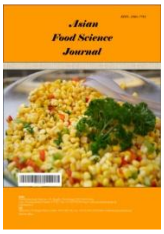

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Effect of "Uchakiri"(*Vitex doniana*),"Uturukpa" (*Pterocarpus santalinoides*) and "Ewa" (*Solanum americanum*) Methanoic Extract on Iron Status of Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Iron deficiency anaemia is a hidden hunger which can affect at every stage of human development especially pregnant women and children.
Aim: The study was carried out to ascertain the effect of "ewa", "uturukpa" and "uchakiri" on iron status of rat.
Methods: The vegetables were purchased from Oriemba market Akpugo in Nkanu West Local Government Area of Enugu State. The vegetables were trimmed, washed with deionized water and drained. The vegetables were ground using electric blender. Methanolic extraction was done using a modified method. All the analysis were carried out using a standard method.
Results: The results obtained were 2.67-3.52 mg iron, 0.52-0.89 mg copper, 1.56-7.43 mg zinc, 2.42-120.70 mg calcium 10.16-39.10 mg magnesium, and 68.80-90.40 mg potassium. The mean serum ferritin, haemoglobin, packed cell volume and RBC all increased gradually after consumption of leaves extract.

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Conclusion: The result showed that ethanol leaf extract of *Vitex doniana*, "ewa" and "uturukpa" posseses anti-anemic potentials that are capable of reducing the risk of iron deficiency anemia in the society.

Keywords: Anaemia; Vitex doniana; Pterocarpus santalinoides; Solanum americanum; vegetables; iron.

1. INTRODUCTION

Iron deficiency anemia is a public health problem that particularly affects young children and pregnant women. According to WHO, 40% of pregnant women and 42% of children under 5 are anemic globally [1]. Nutritional deficiencies, particularly those of iron, vitamins B12 and A, as well as folate, are the main causes of anemia. Infectious disorders like malaria, TB, HIV, and parasitic infections are examples of additional causes. Enrichment with plant ferritin is one approach being studied for enhancing the iron status of people [1].

In particular, non-hem iron is the subject of experiments to increase its bioavailability, remove obstructions, understand its methods of absorption, and promote its use [2]. Iron supplementation in the human diet has become a need [2]. There are several indigenous vegetables, such as bitter leaf, ewa, bubble bush leaf, uchakiri, uturukpa, and others.

In humans, a high vegetable diet has been linked to a decreased risk of cardiovascular disease [3]. Around 31% of ischemic heart disease and 11% of stroke are thought to be caused by low vegetable intake worldwide. Diets deficient in vegetables, complex carbohydrates, and dietary fiber are thought to contribute to 2.7 million annual deaths, ranking among the top 10 risk factors for mortality in the 2007 World Health Report [4].

"Ewa" (Solanum americanum) is a member of the Solanaceae family and is sometimes referred to as American black nightshade and glossy nightshade. Locally, it is known as Gautan kad or Gautan kaaj in Hausa, Oju ologbo in Yoruba, and Anya nwona in Igbo [5]. In some areas of northeastern Nigeria, the plant has been used to cure diarrhea and dysentery.

The tree species known as "Uturukpa" (Pterocarpus santalinoides) belongs to the Fabacea family of legumes. The family Leguminosae includes the genus Pterocarpus, which is widespread across the tropics and subtropics. In the South Eastern region of Nigeria, "Uturukpa" (Ibo) leaves from the Pterocarpus santalinoides tree are used to make soup. Some tribes in Eastern and Southern Nigeria employ the leaf extracts as antibacterial agents and as a remedy for respiratory issues, convulsions, fever, and headaches, as has also been described for Sansevieria trifasciata [6].

Southern Nigeria is home to "Uchakiri" (Vitex doniana), a member of the Verbenaceae family popularly known as the black plum [7]. It is the most common species of the genus and is helpful in the treatment of ailments since it is frequently found in savannah regions [8]. In Nigeria, V. doniana is known by the Hausa, Fulani, Yoruba, and Igbo names "dinyar," "ori nla," "uchakiri," and "galbihi" [9]. The young leaves are made as vegetable soup and cooked alongside other ingredients, including sauces. Tanning agents, anthraquinones, flavonoids, resins, cardiac glycosides, saponins, and alkaloids were discovered through phytochemical investigations in Vitex doniana [10].

The size and scope of the issue need the swift implementation of proven solutions. Therefore, it is crucial to promote locally produced food items in order to diversify diets.

2. MATERIALS AND METHODS

2.1 Procurement of the Raw Materials

The "*ewa", "uturukpa" and "uchakiri*" leaves were purchased from Oriemba market Akpugo in Nkanu West Local Government Area of Enugu State.

2.2 Preparation of Materials

The vegetable samples were separately plucked and sorted by removing extraneous materials and cleaned by washing with deionized water. The vegetables were milled using electric blender until the desired particle size was obtained (150-180 microns).

2.3 Chemical Analysis

The Minerals composition of the vegetables were determined in triplicate using AOAC method [11].

2.4 Preparation of Methanolin Extract

Methanolic extraction was done using a modified method of Bhandari and Kawabata [12]. Five hundred grams of each vegetables were soaked in 4000 ml of methanol and kept overnight. The suspension was filtered through Whatman No.1 filter paper, and the filtrate was diluted to make up to 100ml with methanol. Sample solutions were stored at 4°C in amber bottles and served as the stock solution for subsequent analysis.

2.5 Study Design

The anemia studies were carried out using the Completely Randomized Design (CRD). Rats were randomly assigned to the treatments based on their weights. There were five treatments each replicated five times. The rats were the replicates while the different diets were the treatments.

2.6 Animal Experiment

2.6.1 Animal housing

At the University of Nigeria Nsukka's Department of Veterinary Pathology, 25 adult rats weighing between 40 and 60g were purchased. On the basis of body weight, the animals were separated into 5 groups of 5 rats each. The rats were housed individually in cages to separate urine and feaces on a base tray.

2.6.2 Bioassay

The rats were fed on standard rat chow throughout the experiment. The ewa, uchakiri and uturukpa extract were made to provide 0.11mg/day iron to the rats. The study lasted for 28 days. A 7-day acclimatization, a 7- day inducing of anemia and a 14 day feeding trial. Commercial hematinic- ferrous sulphate was used as positive control. The diets were formulated using AIN-93G (American Institution of Nutrition) method [13]. The rats were weighed prior to access to their respective diets. Group 1 were fed rat chow alone, group 2 rat chow with ferrous sulphate, group 3 rat chow and ewa extract, group 4 rat chow and uchakiri extract and group 5 with rat chow and uturukpa extract. The weight of the animals was recorded each day. Daily food intake and extract were recorded to calculate nutrient intake.

2.6.3 Diet composition

The iron content of extract from 100g sample of each vegetable with100ml of water was used for the study. During the acclimatization period the least quantity of water that was taken by a rat per day was used. The iron need for rat per day is 0.11mg/dl.

Using the dilution of standard solution equation C1VI =C2V2

Where

CI= Initial concentration
C2 = Final concentration
V1= Initial volume
V2 = Final volume

2.7 Blood Sample Collection

Anemia was induced to the rats by collecting 2 millilitres of blood between the hours of 8.00-10.00 am for 7 days of the study. The blood was collected from ophthalmic venous plexus located in the orbital sinus of the rat using a heparinized-capillary tube. Blood was collected on day 0, 7, 12, 17 and 22 for heamatological determinations.

2.7.1 Heamoglobin level

Heaglobin level was determined using the cyanomethamoglobin technique recommended by The International Committee for Standardization in Hematology [14].

2.7.2 Determination of serum ferritin

This was measured by a two-site immune radiometric assay and radioimmuno assay as given by [15].

2.7.3 Determination of the red blood cell in the rat

The red blood cells count was determined by haemocytometry [16].

2.7.4 Determination of pack cell volume of the rat

The packed volume was determined using micro-haematocrit reader according to the method of [17].

2.8 Statistical Analysis

The data generated was subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS, version 20) software. Means was separated using the Turkey's Least Significance Difference (LSD) Test at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Mineral Composition of the Vegetables

The mineral content of the three leafy vegetables is shown in Table 2. The "uturukpa" leaf included 3.01 mg of iron, 0.52 mg of copper, 7.43 mg of zinc, 120.70 mg of calcium, 39.10 mg of magnesium, and 68.80 mg of potassium. The iron content of vitex doniana leaf was 2.67 mg, copper 0.89 mg, zinc 2.31 mg, calcium 7.30 mg, magnesium 10.16 mg, and potassium 75.02 mg. In contrast, "ewa" leaf had 3.52 mg of iron, 0.68 mg of copper, 1.56 mg of zinc, 2.42 mg of calcium, 15.17 mg of magnesium, and 90.40 mg of potassium.

The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had relative iron contents of 3.01 mg, 2.67 mg, and 3.52 mg. Comparing the iron level of all the samples studied to the RDA (mg/day), they were all lower. For men of all ages and postmenopausal women, the Recommended Dietary Allowance (RDA) is 8 mg per day; for premenopausal women, it is 18 mg per day. For men, the average daily consumption of iron ranges from 16 to 18 mg, and for women, it is 12 mg [18]. In the human body, iron is a significant trace element with key functions in hemopoiesis, infection management, and cell-mediated immunity. According to estimates, more than a billion individuals worldwide suffer from iron deficiency anemia, which has been called the most common dietary deficiency [19]. Reduced productivity, behavioral and cognitive deficits, as well as lowered infection resistance, are all effects of iron deficiency.

The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had copper contents of 0.52 mg, 0.89 mg, and 0.68 mg, respectively. For both humans and other animals, copper (Cu) is a crucial trace element. Although the majority of the body's copper is in the cupric ($Cu2+$) form, copper can also exist in the cuprous ($Cu1+$) form [20]. The importance of copper in oxidation-reduction (redox) reactions and the scavenging of free radicals is explained by its ease in accepting and donating electrons [21].

The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had zinc contents of 7.43 mg, 2.31 mg, and 1.56 mg, respectively. Zn levels were moderately high in all of the leafy vegetable samples, with values ranging from 1.56 to 7.43 mg. This figure is comparable to that which [22;23] reported (2010). For healthy immune system and human growth, zinc is a crucial mineral [24]. According to reports, 20% of the world's population may not get enough zinc [25]. Zinc deficiency affects 20% of children under the age of five, 28.1% of mothers, and 43.9% of pregnant women in Nigeria, according to studies [20]. According to research by [26], zinc is essential for the formation of hemoglobin. Anemia and fragile erythrocytes have been linked to zinc deficiency. Additionally, zinc functions as a cofactor for RBC-SOD, defending the cell's integrity from oxidative stress [27].

The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had relative magnesium contents of 39.10 mg, 10.16 mg, and 15.17 mg. According to earlier research on fluted pumpkin and "uturukpa" by [28], "uturukpa" has the greatest magnesium concentration. The levels found in this study, however, were insufficient to reach the RDA, which is 310 mg for women and 400 mg for males between the ages of 19 and 30. [29]. The structure and operation of the human organism depend heavily on magnesium. The average adult's body has 25 grams of magnesium. More than 60% of the body's magnesium is present in the skeleton, followed by 27% in muscle, 6–7% in other cells, and less than 1% outside of cells [30]. The production of blood and the extracellular and intracellular fluids that make up bodily cells need the minerals calcium and magnesium. They contribute to the formation of bones and teeth as well as the control of nerve and muscle activity [31;32].

The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had potassium contents of 68.80 mg, 75.02 mg, and 90.40 mg, respectively. Potassium is a systematic electrolyte that works with sodium to co-regulate ATP. One important intracellular cation that helps to sustain intracellular osmotic pressure is potassium [33]. Potassium is essential for treating sickle cell anemia. It significantly affects heartbeat and aids in the passage of nerve impulses. Cellular potassium loss and dehydration observed in sickle cell anemia were revealed to be caused by abnormal activation of the potassium chloride co transport system [34].

3.2 Nutrient Composition of Rat Chow

Table 3 presents the nutrient composition of the raw chow. From the result, it contained a negligible amount of zinc, 0.10mg of iron and 0.20µg beta-carotene. The nutrient composition of the rat chow were trace amount of zinc, 0.10mg of iron and 0.20µg of beta-carotene respectively. "Chow" means formula feed of pet or laboratory animal. The result of the nutrient content of the rat chow showed that it will not have much effect on the bioassay.

3.3 Mean Serum Ferritin Level of Rat

As could be seen from Table 4, *V.doniana*, “ewa” and “uturukpa” extract significantly elevated the serum concentration of ferritin (19.30, 22.24, and 20.10 mmol/L). Only the rat chow without any administration were significantly reduced in serum level of ferritin (i.e. 11.20 mmol/L) compared to control (24.04mmmol/L). It is obvious from the above data that the vegetable extract caused increased ferritin level. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body; hence, serum ferritin is used as a diagnostic test for iron-deficiency anemia [35]. The increase in serum ferritin of the rat with the plant extract may be due to the presence of concentration of iron in both plants [36].

3.4 Mean Haemoglobin Level of Rat

The rat's mean hemoglobin level is shown in Table 5. According to the study's findings, giving rats extracts from the vegetables V.doniana, "ewa," and "uturukpa" increased their levels of hemoglobin. From day 7 to day 22, all of the rats fed the vegetable extract had significantly higher heamoglobin levels. This might be explained by the heamatopoietic component present in the vegetables. The iron and ascorbate content of the veggies may have contributed to the rise in hemoglobin levels. Your blood's hemoglobin helps carry oxygen from your lungs to your tissues. In muscle cells, myoglobin receives, transports, stores, and releases oxygen. This suggests that large dosages of "ewa" and "uturukpa," an extract from V.doniana, could increase hemoglobin production. Numerous disorders, including thalassemia and iron deficiency anemia, result in the inability to make hemoglobin [37]. Numerous cellular enzymes require iron as a component, and hemoglobin (which contains an iron-containing porphyrin ring) also contains iron as a member of the heme group [38]. Red blood cells hold the majority of the body's iron reserves since they are where hemoglobin is made, which depends on iron. A lack of iron intake or absorption, excessive blood loss from external bleeding, or interference with iron metabolism could all contribute to an iron shortage [39].

3.5 Mean Erythrocytes Level of Rat

The mean level of erythrocytes in rats is shown in Table 6. The amount of red blood cells in the experimental groups increased significantly (p 0.05) after the administration of the plant extracts from V. doniana, "ewa," and "uturukpa." Particularly in the rats that ingested "ewa" extract, a considerable rise in erythrocyte levels was seen. A similar result was obtained by Asuquo (2012) when ethanol leaf extract of yellow mombin was administered to rats [40]. Red blood cells (RBC), also referred to as erythrocytes, carry oxygen to the body's tissues [41]. Carbon dioxide is released by the tissues as oxygen is converted to energy. According to Asaolu et al. [42], red blood cells also carry carbon dioxide to the lungs for exhalation. As a result, when the methanol leaf extract of V. doniana, "ewa" and "uturukpa" was supplied, a significant rise in RBC was seen. Vamsee et al. [43] also discovered a similar outcome after giving anemic rats 400 mg/kg of curry leaf.

3.6 Mean Packed Cell (PCV) Volume Level of Rat

The rat's mean PCV level is shown in Table 7. When rats treated with leaf extracts of V.doniana, "ewa," and "uturukpa" were compared to control rats treated with ferrous sulphate, the packed cell volume (PCV) of the control rats increased significantly (p 0.05). At day 22, the rats' levels of PCV were significantly higher thanks to the leaf extract. This may be because the V.doniana, "ewa," and "uturukpa" leaves have phytochemical content and antioxidant potential [44]. Similarly, "ewa" and "uturukpa" leaves are abundant in phytochemicals and antioxidants, according to [45;46], respectively. Additionally, according to a recent study by [47], "ewa" leaves have antioxidant activity and can both prevent and treat ethanol-induced oxidative stress in Wistar rats. In a similar vein, [48] revealed the pharmacotherapeutic effect of "ewa" leaves on hyperglycemia and lipidemic parameters of alloxan-induced diabetic mice. This effect was attributed to the leaves' antioxidant capability. Their effects on PCV levels could potentially be explained by their antioxidant functions. Therefore, it is probable that human ingestion of both plants can aid in the prevention of anemia, particularly in women who are pregnant or menstrual. It is known that red blood cell counts and other parameters that are below normal ranges are indicative of anemia, while values that are above normal ranges are indicative of polycythemia [49]. As a result, it is possible that the 22-day treatment with all the plants does not have the potential to result in polycythemia. A possible indicator that "ewa" leaves are more effective haematopoietic agents than V.doniana and "uturukpa" leaves is the considerable elevation in PCV that was shown in animals treated with "ewa" leaves compared to those treated with V.doniana and "uturukpa" leaves for 22 days.

Table 1. Composition of experimental diets

GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Rat chow	Rat chow + ferrous Sulphate	Rat chow + ewa leave extract	Rat chow + uchakiri leave extract	Rat chow + uturukpa leave extract

Table 2. Mineral composition of the vegetables (mg/100g)

Sample	Iron	Copper	Zinc	Calcium	Magnesium	Potassium
A	3.01^{b}±0.21	0.52^{c}±0.68	7.43^{a}±0.94	120.70^{a}±0.35	39.10^{a}±0.05	68.80±0.14
B	2.67^{c}±0.10	0.89^{a}±0.18	2.31^{b}±0.53	7.30^{b}±0.94	10.16^{c}±0.38	75.02±0.55
C	3.52^{a}±0.31	0.68^{ab}±0.60	1.56^{c}±0.64	2.42^{c}±0.02	15.17^{b}±0.14	90.40±0.47

Values are mean ± standard deviation of 3 replication
Keys: A = Uturukpa; B = Vitex doniana; C = Ewa

Table 3. Nutrient composition of rat chow (mg/100g)

Nutrients	Rat chow
Zinc	-
Iron	0.10±0.01
B-Carotene	0.20±0.03

Values are mean ± standard deviation of 3 replication

Table 4. Mean serum ferritin (ng/ml) level of rat

	Group 1	Group 2	Group 3	Group 4	Group 5
Days	**Rat chow**	**Rat chow + Ferrous sulphate**	**Raw chow + *V.doniana* extract**	**Raw chow + Ewa extract**	**Raw chow + Uturukpa extract**
0	23.41a±0.30	23.78a±0.13	23.21a±0.26	23.60a±0.38	23.54a±0.40
7	12.33b±0.10	12.40c±0.84	12.32d±0.45	12.28d±0.36	12.37d±0.53
12	12.01bc±0.18	20.20b±0.89	14.01c±0.51	17.29c±0.22	15.86c±0.32
22	11.20c±0.77	24.04a±0.39	19.30b±0.15	22.24b±0.19	20.10^{b}±0.04

Values are mean ± standard deviation of 3 replication

Table 5. Mean haemoglobin (g/dL) level of rat

	Group 1	Group 2	Group 3	Group 4	Group 5
Days	**Rat chow**	**Rat chow + Ferrous sulphate**	**Raw chow + *V.doniana* extract**	**Raw chow + Ewa extract**	**Raw chow + Uturukpa extract**
0	13.81a±0.24	13.76a±0.46	13.98a±0.21	13.84a±0.15	13.90a±0.03
7	7.51a±0.16	7.38c±0.36	7.26cd±0.62	7.40c±0.13	7.33d±0.10
12	7.50d±0.32	12.80b±0.28	8.45c±0.80	10.20b±0.28	9.03c±0.19
22	7.50c±0.11	14.22a±0.68	11.74b±0.47	13.44a±0.83	12.30b±0.11

Values are mean ± standard deviation of 3 replication

Table 6. Mean erythrocytes level of rat (10^6 cell/µl)

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + *V.doniana* extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	7.12a±0.10	7.13a±0.15	7.07^{a}±0.56	7.15^{a}±0.83	7.12a±0.21
7	5.21b±0.20	5.21b±0.18	5.18^{bc}±0.16	5.19^{b}±0.23	5.19b±0.34
12	5.11b±0.14	7.09^{a}±0.56	5.53^{b}±0.13	5.90^{b}±0.68	5.75b±0.60
22	5.10b±0.35	7.11^{a}±0.02	6.18^{b}±0.25	6.53^{a}±0.32	6.13b±0.70

Values are mean ± standard deviation of 3 replication

Table 7. Mean packed cell volume (%) level of rat

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + *V.doniana* extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	45.10a±0.31	45.20a±0.07	45.30a±0.10	45.15a±0.02	45.17a±1.04
7	35.20b±0.14	35.13c±0.16	35.18c±0.30	35.25d±0.58	35.00d±0.54
12	30.00c±0.54	44.85b±0.38	36.28bc±0.43	40.25c±0.22	38.10c±0.33

Values are mean ± standard deviation of 3 replication

4. CONCLUSION

In conclusion, results obtained from the present study indicated that the ethanol leaf extract of *V.doniana*, "ewa" and "uturukpa" possesses anti-anemic potentials and this may be attributed to the phytochemicals, antioxidant and the nutrient content of *V.doniana*, "ewa" and "uturukpa" leaf. The present study, therefore, supports the therapeutic use of the *V.doniana*, "ewa" and "uturukpa" leaf in the traditional medicine for the treatment of anemia. Also, the increase in the haematological parameters observed in rats administered extract from the three host plants suggests that *V.doniana*, "ewa" and "uturukpa" extract contains agents that could stimulate the production of iron, therefore the plant extracts could serve as immune booster.

ETHICAL APPROVAL

All procedures using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria, Nsukka.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Anemia, /Anemia/Anaemia.pdf; 2022.
2. Bejjani S, Pullakhandam R, Punjal R, Nair KM. Gastric digestion of pea ferritin and modulation of its iron bioavailability by ascorbic and phytic acids in caco-2 cells. World Journal of Gastroenterology. 2007; 13:2083–2088.
3. Fattouch S, Caboni P, Coroneo V, Tuberoso C, Angioni A, Dessi S. Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of tunisian pome fruit pulp and peel aqueous acetone extracts. Journal of Agricultural and Food Chemistry. 2008; 56(3):1084-1090.
4. Gonzalez-Aguilar GA, Wang CY Buta, JG. Maintaining quality of fresh-cut mangoes using anti browning agents and modified atmosphere packaging. Journal of Agricultural and Food Chemistry. 2000; 48(9):4204-4208.
5. Ibrahim MA, Nwude N. Plants used in traditional veterinary medical practice in Nigeria. Journal of Veterinary Pharmacology and Therapeutics. 2010; 3(4):261–273.
6. Ogukwe CE, Oguzie EE, Unaegbu C, Okolue BN. Phytochemical Screening on the leaves of *Sansevieria trifasciata*. Journal of Chem. Soc. Nigeria. 2004;29(1): 8–9.
7. Agbafor KN, Nwachukwu N. Phytochemical analysis and antioxidant property of leaf extract of V. doniana and Mucuna pruriens. Biochemistry Research International. 2011;1-6.

8. Dauda BN, Oyeleke SB, Jigam AA, Salihu SO, Balogun MM. Phytochemical and *In-vitro* antibacterial investigation of *Vitex doniana* leaves stem bark and root bark extracts. Australian Journal of Basic and Applied Sciences. 2011;5(7):523-528.
9. Adejumo AA, Alaye SA, Ajagbe RO, Abi EA, Adedokun FT. Nutritional and anti-nutritional composition of black-plum (*Vitex doniana*). Journal of Natural Sciences Research. 2013;3(12):144-148.
10. Ezekwesili CN, Ogbunugafor HA, Ezekwesili-Ofili JO. Antidiabetic activity of aqueous extracts of *Vitex doniana* leaves and cinchona bark in alloxan-induced diabetic rats. International Journal of Tropical Diseases. 2012;2(4):290-300.
11. AOAC. Official methods of analysis. Association of analytical chemists. 18th edition, Washington, D.C. USA. 2010;614-621.
12. Bhandari MR, Kawabata J. Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea Spp*) tubers of Nepal. Food Chem; 2004;88:163-168.
13. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A rodent diet. Journal of Nutrition. 1993;123(11):1939–1951.
14. International Committee for Standardization in Hematology (ICSH). Recommendation for hemoglobinometry in human blood, Br. J. Heamatol. Suppl. 1967;13:17-18.
15. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. J Lab Clin Med. 1989;114(4):368-377.
16. Schalm OW, Jain NC, Carol EJ .Normal values in blood of laboratory fur bearing and miscelloaneous zoo and wild animals, In: O.W.Schalm (Ed.)Vert. Heam, 3rd edition. Lea and Febiger, Philadelphia. 1975;219-283.
17. Coles EH. Veterinary clinical pathology in (1st edition). W.B. Saunders company, Pennsylvania, USA. 1986;25-36.
18. Bhaskaran P. Immunobiology of mild nutrient deficiency. Br. J. Nutr. 2001;85:S75-S80.
Available:http://dx.doi.org/10.1079/BJN2000297
19. Trowbridge F, Martorell M. Forging effective strategies to combat iron deficiency summary and recommendations. Journal of Nutrition. 2002;85:875-880.
20. Dioxon BM, Haris EM. Nigeria food consumption and nutrition survey. 2004; 2001-2003.
21. Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. American Journal of Clinical Nutrition. 1996;63:797S-811S.
22. Asaolu SS, Asaolu MF. Trace metal distribution in Nigerian leafy vegetables. Pakistan Journal of Nutrition. 2010;9(1): 91-92.
23. Ayoola PB, Adeyeye A, Onawumi OO. Trace elements and major minerals evaluation of *Spondias mombin*, *Vernonia amygdalina* and *Momordica charantia* leaves. Pakistan J. Nutrition. 2010;9(8): 755-758.
24. Black R. Mronutrient deficiency: An underlying cause of morbidity and mortality; Bull. World Health Organisation. 2003;8(2):79.
25. Hotz C, Brown KH. International Zinc Nutrition Consultative Group (IZiNCG) technical document No. 1. assessment of the risk of zinc deficiency in populations and options for its Control. Food and Nutrition Bulletin. 2004;25:S94-S203.
26. Nasima A, Raghib A, Abdul WM, Mostafa CGM, Sakhi C, Hai MA. Relationship between zinc and anaemia in chronichaemodialysis patients J Teachers Asso RMC. 2003;16(1):1019-8555.
(6) (PDF) Effect of *Solanum nigrum* methanol leaf extract on phenylhydrazine induced anemia in rats. (Accessed Oct 08 2022).
Available:https://www.researchgate.net/publication/342452597_Effect_of_Solanum_nigrum_Methanol_Leaf_Extract_on_Phenylhydrazine_Induced_Anemia_in_Rats
27. El-Nawawy A, Barakat S, Elwalily T, Abdel-Moneim Deghady A, Hussein M. Evaluation of anemia in severe under nutrition in protein energy malnutrition, East Mediterranean Health J. 2002;8(2-3):281–9.
28. Mephba HD, Eboh L, Banigo EB. Effect of processing treatments on the nutritive compositionand consumer acceptance of some Nigerian edible leafy vegetables. African Journal of Food Agriculture Nutrition and Development. 2007;7(1):23-26. (6) (PDF) Proximate and mineral

composition of Nigerian leafy vegetables. (Accessed Oct 07 2022).
Available:https://www.researchgate.net/publication/272922859_Proximate_and_Mineral_Composition_of_Nigerian_Leafy_Vegetables
29. FNB. Food and Nutrition Board, Institute of medicine: Dietary refrence intakes for calcium, phosphorus, magnesium. Washington DC, National Academy Press; 1997.
30. Shils ME. Magnesium in modern nutrition in health and disease. 9th Ed., edited by Shils ME, Olson JA, Shike M, Ross AC, editor. New York: Lippincott Williams & Wilkin. 1999;169–192.
31. Brody H, Robert M, Bert L, Panja K, Claus L, Olson R. Changes in the red blood cell membrane in protein-calorie malnutrition. Am J Clin Nutri. 2004;31:574-578.
32. Ogbe RJ, Adoga GI, Abu AH. Antianaemic potentials of some extract on phenylhydrazine-induced anaemia in rabbits. J of Medi Plants. 2010;4(8):680-684.
33. Vasudevan M, Sreekumari S. Kannan Vaidyanathan. Bichemistry textbook, Reprint: 2008; Sixth Edition: 2007; 2011. ISBN: 978-93-5025-016-7.
34. Agoreyo FO, Nwanze N. Plasma sodium and potassium changes in sickle cell patients. Inter J Genetics and Mol Biol. 2010;2(2):14-19.
35. Turaskar A. Inhibitory potential of picrorrhiza kurroa royle ex. extract on phenyhydrazine induced reticulocytosis in rats. J Pharma Clin Res. 2010;6(2):215-216.
36. Vamsee VA. Comparative anti anaemic activity of *Murraya koenigii* (inn.) leaves and its combination with *Emblica officinalis* in aluminum chloride induced anaemia using rodents. Inter J Advance Res Med Sci. 2014;1(1):15-24.
37. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and mineral nutrition information system, 1993–2005. Public Health Nutr. 1993;12:444–454.
[PubMed] [Google Scholar]
38. Okonkwo Christopher C, Agu Chidozie Victor, Njoku Obioma U, Abonyi Uchenna, Apeh Victor, Anaduaka Emeka G, Iloabuchi Kenechukwu V, Odo Christian E. Hypoglycaemic and haematinic properties of ethanol leaf extract of artocarpus heterophyllusi in alloxan induced diabetic rats. Afr J Tradit Complement Altern Med. 2015;12(2):144-148
Abvailible:http://dx.doi.org/10.4314/ajtcam.v12i2.21
39. Chernecky C, Berger BJ (eds) Laboratory tests and diagnostic procedures. 3rd Ed. Philadelphia, PA, Saunders. 2001;372–376.
40. Asuquo OR, Ekanem TB, Udoh PB, Eluwa MA. Histomorphological study of the antifertility effect of *Spondias mombin L*. in adult male rats. J Pharm Biol Sci. 2012;3(2):29-34.
(6) (PDF) Haematinic potential of *Spondias mombin* leaf extract in wistar rats. (Accessed Oct 08 2022].
Available:https://www.researchgate.net/publication/289790674_HAEMATINIC_POTENTIAL_OF_SPONDIAS_MOMBIN_LEAF_EXTRACT_IN_WISTAR_RATS
41. Ejoh AR, Tchouanguep MF, Fokou E. Nutrient composition of the leaves and flowers of *Colocasia esculenta* and the fruits of *Solanum melongena*. Plant Food for Human Nutrition. 1996;49:107-112,.
42. Asaolu SS, Adefemi OS, Oyakilome IG, Ajibulu KE, Asaolu MF. Proximate and mineral composition of Nigerian leafy vegetables. Journal of Food Research. 2012;3:214-218,
43. Vamsee VA, Jyothi Y, Rina P, Rajdwip G, Ronak P. Vijay. Comparative anti anemic activity of Azadirachta indica leaves and its combination with Emblica officinalis in phenylhydrazine induced anemia using rats. J Chem Pharm Res. 2015;7(8):1019-1022.
44. Airaodion AI, Ogbuagu U, Ogbuagu EO, Ekenjoku JA, Airaodion EO. Protective effect of ethanolic leaf extract of Moringa oleifera on haematological indices of rats fed with crude oiltreated diet. International Journal; 2019.
45. Swarna J, Ravindhran R. Pharmacognostical and phytochemical evaluation of *Talinum triangulare* (Jacq.) willd. International. Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(2): 249–256.
46. Liao YH, Houghton PJ, Hoult JR. Novel and know constituents from Buddleja species and their activity against leukocyte eicosanoid generation. J. Nat. Prod. 1999; 62:1241–1245.
47. Airaodion AI, Adekale OA, Airaodion EO, Ogbuagu EO, Uloaku Ogbuagu U, Osemwowa EU. Efficacy of combined

crude extract of *Curcuma longa* and *Moringa oleifera* in the prevention of peptic ulcer in albino rats. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019;7(2):1-8.

48. Airaodion AI, Olayeri IM, Ewa AO, Ogbuagu EO, Ogbuagu U, Akinmolayan JD, Agunbiade AP, Oloruntoba AP, Airaodion EO, Adeniji AR, Obajimi OO, Awosanya OO. Evaluation of *Moringa oleifera Leaf* potential in the prevention of peptic ulcer in wistar rats. International Journal of Research. 2019;6(2):579-584.
49. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(Suppl. 1):S62–S69.

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