WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 136-162. Research Article ISSN 2277– 7105

AERIAL CONSTITUENTS OF *FICUS VOGELII* REVERSED PHENYLHYDRAZINE-INDUCED ANEMIA IN ALBINO WISTAR RATS.

***Igile G. O., Okoi U. L., Iwara I. A., Ekpe O. O., Mgbeje B. I. A. and Ebong P. E.**

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B 1115, Calabar, Nigeria.

Article Received on 28 May 2018,
Revised on 18 June 2018,
Accepted on 08 July 2018
DOI: 10.20959/wjpr201814-12888

***Corresponding Author**
Dr. Igile G. O.
Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B 1115, Calabar, Nigeria.

ABSTRACT

Aqueous extract of Ficus vogelii is used locally to treat anemia in children, especially those suffering from Marasmus or kwashiorkor or a combination of both. In this study, leaf extracts of the plant was evaluated on phenylhydrazine-induced anemic rats. Proximate composition showed carbohydrate (43.44±2.84mg/100g), crude fibre (19.11±2.14mg/100g), protein (17.67±1.22mg/100g), fat (3.75±0.07mg/100g), ash (9.27±0.72mg/100g), moisture (6.54±0.05mg/100g), and Energy value (279±11.45Kcal/100g). Vitamins included vitamin C (22.17±1.77 mg/100g), vitamin B1 (2.14±0.08 mg/100g), Vitamin E (1.52±0.25mg/100g), Vitamin B6 (0.89±0.15 mg/100g), Vitamin A (319.05±12.44 IU), and Vitamin K (0.14± 0.02 mg/100g). Mineral elements included calcium (424.24±0.21 mg/100g), magnesium (327.11± 0.52mg/100g), phosphorous (319.77±0.55mg/100g), potassium (38.23±2.11mg/100g), iron (16.09±0.2 mg/100g), sodium (8.50±0.07mg/100g), zinc (11.14 ± 0.02mg/100g), manganese (3.79±0.13 mg/100g), and selenium (0.22±0.04μg/100g). Experimentally, Fifty-six (56) albino Wistar rats of both sexes weighing 150-l80g were shared into five groups of 8 animals each. Groups 1 and 2 served as normal control (NC) and anemic control (AC), and received placebo treatment. Groups 3 and 4 were made anemic by intraperitoneal injection of phenylhydrazine (20mg/kg bw in 20ml distill water), and received 200mg/kg bw and 400mg/kg bw of aqueous crude extract (CE). Group 5 received 200mg/kg bw of multivitamin (MVT). A significant (P<0.05) increase in WBC was observed in groups 3 (6.97±1.71) and 4 (7.47±1.72), when compared to AC (3.13±1.63). In groups 3 and 4, RBC count (4.34±1.54-5.25±0.16), Hemoglobin count

(11.87+05 -13.23+0.54) and Mean corpuscular Hemoglobin (MCH) (25.17 0.33- 27.37 0.76) increased, when compared to AC, 4.27+l.54 (RBC), 5.90+2.95 (hemoglobin), and13.03 6.51(MCH). Lymphocyte (5.40 0.67-6.85 1.35) and Neutrophils (1.57 0.75-3.57 2.78) increased in both groups when compared to AC (2.17 1.10) and (0.03 0.03). It was concluded that nutrient constituents of leaves of FV contributed significantly in reversing anemia, and supports the ethno-medical claims on the use of the plant as anti-anemic remedy.

KEYWORDS: *Ficus vogelii*, proximate composition, Vitamins, Mineral elements, Children, Anemia, phenylhydrazine.

INTRODUCTION

One of the prevailing health conditions among children and adults in sub-Sahara Africa is anemia. Anemia is a condition which develops especially in children due to poor nutrition, lacking in essential vitamins, mineral elements and imbalance in protein and energy, generally referred to as Protein-Energy Malnutrition (PEM).[1] The condition is most prevalent in children between the ages of 1-5years. In adults the condition is mostly associated with disease conditions which include malaria, diabetes, renal disease, diabetic kidney disease, nutritional status, pregnancy, post-menstrual events and anemia associated with ageing.[2]

Poor nutritional education and inadequate Government policies including lack of advocacy contribute significantly to the ignorance of affected populations. Generally peasants populations are ignorant of the dangers of anemia and its poor management.

Anemia is defined as a condition which is characterized by a decreased blood hemoglobin concentration below the normal values, and decreased RBC count. The World Health Organization (WHO)[3], reports that a healthy person has a hemoglobin (Hb) content <130g/L for men and <120g/L for women. Anemia is defined as a condition which is characterized by a decreased Red Blood Cell mass which lacks the ability to transport oxygen to the peripheral tissues.[4]

Another major contributor to anemia is iron deficiency. In Nigeria, plants-based materials and indeed green-leafy vegetables have variously been used to wean children and prevent them from developing anemic conditions.[5]

Anemia is classified into six key types based on the etiology of the specific type. The key types of anemia include iron deficiency anemia, hemolytic anemia, megaloblastic anemia, aplastic anemia, folic acid deficiency anemia and chronic disease anemia.[6]

Some of the effects and presentation of anemia include; impaired cognitive ability, decreased work capacity and increased susceptibility to several diseases including liver, kidney and severe malaria,[2] The human kidney is an essential organ which maintains the body electrolyte composition and function as an excretory organ for waste products from the body[2] The liver is an organ with tremendous importance in metabolism and synthesis. In anemia both the liver and kidneys suffer oxidative stress and affect the hematological indices of the affected individuals.

Nutrition and diets, based on the consumption of green-leafy vegetables and fruits has helped to reverse anemia in populations of sub-Sahara Africa. Plant derived diets are easily available and affordable for the people of the continent including Nigeria. Animal protein is usually expensive for the poor people in Nigeria; therefore indigenous populations subsist predominantly on vegetable based diets. Right from creation, man has benefited immensely from plants, which supply oxygen, food, medicines and shelter.[7] Plants contain phytochemicals that have high therapeutic value which can also be refined to produce ethical drugs.[8] The World Health Organization (WHO)[9], estimates that about 80% of the world's population relies mainly on vegetables for food and medicinal plants for their health care.

Ficus vogelii is a green leafy vegetable which serves as a medicinal plant. The leave of *Ficus vogelii* and *Ficus asperifolia* look alike externally, except for slight difference in venation, and these plants co-occur in most tropical and sub-tropical regions of the world.[10] *Ficus vogelii* is called kujung by the Obudu people in Northern Cross River State. Its leaves are used for the treatment of malaria, diarrhea, dysentery and anemia in traditional medicine.

Ficus vogelii produces fruits which resemble inverted flower.[11] The latex of members of the *Ficus genus* has been reported to give protection against physical assault by pests.[12] *Ficus vogelii* leaf is used by the Obudu and Bekwarra people of Cross River State as a green leafy vegetable, and as a herb in ameliorating anemia and diabetic conditions, as well as other endocrine complications.

Poverty, lack of wholesome foods, under-nutrition and inadequate intake of nutrients in diets in Sub-Sahara African countries are some of the causes of anemia in infants, children and adults. Much of the diets consumed in this region contain inadequate proportion of protein and therefore result in protein energy malnutrition (PEM) in children, including kwashiorkor and marasmus.[13] PEM itself pre-disposes anemia and other endocrine and metabolic dysfunctions in infants and children between the ages of 1-5 years.

Anemia is a significant public health problem in Nigeria affecting people of all ages especially children and pregnant women. Obudu and other ethnic groups in Cross River State, Nigeria, use the leaves of *Ficus vogelii* to boost hemoglobin in blood of anemic children and adults, thus reversing anemic conditions. The aqueous extracts of the fresh leaves are administered to infants and children once daily, and this old tradition has proved very effective in reversing anemia and PEM associated anemic conditions in children. Adults extract the leaves in ethanol or in water, and served at room temperature twice daily to boost their RBC and HB concentrations, and reverse hemogglobinopathies.

There is no scientific information on the proximate and phytochemical composition of the plant, and so far, there are no reports on the biological properties of the plant on human subjects or animal models. Studies have shown that the *Ficus* genus has several medicinal uses including wound healing, anti-diabetes and anti-microbial effects. However, no work has been reported on the anti-anemic properties of *Ficus vogelii.* Claims by ethno-medical practice of the use of *Ficus vogelii* for the treatment of anemia and its complications have not been verified. Orthodox options for the treatment of anemia are expensive and unaffordable by the poor in Sub-Saharan Africa.

Research has shown that anemia can be treated using plant based diets which contain a myriad of vitamins, mineral elements, macro-nutrients and plant phytochemicals, including polyphenols, flavanoids, saponins and carotenoids.

METHODS

Collection of Plant Material

Fresh mature *Ficus vogelii* leaves were harvested from a farm in Obudu Local Government Area of Cross River State of Nigeria. The plant was authenticated by a botanist in the Botany Department of the University of Calabar, Nigeria. Rat chow feed was purchased from Grand

Cereals Limited in Aba, Abia State of Nigeria. All solvents were of BDH grade and were purchased from Grand Chemicals Ltd, Calabar, Nigeria.

Sample preparation for analysis of proximate, mineral elements and vitamins composition

500g of the leaves were washed, cut into small pieces and air-dried at room temperature (27 ± 1.50^oC) for seven days for the determination of proximate composition, mineral elements and vitamins concentrations.

Vitamin analysis

The composition of the water-insoluble vitamins, including riboflavin, thiamine and pyridoxine, were determined by the method described by[14], while ascorbic acid content was determined by the method.[15] Vitamin A concentration was determined by the spectrophotometric method described by.[16]

Mineral *Analysis*

Minerals were determined after the dried powdered samples were first digested with nitric acid and perchloric acid and the filtered aqueous aliquots were used for the determination of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, selenium, chromium, cobalt and manganese content. Potassium and sodium were determined by the Flame photometric method. Iron, copper, zinc, manganese, chromium, cobalt, selenium calcium and magnesium were determined by atomic absorption spectrophotometric method described by.[17,18]

Proximate analysis

The analysis of the proximate composition of *V. calvaona* leaf was carried out using the official methods of analysis of the Association of Official Analytical Chemists (AOAC)[19], and the FAO method[20] as modified by.[21]

Animals and Treatments

Albino Wistar rats of both sexes were purchased from the Department of Biochemistry animal house, University of Calabar. The animals were then allowed to acclimatize for two weeks in the animal house under 12 hour light/dark cycle at ambient temperature ($27\underline{+}2.5^oc$). During this period, animals were allowed free access to rat chow and water *ad lib*.

Animals Grouping and Treatment

Fifty six (56) albino Wistar rats weighing 150-180g were used for the study. They were randomly divided into seven groups of eight (8) animals per group. Group 1 served as the normal control (NC) and was not induced with anemia. Group 2, 3,4, 5, 6 and 7 were induced with anemia using phenylhydrazine.[22] Group 2 served as the anemic control group (AC) and was not treated. Group 3, 4, 5, 6 and 7 were treated. Group 7 served as the standard drug group.

Treatment and Dosage

At the end of the acclimatization, groups 1 and 2 were given placebo 0.20% DMSO. Groups 3, 4, 5, 6 and 7 rats were induced with anemia by intraperitoneal injection of phenylhydrazine (20mg/kg body weight in 20ml of distilled water). Thereafter, the n-hexane, methanol fractions, crude extract and multivitamin mix were administered to the anemic rats for seven days. The rats were weighed daily for the period of the experimentation.

Table 1: Experimental Design.

Group	No of Rats	Designation	Dosage
1	8	Normal Control (NC)	0.20% DMSO
2	8	Anemic control (NA)	0.20% DMSO
3	8	n-hexane fraction (HF)	400mg/kg bw
4	8	Crude extract (CE)	200mg/kg bw
5	8	Crude extract (CE)	400mg/kg bw
6	8	Methanolic fraction (MF)	400mg/kg bw
7	8	Multivite (MV)	200mg/kg bw

Collection and Handling of Blood Samples for Biochemical Analysis

At the end of 7 days treatment, food was withdrawn from the animals for 24 hrs. Animals were then anaesthesized and sacrificed. Anaesthesia was carried out using chloroform vapor in a dessicator and, followed by dissection using surgical forceps and scissors. Blood was collected from the heart via cardiac puncture using sterile syringes and needles for hematological analysis.

Biochemical Analysis

Standard methods involving the use of appropriate analytical kits were used to analyze;

1.0 Hematological indices: HB count, RBC, WBC, PCV, MCV, MCHC, platelet count.

2.0 Body weight was determined by weighing with a laboratory HP top loading balance.

3.0 LD_{50} of the plant extract was determined using.[23]

Statistical Analysis

Data obtained was analyzed for statistical significance using two-way analysis of variance (ANOVA) with a post hoc Dunnet (to compare intra and inter-differences between groups) at $P<0.05$ using statistical package for social sciences (SPSS) and Microsoft Excel. Results were expressed as mean ± SEM, for n=3.

RESULTS

Proximate Composition of Fresh Leaves of Ficus vogelii

The results of the proximate composition of fresh leaves of *Ficus vogelii* as presented in table 2 showed a significant amount of protein (17.67±1.22 g/100gm), carbohydrate (43.44±2.84), crude fat (3.75±0.07), crude fibre (19.11±2.14), moisture (6.54±0.05) and energy value in kcal/100g (279±11.45). *Ficus vogelii* was found to have a significant energy value suggesting that the plant will elicit fast recovery to anemic patients consuming it.

Table 2: Proximate composition of leaf of *Ficus vogelii* (dry matter basis).

Parameters	Result (mg/100g)
Protein	17.67 ± 1.22
Carbohydrate	43.44 ± 2.84
Crude fat	3.75 ± 0.07
Crude fibre	19.11 ± 2.14
Ash content	9.27 ± 0.72
Moisture	6.54 ± 0.05
Energy value (kcal/100g)	279 ± 11.45

Mean ± SEM, n = 3

Mineral Elements Composition of Fresh Leaves of Ficus Vogelii

The results of the mineral elements composition of fresh leaves of *Ficus vogelii* as presented in table 3 showed a significant presence in mg/100g of the following essential mineral elements required by children for fast recovery from anemic conditions: Fe (16.09±0.25), Mn (3.79±0.13), Se (0.22±0.4), Mg (327.11± 0.52), k (38.23±2.11), P (319.77± 0.55), Ca (424.24± 0.21), Na (8.59± 0.07), Zn (11.14± 0.02), Ni (0.49±0.05) and Cu (1.39± 0.12). This study showed that selenium and calcium were the least occurring mineral elements in the plant.

Table 3: Mineral Composition of fresh leaves of *Ficus vogelii*.

Mineral elements	Result (mg/100g)
Fe	16.09 ± 0.25
Mn	3.79 ± 0.13
Se	0.22 ± 0.04
Mg	327.11 ± 0.52
K	38.23 ± 2.11
P	319.77 ± 0.55
Ca	424.24 ± 0.21
Na	8.59 ± 0.07
Zn	11.14 ± 0.02
Ni	0.49 ± 0.05
Cu	1.39 ± 0.12

Values are mean ± SEM, n=3 determinations

Vitamin Composition of Fresh Leaves of *Ficus vogelii*

The results of vitamin composition of fresh leaves of *Ficus vogelii* as presented in table 4 showed the presence of vitamin E (1.79± 0.45mg/100g), vitamin C (22.17± 1.77 mg/100g), Vitamin B_1 (2.14± 0.09 mg/100g), vitamin B_2 (1.75± 0.11 mg/100g), vitamin B3 (1.52± 0.25 mg/100g) Vitamin B_6 (0.89± 0.15 mg/100g), folic acid (1.66± 0.05), vitamin A (319.05± 12.44 mg/100g) and Vitamin K (0.14± 0.02 mg/100g). The result showed that vitamins A and C were the most abundant in the plant. This further reveals the balanced nutrient composition of *Ficus vogelii* necessary for the reversal of anemic conditions in affected patients.

Table 4: Vitamin Composition of fresh leaves of *Ficus vogelii* (mg/100g).

Vitamin	Result (mg/100g)
Vitamin E (mg/100g)	1.79 ± 0.45
Vitamin C (mg/100g)	22.17 ± 1.77
Vitamin B_1 (mg/100g)	2.14 ± 0.09
Vitamin B_2 (mg/100g)	1.75 ± 0.11
Vitamin B_3 (mg/100g)	1.52 ± 0.25
Vitamin B_6 (mg/100g)	0.89 ± 0.15
Folic acid (mg/100g)	1.66 ± 0.05
Vitamin A (IU)	319.05 ± 12.44
Vitamin K (mg/100g)	0.14 ± 0.02

Values are mean ± SEM, n=3 determination

Haematological Indices of Different Experimental Groups.

Table 5 shows the results of the effect of treatment of laboratory animals with various extracts of *Ficus vogelii* on hematological indices including, WBC, RBC, Hb, HCT, MCV, MCHC, MCH, Platelets, Lymphocytes and Neutrophils, for. the different experimental groups. After 28 days of treatment, all extracts of *Ficus vogelii* showed significant recovery from anemic conditions, as there was obvious increases and normalization of all hematological parameters tested. The increases in the various hematological parameters are expressed in bar charts (figures 1-10).

Table 5: Haematological indices of different experimental groups.

MEAN	WBC × 10^3/μL	RBC × 10^6/μL	HGB g/dL	HCT %	MCV Fl	MCH Pg	MCHC g/dl	PLT × 10^3/μL	LYMP×10^3/μL	NEU 103/μL
Normal control	12.03±1.03	7.97±0.57	14.40±0.56	47.93±1.57	60.70±4.78	18.16±0.64	30.13±1.56	712.00±56.58	9.40±0.81	2.10±0.32
Anaemia control	3.13±1.63*,b	4.27±1.54*	5.90±2.95*	23.83±8.69*	55.43±0.62	13.03±6.51	23.27±11.63	578.33±298.16	2.17±1.10*	0.03±0.03
Multi Vit	13.0±1.38a	8.54±0.48a	13.74±0.87a	49.07±1.79a	61.57±3.66	18.02±1.09	31.51±0.98	716.33±55.07	9.20±0.80a	2.33±0.39
Crude EXTR	7.47±1.72*,b	4.34±0.20*,b	11.87±0.5a	39.10±1.79a	90.90±3.5*,a,b	27.37±0.76*,a,b	30.13±0.35	871.67±86.36	5.4±1.15*,a,b	1.57±0.75
n-Hex FRN	8.47±1.18a	4.58±0.14*,b	11.90±0.49a	40.07±1.58a	87.43±1.80*,a,b	25.97±2.85a	29.70±0.49	706.33±70.27	5.40±0.67*,a,b	1.87±0.83
Met FRN	6.97±1.71*,b	5.25±0.16*,b	13.23±0.54a	44.27±2.17a	84.20±1.64*,a,b	25.17±0.33a	29.90±0.31	773.67±72.99	2.86±1.47*,b	3.57±2.78

FRN = Fraction, EXTR = Extract

Values are expressed as mean ± SEM; (n=6)

* Significantly different from NC at $p<0.05$

a significantly different from Anemic control at $p<0.05$

b significantly different from Multivitamin at $p<0.05$

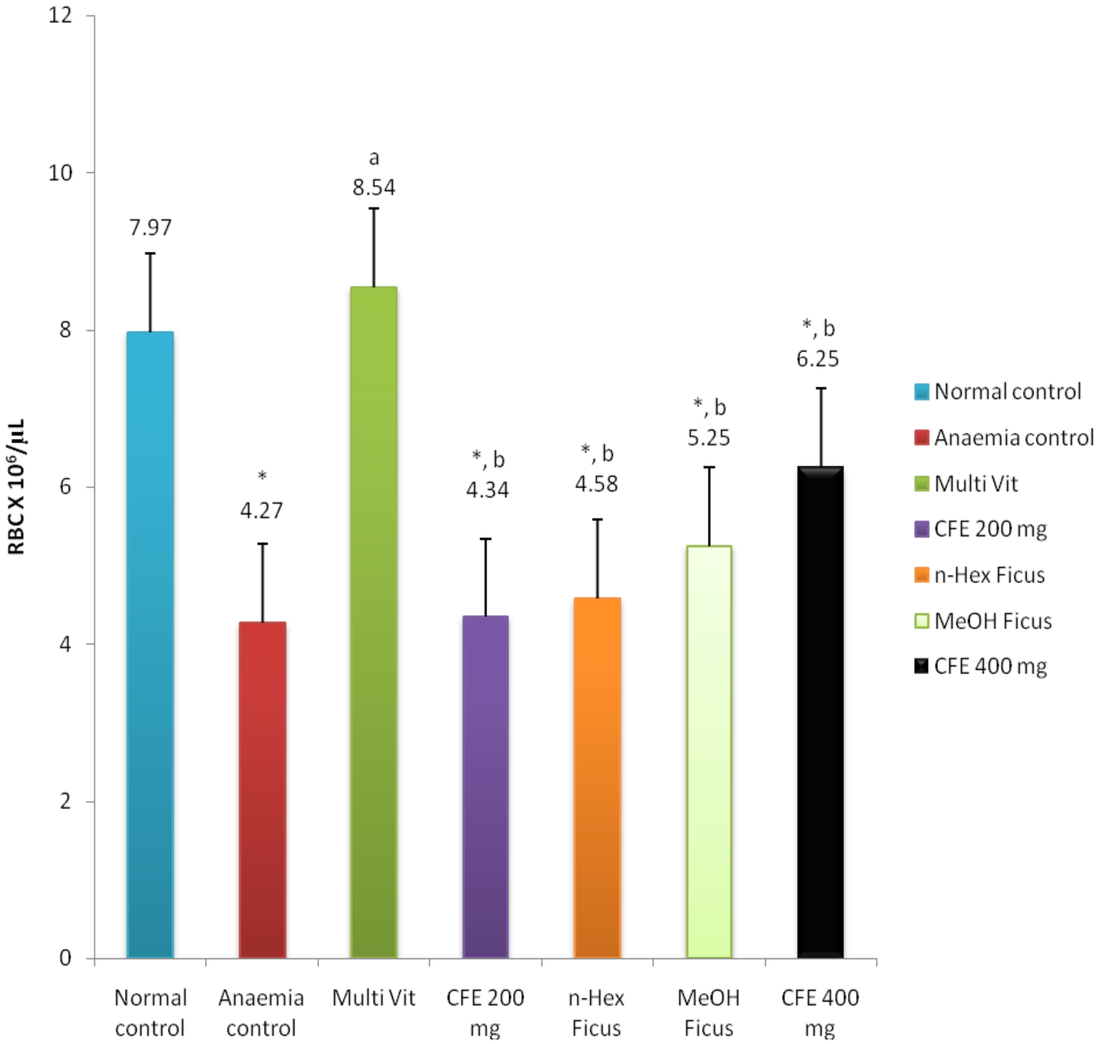

Figure 1: Variation in RBC of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at $p<0.05$

a significantly different from Anaemia control at $p<0.05$

b significantly different from Multi vit at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

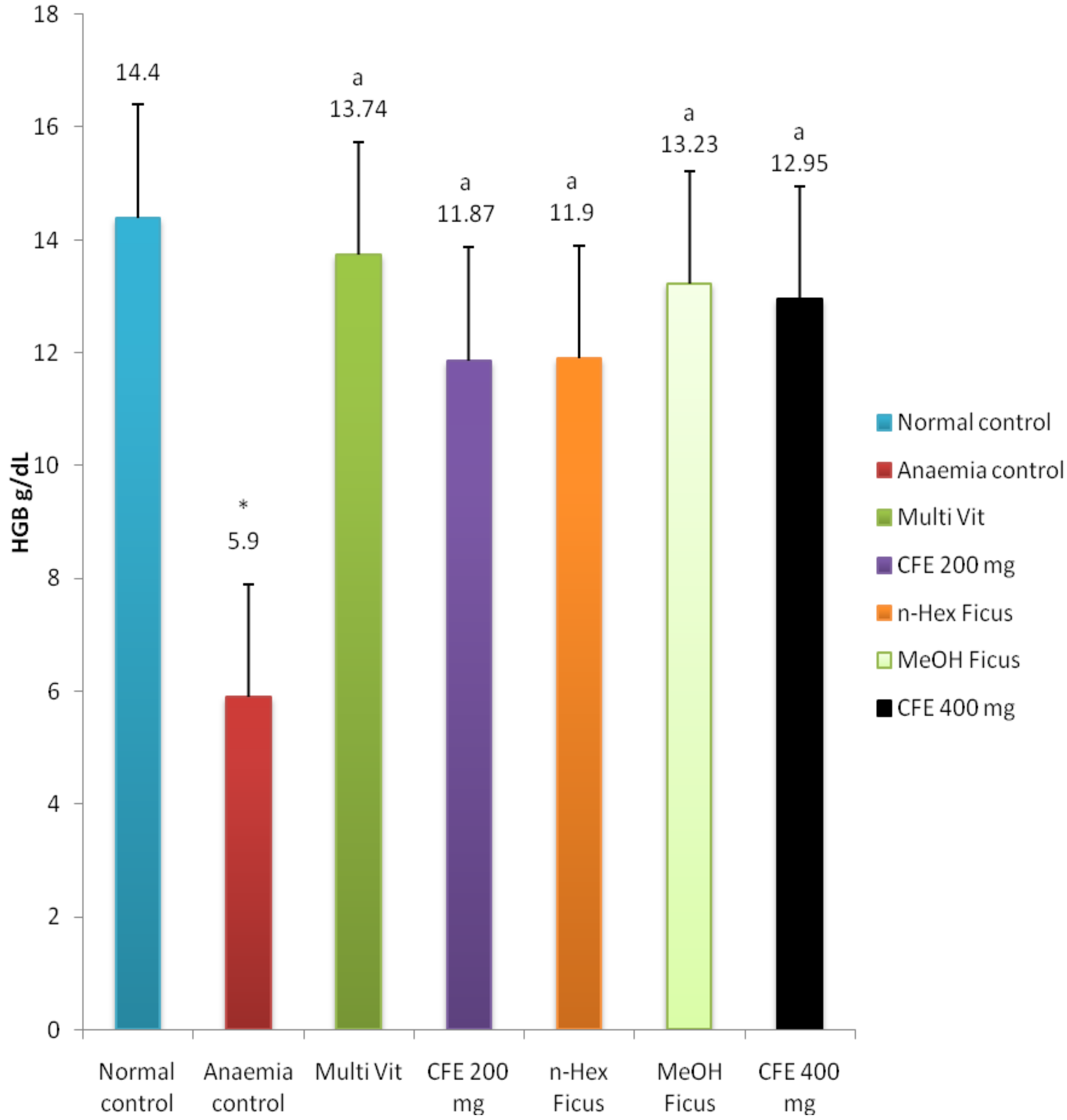

Figure 2: Variation in HGB concentration of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at p<0.05

a significantly different from Anaemia control at p<0.05

b significantly different from Multi vit at p<0.05

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

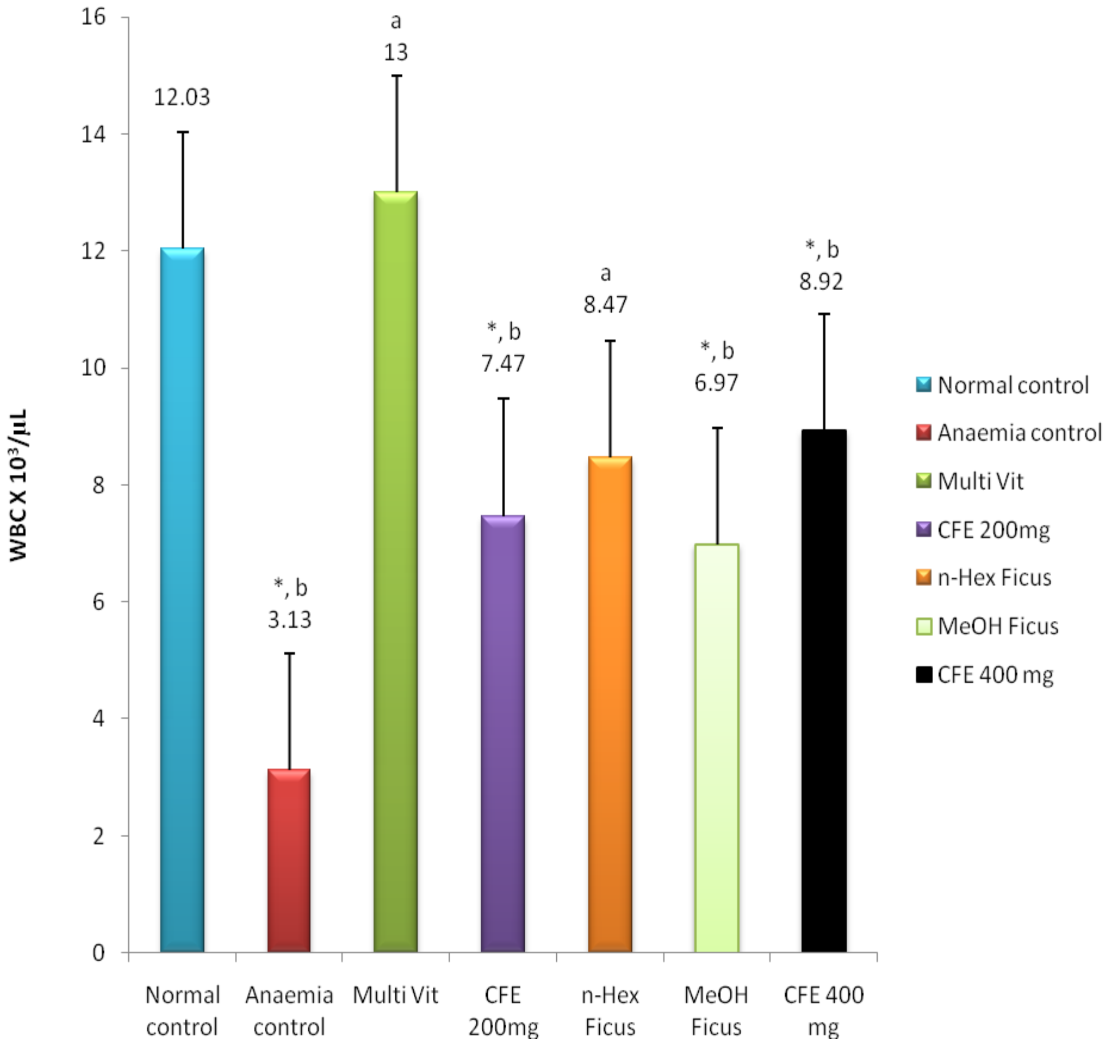

Figure 3: Variation in WBC levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at $p<0.05$

a significantly different from Anaemia control at $p<0.05$

b significantly different from Multi vit at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

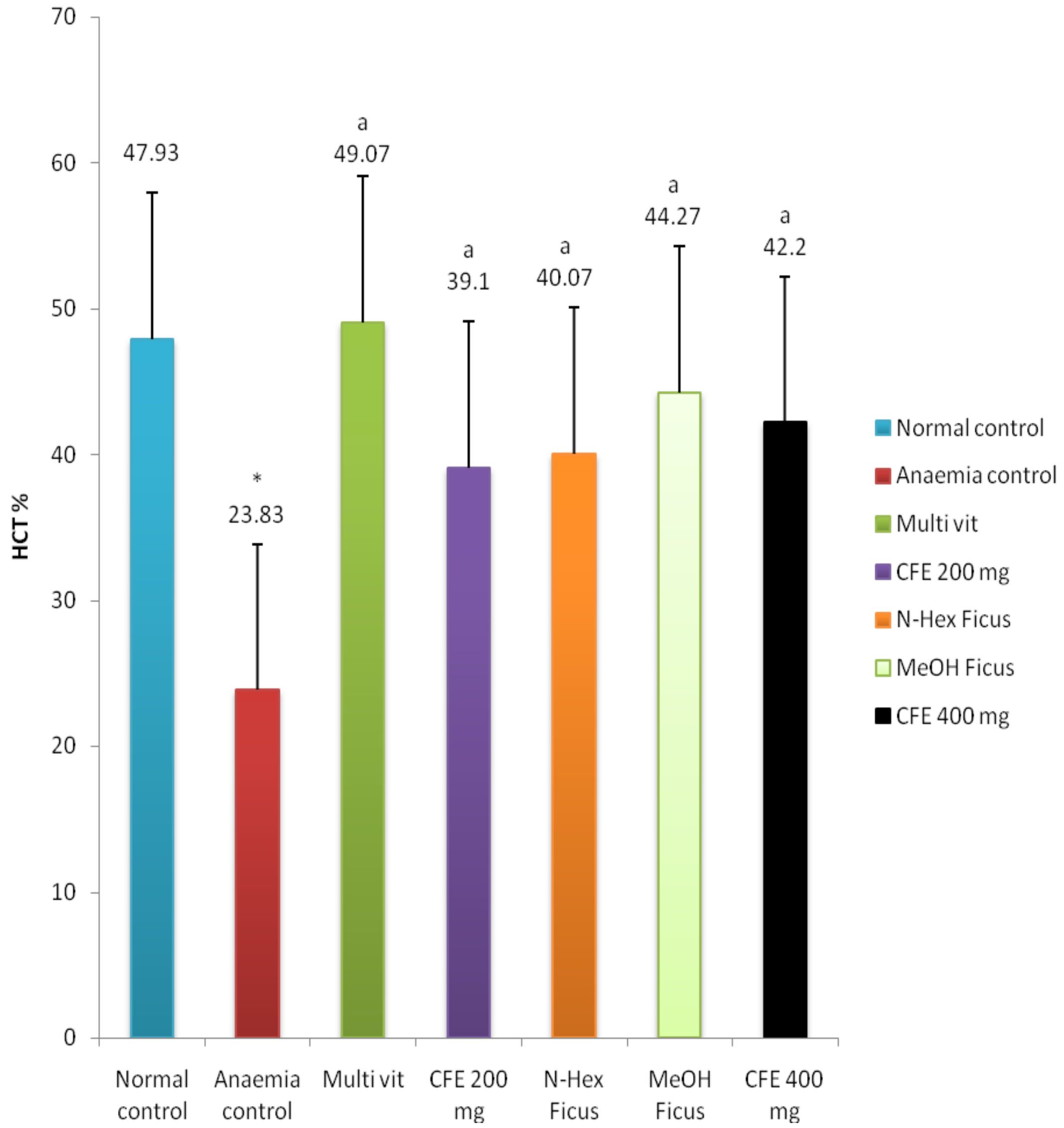

Figure 4: Variation in HCT levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at $p<0.05$

a significantly different from Anaemia control at $p<0.05$

b significantly different from Multi vit at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

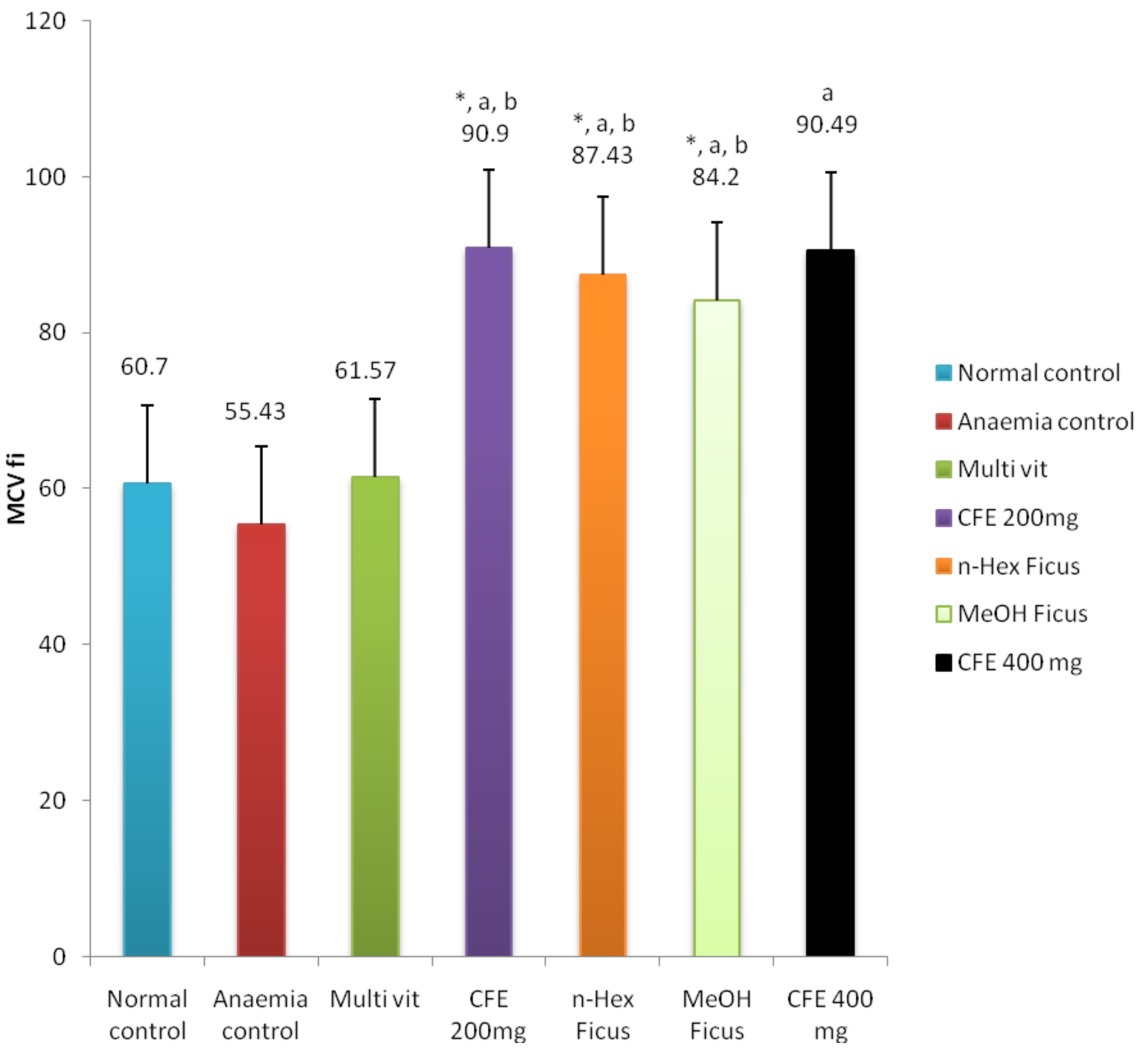

Figure 5: Variation in MCV levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at p<0.05

a significantly different from Anaemia control at p<0.05

b significantly different from Multi vit at p<0.05

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

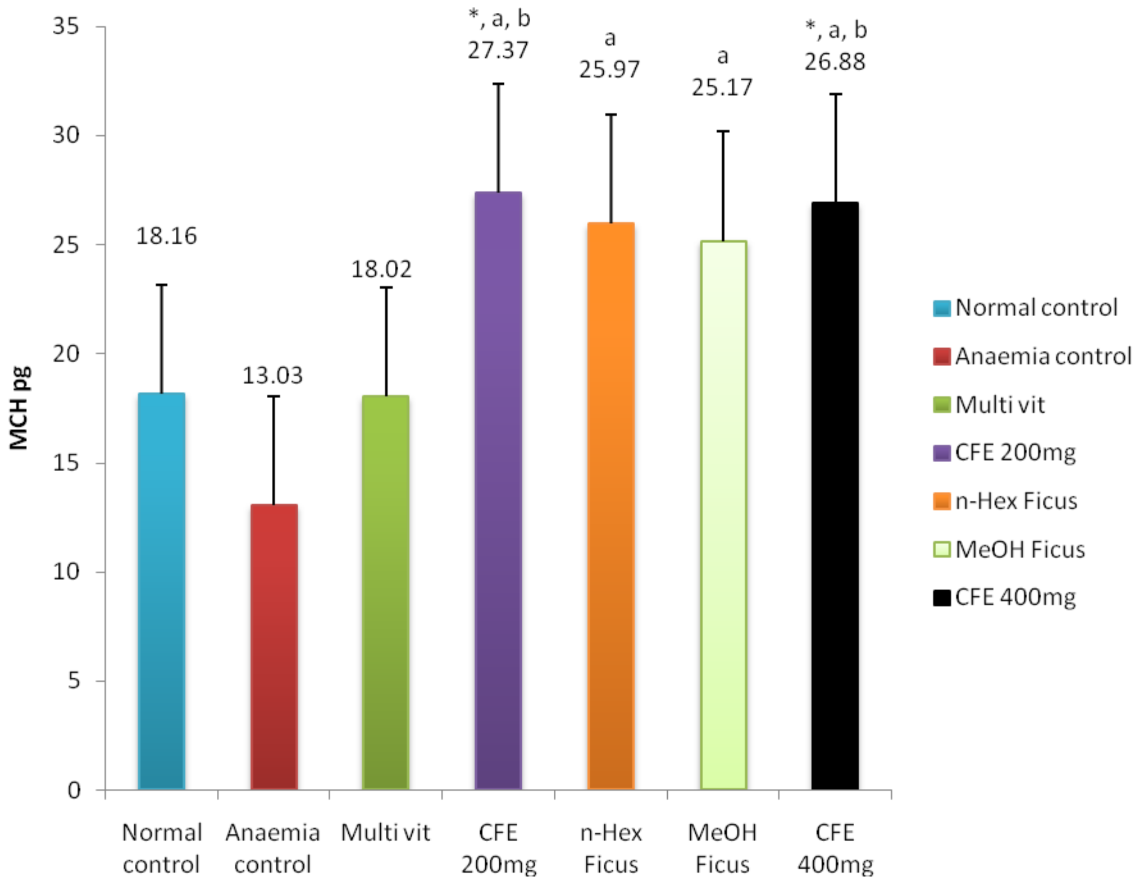

Figure 6: Variation in MCH levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at $p<0.05$

a significantly different from Anaemia control at $p<0.05$

b significantly different from Multi vit at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

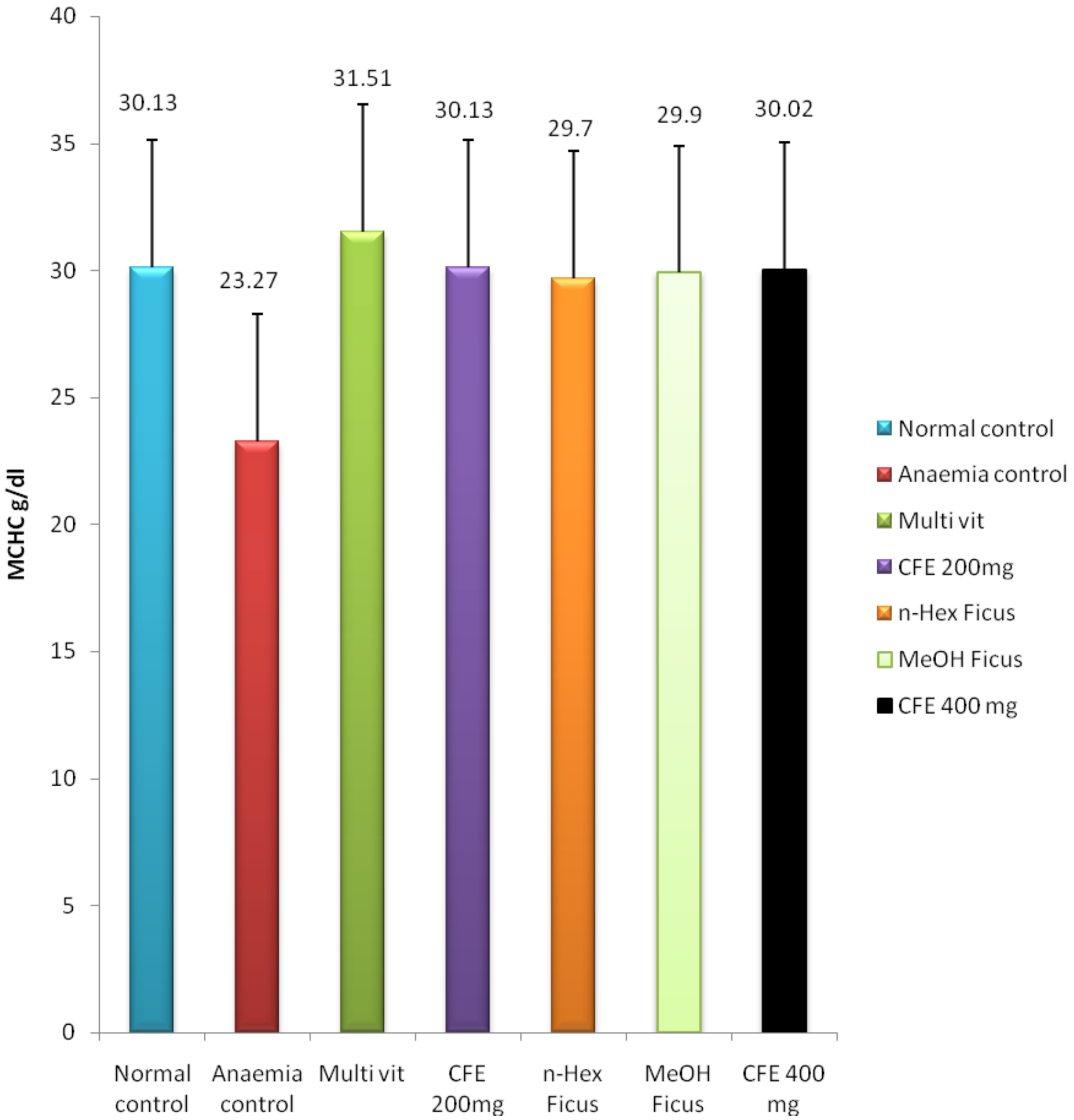

Figure 7: Variation in MCHC (g/dL) levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

No significant difference between groups at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

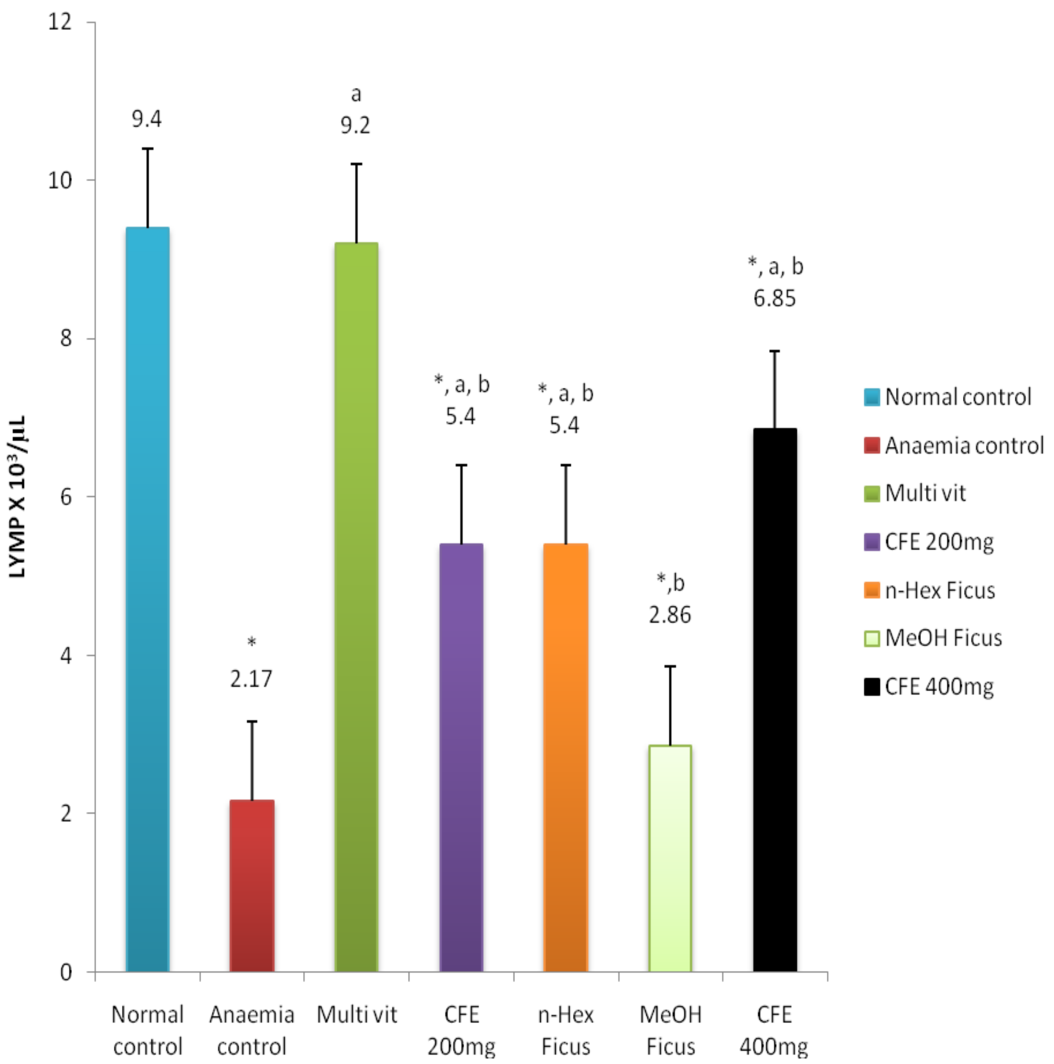

Figure 8: Variation in lymph (g/dL) levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

*Significantly different from NC at p<0.05

a significantly different from Anaemia control at p<0.05

b significantly different from Multi vit at p<0.05

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

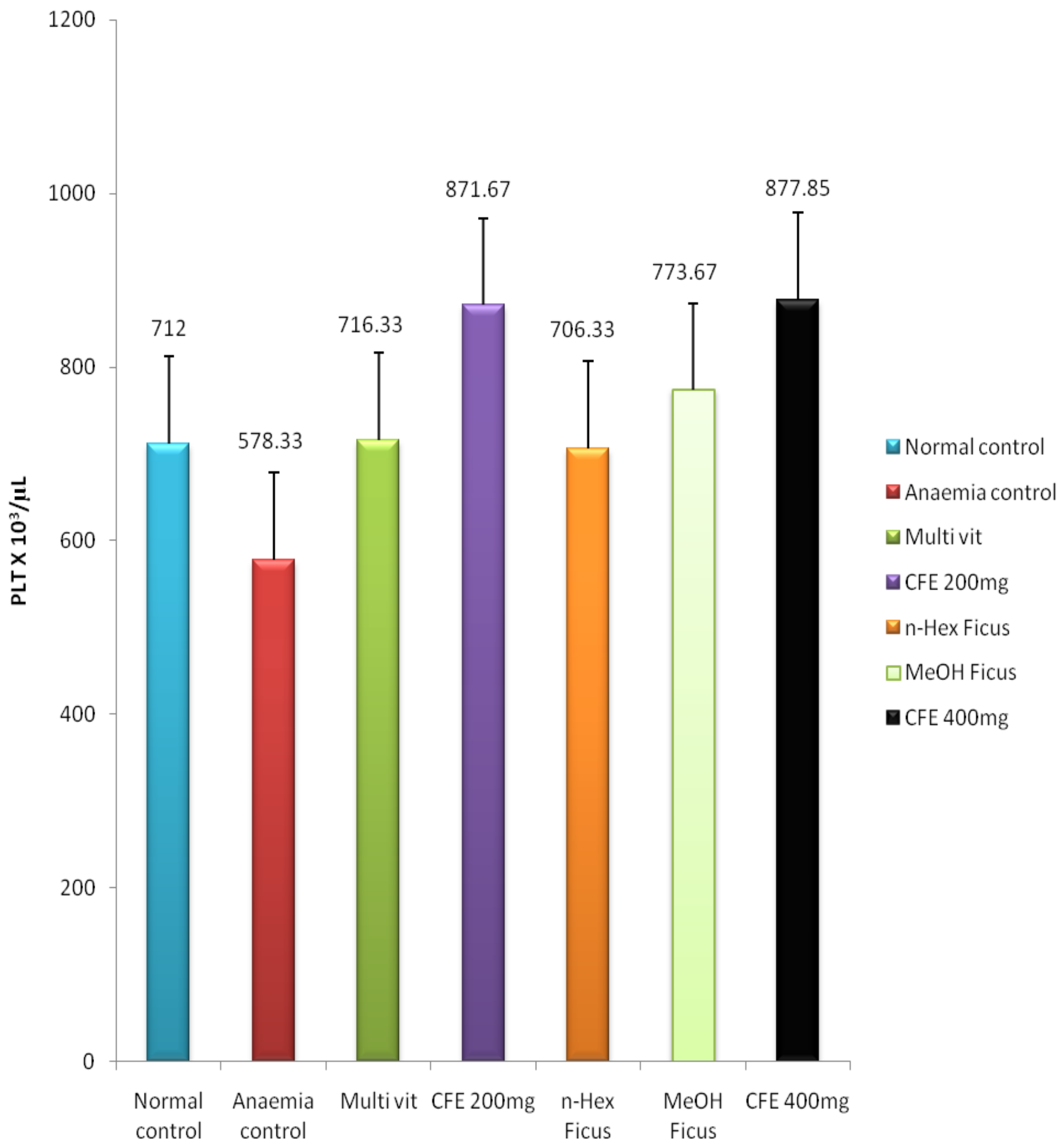

Figure 9: Variation in Platelet (g/dL) levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

No significant difference between groups at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolic Ficus Extract

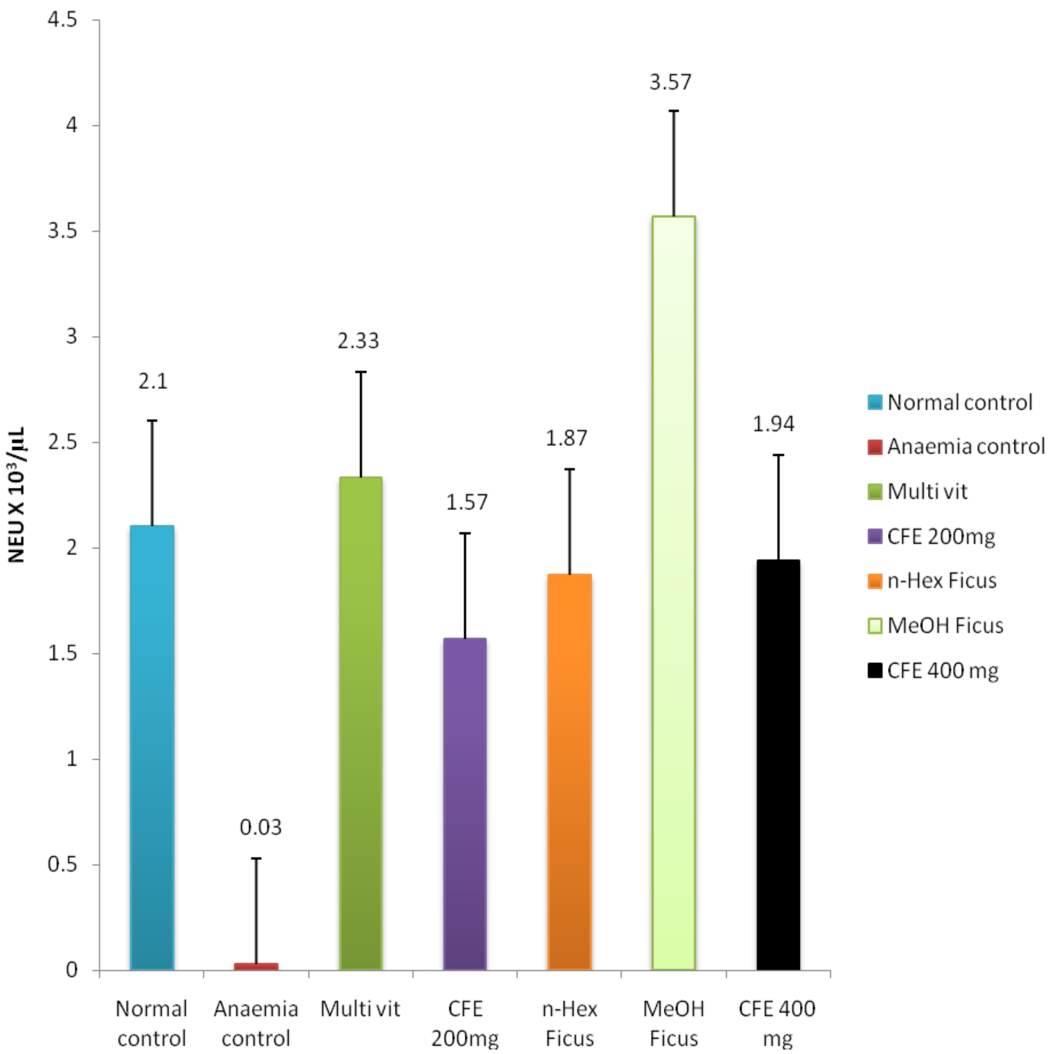

Figure 10: Variation in Neutrophil (g/dL) levels of the different experimental groups.

Values are expressed as mean ± SEM, n=6

No significant difference between groups at $p<0.05$

CFU= Crude Ficus Extract

MeOH= methanolicFicus Extract

DISCUSSION

Plant based diets have been variously reported to confer a myriad of health benefits to the consumers.[21] This is because most plants tissues including green-leafy parts, fruits, nuts and tubers are good sources of vitamins, mineral elements and macro-nutrients. In addition, plants contain important phytochemicals which complement the health benefits provided by micro- and macro-nutrients. In sub-sahara Africa, including Nigeria, indigenous populations have used plants such as *Ficus vogelii* to supplement diets in children and the aged, in order to minimize incidences of anemia and its complications. *Ficus vogelii* is used as a green-leafy vegetable in Northern Cross River State to ameliorate anemic conditions, caused by PEM The aerial parts contain a balanced source of mineral elements and vitamins which combine effectively with the protein and carbohydrate content to reverse PEM and its associated complications in children.

Mineral Elements in *Ficus Vogelii*

Iron was found to occur in *Ficus vogelii* in appreciable amount (16.09±0.25 mg/100g). This confers biochemical benefits in children health who consume the plant by modulating hemoglobin and red blood cells concentration in plasma, thus contributing to preventing anemia in children. The sodium content is low (8.59±0.7 mg/100g) which suggests that the plant can be used in treating and managing heart diseases especially in the aged.[24] Calcium was the most abundant mineral element in *Ficus vogelii* (424.24±0.21 mg/100g). Calcium is needed by children for bones and teeth development. It also plays an important role in maintaining the integrity of the nervous system, heart and the muscles. The plant is also a good source of magnesium (327,11±0.52 mg/100g). Magnesium is has been reported to play a significant role in maintaining normal heart beat and stabilizing cardiac rhythm. The phosphorus content (319.77± 0.55 mg/100g) in the plant was also in very appreciable amount. Phosphorus is needed for the proper functioning of the human kidney. It also plays an important role in nerve impulse transfer. The potassium content in the plant was found to be 38.23± 2.11 mg/100g. Potassium is needed by the body for fluid and electrolyte balance. This is a very important intervention step in ameliorating and reversing anemia especially in children. Potassium also plays an effective role in maintaining body weight. The zinc content was given as 11.14±0.02 mg/100g. Zinc is needed for proper growth and development of the body and the brain in children. It is needed for protein and nucleic acid synthesis[25] and for boosting fertility in men.[26] Manganese content in the plant was also estimated and found to be 3.79±0.13 mg/100g. Manganese plays an important role in proper bone development. It is also needed for carbohydrate and protein metabolism. The copper content was given as 1.39$^{\pm}$ 0.12 mg/100g. Copper is needed by the body for red blood cells formation. It also plays a functional role in the absorption and utilization of iron in the body Copper is also needed for proper bone, connective tissue, brain and heart development and integrity.[27] Nickel content was found to be 0.49$^{\pm}$0.05 mg/100g). Nickel is a trace element which plays an important role in iron absorption from foods. It is also needed for red blood cells formation. The presence of iron, copper and nickel in *Ficus vogelii* as elements which are required for red blood cells formation, oxygen transport, iron absorption and utilization is in line with the claim by the natives that *Ficus vogelii* is useful in the treatment and prevention of anemia. The selenium content was low and was found to be 0.22±0.04 mg/100g. Selenium helps the body to fight diseases and reduces the body's susceptibility to diseases.

Vitamin composition in *Ficus vogelii*:

Vitamin A was found to be the most abundant (319.05±12.44 IU). Vitamin A is needed by the body for good eye sight and for proper growth and development of body tissues. This suggests that the plant can also be effective in maintaining good vision in addition to its anti anemic function. Vitamin C content (22.17±1.77 mg/100g) was also found to be in appreciable amount. Vitamin C is a good antioxidant which neutralizes free radicals from the body. It also helps the body to absorb iron. This suggests that the plant is a good antioxidant and is useful in preventing anemia. Vitamin B1 content was given as 2.14±0.09 mg/100g. Vitamin B1 plays a functional role in releasing energy from foods and is also needed for the proper functioning of the nervous system. Vitamin E content was estimated to be 1.79±0.45 mg/100g. Vitamin E plays an important role in protecting red blood cells. This is in line with the fact that *Ficus vogelii* is useful in preventing and treating anemia. Vitamin E is an antioxidant and is also important in boosting the immune system. Vitamin B2 content was shown to be 1.75±0.11 mg/100g. Vitamin B2 is needed for releasing energy from foods and for proper growth and development of the body. The folic acid content was given as 1.66+0.05 mg/100g. Folic acid is needed by the body for red blood cells formation. This again suggests that the plant has anti-anemic properties. The vitamins B2 content was estimated as 1.52±025 mg/100g. Vitamin B3 is important in releasing energy from foods and for maintaining blood cholesterol levels. The vitamin B6 content was shown to be 0.89±0.15 mg/100g. Vitamin B6 is very essential for red blood cells formation and hemoglobin production. This also infers that *Ficus vogelii* is effective in the prevention and treatment of anemia. Vitamin was found to be in very low amount (o.14±0.02 mg/100g.). Vitamin K is needed by the body for blood clotting, maintaining a healthy skin, bone development and also for preventing heart diseases.

The vitamin composition showed that the plant contains very balanced B-group vitamins which contribute significantly to the reversal of anemic conditions observed in this study, and restoration of general metabolism and body homeostasis. Thereby balancing basal metabolic rate (BMR) and improving thermic effects in children consuming the aerial parts of the plant.

Proximate Composition of Aerial Parts of *Ficus vogelii*:

The energy value of *Ficus vogelii* was calculated to be 279±11.45 Kcal/100g. This suggests that the use of the plant may accelerate quick recovery in anemic or malnourished persons fed with leaves of *Ficus vogelii*. The carbohydrate (43.44±2.8mg/100g), protein (17.67±1.22mg/100g) and fat contents (9.27±072/100g) were high suggesting that *Ficus vogelii* can serve as a good source of food. The high protein content in addition to the protein quality and amino acid composition which will be studied later may have contributed significantly to the anti anemic effects of the plant. Protein is needed by the body for proper growth and development, energy supply and for repairs of worn out tissues.

EFFECT OF TREATMENT OF ANEMIC ANIMALS WITH *FICUS VOGELII*

Effect on White Blood Cells (WBC) count

There was a recovery of WBC count in treatment groups when compared to the slow recovery in anemia control group (3.13± 1.63 x 10^3/µL). Significant recovery ($p<0.05$) was observed in multivitamin group (13.0± 1.38 x 10^3/µL), and the n-hexane group (847± 1.18 x 10^3/µL) when compared to the anemia group (2.13±1.63 x 10^3/µL).

Effect on Red Blood Cells (RBC) count

Normalization of RBC count in anemia patients is an indicator of recovery after treatment. The treatment groups (200mg CE group, 4.34± 0.20 x 10^6/µL), (400mg CE, 6.25± 0.22 x 10^6/µL), n- hexane, 4.58± 0.14 x 10^6/µL) and (methanol, 5.25±016 x 10^6/µL) all showed increase in RBC count when compared with the anemia control (4.27±1.50 x 10^6/µL). The values of RBC count obtained with the multivitamin group (8.54± 0.14 x 10^6/µL) were more significant ($P<0.05$).

Effect on Hemoglobin (Hb) count

Also, normalization of Hb count in anemia patients is an indicator of recovery after treatment. The Hb count in anemia control group (5.90± 2.95 g/dL) significantly decreased ($P<0.05$) when compared with the normal control (14.40± 0.56 g/dL) and treatment groups where the values increased. The multivitamin group (13.74± 0.87 g/dL), and extract groups (200mg CE, 11.87± 0.5 g/dL), (400mg CE, 12.95± 0.54 g/dL), (n-hexane, 11.90±0.49 g/dL) and (methanol, 13.23±0.54 g/dL) all gave increased and significant ($p<0.05$) Hb values and compared with the normal control (14.40± 0.56 g/dL). The increase was significant ($P<0.05$) suggesting that aerial parts of *Ficus vogelii* may substitute clinical drugs used in the treatment of anemia and PEM conditions.

Hematocrit (HCT %) Count

All treatment groups including, multivitamin group (49.05± 1.79%), 200mg CE (39.10± 1.79%), 400mg CE (42.27±2.17%), and the methanol group (44.27± 2.17%) showed significant increase (P<0.05) in HCT values when compared to the anemia control group (23.83± 8.69%), which showed no sign of recovery throughout the 28 days study period.

Effect of treatment on Mean Corpuscular Volume (MCV) Count

The treatment groups also reversed all low values of MCV caused by phenylhydrazine treatment. Values of MCV in 200mg CE (90.90± 3.5), 400mg CE (90.49± 1.50), n hexane group (87.43± 1.80), and methanol group (84.20± 1.64), all increased significantly (P<0.05) when compared with low values in anemia control (55.43±0.62). The values of MCV in treatment groups were significantly higher when compared to the results obtained for multivitamin group (61.57±3.66) and normal control group (60.70±4.78). This showed that aerial parts of *Ficus vogelii* have significant effect on the MCV parameter of hematological indices and may play a major role in the recovery of anemic patients.

Mean Corpuscular Hemoglobin (MCH) Count

Treatment groups including, 200mg CE (27.37±0.76pg), 400mg CE (26.88±0.50 pg), n-hexane (25.97±2.85 pg) and methanol group (25.17±0.33 pg) all increased MCH count significantly (P<0.05) when compared to the anemia control group (13.03±6.51 pg). The increase in MCH values elicited by multivitamin group (18.02± 1.09 pg) was marginal and compared with the normal control (18.16±0.64 pg) group. Again this showed that aerial parts of *Ficus vogelii* have significant effect on the MCH parameter of blood component and may play a major role in the recovery of anemic patients.

Mean corpuscular Hemoglobin Concentration (MCHC) Count

Animals exposed to *Ficus vogelii* treatment including, 200mg CE (30.13± 0.35 g/dl), 400mg CE (30.02±0.05 g/dl), n-hexane group (29.70±0.49 g/dl), methanol group (29.90±0.31 g/dl), and multivitamin group (31.51±0.98 g/dl), all increased MCHC count significantly (P<0.05) when compared to the anemia control group (23.27±11.63 g/dl). The increases were consistent with the results of the multivitamin group and when compared well with the normal control group (30.13±1.58 g/dl).

EFFECT ON ANEMIC CONDITIONS

Groups treated with extracts of *Ficus vogelii* and multivitamin, showed significant reversion and normalization of hematological indices which are key indicators in anemia. The normalized parameters compared well with values of normal control group and multivitamin treated group. The reversion by increases in Hb, RBC and WBC parameters in particular and the physical activeness of treated animals suggests that the anemic test subjects have recovered from the condition. Overall this study suggests that aerial parts of *Ficus vogelii* may substitute multivitamin requirement in anemic conditions especially in children in local communities.

CONCLUSION

This study showed that anemia which was seen to be characterized by decrease in Hb count, HCT, RBC count and other hematological indices was ameliorated and reversed on treatment with extracts and leaves of *Ficus vogelii.* This supports the ethno-medical claims on the use of the plant as anti-anemic remedy. It was further concluded that, the anti-anemic effect may be attributable to the protein, vitamin and mineral elements content of the leaves, and its anti-oxidant phenolic constituents.

REFERENCES

1. Constan T., Alix, E., Dardaine, V. (2000). Protein Energy Malnutrition. Diagnostic methods and epidemiology. Press med, 29(39): 2171-6.
2. David J. and Christopher P. (2001). Price renal function in: The Fundamentals of Clinical Chemistry. 5th edn. Burtis C and Edward R. (edn) Elsevier Ltd. New Deihi. India, 699-701.
3. World Health Organization/UNICEF/UNU. (2001). Iron deficiency anemia: assessment, prevention and control. Geneva world Health Organization (WHO/NHD/0.13).
4. Mboso, O. E., Odey, M.O., Uboh, F. E., Essien, M.N., Chidinma O. and Eyong, E. U. (2014). Erythropoietin Effects of Eremomastax polysperma leaf extracts on female prepubertal and pubertal wistar rats. British Journal of pharmaceutical Research, 4(15): 1833-1839.
5. Abolaji, O. A., Adebayo, A. H., Odesanmi, O. S. (2007). Nutritional qualities of three medicinal plant parts (xylopia aethiopica, Blighia sapida and Parinari polyandra) commonly used by pregnant Women in the Western part of Nigeria. Pak.J. Nutri, 6: 665-668.

6. Adamson, J. W., and Longo D. L. Anemia In: Kasper D. L. Fauci A.S., Loscalzo J. Longo D. L., Brawnwald E. Hawser, S. L., Jameson, J. L. (eds (2008). Harrison;s Principles of Internal Medicine. 17th ed. NY. Mc Graw Hill, 355-372.
7. Mamedov, N. (2002). Medicinal plant studies: History challenges and prospective. Med Aromat plant, 1: el 33.
8. Rassol Hassan, B. A. (2012). Medicinal plants (importance and uses) pharmaceut anal Acta, 3: e139.
9. World Health Organization (WHO) (2000). Department of Nutrition for Health and Development, Nutrition for Health and Development: a global agenda for combating malnutrition.
10. Arbonnier M. (2004). Trees, shrubs and lianas of West African Dry Zones. Margraf Publishers, 3823614193.
11. Sirisha, N., Sreenivasulu, M., Sangeeta, K. and Madhusudhana Chetty C. (2010). International Journal of Pharmtech Research, 2(4): PP 2174-2182.
12. Ephraim P., Pavilalinen, H., Pawlus, A. and Newman R. (2008). Ficus Spp (Fig): Ethnopharmacology, and Potential as anticancer and anti inflammatory agents. Journal of Ethnobotany, 119(2): 195-213.
13. Beers, M., and Berkow R (1999). Nutritional disorders: malnutrition. The Merch manual. 17TH Edn Merck, 28-32.
14. Scalar (2000). Ln: Segregated flow analyzer for analytical process Laboratories, Netherland, *45,55 and 61*.
15. AOAC (1980). Official Methods of Analysis. 14th edn, Association of Official Analytical Chemists, Washington D.C.
16. Pearson D (1976). The Chemical Analysis of Foods. 7th ed., Churchill Livingstone, Edingburg.
17. James CS (1995). Analytical Chemistry of Food. Chapman Hall, New York.
18. AOAC (1990). Official methods of analysis (15th ed.). Washington D.C USA. Association of Official Analytical Chemists inch. 400 - 2200Wilson Boalevard, Arlinton Virginia USA, **2**: 910-928.
19. AOAC. (1984) Official Methods of Analysis 14th ed. Association of official Analytical Chemists.
20. FAO (1986). Manuals of food quality control. Food analysis: general techniques, additives, contaminants and composition. *FAO Food and Nutrition Paper., 14 (7): 203 - 232.*

21. Igile, G. O., Iwara, I.A., Mgbeje, B. I. A., Uboh, F. E., Ebong, P. E. (2013). Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (*Asterecea*). A Green Leafy vegetable in Nigeria. Journal of food research, 2(6).
22. Berger, J (2007). Phenylhydrazine hematotoxicity. J. Applied Biomedicine pgs, 125-130.
23. Lorke, D (1983): A New approach to acute toxicity testing. Arch Toxicol, 54: 275-287.
24. NRC (1999). National Research Council Recommended Dietary Allowance National Academy Press. Washington D.C.
25. Melaku, U., Clive, E. W. and Habtamon, F. (2005). Content of zinc, Iron, Calcium and Their Absorption Inhibitors in Ethiopia. *Journal of Food Composition Analysis, 18**:** 803-817*.
26. Oluyemi, E. A., Akilua, A. A., Adenuya, A. A. and Adebayo, M.B. (2006). Mineral contents of some commonly consumed Nigerian foods. Science focus, 11(1): 153-157.
27. Scheiber, I., Dringen, R., Mercer, J. (2013). Chapter 11: Copper: Effects of Deficiency and overload in Astrid Sigel, Helmut Sigel and Roland Sigel, interactions between essential metal ions and human diseases. Metal ions in life sciences, 13. Springer 359-387.