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ANTIOXIDANT POTENTIALS OF METHANOL LEAF EXTRACT AND CHROMATOGRAPHIC FRACTIONS OF SOLANUM AETHIOPICUM ON CYCLOPHOSPHAMIDE-INDUCED MEGALOBLASTIC ANAEMIA IN RATS

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ABSTRACT
The antioxidant potentials of methanol leaf extract and chromatographic fractions of *Solanum aethiopicum* on cyclophosphamide-induced megaloblastic anaemia in rats was evaluated using standard protocols. The toxicity study, proximate, vitamins and phytochemical analyses of the extracts were investigated. The fractionation and thin layer chromatography yielded five fractions. Proximate constituents results include moisture (39.46±0.08%), dry matter (60.54±0.08), ash content (72.12±0.02%), crude fiber (11.97±0.04%), crude fat (2.56±0.02%), protein content (15.48±0.07%), Important vitamins such as vit. C (53.27±0.05mg/kg), vit. B_9 (60.24±0.50mg/kg), and vit. B_{12} (63.98±0.01mg/kg); and phytochemicals such as alkaloids (5.23±0.02mg/100g), flavonoids (21.49±0.16mg/100g), phenols (25.82±0.26mg/100g), and tannins (3.09±0.04mg/100g), were observed in the sample. The scavenging activities of DPPH, NO and FRAP compared favorably with their respective standards in this study. The acute toxicity (LD_{50}) test revealed that the extract is safe up to the dose of 5000mg/kg.bw. Significantly ($P<0.05$) there was an increment in the value of GPx and a reduction in MDA in all the groups treated with graded doses of the crude extract when compared with the group II. The results also revealed a non-significant ($P>0.05$) increase in GPx, SOD, CAT, and MDA levels in all the groups treated with various fractions (F1-F5), when compared with the standard control group. The observed bioactive constituents of *S. aethiopicum* leaf could be behind the different degrees of efficacy exhibited by the fractions and crude extracts in this study. Therefore, *S. aethiopicum* leaf may possess free radical scavenging potentials at minimal dose that supports its use for the management of cellular oxidation.

KEYWORDS: Antioxidants, Cyclophosphamide, Megaloblastic-anaemia and Phytochemicals.

INTRODUCTION
Medicinal plants contain bioactive organic chemical compounds often referred to as phytochemicals, which play a defensive role against major chronic diseases in both host-metabolic or genetic dysfunctional disease and infectious disease, and found in grains, vegetables, fruits, and other plant products.[1,2,3,4] Phytochemicals perform intermediary metabolic activities, and they function as primary metabolites such as fats and sugars found in all plants, while secondary metabolites are found in a smaller range of plants and provide specialized functions. Secondary metabolites are biomolecules produced for sustainability or adaptation of plants in the environment as a result of many factors, such as protection from drought, pollination, and predation, but are not required for immediate survival of the plants.[5] Secondary metabolites and pigments, because of their healing effects in humans are processed into drugs such as inulin (Dahlias plant), morphine and codeine (Poppy plant), quinine (Cinchona plant), and digoxin (Foxglove plant).[6]

A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Spinacia oleracea, Telfeira occidetallis, Jatropha curcas, Waltheria indica and Solanum nigrum.*[7,8] *Solanum aethiopicum L.* also known as garden egg is a seasonal plant which belongs to the family *Solanaceae. Solanaceae* has over 1000 species worldwide with over 100 species found in Africa (e.g. S. *aethiopicum, S. macrocarpon and S. muricatum).* The leaves of *S. aethiopicum* are used to prepare delicacies

like stew, soup and yam porridge. The leaves of *S. aethiopicum* are oval shaped, wavy margined and alternately arranged. They are about 10–30 cm long and 4–15 cm wide. The leaves have a high content of crude fiber, calcium, iron, zinc, protein, fat, vitamins and phytochemicals.[9] In traditional medicine, *S. aethiopicum* is used in treatment of various ailments such as overweight, constipation, asthma, allergic disease, dyspepsia, swollen joint pain and gastro-esophageal reflux disease.[10] Scientific reports have also demonstrated the potentials of using *S. aethiopicum* as purgative, anti-diabetic, weight reduction, sedative, anti-hyperlipidaemia, anti-inflammatory, anti-anaemic, anti-ulcerogenic and anti-constipation.[10,11] Although, *S. aethiopicum* leaves have been shown to have various health benefits, their effects on cyclophosphamide (CPM) induced megaloblastic anaemia have not been investigated. Therefore, the objective of this study is to evaluate the antioxidant potentials of methanol leaf extract and chromatographic fractions of *S. aethiopicum* on cyclophosphamide-induced megaloblastic anaemia in rats.

MATERIALS AND METHODS

Collection and Identification of plant materials

Fresh leaves of the plant was obtain from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and was identify by Dr. Garuba Omosun, a taxonomist of the Plant Science and Biotechnology Department, Michael Okpara Uiversity of Agriculture, Umudike (MOUAU), as *Solanum aethiopicum*. A sample of the plant was deposited in the herbarium of MOUAU for reference purposes.

Equipment and Reagents Used/Model

Atomic Absorption Spectrometer (AA32ON), Ultraviolet Visible Spectrophotometer (UN 72ON), Hot air oven (DHG 910), Muffle furnace (P5900), Electronic weighing balance (Scout Pro Pu 401) and Electric Blender (BN2033). All chemicals and reagents used are of standard analytical grade. Hydrochloric acid, ferric chloride, tetraoxosulphate (VI) acid, acetic anhydride, Potassium hydroxide methanol, and Ammonia solution were purchased from Jaj Chemical Ltd, China.

Preparation of plant extract

The leaves were destalked from the stem, sorted, and air dried under shade for three weeks. The dried leaves were grinded to obtain ground samples that was stored until needed for further studies.[12]

Quantitative analysis of phytochemicals

The phytochemicals (flavonoids, alkaloids, saponins, tannins, steroids, phenols, cardiac glycosides analysis of the sample was carried out using procedures outlined by Harbone and Sofowora.[13,14]

Proximate composition analysis

The leaves (powder) of *Solanum aethiopicum* were subjected to proximate nutrient composition analysis and components analyzed were moisture, ash, protein, fat, crude fiber and carbohydrate. This was done using standard methods as described by the Association of Official Analytical Chemists.[15]

Vitamin analysis

Vitamin analysis was carried out for vitamin A, B_1, B_2, B_3, B_9, B_{12}, C, D and E using the method of AOAC.[16]

Determination of antioxidant activity (*In vitro*)

Determination of 2, 2-diphenyl-l-picryl hydrazyl (DPPH Assay)

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay was determined using the method described by Banerjee.[17] The free radical scavenging activity of the extract was measured by the decrease in absorbance of methanol solution of DPPH. Different concentration of the plant extracts (20, 40, 60, 80 and 100 mg/mL in methanol) was added at an equal volume (10 mL) to methanol solutions of DPPH (400 mg/mL) and incubated for 30 minutes. The absorbance was measured at 517 nm using spectrophotometer (VIS 721, PEC MEDICAL USA). A different concentration of L-ascorbic acid ((20, 40, 60, 80 and 100 mg/mL) was used as standard antioxidant. The antioxidant activity of the leaf extract was compared with L-ascorbic acid. Values obtained was converted into percentage antioxidant activity using the equation below:

$$\% \text{ DPPH antiradical scavenging capacity} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank} \times 100}{\text{Absorbance of blank}}$$

Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

Ferric Reducing Antioxidant Power (FRAP Assay) was determined using the method described by Banerjee.[17] Various concentration (20, 40, 60, 80 and 100 mg/100 mL of the aqueous leaf extract was mixed with 1mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide in separate test tubes. The reaction mixtures was incubated in a temperature controlled water bath at 50 ^{0}C for 20 min, followed by the addition of 1 mL of 10 % trichloroacetic acid. The mixtures was centrifuged for 10 min at room temperature. The supernatant obtained (1 mL) was added with 1 mL of de-ionized water and 200 µL of 0.1 % $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide which was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. L-ascorbic acid was used as standard. The reducing power was expressed as an increase in A700 nm after blank subtraction.[17] Percentage inhibitory activity was calculated as follows:

$$\% \text{ inhibitory activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control.}}$$

Determination of nitric oxide radical scavenging activity

Nitric oxide was determined using the method described by Banerjee.[17] Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5µM) in phosphate buffered saline (PBS) was mixed with 3.0 ml of different concentrations (20-100µg/mL) of the drug and incubated at 25^0C for 150mins. The solution formed was treated with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylenediamine dihydrochloride). The absorbance of resulting chromophore from diazotization and subsequent coupling with naphthylenediamine was absorbed at 546 nm. Control reaction was also carried out in between potassium nitrite and Griess reagent and absorbance was measured.

The percentage of nitric oxide radical scavenging was calculated as

NO scavenged (%) = $[(A_{control}-A_{test})/A_{control}] \times 100$

$A_{control}$ = absorbance of control reaction; A_{test} = absorbance in presence of sample of extracts.

Rutin was used as standard for nitric oxide radical scavenging activity.

Column fractionation

Principle: Molecules are separated because they differ in the extent to which they are distributed between the mobile and stationary phase. Molecules that show weak affinity for the sorbent spend more time in the mobile phase and are easily eluted from the system.[17]

Animal housing

Healthy albino rats of mean weight of (80 ± 10 g) was used for the study. All animals were kept in aluminum cages in the animal house, of the Department of Biochemistry under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

Acute toxicity study of *S. aethiopicum* methanol crude extract

Lethal dose (LD_{50}) determination was conducted using the Lorke's method. Nine rats were divided into 3 groups of 3 rats each. The first group received the extract orally at a dose of 10 mg/Kg b.wt; group 2 received the extract at a dose of 100 mg/Kg b.wt orally, while the last group received the extract at the dose of 1000 mg/Kg body weight. The animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours. In the second phase 9 rats were divided into 3 groups of 3 rats each. The extract were administered at the dose of 1600, 2900, and 5000 mg/Kg b.wt orally respectively, based on the result of the first phase, and the final, LD_{50} was calculated as the square root of the geometrical mean of highest non lethal dose and the lowest lethal dose.[19]

Then the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

Induction of megaloblastic anaemia

A method described by Berger,[20] was used for this study. The animals of Groups II to VI received cyclophosphamide (30mg/kg b.wt) at two days interval for fourteen days via intraperitoneal route.

Experimental design

In phase one (1), thirty (36) adult wistar rats of body weight between 57.8g and 95.2g were used for the study. The animals for the study were randomly selected into six (6) groups of six (6) rats each. Groups (1), (2) and (3) were the control groups while groups (4), (5) and (6) were the test groups. Group 1 is the normal control that received feed and water, group 2 is the negative control group (untreated group) that was induced with 30mg/kg b.wt of cyclophosphamide (i.p). Group 3 is the standard control that was induced and treated with standard drugs (levofolinate 20mg/kg.bw and cyanocobalamin 0.2mg/kg.bw). Test groups (4), (5) and (6) were induced and was orally given 250mg/kg.bw low dose, 500mg/kg.bw mid dose and 1000mg/kg.bw high dose of *S. aethiopicum* methanol leaves extract respectively for treatments. At the end of the two weeks' treatment period, the rats were anaesthetized using chloroform. The blood sample was collected by cardiac puncture using 5ml syringe. About 1ml of blood was collected into an EDTA sample bottle for haematological assay while 3mls were put into the plain bottles labeled accordingly for all the groups and some biochemical parameters were determined.

In phase two (2), 48 animals in eight (8) experimental groups of six rats (n=6) each was used.

Groups 1- 3 was the same as in phase 1.

Groups 4- 8 received 30mg/kg.bw bioactive fractions 1-5 of *S. aethiopicum* for 14 days.

Blood Collection and Analysis

About 1ml and 4 ml of blood was collected from each rat into EDTA bottles and plain bottles without EDTA (for serum) respectively by cardiac puncture with 5ml syringe. The 1 ml blood was thoroughly mixed with EDTA to avoid coagulation and used for haematological tests. The 4 ml blood was allowed to clot at room temperature and then serum was collected after centrifugation at 1000 rpm for 10 min. Sera samples was stored in the refrigerator at low temperature until when used for biochemical assays.

Determination of *invivo* antioxidant status

Superoxide dismutase (SOD) activity in the tissue was estimated by the method of Kakkar.[21] Catalase (CAT) activity was evaluated by the method of Aebi,[22] and reduced glutathione (GSH) by Aebi.[22] Lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) according to the method described by Ohkawa.[23] The released MDA served as an index of lipid peroxidation. Vitamin C and E was determined by the method of Aebi.[22] Glutathione peroxidase (GPX) was evaluated by the method of Kakkar.[21]

Statistical analysis

The results obtained was analyzed statistically using one-way analysis of variance (ANOVA) to get the grouped mean which was used to determine the significant difference between the group means at 95% level of confidence, using the statistical products and service solutions (IBM SPSS Statistics 22.0), and $P<0.05$ was considered significant.

RESULTS

The results of the present study are presented in the Tables below.

Table 1: Qualitative and quantitative phytochemical constituents of *S. aethiopicum* leaves.

Phytochemical parameter	Qualitative	Quantitative (mg/100g)
Alkaloids	+	5.23±0.02
Tannins	+	3.09±0.04
Flavonoids	+++	21.49±0.16
Phenols	+++	25.82±0.26
Terpenoids	+	2.91±0.02
Saponins	++	7.31±0.03
Cardiac glycosides	+	0.25±0.01
Steroids	+	1.67±0.02

Quantitative results are expressed as Mean ± Standard deviation of triplicate determinations, while + = Low concentration; ++ = moderate concentration; and +++ = high concentration for qualitative results.

Table 1 shows the qualitative and quantitative phytochemical constituents of *S. aethiopicum* leaves. The qualitative results shows the presence of alkaloids, tannins, flavonoids, phenols, terpenoids, saponins, cardiac glycosides and steroids, while the quantitative results show the concentrations of phytochemicals found present as 5.23±0.02 mg/100g for alkaloids, 3.09±0.04 mg/100g for tannins, 21.49±0.16 mg/100g for flavonoids, 25.82±0.26 mg/100g for phenols, 2.91±0.02 mg/100g for terpenoids, 7.31±0.03 mg/100g for saponins, 0.25±0.01 mg/100g for cardiac glycosides, and 1.67±0.02 mg/100g for steroids.

Table 2: Results of Proximate composition of *S. aethiopicum* leaves.

Parameter	Value
Moisture (%)	39.46±0.08
Dry matter	60.54±0.08
Ash content (%)	72.12±0.02
Crude fiber (%)	11.97±0.04
Crude fat (%)	2.56±0.02
Protein content (%)	15.48±0.07
Carbohydrate (%)	62.87±0.09
Energy (kcal/100 g)	873.37±1.61

Values are expressed as Mean ± Standard deviation of triplicate determinations

The proximate composition of *S. aethiopicum* leaves as presented in Table 2 reveals the presence of moisture (39.46±0.08 %), dry matter (60.54±0.08), ash content (72.12±0.02 %), crude fiber (11.97±0.04 %), crude fat (2.56±0.02 %), protein content (15.48±0.07 %), carbohydrate (62.87±0.09 %), and energy (873.37±1.61 kcal/100g).

Table 3: Results of Vitamin composition of *S. aethiopicum* leaves.

Vitamins	Concentration (mg/kg)
A	5.56±0.03
E	14.41±0.05
C	53.27±0.05
D	3.13±0.01
B1	0.02±0.00

B2	0.02±0.00
B3	0.61±0.00
B9	60.24±0.50
B12	63.98±0.01

Values are expressed as Mean ± Standard deviation of triplicate determinations.

The vitamin composition of *S. aethiopicum* leaves as presented in Table 3 reveals the presence of vitamin A (5.56±0.03 mg/kg), vitamin E (14.41±0.05 mg/kg), vitamin C (53.27±0.05 mg/kg), vitamin D (3.13±0.01 mg/kg), vitamin B1 (0.02±0.00 mg/kg), vitamin B2 (0.02±0.00 mg/kg), vitamin B3 (0.61±0.00 mg/kg), vitamin B9 (60.24±0.50 mg/kg), and vitamin B12 (63.98±0.01 mg/kg)

Table 4: Results of DPPH radical scavenging activity of *S. aethiopicum* leaf extract.

Concentration (µg/ml)	Leaf extract % inhibition	Standard (Ascorbic acid) % inhibition
25	17.42±0.08^{a}	20.19±0.02^{a}
50	29.25±0.16^{b}	27.77±0.08^{b}
100	58.63±0.14^{c}	53.38±0.04^{c}
200	65.81±0.17^{d}	69.83±0.07^{d}
400	78.67±0.50^{e}	83.53±0.19^{e}

Values of % scavenging activity are expressed as Mean ± Standard deviation of triplicate determinations. Values with different letters of alphabets are statistically significant (P < 0.05).

Table 4 shows that the DPPH scavenging activity of *S. aethiopicum* leaf extract ranged from a percentage inhibition of (17.42±0.08^{a}) to (78.67±0.50^{e}) over a concentration range of 25-400 (µg/ml)**,** against the standard with scavenging activity range in percentage of (20.19±0.02^{a}) to (83.53±0.19^{e}) over a concentration range of 25-400 (µg/ml)**.**

Table 5: Results of Nitric oxide radical scavenging activity of *S. aethiopicum* leaf extract.

Concentration (µg/ml)	Leaf extract % inhibition	Standard (Ascorbic acid) % inhibition
25	6.72±0.02^{a}	11.78±0.06^{a}
50	23.58±0.15^{b}	20.61±0.23^{b}
100	35.74±0.06^{c}	41.24±0.02^{c}
200	51.83±0.28^{d}	58.76±0.10^{d}
400	69.15±0.05^{e}	73.89±0.23^{e}

Values of % scavenging activity are expressed as Mean ± Standard deviation of triplicate determinations. Values with different letters of alphabets are statistically significant (P < 0.05).

Table 5 shows that the nitric oxide scavenging activity of *S. aethiopicum* leaf extract ranged from a percentage inhibition of (6.72±0.02^{a}) to (69.15±0.05^{e}) over a concentration range of 25-400 (µg/ml)**,** against the standard with scavenging activity range in percentage of (11.78±0.06^{a}) to (73.89±0.23^{e}) over a concentration range of 25-400 (µg/ml).

Table 6: Results of FRAP activity of *S. aethiopicum* leaf extract.

Concentration (µg/ml)	Leaf extract % inhibition	Standard (Ascorbic acid) % inhibition
25	4.56±0.08^{a}	12.37±0.06^{a}
50	17.69±0.07^{b}	28.47±0.12^{b}
100	32.50±0.19^{c}	44.28±0.16^{c}
200	59.28±0.24^{d}	61.41±0.08^{d}
400	65.42±0.07^{e}	72.58±0.14^{e}

Values of % scavenging activity are expressed as Mean ± Standard deviation of triplicate determinations. Values with different letters of alphabets are statistically significant (P < 0.05).

Table 6 shows that the FRAPP activity of *S. aethiopicum* leaf extract ranged from a percentage inhibition of (4.56±0.08^{a}) to (65.42±0.07^{e}) over a concentration range of 25-400 (µg/ml)**,** against the standard with scavenging activity range in percentage of (12.37±0.06^{a}) to (72.58±0.14^{e}) over a concentration range of 25-400 (µg/ml).

Table 7: Phase 1 Toxicity (LD_{50}) results of crude methanol extract of *S. aethiopicum* leaves.

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

Toxicity (LD_{50}) results of crude methanol extract of *S. aethiopicum* leaves as shown in Table 7 shows that no death was recorded among the experimental animals in phase 1 with dosages (10-1000 mg/kg body weight) of the extract.

Table 8: Phase 2 Toxicity (LD_{50}) results of crude methanol extract of *S. aethiopicum* leaves.

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5000	0/3	Animals were calm for about 30 hours, but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

LD_{50}> 5000 mg/kg body weight

Toxicity (LD_{50}) results of crude methanol extract of *S. aethiopicum* leaves as shown in Table 8 shows that no death was recorded among the experimental animals in phase 2 with dosages (1600-5000 mg/kg body weight) of the extract.

Table 9: Effect of crude extract on serum antioxidant parameters in cyclophosphamide-induced megaloblastic anaemic rats.

Groups	Treatment	GSH (mg/dl)	GPx (u/l)	SOD (u/l)	CAT (u/l)	MDA (mMol/L)
1	Normal control	9.47 ± 0.21^{b}	46.33 ± 1.45^{c}	29.33 ± 1.45^{b}	18.67 ± 0.88^{ab}	0.28 ± 0.02^{a}
2	CPM 30 mg/kg bw only (Megaloblastic anaemia control)	7.56 ± 0.18^{a}	33.00 ± 1.53^{a}	24.67 ± 1.45^{a}	15.67 ± 0.88^{a}	0.55 ± 0.02^{d}
3	Vit.B9/Vit.B12 Combi (20 mg/kg bw) + CPM (30 mg/kg bw)	8.85 ± 0.25^{b}	38.00 ± 1.15^{ab}	27.33 ± 0.88^{ab}	16.67 ± 0.88^{a}	0.40 ± 0.01^{c}
4	SA crude extract (250 mg/kg bw) + CPM (30 mg/kg bw)	9.12 ± 0.05^{b}	41.33 ± 4.10^{bc}	26.33 ± 0.88^{ab}	16.00 ± 0.58^{a}	0.35 ± 0.02^{bc}
5	SA crude extract (500 mg/kg bw) + CPM (30 mg/kg bw)	9.31 ± 0.05^{b}	41.67 ± 1.45^{bc}	28.33 ± 0.67^{ab}	18.67 ± 0.88^{ab}	0.34 ± 0.01^{b}
6	SA crude extract (1000 mg/kg bw) + CPM (30 mg/kg bw)	9.57 ± 0.46^{b}	43.00 ± 0.58^{bc}	30.00 ± 1.53^{c}	20.00 ± 1.15^{b}	0.30 ± 0.01^{ab}

Results are presented as Mean ± SEM. Means with the same letters in the same column are not statistically significant, while those with different letters are statistically significant ($P < 0.05$).

Group 1: Normal control rats
Group 2: Megaloblastic anaemia control rats administered cyclophosphamide (CPM 30 mg/kg b.w).
Group 3: Standard control rats administered cyclophosphamide (30 mg/kg b.w) + Vit.B9/Vit.B12 Combi (20 mg/kg bw).
Group 4: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + *S. aethiopicum* leaf extract (250 mg/kg b.w).
Group 5: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + *S. aethiopicum* leaf extract (500 mg/kg b.w).

Group 6: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + *S. aethiopicum* leaf extract (1000 mg/kg b.w).

Table 9 shows the effect of crude extract on serum antioxidant parameters in cyclophosphamide-induced megaloblastic anaemic rats. The results revealed that GPx values were significantly ($P < 0.05$) higher in all the groups treated with *S. aethiopicum* leaf extract 250mg/kg.bw; 500mg/kg.bw and 1000mg/kg.bw when compared with the negative control group induced but not treated. It further revealed that MDA values decreased significantly ($P < 0.05$) in all the groups treated with *S. aethiopicum* leaf extract 250mg/kg.bw. 500mg/kg.bw and 1000mg/kg.bw when compared with the negative control group induced but not treated.

There was a non-significant ($P > 0.05$) increase in CAT levels in the groups treated with Vit.B9/Vit.B12 combi and 250mg/kg.bw of extract, when compared with the negative control group induced but not treated.

Similarly, there was a non-significant ($P > 0.05$) increase in GSH levels in all the groups treated with *S. aethiopicum* leaf extract 250mg/kg.bw; 500mg/kg.bw and 1000mg/kg.bw when compared with the standard control group treated Vit.B9/Vit.B12 combi.

Finally, there was a non-significant ($P > 0.05$) increase in SOD values in the groups treated with *S. aethiopicum* leaf extract 250mg/kg.bw and 500mg/kg.bw**,** when compared with the standard control group treated Vit.B9/Vit.B12 combi.

Table 10: Effect of fractions on serum antioxidant parameters in cyclophosphamide-induced megaloblastic anaemic rats.

Groups	Treatment	GSH (mg/dl)	GPx (u/l)	SOD (u/l)	CAT (u/l)	MDA (mMol/L)
1	Normal control	9.86 ± 0.11^{cd}	43.33 ± 2.03^{c}	29.33 ± 0.88^{c}	18.67 ± 0.67^{a}	0.31 ± 0.01^{a}
2	CPM 30 mg/kg bw only (Megaloblastic anaemia control)	7.48 ± 0.29^{a}	31.33 ± 1.33^{a}	21.67 ± 0.88^{a}	15.67 ± 0.33^{a}	0.59 ± 0.02^{d}
3	Vit.B9/Vit.B12 Combi (20 mg/kg bw) + CPM (30 mg/kg bw).	8.87 ± 0.05^{b}	38.67 ± 0.88^{b}	27.33 ± 0.88^{bc}	17.00 ± 1.15^{a}	0.36 ± 0.02^{abc}
4	F1 (500 mg/kg bw) + CPM (30 mg/kg bw)	8.95 ± 0.26^{b}	38.00 ± 1.00^{b}	25.33 ± 0.33^{b}	17.00 ± 0.58^{a}	0.41 ± 0.01^{c}
5	F2 (500 mg/kg bw) + CPM (30 mg/kg bw)	9.32 ± 0.19^{bc}	37.67 ± 1.33^{b}	26.67 ± 0.67^{b}	17.00 ± 1.73^{a}	0.37 ± 0.01^{bc}
6	F3 (500 mg/kg bw) + CPM (30 mg/kg bw)	9.62 ± 0.29^{cd}	37.67 ± 1.20^{b}	25.67 ± 0.88^{b}	16.67 ± 0.67^{a}	0.36 ± 0.01^{bc}
7	F4 (500 mg/kg bw) + CPM (30 mg/kg bw)	10.26 ± 0.21^{d}	40.33 ± 0.67^{bc}	27.67 ± 0.67^{bc}	18.33 ± 0.33^{a}	0.35 ± 0.03^{ab}
8	F5 (500 mg/kg bw) + CPM (30 mg/kg bw)	9.95 ± 0.15^{cd}	38.33 ± 0.67^{b}	26.00 ± 0.58^{b}	17.33 ± 1.45^{a}	0.40 ± 0.01^{bc}

Results are presented as Mean ± SEM. Means with the same letters in the same column are not statistically significant, while those with different letters are statistically significant ($P < 0.05$).

Group 1: Normal control rats
Group 2: Megaloblastic anaemia control rats administered cyclophosphamide (CPM 30 mg/kg b.w).
Group 3: Standard control rats administered cyclophosphamide (30 mg/kg b.w) + Vit.B9/Vit.B12 Combi (20 mg/kg bw).
Group 4: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + F1 (500 mg/kg bw)
Group 5: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + F2 (500 mg/kg bw)
Group 6: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + F3 (500 mg/kg bw)
Group 7: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + F4 (500 mg/kg bw)
Group 8: Anaemic rats administered cyclophosphamide (30 mg/kg bw) + F5 (500 mg/kg bw)

Table 10 displays the effect of fractions on serum antioxidant parameters in cyclophosphamide-induced megaloblastic anaemic rats. It revealed that the GSH levels is significantly ($P < 0.05$) higher in all the groups treated with various fractions (F1-F5), when compared with the negative control group induced but non-treated.

Again the results revealed a non-significant ($P > 0.05$) increase in GPx, SOD, CAT, and MDA levels in all the groups treated with various fractions (F1-F5), when compared with the standard control group treated Vit.B9/Vit.B12 combi.

DISCUSSION AND CONCLUSION

Discussion

Anaemia is a well-known life threatening condition. It may be caused by excessive blood loss, haemolysis, and deficiency with RBC synthesis (due to iron deficiency) or deficiency of certain vitamins like cobalamin and folate (megaloblastic anaemia). The synthetic drugs available for its management/cure are not without their side effects, they may also be inaccessible and cost ineffective to some rural sufferers. All these limitations of the synthetic drugs have necessitated researches focusing on less toxic herbal therapies known for their

anti-anaemic efficacies as claimed by ethno medicinal practitioners. It is in the strength of this premise that this work was designed to investigate the antioxidant potentials of methanol leaf extract and chromatographic fractions of *Solanum aethiopicum* on cyclophosphamide-induced megaloblastic anaemia in rats. It also studied the possible synergistic antioxidant effects of the plant extract and attempted the determination of the secondary metabolites present in the plant leaves that may be responsible for the management of cellular oxidation. Effects of medicinal plants may involve one or more active components which are known as phytochemicals. Phytochemicals are plant derived chemical compounds that can be used as therapeutic agents. They are responsible for different colours, flavours, smell and other organoleptic properties.[24]

The results of the qualitative and quantitative phytochemical analyses of *S. aethiopicum* are shown in table 1. It is obvious from the result that the leaves are rich in phenols, flavonoids, saponins, steroids, terpenoids, tannins, alkaloids and cardiac glycosides. The presence of these biological active compounds suggest that plants could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals.[24]

Flavonoid and phenol contents were appreciably high. Both have been shown to augment humoral response by stimulating the macrophages and lymphocytes involved in antibody synthesis. Reports indicate that several types of flavonols stimulate human peripheral blood leucocyte proliferations,[25] and possess antibacterial, anti-inflammatory, anti-allergic, anti-viral and anti-neoplastic activities. Flavonoids and phenols also function as reducing agents, free radical scavengers and quenchers of singlet oxygen formation,[25] thus very useful in counteracting the free radicals generated by immune depressants. Alkaloid content was equally observed. Alkaloids being a class of secondary metabolites and with their wide range of pharmacological activities including antimalarial, anticancer, antibacterial and anti-hyperglycemic activities, have been of essence in drug design.[24] Tannins have been shown to play major roles as antidiarrheal and anti-hemorrhagic agent.[24] The terpenoids have been shown to decrease blood sugar levels in animal studies. Glucosides may be crucial in the transduction of intracellular signals mediated by neurotransmitters, hormones and neuromodulatory receptors. When activated, these molecules can act on several intracellular targets. Saponins and terpenoids can exhibit astringent properties; they can be used as foam enhancing agents in beverages. They equally possess anti-inflammatory and antioxidant effects. They act as immune-adjuvants in vaccines.[24] Hence, the leaf extract of *S. aethiopicum* could be of great importance to human health.

The result of the proximate analyses revealed the presence of all the major food components as shown in table 2. This is similar to those of Aja,[25] except that a much higher energy and carbohydrate values were recorded from this work. The protein and fiber contents are equally much higher than that which was reported by Aja.[25] Adequate intake of fiber in diet facilitates digestion, aids absorption of trace elements in the gut, reduces absorption of cholesterol, and facilitates efficient elimination of wastes.[26] The protein content is high since it is able to contribute 15.48% of its calorific value as protein as compared with 12% minimum standard.[27] The high value of ash (72.12%) indicates a high index of minerals in the leaves and as such a good source of minerals.

The vitamin content of *S. aethiopicum* was presented in table 3. The high value of vitamin B_9 (60.24±0.50mg/kg) and vitamin B_{12} (63.98±0.01mg/kg) as shown is an indication that *S. aethiopicum* leaf can ameliorate megaloblastic anaemia which occurs as a result of deficiencies in folate and cobalamin. Vitamin C content was equally high (53.27±0.05mg/kg) in *S. aethiopicum* leaves. Vitamin C helps in healing process and helps the body fight infections. *S. aethiopicum* contains adequate amounts of antioxidants (when compared with most Nigerian and European leafy vegetables), they help in mopping up free radicals in the body. The value of vitamins are in line with the findings of Opara and Udourioha.[28] who showed that *S. aethiopicum* leaves contain medicinal values when compared with other leafy vegetables in the sense that they help in fighting anaemia.

S. aethiopicum leaf extract had significant DPPH activity which increased with increase in concentration in the 25-400 (µg/ml) range though lower than the standard (table 4). The nitric oxide activity of the leaf extract (table 5) equally increase with increase in concentration in the 25-400 (µg/ml) range, though lower than the standard. FRAP activity of the present study increased with increase in concentration in the 25-400 (µg/ml) range though lower than the standard (table 6).

Acute toxicity (LD_{50}) of *S. aethiopicum* leaf extract in the two phases (tables 7 and 8) showed that no animal died even at higher doses, which could signify the non-toxic nature of the leaf extract and invariably the leaves of *S. aethiopicum* in general. The non-existence of any form of acute toxicity could be attributed to the fact that this plant is a food of value for man and has been used as such over the years with no report of any form of toxicity or mortality. It may therefore be said that the extract is completely safe since no death was observed after the administration even at a dose of 5000mg/kg.bw. A similar conclusion was drawn in other researches involving acute toxicity test in rats.[25,29] This conclusion is in line with the OECD guideline for acute toxicity studies, which emphasizes that mortality is the expected end point of acute toxicity and that non observance of mortality within a population treated with a dose range of

the substance at which mortality is expected indicates tolerance or lack of acute toxicity.[30]

Antioxidants are a class of compounds of great interest for the pharmaceutical industry and biochemists known for their capacity to reduce hurt caused by some reactive species: oxygen, nitrogen or even chlorine. The discovery of the role of free radicals in several diseases is offering a new way for health care. A great interest was observed in the research performed in order to achieve the replacement, partial or total, of synthetic antioxidants with natural ingredients. The organisms are well protected against free radical damage due to the presence of oxidative enzymes or chemical compounds.[25] This study has examined changes in activities of antioxidant parameters associated with CPM-induced megaloblastic anaemic rats treated with crude extracts of *S. aethiopicum* (table 9). The result revealed that GPx values were significantly ($P<0.05$) higher in all the groups treated with *S. aethiopicum* leaf extract, when compared with the negative control group. However, the MDA values decreased significantly ($P<0.05$) in all the groups treated with *S. aethiopicum* leaf extract, when compared with the negative control group. There was a non-significant ($P>0.05$) increase in CAT levels in the group treated with the standard drug and 250mg/kgb.w extract when compared with the negative control group. GSH and SOD levels were non-significantly ($P>0.05$) higher in all the groups treated with *S. aethiopicum* leaf extract, when compared with the standard control group. *S. aethiopicum* has been shown to be rich in phenolic compounds; flavonoids and ascorbic acid have been found in plants which show antioxidant activities.

In the results of the fractions (table 10), GPx, SOD, CAT and MDA levels in all the groups treated with various fractions (F1-F5), revealed a non-significant ($P>0.05$) increment when compared with the standard control group. GSH levels were significantly ($P<0.05$) higher in all the groups treated with various fractions (F1-F5), when compared with the negative control group. MDA is a well known indicator of oxidative stress in cells and tissues called lipid peroxidation.[25]

CONCLUSION

All the data obtained from this study showed strong preliminary evidence that *S. aethiopicum* leaf extracts have anti-oxidative effects against megaloblastic anaemia induced by cyclophosphamide as proven by several biochemical and anti-oxidant analyses. The effects of crude extract were comparable to that of vitamin B_9/B_{12} combi used as standard drug.

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