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Trace Element Composition and Anti-anaemic Effects of the Ethanol Extract of *Harungana madagascariensis* Lam. Ex Poiret. in Phenyl Hydrazine Induced Anaemia in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAS designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author ACM managed the analyses of the study. Author OEA managed the plant identification. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the acute toxicity, a phytochemical constituent of the ethanol extract of the fruit of *Harungana madagascariensis*, as well as its trace element composition and anti-anaemic effects.
Materials and Methods: Lorke's method was used to evaluate acute toxicity and energy dispersive x-ray fluorescence (EDXRF) spectrometer for elemental composition analysis. Thirty-six albino rats were divided into 6 groups of 6 animals each. Anaemia was induced by intraperitoneal administration of phenyl hydrazine hydrochloride (80 mg/kg) in 5 groups while the 6th group served as non-anaemic group (normal control). Administration of extract was done for 21 consecutive days orally at doses of 250, 500 and 1000 mg/kg respectively to groups I, II and III, group IV received

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vitamin B_{12} while group V served as the negative control. Haematological parameters (PCV, HB and RBC) were evaluated.

Results: Phytochemical screening revealed the presence of flavonoids, tannins, saponins and free anthraquinones. The extract contains sodium, iron and zinc in proportions of 135.09 ppm, 15.52 ppm and12.68 ppm respectively as well as copper, chromium and other elements.

The results showed that the extract at 500 mg/kg significantly ($P<0.05$) increased the PCV, Hb and RBC (55.33%, 18.47 g/dl and 6.17 $\times 10^{12}$) more than the 1000 mg/kg dose when compared to the controls.

Conclusion: The findings showed that this extract possesses anti-anaemic activity which may be attributed to its phytochemical constituents and trace elemental composition.

Keywords: Harungana madagascariensis; anaemia; phenyl hydrazine; trace; element.

1. INTRODUCTION

Anaemia is not a disease disorder but is rather a condition that results from a number of different pathologies. World Health Organisation defines anaemia in adults as haemoglobin less than 13g/dl for males and less than 12 g/dl for females. Anaemia can also be defined as a decrease in the red blood cell mass of the blood. Red blood cells help to deliver oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs and they carry out this function by using haemoglobin. Hence anaemia impairs the body's ability for gaseous exchange by decreasing the number of red blood cells available for transporting oxygen and carbon dioxide [1].Anaemia is a public health problem that affects populations in both developed and under developed countries. Its primary causes include iron deficiency, vitamin B_{12} deficiency, folate deficiency and other conditions such as malaria, parasitic infection, inherited disorder and haemoglobinopathies [2]. Anaemia in older adults can be attributed to renal failure and chronic inflammation. About one-third of anaemia is unexplained [3]. The most important complications of severe anaemia arise from tissue hypoxia, shock, hypotension and coronary or pulmonary insufficiency. This is most commonly seen in older individuals with the underlying pulmonary disease and cardiovascular disease [1]. Trace element deficiency and anaemia are major problems, especially amongst children and women with low resources. .Anaemia due to iron deficiency may be complicated by a deficiency of one or more additional micro nutrients. Trace elements such as selenium, zinc and copper are essential nutrients that have regulatory, immunological and anti-oxidant functions due to their role as essential components or cofactor of an enzyme involved in metabolism [4]. A typical example of the economic benefits of research into the nutritional significance of trace elements is the discovery of the importance of cobalt for ruminant animals. Extension of this discovery leads to the synthesis of vitamin B_{12} from cobalt which resulted in similar benefits for human health, notably the conquest of pernicious anaemia [5].

Application of the knowledge of trace elements nutrition to human health problem depends on a clear understanding of the events that link molecular and biochemical mechanisms to clinical manifestations of deficiency [6].Trace elements are often suspected of being the missing link in some of the unexplained human diseases such as atherosclerosis, hypertension and ischemic heart disease [7]. Anaemia is very common and its incidence is on the increase, hence the need to prevent or seek cost-effective and better treatment [8]. The unopened buds of *Harungana madagascariensis* are used traditionally in the treatment of anaemia and skin diseases in Nigeria [9]. This study evaluated the ethanol extract of *H. madagascariensis* fruit for its anti-anaemic and trace elemental composition.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh fruit of *H. madagascariensis* was collected from Aluu community around the University of Port Harcourt. The plant sample was authenticated Dr Chimezie Ekeke of the Botany department of the University of Port Harcourt with herbarium number UPH/V/1,219.

2.2 Preparation of Extract

The fruit of *H. madagascariensis* was dried under shade for about 3 weeks. The air-dried fruit material was reduced to powder form

mechanically and macerated for 72 hours using absolute ethanol. The rotary evaporator was used to concentrate the extract after which it was evaporated to dryness over a water bath at a temperature of 45°C and stored in a refrigerator.

2.3 Animals Used

Albino rats of the average weight of 170 g of both sexes were obtained from the animal house of the Department of Pharmacology, Faculty of the Pharmaceutical Sciences University of Port Harcourt. The animals were maintained at room temperature for two weeks in the animal house to acclimatize. The animals were housed in six cages each containing five animals and were fed with standard diet and water *ad libitum.* Ethics approval was obtained from the Research ethics committee of the University of Port Harcourt.

2.4 Drugs and Chemical Used

Absolute ethanol 95% (JHD, Guangdong Guanghua Shanton china Sci-Tech Company Ltd). Phenyl hydrazine hydrochloride (J.T. Baker; A division of Mallinckrodt Baker, Inc. USA). HB_{12} ®syrup (Ecomed Nigeria Ltd under license from Krispine Laboratories, England).

2.5 Phytochemical Screening

Phytochemical studies were carried out on the ethanol extract of the fruit of *H. madagascariensis* for the presence of biologically active constituents. All procedures used were taken from Harborne [10].

2.6 Acute Toxicity Evaluation

This was done according to Lorke [11] method.

2.7 Evaluation of Anti-Anaemic Activity

The method of [12] was adopted and modified. The rats were divided into 6 groups of 6 animals each. Anaemia was induced by intraperitoneal administration of 80 mg/kg phenyl hydrazine hydrochloride into all the rats except group VI. Group I, II and III animals was treated with the extract at doses of 250,500 and 1000 mg/kg respectively. Group IV was administered with vitamin B_{12} syrup (positive control) while group V was given deionized water and served as the negative control. Group VI served as a normal and untreated group in which anaemia was not induced (non-anaemic). On day 0, blood samples were obtained in all the groups for PCV, HB and RBC before the induction of anaemia. Anaemia was allowed to establish within 24 hours after which blood samples were withdrawn and taken as day 1. Treatment commenced after this and all administrations were done through the oral route for 21 consecutive days. Blood was collected from the tail tip of the animals on days 0, 1,7,14 and 21 for the measurement of PCV, HB and RBC.

2.8 Haematological Evaluation

All haematological evaluations were carried out according to the protocols of [13].

2.8.1 Packed cell volume (PCV)

Anticoagulated blood was aspirated into a capillary tube and the end sealed with plasticine. The blood was centrifuged in a haematocrit centrifuge for 5 minutes at 10000 RP and read with haematocrit reader in percentage.

2.8.2 Haemoglobin (Hb) cyanomethaemoglobin method

0.02ml of blood was added to 5ml of Drabkin's solution, mixed and allowed to stand for 10minutes for full-colour development. The mixture was read in a colourimeter at 546nm against a blank Drabkins solution.

Calculation=Absorbance of test/Absorbance of standard x concentration of standard (g/dl)

2.8.3 Red blood cell (RBC)

A 1:200 dilution of blood in red blood cell dilution fluid was mixed and allowed to stand for 5 minutes. The suspension was mixed and used to fill the counting chamber with a fixed coverslip. The red blood cells were read and counted from x10magnification. Reading included the middle 16 squares

Readings = cells x 10^{12}/L

2.9 Elemental Composition Analysis

The elemental analysis of the plant material was performed by an energy dispersive x-ray fluorescence (EDXRF) spectrometer. The Spectrometer consisted of a Siemens FKO-04 tube with Mo anode, a Kristalloflex 710H x-ray

generator installed in the centre for Energy research and development, Obafemi Awolowo University, Ile-Ife. The equipment is operated under QXAS (Quantitative X-ray Analysis System) software (QXAS, Manual 1993).The sample and standard formulated pellet was irradiated for 20 minutes in a fixed tube at 30 kv and 10 Ma. The sample was analysed before the standard. All the values obtained are results of an average of three measurements on the plant extract [12].

2.10 Statistical Analysis

The results are presented as mean± SEM. Statistical analysis of data was done using Microsoft office excel 2013 [14]. Student's t-test was done to determine the significance of the difference between the control groups and the treated groups. Values of $P<0.05$ were considered statistically significant.

4. RESULTS

4.1 Acute Toxicity Result

From the oral acute toxicity test, it was observed that the lethal dose (LD_{50}) of the ethanol extract of the fruit of *Harungana madagascariensis* is greater than 5000 mg/kg since no death was recorded. This implies that the fruit of the plant is relatively safe since its LD_{50} is greater than 5000 mg/kg [11]. Restlessness was observed in the group of albino rats that received 10,100, and 1000 mg/kg.

4.2 Phytochemical Screening of the Ethanol Fruit Extract of *H. madagascariensis.*

Table 1. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, carbohydrates and anthraquinones were revealed while phlobatannins was absent

Chemical constituent	Observation
Alkaloids	+
Anthraquinones(free)	+
Steroid/triterpenoids	+
Carbohydrate	+
Saponins	+
Tannins	+
Flavonoids	+
Phlobatannins	-

+=present, - =absent

Table 2. Trace element composition of the ethanol fruit extract of *H. madagascariensis* Iron, zinc, sodium and copper were found to occur in higher proportions when compared with other elements

Element	Amount
Calcium	0.06%
Magnesium	0.05%
Potassium	0.71%
Sodium	135.09ppm
Manganese	0.68ppm
Iron	15.52ppm
Copper	2.84ppm
Zinc	12.68ppm
Nickel	0.12ppm
Cadmium	0.11ppm
Chromium	0.37ppm

5. DISCUSSION

Iron, zinc and manganese are important co-factors for enzyme activities. Deficiency of any of these elements may be detrimental to both plants and animals. Therefore accurate quantitative analysis of the elemental content of plant is very important, as a contribution to increasing concern about their potential effect on human health [15]. Phytochemical screening of the H. madagascariensis fruit extract revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones and steroids. Alkaloids and saponins have been reported to possess anti-anaemic potentials [16]. Saponins inhibit platelet aggregation or thrombosis while vitalizing and promoting blood circulation [17,18]. Saponins are membrane active agents that lyse red blood cells, this effect can be overcome by producing a glycosidic enzyme that cleaves some of the terminal sugar of the saponin thereby detoxifying it [19]. This detoxification enhanced proper utilization of iron contained in the extract to synthesize haemoglobin for new red blood cells. It can, therefore, be said that the anti-anaemic potential of the ethanol fruit extract of *Harungana madagascarienses* can be attributed to its saponin content. Trace elemental analysis of the fruit extract revealed the presence of calcium, magnesium, potassium, sodium, manganese, iron, copper, zinc, nickel, cadmium and chromium. The elements occurred in different proportions, most significant were sodium, iron and zinc in proportions of 135.09 ppm, 15.52 ppm and 12.68 ppm respectively. This can be corroborated with the report of [20], which stated that the prominent iron content of

35.69 and 35.21 mg/100 g found in the root bark of *Bridelia cathartic* and *Lannea stuhlmannii,* respectively were responsible for their use in the treatment of anaemia in East Africa. Phenyl hydrazine was administered intraperitoneally to induce anaemia as it has been reported to induce haemolytic anaemia [21]. Plant extract at 500 mg/kg significantly ($P<0.05$) increased the packed cell volume, haemoglobin and red blood cell (55.33%, 18.47 g/dl and 6.17 $\times 10^{12}$) when compared to the controls and did so to a greater degree than 1000 mg/kg dose but the increase was non-dose dependent. The findings of this study can be correlated with the reports of [22] that the aqueous extract of *Spinacia oleracea* leaf increased the PCV, Hb, RBC in phenylhydrazine anaemic induced rats and that of [23], which reported an increase in PCV, Hb, RBC, MCV and MCHC in phenylhydrazine-induced anaemic rabbits treated with *Jatropha tanjorensis* Ellis & Saroja.

Fruit extract of *Harungana madagascriensis* possesses anti-anaemic activity which may be due to its phytochemical constituents such as saponins, alkaloids and flavonoids. This may be corroborated by the report of [24] that the beetroot extract raised the level of haemoglobin and erythrocyte and attributed this activity to its vitamin and mineral constituents. *H. madagascariensis* anti-anaemic activity may also be attributed to the presence of elements such as iron (necessary for the build-up of red corpuscles), zinc (formation of haemoglobin), manganese (necessary for growth) and copper which aids the absorption of iron.

Table 3. Effects of ethanol fruit extract of *H. madagascariensis* on packed cell volume of phenyl hydrazine induced albino rats in days. (Mean±SEM). There was no statistically significant (*P*=0.05) difference between the 250mg/kg extract treated group and the controls throughout the study. The 500 mg/kg extract concentration showed a significantly higher increase in PCV than the controls on days 14 and 21 while the 1000 mg/kg extract concentration indicated no significant increase in PCV on days 7 and 14 than the controls

Treatment	Dose	Packed cell volume (%) in days				
	(mg/kg)	0	1	7	14	21
Extract	250	40.80±0.58	28.20±1.71	41.80±2.33	42.75±1.93	47.50±1.55
	500	40.40±2.46	34.00±4.97	37.75±3.42	52.50±1.44*	55.33±0.67*
	1000	41.00±1.58	26.00±2.00*	33.33±7.26*	37.50±5.50*	45.50±0.50
Vitamin B 12	100µg	37.20±0.92	31.40±6.39	43.00±1.08	38.00±5.73	38.00±5.73
Anaemic untreated (Distilled water)	2ml	43.80±2.20	30.33±1.86	41.67±2.85	38.67±2.33	48.00±3.21
Normal control	2ml	40.60±2.14	31.20±3.67	38.60±3.75	51.40±4.12	44.40±2.84

**P<0.05, n=5*

Table 4. Effect of ethanol fruit extract of *Harungana madagascariensis* on the haemoglobin level of phenyl hydrazine the albino rats in days. (Mean±SEM). There was a statistically significant (*P*=0.05) increase in haemoglobin level at 500 mg/kg dose of the extract on days 14 and 21 when compared to the controls

Treatments	Dose	Haemoglobin (g/dl) in days				
	(mg/kg)	0	1	7	14	21
Extract	250	13.60±0.20	9.38±0.59	14.96±1.15	14.25±0.65	15.88±0.55
	500	13.48±0.82	11.35±1.77	12.58±1.15	17.53±0.50*	18.47±0.24*
	1000	13.60±0.68	8.67±0.67*	10.57±1.99*	12.75±2.05*	15.15±0.15
Vitamin B12	100 µg	12.36±0.29	10.52±0.94	14.35±0.37	14.30±2.47	14.30±2.47
Anaemic untreated (Distilled water)	2 ml	14.62±0.76	10.10±0.56	13.93±0.79	13.23±0.79	16.10±1.08
Normal control	2 ml	14.74±1.19	10.40±1.22	12.88±0.25	18.00±2.20	14.62±0.84

**P<0.05, n=5*

Table 5. Effect of ethanol fruit of *Harungana madagascariensis* on red blood cell count of phenylhydrazine-induced albino rats in days. (Mean±SEM).The extract showed a statistically significant (*P*=0.05) difference in red blood cell values at 500 and1000mg/kg on days 21 and 14 respectively when compared to the controls

Treatment	Dose (mg/kg)	Red blood cell count (10^{x12}) in days				
		0	1	7	14	21
Extract	250	4.50±0.05	3.16±0.19	4.70±0.26	4.75±0.18	5.30±0.18
	500	4.50±0.60	3.90±0.58	4.23±0.36	5.83±0.15	6.17±0.09*
	1000	4.68±0.23	2.93±0.23*	3.63±0.65*	4.20±0.60*	5.10±0.10
Vitamin B12	100 µ	4.00±0.32	3.50±0.32	4.78±0.11	4.80±0.83	5.13±0.22
Anaemic untreated (Distilled water)	2 ml	4.48±0.37	3.40±0.20	4.70±0.31	4.33±0.26	5.33±0.35
Normal control	2 ml	4.56±0.21	3.58±0.39	4.38±0.07	5.74±0.42	4.90±0.30

**P<0.05, n=5*

6. CONCLUSION

The ethanol extract of the fruit of *Harungana madagascariensis* possesses anti-anaemic activity. Further pharmacological studies will be carried out through fractionation of the extract with different solvents to identify where the activity lies and the active constituents will be isolated and characterized.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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