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Chemical Composition and Evaluation of Methanol Leaf Extract of *Combretum dolichopetalum* on Body Weights and Haematological Indices of Phenylhydrazine Induced-anaemic Rats

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Abstract

Anaemia is a serious health challenge in developing countries. This study evaluated the chemical composition of *Combretum dolichopetalum* (CD) leaves, the effect of its methanol extract on the body weights and haematological indices of phenylhydrazine induced-anaemic rats. Fresh matured leaves of CD were collected, identified and authenticated. Phytochemicals, proximate compositions, mineral elements, anti-nutritional factors and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were determined. A total number of twenty (20) mature inbred apparently healthy male albino rats of the Wistar strain (100-150 g) were used for this study. Anaemia was induced by administering phenylhydrazine to rats in Groups 2, 3 and 4 comprising of five rats per group. Group 1 (non-anaemic control) (n = 5) and Group 2 (anaemic control) received distilled water respectively while Groups 3 and 4 were administered 200 and 400 mg/kg of CD leaf extract orally using oropharyngeal cannula once per day for 28 days. Blood samples were obtained for haematological analysis using standard methods. The result showed a significant increase in Red Blood Cell (RBC), Packed Cell Volume (PCV) and haemoglobin concentration in Groups 3 and 4 compared with the anaemic group. Chemical analysis showed the presence of some phytochemicals, proximate compositions, mineral elements and anti-nutritional factors. GC-MS analysis showed the presence of nine (9) compounds. This study indicates that CD is nutritionally rich and contains some important bioactive principles that support its anti-anaemic properties.

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Keywords: Anaemia, *Combretum dolichopetalum*, Haematological Parameters, Mineral Elements, Phytochemicals, Proximate Compositions

1. Introduction

Anaemia has been defined as a reduction in the oxygen-carrying throughput of blood[1] and it results from a deficiency of haemoglobin in the blood. This condition is appertaining to a decrease in haemoglobin level (<13 g/dL for males and <12 g/dL for females)[2]. The ubiquity of anaemia was superlative in South Asia, Central and West Africa[3]. Anaemia is ignored in most developing countries like Nigeria, even though it is of utmost prevalent public health conditions[4]. Studies have shown that it affects the whole being of adults, children and pose risk to pregnant women and affect neonatal growth and development[3]. Medicinal plants and their products have increasingly been found to be important as complementary/alternative medicine in most countries especially the developing ones due perhaps to the fact that they are cheap, easily available and effective[5]. Studies have shown that some medicinal plants are traditionally used to treat and manage anaemia. They include *Telfeira occidentalis, Psorospermum ferbrifugum, Jatropha curcas, Flacourtia flavenscens, Brillantasia nitens and Hibiscus sabdariffa*[6,7].

Combretum dolichopetalum is a plant that is indigenous to Africa and which is commonly found in the Eastern part of Nigeria. It is known as "achichanza" (food of the sunbird) in Igbo land and "okoso" in the Edo State of Nigeria[8]. The plant serves as food and they have different medicinal importance. As for food, the leaves are cooked and the content turns red which is prepared as soup for drinking[9]. Some folkloric uses include wound healing, antiulcer and antidiarrhea activity[10,11]. We had earlier studied the acute and sub-acute toxicity studies on *Combretum dolichopetalum* leaves in experimental animals[12]. Also, there are claims that this plant is used traditionally to manage and treat anaemia although its scientific basis has not been documented. Phytochemicals are plant-derived chemicals that confer different biological properties to plants[13]. In addition to their biological properties, these bioactive ingredients have been seen to be relevant in the pharmaceutical companies for the production of drugs[13]. Gas Chromatography-Mass Spectrometry (GC-MS) analysis provides a reliable and reproducible analytical protocol for authentication, quantitation and characterization of bioactive principles from herbal extracts[14].

In the traditional usage of *C. dolichopetalum* leaves in Nigerian and African ethnomedicine for the treatment of different ailments, practitioners often use different polar solvent extracts of the plant to prepare their traditional medicines. However, information on the bioactive constituents that confer this plant with its pharmacological potentials is scarce in literature and holistic study on the nutritional composition of this plant is also limiting in literature. As a step to validate the claim on the usage of this plant in the treatment of anaemia, the present study investigated the effect of *C. dolichopetalum* leaves on body weights and haematological indices of phenyl hydrazine-induced anaemic rats and also determined the chemical composition of the plant.

2. Material and Methods

2.1 Plant Materials

Fresh matured leaves of *C. dolichopetalum* were located and collected in 2017 from its natural habitat in Nsukka, Enugu. The plant samples were authenticated by Mr. C. J. Onyeukwu, a taxonomist of the Plant Science and Biotechnology Department of the University and the voucher specimen (UNH No.49a) of the plant was deposited at the herbarium.

2.2 Preparation of Extract

The leaves were washed and air-dried at room temperature for 7 days, thereafter ground to a coarse powder using an electric blender (model ms-233, China). About one gram (1 g) of the powder was used to determine the phytochemicals, proximate and mineral components, the remaining quantities of the powder (2 kg) were extracted for 48 h with methanol in a Soxhlet extraction following the method of Jensen[15]. Following the extraction, the extract was collected and dried at low temperature (40^{0}C) to obtain a pasty dark-green extract which was used for animal experiments and GC-MS analysis.

2.3 Phytochemical Analysis

The alkaloid was determined using the method of Harbone[16]. Tannins and saponins were measured using the method of Association of Official Analytical Chemists (AOAC)[17]. Flavonoids were analyzed using the method of Bohamand Kocipai[18]. The method of Sofowora[19] was used for the determination of cardiac glycosides. Terpenoids were assayed following the method of Ferguson[20]. Phytates was analyzed using the method of Thompson and Erdman[21]. The determination of total oxalate was performed using

the method of Nile and Park[22]. The hydrogen cyanide was measured using the method of Onwuka[23].

2.4 Proximate Analysis

Moisture, ash, protein, lipid, and crude fibre were measured using the method of AOAC[17] while total carbohydrate content was calculated.

2.5 Mineral Analysis

An Atomic Absorption Spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used to analyze the Calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu), Lead (Pb) and Iron (Fe) contents of the flours; the flame photometric method was employed for the analysis of sodium and potassium[20]; the molybdate method[23] was adopted to analyze phosphorous while the method of AOAC[17] was employed to measure Manganese.

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Characterization of the bioactive constituents of the *C. dolichopetalum* extract was done using GC-MS QP2010 Plus (Shimadzu, Japan). The methods of Kalu *et al.*[24] and Otuokere *et al.*[25] were adopted.

2.7 Animal Experiments

Twenty (20) mature inbred apparently healthy male albino rats of the Wistar strain (100-150 g) were procured from the Animal House, Department of Physiology, University of Nigeria Enugu Campus. The rats were acclimatized to their feed (Vital feed, Nigeria) and water (which they had access to *ad libitum*) for two weeks before the commencement of the experiment. The study protocol was approved by the College of Medicine Research Ethics Committee of the University with protocol number 026/02/2017. The study was carried out under the established institutional guidelines and the NIH guidelines on the use of experimental animals.

2.8 Induction of Anaemia

Freshly prepared phenylhydrazine hydrochloride solution (prepared in 0.1 M potassium phosphate buffer, pH 7.4) was injected intraperitoneally to fifteen of the rats at a dosage of 80 mg/kg body after an overnight fast while the remaining five rats were taken as the non-anaemic group. After 48 h, blood was collected from the tail vein and Packed Cell Volume (PCV) was analyzed using the procedure described by Ochei and Kolhatkar[26] before the commencement of the extract administration. The phenylhydrazine-treated rats with PCV < 30% was considered to be anaemic and was used for the study.

2.9 Experimental Procedure

Phenylhydrazine-treated rats with the stable anaemic condition were randomly divided into three subgroups (Groups 2, 3 and 4) comprising of five rats per group while the non-anaemic group formed the first group as follows:

Group 1: Non-anaemic control received normal rat chow and distilled water,
Group 2: Rats which also received distilled water,
Group 3: Anaemic rats received 200 mg/kg body weight of *C. dolichopetalum* extract.
Group 4: Anaemic rats received 400 mg/kg body weight of *C. dolichopetalum* extract.
The body weights of the rats were recorded. All administration was done orally using an oropharyngeal cannula once per day for 28 d, after which the rats were anaesthetized with 2% sodium pentobarbital (75 mg/kg) intraperitoneally. Venous blood was obtained via the orbital and poured into EDTA tubes for haematological studies. The doses of the extracts that were administered were chosen following acute toxicity testing[12] that showed the extract not to be toxic even at 5000 mg/kg body weight.

2.10 Haematological Parameters

Red Blood Cell count (RBC), Packed Cell Volume (PCV), Haemoglobin concentration (HGB), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), White Blood Cell count (WBC), and Platelets Count (PLT) were assayed for using Coulter Ac-T 5Diff AL, (Beckman Coulter, Inc. Port Matilda, Pennsylvania, USA).

2.11 Statistical Analysis

Results were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). One-way ANOVA following Turkey post hoc test was used for data comparison and values were regarded as significant at $p<0.05$. Results were expressed as mean ± Standard Error of the Mean (SEM).

3. Results

The result revealed the presence of phytochemical contents of the extract. They include; 1.72% alkaloid, 0.14% tannin, 2.67% saponin, 2.03% flavonoid, 0.66% glycosides, 0.46% terpenoids, 3.17% phytate, 0.66% hydrogen cyanide and 8.22% oxalate respectively. The proximate composition of *C. dolichopetalum* showed that the plant contained: 92.68% dry matter, 13.51% protein, 8.81% lipid, 7.32% moisture, 7.13% crude fibre, 3.05% ash and 60.41% carbohydrate.
Table 1 showed the mineral composition of *C. dolichopetalum* reported in mg/100 g. Result showed it contained 12.81

mg/100 g phosphorous, 752.55 mg/100 g calcium, 109.65 mg/100 g magnesium, 152.34 mg/100 g potassium, 56.74 mg/100 g sodium, 6.61 mg/100 g zinc, 18.47 mg/100 g iron, 5.9 mg/100 g manganese, 0.11 mg/100 g lead and 0.37 mg/100 g Cu.

Table 1. Mineral contents of *C. dolichopetalum*

Mineral	Composition (mg/100g)
Phosphorus	12.81 ± 0.012
Calcium	752.55 ± 0.012
Magnesium	109.65 ± 0.012
Potassium	152.34 ± 0.012
Sodium	56.74 ± 0.012
Zinc	6.61 ± 0.012
Iron	18.47 ± 0.012
Manganese	5.9 ± 0.012
Lead	0.11 ± 0.012
Copper	0.37 ± 0.017

Values in the table are reported as means ± SEM of triplicate experiments

GC-MS chromatogram of *C. dolichopetalum* indicated presence of nine compounds (Table 2) and their structures (Figure 1). The identified compounds had their percentage occurrence as: 3-fluoro-5-[1-hydroxy-2-(methylamino) ethyl]phenol (3.577%), dextroamphetamine (3.607%), (R)-(-)-2-Amino-1-Propanol (4.226%), 2-(methylamino) ethanesulfonic acid (N-methyltaurine) (4.407%), 2-(aziridine-1-yl) ethanamine (4.033%) and bis(2-methylpropyl) benzene-1,2-dicarboxylate (35.062%), 1-Cyno-[2-(2-Phenyl-1,3-Dioxolan-2-yl) ethyl] pentyl ester (4.273%), 1-Guanidinosuccinimide (11.350%) and 1-(4-methylphenyl)propan-2-amine (aptrol) (3.696%).

There were no significant differences ($p>0.05$) in the initial body weights in the respective groups. In contrast, the final body weights of Group 2 rats (anaemic control) (that recorded 0.70% increase in body weight) were significantly decreased when compared with Group 1 (that recorded 15.89% increase in weight). The effect of administering *C. dolichopetalum* to rats in Groups 3 and 4 resulted in significant increases in their body weights (13.19 and 19.13% increases respectively) compared with Group 2 rats.

Table 2. Bioactive constituents of *C. dolichopetalum* identified using GC-MS

S/N	Retention time (min)	Peak area (%)	Molecular weight (g/mol)	Chemical Formulae	Chemical Compound	%Content
1	2.279	44.0	185.0	$C_9H_{12}FNO_2$	3-fluoro-5[1-hydroxy-2-(methylamino) ethyl] phenol	3.577
2.	4.569	44.0	135.0	$C_9H_{13}N$	Dextroamphetamine	3.607
3.	5.895	44.0	75.0	C_3H_9NO	(R)-(-)-2-Amino -1-Propanol	4.226
4.	7.575	44.0	139.0	$C_3H_9NO_3S$	2- (methylamino) ethanesulfonic acid(N-methyltaurine)	4.407
5.	8.623	44.0	86.0	$C_4H_{10}N_2$	2- (aziridine-1-yl) ethanamine	4.033
6.	9.168	149.0	278.0	$C_{16}H_{22}O_4$	Bis(2-methylpropyl) benzene-1,2-dicarboxylate	35.062
7.	9.265	149.0	347.0	$C_{19}H_{25}NO_5$	1-Cyno - [2-(2-Phenyl -1,3-Dioxolan-2-yl) ethyl] pentyl ester	4.273
8.	10.773	44.0	141.0	$C_5H_7N_3O_2$	1-Guanidinosuccinimide	11.350
9.	14.560	44.0	149.0	$C_{10}H_{15}N$	1-(4-methylphenyl) propan-2-amine (aptrol)	3.696

Table 3. Body weights of rats

Groups	Initial Weight (g)	Final weight (g)	% weight difference
Group 1	136.0 ± 9.64	161.7 ± 16.50	15.89
Group 2	142.0 ± 1.29	143.0 ± 3.35[a]	0.70
Group 3	158.0 ± 4.54	182.0 ± 22.20[b]	13.19
Group 4	186.0 ± 11.60	230.0 ± 8.89[b]	19.13

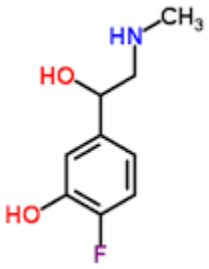
Values are mean ± standard error of the mean (SEM). [a] = p< 0.05 versus normal control (group 1); [b] = p<0.05 versus anaemic control (Group 2).

There were significant decreases (p<0.05) in RBC, PCV and haemoglobin concentration of Group 2 rats when compared to Group 1 but no significant changes (p>0.05) in WBC, MCV, MCH, MCHC and platelet concentrations of Group 2 rats compared to Group 1. The effect of administering *C. dolichopetalum* extract at 200 and 400 mg/kg bwt to the anaemic rats of Groups 3 and 4 significantly increased (p<0.05) their RBC, PCV and haemoglobin concentrations of the rats when compared to the control groups. The effect of administering *C. dolichopetalum* extract did not significantly affect their WBC, MCV, MCH, MCHC and platelet concentrations compared to the anaemic group.

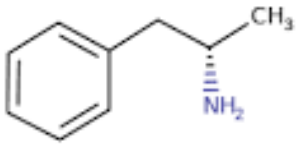
Table 4. Haematological profile of rats

Parameters	Group 1	Group 2	Group 3	Group 4
WBC X 10^9/L	6.20 ± 0.71	5.05 ± 0.83	5.90 ± 0.19	5.75 ± 0.90
RBC X 10^{12}/L	5.03 ± 0.26	4.83 ± 0.07[a]	5.34 ± 0.20[b]	6.18 ± 0.18[ab]
PCV (L/L)	30.20 ± 1.58	26.40 ± 0.50[a]	42.70 ± 0.66[ab]	44.4 ± 1.48[ab]
HGB (g/dL)	10.20 ± 0.47	8.60 ± 0.10 [a]	14.40 ± 0.37[b]	14.80 ± 0.51[b]
MCV (fL)	58.60 ± 1.02	66.30 ± 0.69	63.40 ± 2.83	66.60 ± 0.84
MCH (pg)	21.80 ± 0.34	23.70 ± 0.15	22.60 ± 0.80	23.60 ± 0.20
MCHC (g/dl)	36.10 ± 0.47	35.90 ± 0.48	35.60 ± 0.35	35.40 ± 0.18
PLT X 10^9/L	701.00 ± 147	893.00 ± 21.30	860.00 ± 100	870.00 ± 150

Values are mean ± standard error of the mean (SEM). [a] = p< 0.05 versus normal control (group 1); [b] = p<0.05 versus anaemic control (Group 2).



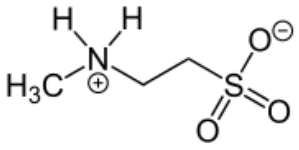
(1). 3-fluoro-5[1-hydroxy-2-(methylamino) ethyl] phenol



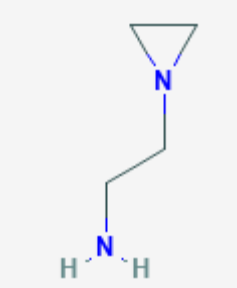
(2). Dextroamphetamine



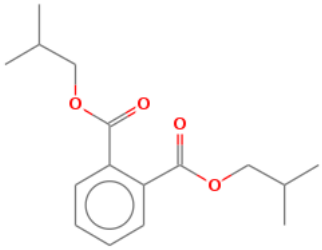
(3). Amino propanol



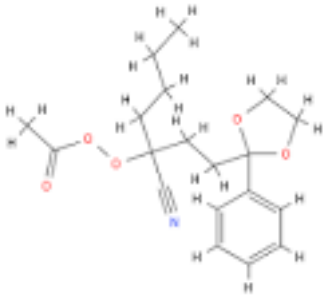
(4). N-Methyltaurine



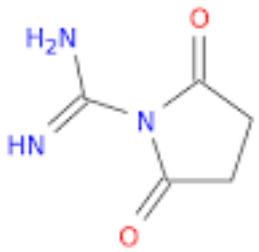
(5). N- β (aminoethyl) ethylenimine



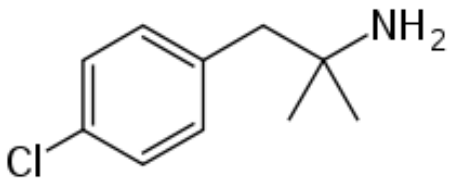
(6). 1, 2- benezenedicarboxylic acid, bis (2- methylpropyl) ester



(7). Ethaneperoxic acid, 1-Cyno-1, Chlorophenethylamine



(8). I-Guanidinosuccinimide



(9). [2-(2-phenyl-1, 3-dioxolan-2-yl) pentyl ester

Figure 1. Structures of compounds identified with GC-MS.

4. Discussion

Alkaloids serve as basic medicinal agents due to their anti-anaemic, analgesic, antispasmodic and anti-bacterial properties[13,27,28]. Tannins are water-soluble high molecular weight phenolic compounds that are found in many plants and which confer on these plants, wound healing properties[13,29]. Saponins inhibit the reabsorption of cholesterol, facilitating its excretion from the body[13]. Studies have shown that they have anti-anaemic characteristics[28]. Flavonoids are a class of polyphenolic compounds that have been reported to have antioxidant activity[30].

Cardiac glycosides are a group of inotropic agents that are reportedly being used to alleviate cardiac problems associated with hypertension[31]. Terpenoids are naturally occurring aromatic compounds, obtain from terpenes that confer many plants with their flavour, colour and antiviral properties[29]. Phytate affects the bioavailability of minerals due to the formation of stable phytate complexes, inhibiting phytase enzymatic actions and also makes proteins unavailable for digestion[32]. Despite the negative effects of dietary phytates, they also offer some advantages such as antioxidant and hypocholesterolemic activity, as well as prevention of renal stone formation[32].

Cyanogenic glucosides are compounds that yield glucose, hydrogen cyanide and aldehyde or ketone upon hydrolysis with an acid or enzyme. Dietary exposure to cyanide occurs upon consumption of foodstuffs that are rich in endogenous

cyanide in the form of cyanogenic glucosides. This cyanide could be lethal as it intercalates with cytochrome oxidase for aerobic function[33]. This present study revealed that the plant contained low amounts of tannins, cardiac glycosides, terpenoids and hydrogen cyanide.

Oxalates have been reported to bind to calcium, iron and magnesium, forming insoluble salts in the digestive tract and thereby decreasing the bioavailability of these minerals[22]. Since diet has been found to play an important role in the formation of kidney stones arising from oxalate, avoiding excessive consumption of high-oxalate containing foods and consuming calcium-containing dairy foods are reported as relevant ways of preventing the formation of these kidney stones arising from oxalate[22]. However, the values that were obtained for oxalate in this study may not give any reason for concern going by the permissible range of oxalates in foods.

The high dry matter content of the leaf is relevant as this portends increased shelf life due to low moisture content which will therefore lead to the inactivation of microorganisms. The total protein contents of this plant as quantified from its total nitrogen content reveals that this plant contains significant quantities of proteins. Although vegetables are known to contain small quantities of lipids, this plant was found to contain considerable amounts of lipids. This increased lipid content could arise from the very low amount of moisture in the plant.

Studies have shown that diets containing crude fibre are beneficial and help to delay the digestion of starch to simple sugars via inhibition of pancreatic amylase, which is crucial in the management of diabetes[33]. This present study revealed that this plant contains considerable amounts of crude fibre which could be helpful to people living with diabetes mellitus.

Ash is produced from the inorganic residue and a reliable indicator of the total amount of minerals present in food. The considerable amount of ash in this plant suggests that it could contain some considerable amounts of minerals. Besides, the considerable amount of carbohydrates in this plant suggests that it could supply some good number of calories to the consumer.

Phosphorus is one of the minerals readily available in the human body[34]. Deficiencies in phosphorus could result in rickets and osteomalacia as well as other bone deformities. The results that were obtained for phosphorous suggest that this plant contains some appreciable quantities of this mineral.

Calcium is required for the formation of bones, plays key roles in blood clotting, cell signalling, muscle and nerve functions. The results of the present study revealed that *C. dolichopetalum* extract contained higher amounts of Ca than other mineral elements that were investigated. The Recommended Daily Allowance (RDA) of Ca is 0.5 g/day for adults[35]. Going by this, the results of this present study revealed that consumption of 100 g of this vegetable will meet this requirement and this is an important finding in this present study.

Magnesium is important in muscle relaxation[13]. Results of this study revealed that the plant contained considerable amounts of Mg. Potassium is important for cardiac and skeletal contraction and gastrointestinal function[35]. Results obtained in this study also showed that the plant contained considerable amounts of potassium. Sodium is highly needed for the regulation and maintenance of blood pressure and blood volume. The findings of this study revealed that the plant contained moderate amounts of sodium. Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells[35]. The requirement for Zn in adults and children is 10 mg/day. Going by the values that were obtained for Zn in this study, suggest that this plant is a good fount of Zn.

The World Health Organization reported iron deficiency as the 6th leading cause of illness and diseases in low-income countries[34]. Several factors could be attributed to this deficiency some of which includes: Low bioavailability of iron in foods and others. The findings of this study reveal that this plant is a good source of iron and could meet the daily requirement.

Manganese is a mineral that is required for the growth of bones, as well as in carbohydrate and lipid metabolism[34]. At the moment, there is no established recommended daily allowance for manganese, it was estimated that adult males and females require 2.3 and 1.8 mg per day respectively while an upper limit of 11 mg per day was reported for adults[34]. Following from above, the present study showed that Mn levels in this plant could be considered to be adequate to meet with the RDA for Mn in adult females and males.

Lead is a chemical element and heavy metal which can harm the production of blood cells, cause kidney and brain damage and lead to toxicity to the other tissues. Consumption of foods containing lead is the major source of exposure for the general population[36]. Going by the permissible range of lead in foods (0.2 mg/kg or 20 mg/100 g), the amount of Pb in this plant is too low to confer any toxic effect to the user[36].

Cu is needed for absorption of iron and incorporation of iron into haemoglobin; co-factor for Vitamin C during hydroxylation reactions as well as tyrosinase activity. The daily requirement for Cu for an adult is 1.5 to 3 mg/kg[35]. Going by this, the present study showed that the level of Cu in this plant can be considered to be adequate to meet with the RDA for Cu in humans.

3-Fluoro-5-[1-hydroxy-2-(methylamino) ethyl] phenol and dextroamphetamine are reportedly used for the treatment of anaemia, management of depression, increase in satiety and as aphrodisiacs[37,38]. The existence of these compounds suggests that the plant could have potentials for the management of depression, useful as an aphrodisiac and further justifies the use of traditional use in the management and treatment of anaemia.

(R)-(-)-2-Amino-1-Propanol and 2-(methylamino) ethanesulfonic acid (N-methyltaurine) was reported to function in blood boosting, blood and liver detoxifiers respectively[39]. The presence of (R)-(-)-2-Amino-1-Propanol in *C. dolichopetalum* further justify its traditional usage in Nigerian ethnomedicine in the treatment of anaemia.

2-(aziridine-1-yl) ethanamine and bis(2-methylpropyl) benzene-1,2-dicarboxylatewere reported to possess antimicrobial, anti-inflammatory and anti-oxidative properties. Bis(2-methylpropyl) benzene-1,2-dicarboxylate also serves as a food additive. The presence of these compounds in the extract suggests that the plant could possess antimicrobial and antioxidant and anti-inflammatory properties.

1-Cyno-[2-(2-Phenyl-1,3-Dioxolan-2-yl) ethyl] pentyl esteris suggested to be a cyano compound and it may be an active antimicrobial agent and insecticide[40]. It further affirms potential antimicrobial properties and as well as insecticidal properties. The rest of the compounds (1-Guanidinosuccinimide and 1-(4-methylphenyl) propan-2-amine) that were isolated from the extract were found to have unknown biological properties.

Changes in body weights are important parameters for the evaluation of first signs of toxicity[8]. The decreased body weight of the anaemic rats concerning the control group may be due to phenylhydrazine induced toxicity. Administration of the extract possibly blunted phenylhydrazine induced toxicity on the animals, leading to their rapid weight gain.

The hematopoietic system is one of the targets of toxic compounds which make it an important marker of physiological and pathological state in both humans and animals[41]. As seen in this study, administration of phenylhydrazine decreased the RBC, PCV and Hb concentration of the rats, indicating their anaemic condition. *C. dolichopetalum* leaf at 200 and 400 mg/kg increased the RBC, PCV and Hb concentration of the rats, which suggests the ability of this plant to stimulate hematopoiesis, attributable to phytochemical and nutrient constituents including flavonoids, iron, magnesium and zinc. These constituents are known to have a haemopoietic property that affects bone marrow and the production of blood cells.

Furthermore, the non-significant differences in the WBC, MCV, MCH, MCHC and platelet concentrations of rats, suggests that phenylhydrazine or the extracts did not affect these parameters in rats.

The bioactive phytochemicals with anti-anaemic properties that were found in *C. dolichopetalum* may have contributed to its reversal of phenylhydrazine induced anaemia in rats as seen in this study. Further studies in toxicity are highly needed and amelioration of anaemia in pregnancy.

5. Conclusion

This study has shown the potentials of *C. dolichopetalum* in reversing anaemia induced by phenylhydrazine, thereby validating its traditional usage in the treatment of anaemia. This study discovers the possible reversal of phenylhydrazine induced anaemia in rats by *Combretum dolichopetalum* which validates the traditional usage of this plant in Nigerian ethnomedicine for the treatment of anaemia.

6. References

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