protein and fats is also facilitated by iron. Magnesium is very essential in calcium metabolism in bones and prevention of circulatory diseases. It also aids in regulating blood pressure and insulin release (Onyiriuka *et al*, 1997; Umar *et al*, 2005). Copper is important in the body for enzyme production and biological electron transport. The rich mineral content of the extract would therefore offer better nutritional value.

In this study, the effect of *P.biglobosa* was investigated on the haematological parameters of phenylhydrazine-induced anemia in wistar rats. Phenylhydrazine negatively affected some haematological parameters: packed cell volume (PCV), Hb, red blood cell count (RBC)/haematocrit as well as reticulocytes. This is in support of previous works (Abisola *et al*.,2017; Stern, 1989; Berger 2007; WHO, 2000;Ashour, 2014). Previous studies have reported that PHZ results in hemolytic anaemia consequent on red cell damage with secondary involvement of the liver and spleen. (Stern 1989; WHO 2000; Umoren *et al*.,2020).

Administration of *P.biglobosa*, was observed to improve the level of haematological parameters, in the present study. In addition, the observed increase in RBC count following administration of *P.biglobosa* extract resulted in increase in MCV levels while increase in Hb levels resulted in increased MCH and MCHC profiles. Since MCHC, MCH and MCV profiles relate to individual red blood cell count while hempglobin and hematocrit profiles relate to the total population of red blood cells in the blood, it could thus imply that though the extract may stimulate the production of red blood cells and hempglobin (Adebayo *et al*., 2005; Umoren *et al*.,2020), the extract could have stimulated erythropoietin release (humoral regulators of RBC production) in the kidney (Degruchy ,1976).

The mechanism of action of *P.biglobosa* in restoring the red blood cell (RBC) level may be due to its profile of important trace elements, iron, and proteins (Cook *et al*., 2000; Lockeett *et al*., 2000).

Iron, which is commonly deficient in many plant-based diets, was found in abundance in the seeds of *P biglobosa*. Iron is a necessary component of hempglobin and myoglobin for oxygen transport and cellular processes of growth and division. Iron forms an integral part of hemoglobin. (Kozat, 2007).

The presence of phytochemicals like flavonoids, tannins, alkaloids in the *P biglobosa* seeds extracts could also be responsible for the haemopoietic stimulating effects (Ohlsson and Aher,2006). Flavonoids have been found to protect erythrocytes from oxidative damage and have various benefits for human health due to their antioxidant and free-radical scavenging activities (Grassmann,2005; Ren,2003; Chahar *et al*.,2011).

They have also been reported to exhibit antioxidant activity (Ramanathan *et al*., 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988).

Alkaloids have been known to inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase thereby

causing an accumulation of cAMP levels which stimulates protein phosphorylation and synthesis with a possible enhancement of erythropoiesis (Ndem *et. al*., 2013). Therefore, alkaloids and flavonoids present in the extract may act as antioxidants to protect and prevent damage to red blood cells by free radicals or highly reactive oxygen species (Asgary *et al.,* 2005). This phytochemical composition lends credence to the effectiveness of the traditional use of plants having anti-anaemic effect (N'guessan *et al*.,2010).

It can be concluded that the hematinic effect observed from this study could result from the rich nutritional content of the seeds of *P biglobosa*.

REFERENCES

Abbiw, D.K. (1990). Useful Plants of Ghana. Intermediate Technology Publications and the Royal Botanic Gardens, Kew. UK. p. 337.

Abisola, T.,,Victor, D.D., and Chidinma, I.,E (2017). Effects of the methanolic extract of the rind of *Citrullus lanatus*(watermelon)on some erythrocyte parameters and indices of oxidative status in Phenylhydrazine – treated male Wistar rats.*Journal of African Association of Physiological Sciences.5(1):22 –28.*

Adebayo, J.O., Adesokan, A.A, Olatunji, L.A., Buoro, D.O., Soladoye, A.O. (2005) Effects of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. *He Journal of Biochemistry* 1: 45-50.

Adeyeye, E.I., Fagbohun, E.D. (2005) Proximate, mineral and phytate profiles of some selected spices found in Nigeria. Pak J Sci Ind Res 48: 14-22 31.

Alabi, D.A. (1993) *Parkia biglobosa*, 'an endangered species' International Conference *Proceedings on Lost Crops and Trees in Africa* 3: 265-285 12.

Alabi, D.A., Akinsulire, O.R., Sanyaolu, M.A. (2004) Nutritive and Industrial utility values of African Locust bean seeds. Parkia biglobosa (JACQ) *Benth. Proc Sci Ass Nig* 25:105-110

Anna, T , Kinga, W. and Wojciech, M. W (2019). Digestion Procedure and Determination of Heavy Metals in Sewage Sludge—An Analytical Problem .*Sustainability* 11:1753

Anupam, J., Aditya, G., Ankita, A., Divya, B., Nazneen, D. (2014)

Nazneen Dubey Protective Effects of Beetroot Extract against Phenyl Hydrazine Induced Anemia in Rats *Pharmacognosy Journal* 6:5.1-4.

AOAC Official Methods of Analysis of AOAC International, 16th ed. (1995)

AOAC (Association of Official Analytical Chemists) (1999) ''Official Method of Analysis'' 4th Edition (129-135), Washington D.C. U.S.

Asase, A., Alfred, A., Yeboah, O., Odamtten, G.T., Simmonds, S.J. (2005). Ethnobotanical study of some Ghanaian antimalarial plant. *J. Ethnopharmacol*., 99, 273-279.

Asgary, S., Naderi, G., Askari, N. (2005). Protective effect of flavonoids against red blood cell hemolysis by free radicals. *Exp Clin Cardiol*. 10(2):88-90

Ashour, T.H. (2014). Hematinic and anti-anemic effect of Thymoquinone against phenyl hydrazine-induced hemolytic anemia in rats. *Res J Med Sci.* 8(2):67-72.

Asrie, F. (2017). Prevalence of anemia and its associated factors among pregnant women receiving antenatal care at Aymiba Health Centre, norwest Ethopia. *J Blood Med.* 8: 35-40

Banwo, G.O., Abdullahi, I., and Durguryil, M. (2004). "The Antimicrobial Activity of the Stem Bark of Parkia clappertoniana keay Family Leguminosae Against Selected Microorganisms", *Nig. J. Pharm*., 3(1) pp.16-22.

Berger, J. (2007). Phenyl hydrazine haematotoxicity. *J Appl Biomed* 5: 125-130.

Builders, I.M., Wudil, M., Wannang, J.C., Aguiyi, J.C. (2007). Ethnobotanical survey report of antimalarial plants used in ten local government areas in north central Nigeria. University of Jos, department of pharmaceutical sciences achieve: UniJ/Pharmsci/21/Et24/2007.

Campbell, P.G (1980). African locust bean (Parkia Species) and its West African fermented food product, dadawa. Ecology of food and Nutrition. Pp 123-132

Chahar, M.K., Sharma, N., Dobhal, M.P., Joshi, Y.C. (2011) Flavonoids: A versatile source of anticancer drugs. *Pharmacogn Rev* 5: 1-12.

Cook,J.A.,VanderJagt,D.J.,Pastuszyn,A.,Mounkaila,G., Glew,R.S.(2 000). Nutrient and Chemical compositions of 13 wild plant Foods of Niger Journal of Food Composition and Analysis 13.83-92.

Degruchy, G.C. (1976) Clinical haematology in medical practice. Blackwell Scientific Publication 3: 7.

Derrell Clark, J., Gerald F.G., Janet C.G, Michale E.K.,Dennis F.K. (1997) The 1996 Guide for the care and use of Laboratory Animals. *Institute for Laboratory Animal Research Journal*.38:1 41-48.

Dike, E.N., Odunfa, S.A. (2003) Microbiological and biochemical evaluation of a fermented soyabean product-soyadawadawa. *J Food Sci Tech* 40: 606-610.

Grassmann, J. (2005) Terpenoids as plant antioxidants. Vitam Horm 72: 505-535.

Guil-Guerrero, J.L., Antonin, G., Rodriguez-Garcla, I., Torija-Issa, M. (1998) *J Sci Food Agric* 76: 628- 632.

Heuzé, V., Thiollet, H., Tran, G., Edouard, N.,Lebas, F. (2019). African locust bean (Parkia biglobosa and Parkia filicoidea). Feedipedia, a programme by INRA,CIRAD,AFZ and FAO. https://www.feedipedia.org/node/268 .10:22.

Ikenebomeh, M.J., Kok, R. (1984). Mass balance of the processing and fermentation of the African locust bean (Parkiafilicoidea Welw.). *J Can Inst Food Sci Tech* 17: 48-50. 27.

Institute for Laboratory and Animal Research (1996). Guide for the care and use of laboratory animals. Seventh Edition. National Academies Press. Washington DC, USA.

Jullian, V., Bourdy, G., George, G., Maurel, G., Sauvain, M. (2006). Validation of use of a traditional antimalarial remedy from French Guiana, Zanthoxylum rhoifolium Lam. *J. Ethnopharmacol*., 106, 348-352.

Kale, O.E., Awodele, O., Akindele, A.J. (2019) Acridocarpus Smeathmannii (DC.) Guill. & Perr. Root enhanced reproductive behaviour and sexual function in male Wistar rats: biochemical and pharmacological mechanisms. *J Ethnopharmacol*. 230:95-108.

Keerti, P, Anil, K.M., Akansha, J., and Singh, R.K. (2014). Molecular Mechanism of Phenylhydrazine induced Haematoxicity:Areview American Journal of Phytomedicine and clinical therapeutics.2(3) 390-394.

Kozat, S. (2007). Serum T3 and T4 concetrations in lambs with nutritional myodegeneration. *J. Vet. Intern. Med.*, 21(5): 1135-7.

Lockeett, C.T., Calvert, C.C. and Grivetti,L.E. (2000).Energy and micronutrient composition of dietary and Medicinal wild plants consumed during drought: Study of Rural Fulani, Northestern Nigeria. International *Journal of Food Sciences and Nutrition*. 51:195-208.

Luangaram, S., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan, V., Pannangpetch, P. (2007). Protective effects of quercetin against phenylhydrazine-induced vascular dysfunction and oxidative stress in rats. *Food Chem Toxicol*. ;45: 448-455.

McMillan, D.C., Powell, C.L., Bowman, Z.S., Morrow, J.D., Jollow, D.J. (2005). Lipids versus proteins as major targets of pro-oxidant, direct-acting hemolytic agents. *Toxicol Sci*.;88:274-283

National Academy of Science (1979). Tropical legumes, resources for the future Washington, D.C. Pp 1-4

Ndem, J.I., Otitoju, O., Akpanaiabiatu, M.I., Uboh, F.E., Uwah, A.F., Edet, O.A. (2013). Haematoprotective property of Eremomastax Speciosa (Hochst.) on experimentally induced anemic Wistar rats. *Ann Biol Res*. 4 (6):356-360

N'guessan, K., Kouassi, K.H, Ouattara, D. (2010). Plants used to treat anaemia, in traditional medicine, by Abbey and Krobou populations, in the South of Côte-d'Ivoire. *Journal of Applied Sciences Research*, 6(8): 1291-2197.

Njar, V.C.O., Alao, T.O., Okogun, J.I., Holland, H.L. (1993). 2 methoxy cathin-6- one: A new alkaloid from the stem wood of Quassia amara. *Planta Med*. 59:259-261.

Obizoba, I.C. (1998). Fermentation of African locust bean. Text on nutritional qualityof plant fruits. (Edn) Osagie, Eka (2000). Post-harvest Res. Unit. Dept. Biochem. Uniben, Nigeria. Pp. 160-198.

Odunfa, V.S.A. (1983). Carbohydrate changes in fermenting locust bean (Parkia filicoidea) during preparation. *Qual Pl Hum Nutr* 32:45-52 11.

Ogbe. R.J., Adoga, G.I., and Abu, A.H. (2010) .Antianaemic potentials of some plant extracts on Phenylhydrazine-induced anaemia in rabbits. *Journal of Medicinal Plants Research*.4(8):680-684.

Ohlsson, A., Aher, S.M. (2006) Early erythropoietin for presenting red blood cell transfusion in preterm and/ or low birth weight infants. *Cochrane Database System* 1: 3.

Okpala, J.O (1990). Analysis of some macro nutrients and food substances in the matured fruit parts of Parkia biglobosa. *Nig J Bot*, 3: 209-214.

Onyiriuka, S.O., Nwadinigwe, C.A., Nwaji, M.N., Akpansi, L.E.S., Okoro, U.C. (1997) Principle of Organic Chemistry. 1st Edn.,pp: 12, 206-209.

Raji, Y. (1995). Hormone and Drug stimulation of testosterone secretion in rat leydig cell. PhD Thesis. Department of Physiology, University of Ibadan XXVIII +227.

Ramanathan, R., Lau, K.K. and Das, N.P. (1989). Antiperoxidative action of flavonoids and related products in ground pork (Abstract). Proceedings of III Int. Symp. On flavonoids in Biology and Medicine. Singapore: Pp.56.

Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L. (2003) Flavonoids: promising anticancer agents. *Med Res Rev* 23: 519-534.

Robak, K. and Gryglewski, R.J. (1988). Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol*., 37:837 – 841.

Shao, M. (2002). M.Sc. thesis. Michigan Technological University, Michigan,USA.

Soetan, K.O., Lasisi, O.T., and Agboluaje, A.K. (2011). "Comparative Assessment of in-vitro Anthelmintic Effects of the Aqueous Extracts of the Seeds and Leaves of the African Locust Bean (Parkia biglobosa) on Bovine Nematode Eggs," *J. Cell Ani. Bio.*, 5(6) pp. 109-112

Stern, A. (1989). Drug-induced oxidative denaturation in red blood cells. Semin Hematol. 26(4):301-306.

Stevens, G.A., Bennett, J.E., Hennocq, Q. , *et al* (2015). Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. *Lancet Glob Health*. ;3:e528-e536.

Umar, K.J., Hassan, L.G., Garba, H.J. (2005) Proximate and mineral compositions of M. miristica. *Chemclass* J 3: 81-84.

Umoren, E.B., Ben-Azu, B., Kolawole, T.A., Adebayo, O.G.,Wopara, I., Uzokwe, J.I., and Obembe, A.O. (2020). *Hibiscus sabdariffa* aqueous leaf extract reverses hematological alterations in phenylhydrazine anemic Wistar rats.*International Journal of Biochemistry Research & Review*. 29(4): 30-38.

Victor, O., Henry, A.U., Nathaniel, B., Emmanuel, K., Joel, R.U., and Akafa, R. (2020). Prevalence, Severity and Correlates of Anemia in Pregnancy among Antenatal Attendees in Warri, South-Southern Nigeria: Across- Sectional and Hospital- Based Study*Anemia*. Doi: 10.1155/2020/1915231.

World Health Organization (2000). PHENYLHYDRAZINE: Concise International Chemical Assessment Document; 19. WHO, Geneva.