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(RESEARCH ARTICLE)



## Haematological profile of albino rats exposed to polar leaf extracts of *Portulaca oleracea* Linn.

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### Abstract

Although the use of medicinal plants is becoming popular globally, some of these plants which are purported to be safe are not without side effects or toxicity. *Portulaca oleracea*, Linn. is among the medicinal plants used globally in the treatment of diseases and management of health challenges. The dearth of information on the toxicity of *Portulaca oleracea* in a long term use prompted this study which investigated the sub-chronic effect of the oral administration of polar (aqueous methanol) leaf extracts of *Portulaca oleracea* on haematological parameters in male albino rats. Sixty-four animals were randomly divided into 4 groups of 16 rats each. Group 1 (Control) received 0.5 ml of 20% Tween 80 (vehicle), Groups 2, 3 & 4 received 125, 250 & 500 mg/kg bw of the extract respectively for 60 days by oral gavage. On days 14, 28, 42 and 60; four rats from each group were anaesthetized and blood samples were collected for haematology. No significant ( $p > 0.05$ ) variation occurred in the mean values of PCV, hemoglobin concentration, RBC count, platelet count and differential leucocyte count relative to the control throughout the 60-day duration. Significant ( $p < 0.05$ ) increase in WBC count was recorded on day 42 in the 500mg treated group. Oral administration of polar leaf extract of *P.oleracea* as used in this study had no injurious effect on haematological parameters in a long term treatment of 60 days; thus can be said to be non-toxic to blood parameters.

**Keywords:** *Portulaca oleracea*; Haematology; Polar extract; Aqueous methanol; Phytochemical

### 1. Introduction

In the last few years, the use of medicinal plants for therapeutic purposes has been on the increase globally. It is evaluated that about 80% of the world population depend mainly on medicinal plants for their health care delivery system [1]. Extracts from medicinal plants which are a combination of diverse phyto-constituents that interact with several molecular targets in an individual or organism to elicit physiological activities or pharmacological response [2] are currently prepared as tablets, snips, tinctures, sauces, oral sprays, encapsulated powders and lozenges. In developing countries like Nigeria, it is a common practice that plant products or remedies are administered over a long period of time without due attention to the likely toxicity or side effects.

*Portulaca oleracea* Linn. (Figure 1) commonly called purslane, a member of family Portulacaceae, a warm climate green herb, with obovate leaves, small yellow flowers, and branched succulent stems which are decumbent near the base [3], is one of the medicinal plants with several therapeutic benefits. It has different names in various ethnic groups in Nigeria. It is known as “Ntioke”, or “Idiridi” in Igbo; “Esan omode” or “Papasan” in Yoruba; “Babbajibji” or

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“Halshensaniya” in Hausa and “Eferemakara” in Efik [4-5]. The use of *Portulaca oleracea* in folk medicine dates back to ancient times. It has been listed by the World Health Organization (WHO) as one of the most commonly used medicinal plants, which has given it the name, ‘Global Panacea’ [6]. All parts of the plant, especially the leaves and stems, are useful as remedies for many ailments and they are usually used in fresh or dried state. *P. oleracea* Linn. is used to treat different health challenges such as scurvy, urinary disorder, haemorrhoids, fever, headache, wounds and sores [7]. The leaf juice and tea are used for treating ear aches, stomach aches and headaches [8]. Crushed leaves are applied topically for treatment of burns, swellings, erysipelas, eczema, insect and snake bites [9-10]. The leaf extracts have been shown to possess antidiabetic activity [11-12], antioxidant effects [13-14] and wound healing properties [15]. However, information on the toxicity of *Portulaca oleracea* leaf extract in long term use with regards to haematological profile is scarce. This study was therefore designed to investigate the effect of polar leaf extracts of *Portulaca oleracea* on haematological parameters.



**Figure 1** *Portulaca oleracea* Linn.

## 2. Material and methods

### 2.1. Plant material and authentication

Fresh leaves of *Portulaca oleracea* were collected from Alakahia axis of Port Harcourt, Nigeria, from the months of December, 2017 to February, 2018. The plant was identified by Dr. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with the number UPH/V/1,302.

### 2.2. Preparation of plant extract

After collection of the plant, the leaves were shade-dried at room temperature to constant weight over a period of six weeks. The dried leaves of *P. oleracea* were weighed and grinded to fine powder. The cold maceration method following the successive solvent extraction approach was used for extraction with the polar extract obtained by using 80% aqueous methanol after prior exhaustive removal of non-polar constituents by exhaustive extraction with chloroform. Briefly, a 500 g portion of the powdered leaves of *P. oleracea* was first extracted by maceration in 1.5 Litres of chloroform for 72 hours (with fresh replacement of solvent every 24 hours) to extract out the non-polar constituents of the plant material. The resulting non-polar constituent free marc after air-drying in a fume cupboard to remove residual chloroform, was further macerated by soaking in 1.5 Litres of 80% aqueous methanol (ratio of methanol to distilled water is 4:1) for 72 hours, with fresh replacement of solvent every 24 hours. The combined 80% aqueous methanol filtrate obtained by filtration with Whatman’s No. 1 filter paper, was concentrated with rotary evaporator (Model No: RE-52A) at 45 °C *in vacuo* and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments) at 45°C. The dried aqueous methanol leaf extract of *Portulaca oleracea* (AMLEPO) obtained was stored in a desiccator. All reagents used were of analytical grades.

### 2.3. Preliminary phytochemical screening

This was done following the method of Harborne [16] and Houghton and Raman [17] with modification as briefly outlined below:

#### 2.3.1. Test for alkaloid

A 0.5 g portion of AMLEPO was stirred, with 5 ml of 5% HCl on a steam bath and then filtered. The filtrate was divided into four equal portions in four separate test tubes labeled A-D and used for the following tests: - (a) To test tube labeled

A, 2-3 drops of Meyer's reagent was added. A cream coloured precipitate indicates the presence of alkaloid. (b) To test tube labeled B, 2-3 drops of Dragendorff's reagent was added. Orange-red or red coloured precipitate indicates the presence of alkaloid. (c) To test tube labeled C, 2-3 drops of Hager's reagent was added. Yellow coloured precipitate was considered positive for alkaloid. (d) To test tube labeled D, 2-3 drops of Wagner's reagent was added. Reddish-brown coloured precipitate was taken as evidence of the presence of alkaloid.

### 2.3.2. Test for tannin (phenolics)

A 0.5 g portion of AMLEPO was stirred with 10ml of distilled water, heated and filtered. 3 drops of 5% alcoholic ferric chloride reagent was added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence of the presence of tannin.

### 2.3.3. Test for flavonoid – shinoda reduction test

A 0.5g portion of AMLEPO was stirred with 10 ml of distilled water, heated and filtered. Few pieces of magnesium metal were added to the filtrate followed by the addition of 2 ml of conc. HCl. Formation of orange-red, crimson or magenta colouration shows the presence of flavonoids.

### 2.3.4. Test for saponins – frothing test

A 0.5 g portion of AMLEPO was stirred with 10 ml of distilled water, heated and shaken vigorously in a test tube. Frothing that persisted for more than 10 mins was taken as preliminary evidence for the presence of saponin.

### 2.3.5. Test for phlobatanin

A 0.5 g portion of AMLEPO is stirred with 10ml of distilled water, heated and filtered. 1% HCl was added to the filtrate and boiled. Red coloured precipitate indicates the presence of phlobatanin.

### 2.3.6. Test for carbohydrates

#### Molisch's test

A 0.5 g portion of AMLEPO was stirred and heated with 10 ml of distilled water. 2 drops of 10% 2-naphthol was added to the mixture. The test tube was inclined at an angle of 45° followed by an addition of 2ml of Conc. H<sub>2</sub>SO<sub>4</sub> slowly. Deep violet ring at the interface indicates presence of carbohydrate.

#### Fehling solution test (for reducing sugar)

2ml of well mixed Fehling's solution A and B solutions was added to 2 ml of AMLEPO solution and the mixture heated on a boiling water bath. Formation of brick-red coloured precipitate indicates presence of reducing sugars.

### 2.3.7. Test for anthraquinones

10 ml of chloroform was added to 0.5 g of AMLEPO, shaken and filtered. 1 ml of 10% aqueous NH<sub>3</sub> solution was added to the filtrate and shaken. The presence of pink, or violet colour in the ammonical layer indicates the presence of anthraquinones.

### 2.3.8. Test for triterpenoids/steroids

#### Lieberman-Burchard test

10 ml of chloroform was added to 0.5 g of AMLEPO, shaken and filtered. 2 ml of acetic anhydride was used to dissolve the extract. The solution was dipped in ice after which 1ml of conc. H<sub>2</sub>SO<sub>4</sub> was poured carefully down on the side of the test tube to form a layer. A colour change of violet to blue-green indicates the presence of steroidal nucleus, while pink-red colouration indicates triterpenoid nucleus.

#### Salkowski test

10 ml of chloroform was added to 0.5 g of AMLEPO, shaken and filtered. Conc. H<sub>2</sub>SO<sub>4</sub> was carefully added to form a layer. Reddish-brown colouration at the interface indicates the presence of steroidal nucleus.

### 2.3.9. Test for cardiac glycosides

#### Keller-Killiani test (for deoxy-sugar)

0.5 g of AMLEPO was dissolved in 2 ml of glacial acetic acid containing one drop of ferric solution and underplayed with 1 ml of Conc. H<sub>2</sub>SO<sub>4</sub>. Formation of a brown ring at the interface indicates the presence of deoxy- sugar characteristics of cardenolides.

#### Kedde test (for lactone ring)

0.5 g of AMLEPO was shaken with 10ml of methanol and filtered. To 2 ml portion of the filtrate was added 1 ml of 2% 3, 5-dinitrobenzoic acid followed by 1 ml of 20% NaOH solution. A violet colouration indicates the presence of lactone ring, a characteristic of cardenolides.

## 2.4. Acute toxicity study

The acute toxicity of the extracts was evaluated according to the method of Lorke [18].

## 2.5. Animals

Sixty-four (64) sexually mature male rats weighing an average of 200 g, procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

### 2.5.1. Experimental procedure

Following acclimatization, the animals were randomly assigned to four (4) groups of sixteen (16) animals each for treatment as follows:

Group 1 (Control) received 0.5 ml of 20% Tween 80 (vehicle).

Group 2 received 125 mg/kg body weight of extract

Group 3 received 250 mg/kg body weight of extract

Group 4 received 500 mg/kg body weight of extract

Administration of extract and vehicle was by oral gavage daily for 60 days. Animal's weight was taken weekly and the dose adjusted accordingly. On days 14, 28, 42 and 60; four rats from each group were anaesthetized and blood samples were collected by cardiac puncture into EDTA bottles. The collected blood samples were used for the estimation of haematological parameters such as packed cell volume (PCV), hemoglobin concentration (HB), red blood cell count (RBC), white blood cell count (WBC), platelets count and differential cell count according to Cheesbrough [19]. The red blood cells (RBC), white blood cells (WBC) and platelets counts were determined by the improved Neubauer haemocytometer method. The hemoglobin (Hb) concentration was determined according using the cyanomethemoglobin technique. The packed cell volume (PCV) was determined by the microhaematocrit method. Differential leucocyte count was used to determine the distribution of the various white blood cells in the circulating blood.

## 2.6. Statistical analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean  $\pm$  SEM and data were assessed by one-way ANOVA followed by the Tukey post-test. The significance level was set at  $p < 0.05$ .

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## 3. Results

### 3.1. Phytochemical screening

Phytochemical screening of polar (aqueous methanol) leaf extract of *Portulacaoleracea* (AMLEPO) indicated the presence of carbohydrate, saponins, cardiac glycoside, triterpenoids, steroids, anthraquinones and alkaloids (in trace amounts).

**Table 1** Phytochemical elements of polar (aqueous methanol) leaf extract of *Portulaca oleracea*

Tests	Phytochemical Elements	Result
Wagner's Reagent Drangendorff's Reagent Meyer's Reagent Hager's Reagent	Alkaloid	+ + - -
Ferric Chloride	Tannin	-
Shinoda Reduction Test	Flavonoid	-
Molisch Test Fehling's Test	Carbohydrate	+ +
Frothing Test	Saponins	+
Phlobatanins Test	Phlobatanins	-
Boutrager's Test	Anthraquinone	+
Lieberman's Test Salkowski's Test	Triterpenoids / Steroids	+
Keller-Killiani's Test Kedde Test	Cardiac glycoside	+

### 3.1. Acute toxicity study

Acute toxicity test did not show any mortality, morbidity or other apparent signs of toxicity at the doses used which indicates that the extract is not toxic at the maximum dose of 5000 mg/kg bw. With this in mind, 1/40<sup>th</sup>, 1/20<sup>th</sup> and 1/10<sup>th</sup> of this maximum dose (5000 mg/kg) was adopted for the study which gave rise to 125, 250 and 500 mg/kg doses of the extract used in the treatment groups.

### 3.2. Haematological parameters

The haematological changes produced in experimental rats given 125, 250 and 500 mg/kg bw doses of the aqueous methanol extract is presented in Tables 1-4. During the 60-day study period, no significant ( $p>0.05$ ) change was recorded in the mean PCV and mean Hemoglobin levels of the test groups relative to the control (Table 2).

In the 500 mg/kg bw treated rats (group 4), there was a significant ( $p<0.05$ ) decrease in mean RBC count on day 14 and significant ( $p<0.05$ ) increase in mean WBC count on day 42 in relation to control (Table 3). No significant ( $p>0.05$ ) variation occurred in the mean RBC count and mean WBC count in the other test groups in comparison to the control throughout the 60-day period of treatment. However, WBC count values were higher in all the test groups than the control on day 14, 28, 42 and 60 of treatment.

There was no significant ( $p>0.05$ ) difference in the mean platelet count relative to the control on day 14, 28, 42 and 60. However, the platelet count showed higher values in all the treatment groups than the control throughout the 60 days (Table 4). The differential leucocyte count which involves the neutrophil count, eosinophil count, lymphocyte count and monocyte count did not change markedly ( $p>0.05$ ) with aqueous methanol leaf extract of *P. oleracea* treatment (Tables 4 and 5).

**Table 2** Effect of different doses of AMLEPO on PCV and hemoglobin level

Parameter	Duration							
	PCV (%)				Hemoglobin (g/dL)			
Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group 1 (control)	42.75±1.80	40.25±1.03	39.75±1.03	44.25±0.48	14.25±0.63	13.25±0.25	13.00±0.41	14.75±0.25
Group 2 (125 mg/kg)	38.75±1.55	35.25±1.93	37.00±1.78	43.33±1.67	12.75±0.48	11.75±0.75	12.25±0.48	14.33±0.67
Group 3 (250 mg/kg)	40.50±0.87	40.25±1.03	42.00±1.08	46.50±0.87	13.25±0.25	13.50±0.29	14.00±0.41	15.50±0.29
Group 4 (500 mg/kg)	36.50±2.36	40.50±0.50	36.25±1.38	44.00±0.71	12.00±0.71	13.25±0.25	12.25±0.48	14.75±0.25

Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group 1 (control). Superscript 'a' indicate significant difference (at p<0.05)

**Table 3** Effect of different doses of AMLEPO on red blood cell (RBC) and white blood cell (WBC) count

Parameter	Duration							
	RBC (X10 <sup>12</sup> /L)				WBC (X10 <sup>9</sup> /L)			
Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group 1 (control)	7.00±0.41	6.00±0.00	6.00±0.00	6.25±0.25	5.00±0.41	5.75±0.25	5.50±0.96	7.75±0.85
Group 2 (125 mg/kg)	6.00±0.00	5.25±0.48	5.75±0.25	6.00±0.00	5.25±0.75	10.25±1.75	9.75±1.31	5.33±0.67
Group 3 (250 mg/kg)	6.00±0.00	6.00±0.00	6.25±0.25	6.75±0.25	6.25±1.03	8.25±2.66	8.50±1.04	9.25±1.38
Group 4 (500 mg/kg)	5.75±0.25 <sup>a</sup>	6.00±0.25	5.50±0.29	6.00±0.00	5.75±0.85	6.25±0.00	10.0±0.82 <sup>a</sup>	6.00±1.08

Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group 1 (control). Superscript 'a' indicate significant difference (at p<0.05)

**Table 4** Effect of different doses of AMLEPO on platelet and neutrophil count

Parameter	Duration							
	Platelet (X10 <sup>9</sup> /L)				Neutrophil (%)			
Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group 1 (control)	260.00±22.73	265.00±15.55	255.00±17.08	247.50±0.85	32.00±2.86	24.25±2.17	26.75±3.50	27.50±3.23
Group 2 (125 mg/kg)	295.00±10.41	280.00±23.45	295.00±21.02	256.67±24.04	28.50±2.72	23.25±2.69	28.00±2.86	29.00±3.79
Group 3 (250 mg/kg)	300.00±25.50	295.00±12.58	280.00±7.07	275.00±22.55	31.00±1.68	30.00±4.06	29.75±2.78	31.25±3.12
Group 4 (500 mg/kg)	235.00±15.00	310.00±1.03	282.50±25.62	275.00±16.58	28.50±3.28	32.00±21.60	28.25±4.25	33.75±3.38

\* Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group 1 (control). Superscript 'a' indicates significant difference (at p<0.05).

**Table 5** Effect of different doses of AMLEPO on lymphocyte, eosinophil and monocyte count

Parameter	Duration											
	Lymphocyte (%)				Eosinophil (%)				Monocyte (%)			
Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group 1 (control)	65.00±3.00	73.25±2.84	70.00±3.54	69.25±2.69	0.75±0.48	0.50±0.50	0.75±0.48	1.00±0.41	2.25±0.25	2.00±0.71	2.50±0.87	2.25±0.85
Group 2 (125 mg/kg)	69.50±3.33	75.00±3.54	69.75±2.78	68.33±4.41	0.50±0.50	0.75±0.75	0.25±0.25	0.67±0.67	1.50±0.87	1.25±0.75	2.00±0.71	2.00±1.00
Group 3 (250 mg/kg)	66.75±1.97	66.75±3.50	68.75±2.39	65.50±2.63	0.50±0.29	0.75±0.48	0.25±0.25	0.75±0.48	1.75±0.63	2.50±0.87	1.25±0.75	2.50±0.87
Group 4 (500 mg/kg)	69.00±3.34	65.75±2.16	53.00±15.65	64.25±2.17	1.00±0.58	0.25±2.17	0.75±0.48	0.75±0.48	1.50±0.87	2.00±0.25	2.25±0.85	1.25±0.75

\* Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group 1 (control). Superscript 'a' indicate significant difference (at p<0.05)

#### 4. Discussion

Extraction of phytoconstituents from plant materials is achieved using various extraction techniques. Different types of solvents are usually employed in extraction process. This is due to the fact that the type of solvent used determines to a large extent the extract yield, the available biologically active compounds, as well as the resulting pharmacological activities of the plant materials [20]. Preparations used in ethnomedicine are usually aqueous based, hence the choice of polar extract of *P.oleracea* leaf for this study using aqueous methanol as the solvent. According to Widyawati *et al.* [21] methanol and ethanol can dissolve polar compounds such as sugar, amino acid and glycoside [17], phenolic compounds with low and medium molecular weights and medium polarity [22], aglycones of flavonoid [23], anthocyanin, terpenoid, saponin and tannin [24]. The present study was designed to evaluate the effect of polar leaf extracts of *Portulaca oleracea* on haematological parameters.

Blood is a vital tool used in assessing the physiological and pathological state of vertebrates [25]. Blood constituents (Haematological parameters) change relative to the health status of the organism [26] and this may be due to alterations in cellular integrity, membrane permeability and metabolism or even exposure to toxic chemicals [27]. In this study, *P.oleracea* leaf extracts had no significant effect on mean PCV, hemoglobin concentration and platelet count. However, the significantly lower RBC count recorded on day 14 in group 4 (500mg/kg bw group) suggests that although the red cell count declined at a shorter duration of 14 days, the continued administration of the extract for additional 42 days increased the RBC count which correlated with the non-significant variation in RBC count of the group on day 28, 42 and 60. This reduction in RBC count may be attributed to the unprecedented increase in the control group (group 1) on day 14 (7.00) which resulted in a seemingly reduced RBC count in group 4 on day 14 (5.75). On this premise, it is therefore suggested that the reduction in RBC count may have occurred by chance hence the non-significant variation in the PCV and hemoglobin conc. of that group on day 14. Obinna and Kagbo [28] had reported that an alteration in one parameter alternately alters another since according to Schalm *et al* [25] there is a direct relationship between RBC, PCV and Hemoglobin concentration. Thus, haematocrit, hemoglobin level and RBC count are used to screen for anaemia [29]. Anaemia is a reduction in the number of circulating hemoglobin, red blood cells and/or both which usually results from excessive erythrocyte destruction, erythrocyte loss, or reduced production of erythrocyte. PCV represents the percentage of RBC in the blood. This finding is in agreement with a similar work by Guha *et al* [30] who reported that polar extracts of *Cyanthillium cinerum* (using both methanol and water as solvents) has *in vitro* protective effect against H<sub>2</sub>O<sub>2</sub> - induced haemolysis in human erythrocytes.

AMLEPO produced a significant increase in white blood cell (leucocyte) count only at the dose of 500 mg/kg bw on day 42 of treatment with no significant effect on the differential leucocyte count. An elevated leucocyte count is not only an indication for infection or stress but also denotes a reaction to a therapeutic agent especially steroids that causes increased leucocyte production [31]. This result suggests that *P. oleracea* leaf extracts may have the capacity to increase white blood cell production and could be attributed to the phyto-steroidal constituents present in the extracts. According to Soetan *et al* [32], animals with elevated leucocyte count are capable of producing antibodies in the process of phagocytosis and as such are conferred with high degree of immunity.

In comparison with the work of Oyedeji and Bolarinwa [33] who reported a non-significant variation in the haematological parameters of rats administered with 25, 50 and 75 mg/kg bw doses of methanol extract of aerial parts of *P.oleracea* for 30 days, the findings of this study which involved the administration of AMLEPO is in agreement with their finding. However, the present study showed that the extract can stimulate leucocyte production when the duration of exposure is elongated as was observed on day 42 at the highest dose of 500 mg/kg bw. Contrary to our finding, Shafi and Tabassum [34] reported significant decrease in WBC count in mice at the dose of 400 mg/kg bw after a 14-day treatment with 50% ethanol extract of the whole plant. This disparity could be associated with the plant parts used in the studies since the distribution of phytochemicals in different plant parts differ. This assertion is supported by the work of Ezeabara *et al* [35] which demonstrated that the phytochemicals and nutrients found in various parts of *Portulaca oleracea* were in different concentrations. In our previous study carried out to investigate the hepatic and renal toxicity of chloroform (non-polar) and aqueous methanol(polar) leaf extracts of *Portulaca oleracea* on biochemical profile of albino rats, it was found that both extracts did not cause toxicity to the liver and kidney parameters assessed [36].

#### 5. Conclusion

Based on the result of this study, it could therefore be concluded that polar leaf extracts of *Portulaca oleracea* as used in the study had no deleterious effect on the haematological parameters and thus can be said to be non-toxic to blood



parameters. However, it is recommended that further research should be carried out to isolate the bioactive principle(s) and identify the mechanism of increased leucocyte production associated with the polar leaf extract of this plant.

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## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

None was declared

### *Statement of ethical approval*

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. The rats for the study were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

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