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Research Article

Hepato-protective Potentials of methanol leaf extract and chromatographic fractions of *Solanum aethiopicum* on Cyclophosphamide-induced Megaloblastic Anaemia in Rats

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ABSTRACT
The hepato-protective potentials of methanol leaf extract and chromatographic fractions of *Solanum aethiopicum* on cyclophosphamide-induced megaloblastic anaemia in rats were evaluated using standard protocols. The extracts toxicity study, proximate, vitamins and phytochemical analyses 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 fibre (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 results revealed a significant ($P < 0.05$) increase in total protein, albumin, and globulin concentrations in all the groups treated with various doses of *S. aethiopicum* leaf extract when compared with group II rats. ALT, AST, ALP and total bilirubin levels significantly ($P < 0.05$) decreased in all the groups treated with various doses of *S. aethiopicum* leaf extract when compared with group II rats. The result further revealed a significant ($P < 0.05$) decrease in ALT, AST, ALP and Total bilirubin concentrations across all the groups treated with the five fractions in comparison with group II rats. 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 has beneficial hepato-protective properties in rats at a therapeutic dose that supports its use in the treatment of hepatic diseases.

Keywords: Hepato-protective; Cyclophosphamide; Megaloblastic-anaemia; Proximate; Phytochemicals and Chromatography

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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 are found in grains, vegetables, fruits, and other plant products (Khan *et al.,* 2009; Nayak *et al.,* 2005; Gbolade *et al.,* 2008 and Esposito *et al,* 2016). 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 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) (Agunu *et al.,* 2011).

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* (Luka *et al.*, 2014; Dina *et al.,* 2016). *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 (Komlaga *et al.,* 2014). In traditional medicine, *S. aethiopicum* is used in the treatment of various ailments such as being overweight, constipation, asthma, allergic disease, dyspepsia, swollen joint pain and gastro-esophageal reflux disease (Anosike *et al.,* 2012). Scientific reports have also demonstrated the potential of using *S. aethiopicum* as purgative, anti-diabetic, weight reduction, sedative, anti-hyperlipidaemia, anti-inflammatory, anti-anaemic, anti-ulcerogenic and anti-constipation (Anosike *et al.,* 2012; Edijala, *et al.,* 2005). 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 obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and was identified by Dr. Garuba Omosun, a taxonomist of the Plant Science and Biotechnology Department, Michael Okpara University 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 ground to obtain ground samples that were stored until needed for further studies (Trease and Evans, 1989).

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, 1973; and Sofowora, 1993).

Proximate Composition Analysis

The leaves (powder) of *Solanum aethiopicum* were subjected to proximate nutrient composition analysis and the 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 (AOAC, 2005).

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 1990).

Column Fractionation

Principle: Molecules are separated because they differ in the extent to which they are distributed between the mobile and stationary phases. Molecules that show weak affinity for the sorbent spend more time in the mobile phase and are easily eluted from the system (Banerjee *et al*., 2008).

Animal Housing

Healthy albino rats of a mean weight of (80 ± 10 g) were 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 24 hours. In the second phase, 9 rats were divided into 3 groups of 3 rats each. The extract was 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 the highest non-lethal dose and the lowest lethal dose (Lorke, 1983).

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.

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 were 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-week treatment period, the rats were anaesthetized using chloroform. The blood sample was collected by cardiac puncture using a 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 were used in eight (8) experimental groups of six rats (n=6) each.

Groups 1- 3 were the same as in Phase 1.

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

Induction of Megaloblastic Anaemia

A method described by Berger (1983), was used for this study. The animals of Groups II to VI received cyclophosphamide (30mg/kg b.wt) at two-day intervals for fourteen days via the intraperitoneal route.

Blood Collection and Analysis

About 1ml and 4 ml of blood were collected from each rat into EDTA bottles and plain bottles without EDTA (for serum) respectively by cardiac puncture with a 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 were stored in the refrigerator at low temperature until when used for biochemical assays.

Determination of Liver Function Parameters

Assay of Alanine Amino Transferase (ALT) activity

Serum ALT activity was estimated by the method of (Reitman and Frankel, 1957).

Assay of Serum Aspartate Aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel, (1957).

Alkaline Phosphatase (ALP) Activity Assay

Determination of serum ALP activity was carried out using the method by Reitman and Frankel (1957).

Determination of Albumin

Albumin was determined according to George (1939), method.

Determination of Serum Total Protein

Serum total protein was determined by the biuret reaction as proposed by George, (1939).

Determination of Serum Total Bilirubin (T. bil) Concentration

The total serum bilirubin concentration was determined using the method of Jendrassik and Grof, (1938).

Statistical Analysis

The results obtained were 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 shows the qualitative and quantitative phytochemical constituents of *S. aethiopicum* leaves. The qualitative results show 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.

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 fibre (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).

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).

Toxicity (LD_{50}) results of crude methanol extract of *S. aethiopicum* leaves as shown in Table 4 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 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 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

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.

Table 4: 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 5 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 6 shows the results of the effect of crude extract on liver function parameters in cyclophosphamide-induced megaloblastic anaemic rats. The results revealed significant ($P < 0.05$) increase in the concentrations of total protein, albumin, and globulin in all the groups treated with various doses of *S. aethiopicum* leaf extract when compared with the animals in the negative control group induced but not treated.

Again, concentrations of ALT, AST, ALP and total bilirubin significantly ($P < 0.05$) decreased in all the groups treated with various doses of *S. aethiopicum* leaf extract when compared with the animals in the negative control group induced but not treated.

Table 7 shows the results of the effect of fractions on liver function parameters in cyclophosphamide-induced megaloblastic anaemic rats. The results revealed a non-significant ($P >0.05$) increase in total protein and globulin concentrations of groups treated with fraction 1 and fraction 2, when compared with the negative control group induced but non-treated. However, there is a significant ($P<0.05$) increase in total protein concentrations of groups treated with fraction 4 and fraction 5, when compared with the negative control group induced but non-treated.

The result further revealed a significant ($P < 0.05$) decrease in ALT, AST, ALP and Total bilirubin concentrations in across all the groups treated with the five fractions in comparison with the negative control group induced but non-treated.

Table 5: 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

Table 6: Effect of crude extract on liver function parameters in cyclophosphamide-induced megaloblastic anaemic rats

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT (u/l)	AST (u/l)	ALP (u/l)	Total bilirubin (mg/dl)
1	Normal control	7.07±0.10^{d}	4.07±0.10^{c}	3.00±0.02^{c}	27.00±1.53^{a}	33.67±1.86^{a}	73.33±2.19^{a}	0.60±0.02^{a}
2	CPM 30 mg/kg bw only (Megaloblastic anaemia control)	5.02±0.11^{a}	2.70±0.16^{a}	2.32±0.06^{a}	110.00±5.51^{d}	138.33±1.76^{d}	168.33±7.31^{c}	1.37±0.08^{d}
3	Vit.B9/Vit.B12 Combi (20 mg/kg bw) + CPM (30 mg/kg bw)	5.82±0.15^{bc}	3.13±0.08^{b}	2.68±0.08^{b}	68.33±4.91^{bc}	79.33±2.60^{b}	98.00±2.65^{b}	0.82±0.02^{b}
4	SA crude extract (250 mg/kg bw) + CPM (30 mg/kg bw)	5.71±0.14^{b}	3.05±0.02^{ab}	2.66±0.16^{b}	74.00±2.65^{c}	100.00±1.15^{c}	105.67±3.48^{b}	1.11±0.01^{c}
5	SA crude extract (500 mg/kg bw) + CPM (30 mg/kg bw)	6.21±0.20^{c}	3.43±0.16^{b}	2.79±0.05^{bc}	67.67±1.45^{bc}	99.00±3.06^{c}	109.33±2.03^{b}	1.05±0.02^{c}
6	SA crude extract (1000 mg/kg bw) + CPM (30 mg/kg bw)	6.03±0.16^{bc}	3.39±0.20^{b}	2.65±0.08^{b}	62.00±2.52^{b}	83.33±1.76^{b}	98.33±6.01^{b}	0.86±0.02^{b}

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 ($P < 0.05$).

Globulin = total protein - albumin

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 7: Effect of fractions on liver function parameters in cyclophosphamide-induced megaloblastic anaemic rats

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT (u/l)	AST (u/l)	ALP (u/l)	Total bilirubin (mg/dl)
1	Normal control	7.07±0.10^{d}	4.07±0.10^{c}	3.00±0.02^{c}	28.67±1.33^{a}	36.33±1.86^{a}	72.67±1.76^{a}	0.64±0.03^{a}
2	CPM 30 mg/kg bw only (Megaloblastic anaemia control)	5.02±0.11^{a}	2.70±0.16^{a}	2.32±0.06^{a}	120.33±2.60^{e}	138.67±6.77^{f}	151.33±4.81^{e}	1.30±0.02^{e}
3	Vit.B9/Vit.B12 Combi (20 mg/kg bw) + CPM (30 mg/kg bw)	5.82±0.15^{bc}	3.13±0.08^{b}	2.68±0.00^{8}	72.33±1.45^{c}	77.00±3.21^{bc}	103.00±3.79^{c}	0.79±0.03^{b}
4	F1 (500 mg/kg bw) + CPM (30 mg/kg bw)	5.25±0.09^{a}	3.13±0.04^{b}	2.13±0.12^{a}	83.00±1.53^{d}	106.00±3.79^{e}	112.67±4.10^{d}	1.06±0.02^{d}
5	F2 (500 mg/kg bw) + CPM (30 mg/kg bw)	5.34±0.22^{a}	3.22±0.17^{b}	2.12±0.06^{a}	81.67±2.96^{d}	96.00±3.79^{de}	112.67±2.33^{d}	1.02±0.02^{cd}
6	F3 (500 mg/kg bw) + CPM (30 mg/kg bw)	5.47±0.14^{ab}	3.15±0.07^{b}	2.32±0.08^{a}	78.33±2.40^{cd}	87.67±2.73^{cd}	104.33±0.88^{cd}	0.97±0.05^{c}
7	F4 (500 mg/kg bw) + CPM (30 mg/kg bw)	6.15±0.14^{c}	3.27±0.07^{b}	2.88±0.08^{bc}	63.67±2.40^{b}	70.67±2.33^{b}	92.67±1.45^{b}	0.81±0.02^{b}
8	F5 (500 mg/kg bw) + CPM (30 mg/kg bw)	5.81±0.14^{bc}	3.11±0.05^{b}	2.70±0.10^{b}	72.67±2.03^{c}	78.67±2.73^{bc}	101.33±1.76^{bc}	0.87±0.03^{b}

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 ($P < 0.05$).

Globulin = total protein – albumin

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)

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 research 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 hepato-protective potentials of methanol leaf extract and chromatographic fractions of *Solanum aethiopicum* on cyclophosphamide-induced megaloblastic anaemia in rats. 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, smells and other organoleptic properties (Edeoga *et al.,* 2005).

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 biologically active compounds suggests that plants could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals (Edeoga *et al.,* 2005).

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 proliferation (Aja *et al*, 2000), 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 (Aja *et al*, 2000), 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 the essence in drug design (Edeoga *et al.,* 2005). Tannins have been shown to play major roles as antidiarrheal and anti-hemorrhagic agents (Edeoga *et al.,* 2005). 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 (Edeoga *et al,* 2005). 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 above. This is similar to those of Aja *et al*, (2000), except that a much higher energy and carbohydrate values were recorded from this work. The protein and fibre contents are equally much higher than that which Aja *et al*, (2000) reported. Adequate intake of fibre in the diet facilitates digestion, aids absorption of trace elements in the gut, reduces the absorption of cholesterol, and facilitates efficient elimination of wastes (Bender-David, 2013). The protein content is high since it can contribute 15.48% of its calorific value as protein as compared with the 12% minimum standard (Ugbogu *et al*, 2019). The high value of ash indicates a high index of minerals in the leaves and as such a good source of minerals.

The high value of vitamin B_9 and vitamin B_{12} 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 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 (2023), who showed that *S. aethiopicum* leaves contain medicinal values when compared with other leafy vegetables in the sense that they help in fighting anaemia.

Acute toxicity (LD_{50}) of *S. aethiopicum* leaf extract in the two phases 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 tests in rats (Aja *et al*, 2000; Ijioma *et al,* 2014). 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 (OECD 2001).

Liver function tests are groups of blood tests that give information about the state of the liver. The liver is a vital organ that functions in detoxification, storage and other biochemical metabolisms necessary for the body (Mbuh *et al*., 2003). This study equally examined the changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, globulin, albumin and total proteins as presented in the results above. The concentrations of globulin, albumin and total proteins increased significantly in all the groups treated with various doses of *S. aethiopicum* leaf extract, when compared with the animals in the negative control group. Again, concentrations of ALT, AST ALP and total bilirubin significantly decreased in all the groups treated with various doses of *S. aethiopicum* leaf extract, when compared with the animals in the negative control group. This agrees with a near similar study (Aja *et al*., 2022) on *Whitfieldia lateritia* (another popular blood boosting plant). ALT is purely cytoplasmic, catalyzing the transamination reaction (Mauro *et al*, 2006). The decrease in ALT concentration may be because of the administration of the induction agent CPM. Any type of liver cell injury can reasonably increase ALT levels.

A non-significantly increase in total protein and globulin concentrations of groups treated with fraction 1 and fraction 2 when compared with the animals in the negative control was revealed in the result above. However, there was a significant increase in total protein concentration in groups treated with fraction 4 and fraction 5 when compared with the animals in the negative control group. The result further revealed a significant decrease in ALT, AST, ALP and total bilirubin concentrations in all the groups treated with the five fractions in comparison with the negative control group. Uncongugated bilirubin is a breakdown product of haem (a part of haemoglobin red blood cells). This is in line with the findings of Shivaraji *et al,* (2016), whose work showed that when total bilirubin levels exceeds 17µmol/L, it indicates liver disease, when total bilirubin levels exceeds 40µmol/L, bilirubin deposition at the sclera, skin and mucous colour occurs.

CONCLUSION

All the data obtained from this study showed strong preliminary evidence that *S. aethiopicum* leaf extracts have hepato-protective potentials as proven by several biochemical, and liver marker enzyme activities. Accordingly, the extract can be used as an effective herbal product for the prevention of hepatic diseases and liver related issues. It is believed to be due to its phytochemicals like phenols, flavonoids etc. that contributed to its efficiency.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approval

The National Institute of Health (NIH) approved guideline for the care and use of laboratory animals was adopted for this study.

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Author Contributions

PNO and SIE designed the study, wrote the protocol, and supervised the work. OAA performed all the laboratory work. SIE performed the statistical analysis and managed the analyses of the study. OAA wrote the first draft of the manuscript. OAA, PNO and SIE performed the literature search. PNO read and approved the final draft of the manuscript.

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