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Effects of different Extracts of *Phyllantus amarus* on selected haematological and haemostatic parameters of Leukemic Wistar Rats

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Abstract
The use of plants, plant extracts or plant-derived chemicals in the treatment of diseases is a therapeutic modality that has been explored for centuries. Cancer is the leading cause of mortality worldwide and most of the chemotherapeutic agents have been reported to exhibit several normal tissue toxicities, accompanied by undesirable side effects. This study aimed to assess the effects of different extracts from *Phyllantus amarus* prepared through different drying methods on haematological and haemostatic parameters in benzene-induced leukemia in Wistar rats. A total of 25 rats were used for the study. The rats were divided into five groups (A-E), with group A serving as the control and other groups receiving Benzene to induce leukemia, followed by increasing doses of *Phyllantus amarus* extracts. Haematological and haemostatic parameters were assessed using the Sysmex 2000 haematological analyzer and blood cell morphology, while the Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) were assessed manually. The antioxidant properties of the aqueous extract were greater than those of the methanolic and ethanolic extracts. The ethanol extract of *P. amarus* had a significant effect on the RBC count, haemoglobin, and PCV. The values of PT and APTT, though reduced in the test groups, were still higher than the normal ranges. The platelet count value showed no effect of extracts on platelet concentration studied. This study suggests that *Phyllantus amarus* extracts may have potential therapeutic effects on leukemia and warrants further investigation.

Keywords*: Phyllantus amarus, leukemia, haematological parameters, haemostatic parameters, antioxidant properties.*

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Introduction

Haematological malignancies are a group of cancers that arise from a malignant transformation of cells of the bone marrow or lymphatic system (1). They are primary cancers of the blood and blood forming organs i.e. the bone marrow and lymphoid tissues. Leukemias are a group of haematological malignancies that come from blood-forming tissues. The name of the disease is derived from the Greek word 'leukos' for 'white blood' (2). In this condition, an unusually large number of immature white blood cells (WBCs) is produced by the bone marrow. These leukemic WBCs eventually take the place of the normal ones, leading to the clinical manifestation of anemia, which leaves the body more susceptible to infection (2).

Benzene is a colourless, flammable liquid with a sweet odour (3). It is known to cause cancer especially leukemia based on evidence from studies in both humans and laboratory animals by causing chromosomal changes in bone marrow cells and such changes are commonly found in leukemic cells (3).

The herb *Phyllantus amarus* also called Eyin olobe in Yoruba, Western Nigeria; geeron tsutsaayee in Hausa, Northern Nigeria and Enyikwonwa in Igbo, Eastern Nigeria (4-6), is a member of the family Euphorbiaceae. It is a small, erect, annual herb which possesses a large array of phytochemicals that are credited to its leaves, stem and roots. Worldwide, the herb has been utilized widely over the years because of its rich medicinal component (7). *P.amarus is* traditionally used among different ethnic groups in Nigeria for the treatment of jaundice, diarrhea, dysentery, diabetes, fevers, urogenital diseases, ulcers, sores, boils and wounds (7). Findings from previous studes have revealed that extracts from different parts of *P. amarus* demonstrated anti-oxidant, anti-inflammatory, hypocholesterolemic, anti-carcinogenic and anti-HIV potential (8).

The immune system defends the body against invading pathogens and mediators which are released subsequently, and both of which cause diseases (9). The immune system may be disturbed by various physical, chemical, and biological agents, such as exposure to corrosive chemicals, excessive amounts of x-rays, sunlight and radioactive materials, extremes of cold and heat, or by pathogenic microorganisms and mechanical trauma (10, 11). In addition, there is a close relationship between oxidative stress, inflammatory response, and immune system (9). Excessive oxidative stress and inflammation may result in the activation and increase of CD8+ lymphocytes, macrophages, neutrophils, and epithelial cells, as well as inflammatory mediators (IL-8, IL-6, TNFα and TGF-β) (12).

Phytochemical tests on the aqueous and methanolic extracts of the herb have shown it to contain *flavonoids, tannins, saponins, glycosides and cardiac glycosides (13). There has been little research on the* effects of different Extracts of *Phyllantus amarus* on selected haematological and haemostatic parameters of Leukemic Wistar Rats, which was what necessitated this study.

Materials and methods

Study area

The study was carried out at Kwara State University, Malete, Nigeria.

Collection, Identification, and Preparation of Plant Sample

Fresh leaves of *Phyllantus amarus* were collected from a garden in Ilorin, Kwara State. The plant was identified and authenticated by the Plant Science Department of the University of Ilorin, Ilorin, Kwara State, Nigeria. *The plant was assigned with authentication number:* UILH/001/1109/2021. It was thereafter deposited in the herbarium of the Department of Medical Laboratory Science, Kwara State University, Malete. The leaves were rinsed with tap water to remove dirt and air-dried at ambient temperature (25 ± 2 ºC) for 2 weeks. Then, 200 g of the dried leaves was milled to a fine powder using an electric grinder.

Preparation of the extracts (14)

The fresh leaf of *Phyllanthus amarus* was collected from the campus of Kwara State University, Malete. It was rinsed in clean water to remove dirt, and was divided into three parts: first part was dried at room temperature (26ºC) for a period of 2weeks; the second part was dried under the sun, while the third part was dried inside the oven. The dried leaves were milled to fine powder using manual engine grinder and 200g of the plant was obtained from each of the drying methods. The weighed quantity from room dried was soaked in 500ml of Distilled water, the sundried was soaked in 500ml of methanol and the oven dried was soaked in 500ml of ethanol, each for 48hours. They were then filtered with WhatmanNo.1 filter paper to separate the filtrate from the residue. The total ratio of the extract to solvent used was 1:5. The aqueous extract was then freeze-dried in the laboratory to concentrate the extract, while the methanol and ethanol extracts were taken to a rotary evaporator to concentrate the extract. The extracts were then later stored in a refrigerator at 4ºC before use for the study.

Phytochemical Analysis

The *Phyllantus amarus* extracts were subjected to standard phytochemical analyses to determine different constituents, including alkaloids, tannins, flavonoids, and phenolics (15,16).

Antioxidant Assessment

The antioxidant activity in the *Phyllantus amarus* extracts were analyzed utilizing different methods like, 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and reducing power (RP) assay. (17).

Experimental Animals

Twenty-five Wistar rats, weighing 120–200 g, used for this study were purchased from an animal farm in Ilorin, Nigeria. The rats were kept in a spacious and well-ventilated cage at ambient temperature (25 ± 2 ºC) and under natural dark/light cycles. They were allowed to acclimatize for 5 days and were given feed and water *ad libitum*.

Induction of Leukemia in experimental animals

Leukemia was induced into eighteen Wistar rats by injecting 0.2 ml of diluted benzene (5ml of benzene into 95ml of methanol) solution intravenously through the tail thrice a week for three

weeks. Leukemia developed in 92% of rats within 2 to 3 weeks after the last benzene injection and further observed for leukemia development in appropriate rat groups. Leukemia burden was assessed using indicator parameters such as total leukocyte, red blood cell count, haematocrit, and haemoglobin concentration (18).

Experimental Design

This was an experimental study on the assessment of Haematological and haemostatic parameters in Wistar rats with Leukemia, following the administration of different extracts of *Phyllantus amarus.* Adult Wistar rats weighing 120–200 g was used in the study. The rats were selected at random and divided into 5 groups (groups A, B, C, D, and E). Each group had 5 animals. All the rats were numbered group-wise and individually.

Group A was labelled as "normal control" and given only rat feed and water.

Group B was labelled as "positive control" and given rat feed, water, and 0.2 mL of Benzene.

Group C received feed, water, 0.2 mL of Benzene, and 200 mg of aqueous extract of *Phyllantus amarus* (prepared by room drying) for 3 weeks.

Group D received feed, water, 0.2 mL of Benzene, and 200 mg of methanolic extract of *Phyllantus amarus* (prepared by oven drying) for 3 weeks.

Group E received feed, water, 0.2 mL of Benzene, and 200 mg of ethanolic extract of *Phyllantus amarus* (prepared by sun drying) for 3 weeks.

This was done using an oral gavage once daily (9–10 AM).

Animal Sacrifice

The animals were sacrificed 12 hours after the last treatment. Whole blood was collected from the heart via cardiac puncture into tubes containing ethylene di-amine tetra acetate (EDTA)using a sterile syringe and needle and dispensed into respective dry specimen bottles that were labelled accordingly. The haematological and haemostatic analyses were carried out as the blood sample was collected.

Laboratory Analysis

Full Blood Count

FBC was determined using a Sysmex haematology autoanalyzer manufactured in Japan (19). The blood cell morphology was assessed microscopically.

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) Assays

The plasma levels were determined manually using the CoagTHREE kits from AGAPPE Diagnostics, according to the method used in a previous study (20).

Results

Phytochemical Concentration in different Phyllantus amarus extracts subjected to different drying methods

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Table 1 shows the concentration of the different phytochemical constituents in the *Phyllantus amarus* extract. This result revealed that the total Phenolics had the highest concentration in the aqueous extract (27.46±0.75 GAE mg/g), and the lowest concentration in the ethanolic extract of *Phyllantus amarus* (18.15±0.08 GAE mg/g). The mean level of the total Phenolics was significantly higher in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (27.46±0.75 GAE mg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (21.36±0.28 GAE mg/g and 18.15±0.08 GAE mg/g; p=0.001 respectively in each case). The mean level of the total Phenolics was also significantly higher in the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) subjects (21.36±0.28 GAE mg/g) compared to the group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (18.15±0.08 GAE mg/g) (p=0.004).

For the Tannins, the highest concentration was also observed in the aqueous extract (35.12±0.98 TAE mg/g), and the lowest concentration in the ethanolic extract of *Phyllantus amarus* (23.26±0.15 TAE mg/g). The mean level of the tannins was also significantly greater in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (35.12±0.98 TAE mg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (28.79±0.29 TAE mg/g and 23.26±0.15 TAE mg/g; p=0.001 respectively in each case). The mean level of the tannins was also significantly higher in the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) subjects (28.79±0.29 TAE mg/g) compared to the group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (23.26±0.15 TAE mg/g) (p=0.001).

The mean level of the flavonoids was highest in the aqueous extract (10.82±0.21 QE mg/g), and lowest in the ethanolic extract of *Phyllantus amarus* (7.47±0.04 QE mg/g). The mean level of the flavonoids was also significantly greater in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (10.82±0.21 QE mg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (7.98±0.18 QE mg/g and 7.47±0.04 QE mg/g; p=0.005 and p=0.004 respectively in each case). The mean level of the flavonoids in group D subjects (7.98±0.18 QE mg/g) was not statistically significant when compared to that of group E subjects (7.47±0.04 QE mg/g) (p=0.058).

For the Saponins, the mean concentration was highest in the aqueous extract (27.50±0.18 DE mg/g), and lowest in the ethanolic extract of *Phyllantus amarus* (18.59±0.42 DE mg/g). The mean level of the saponins was significantly higher in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (27.50±0.18 DE mg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (21.11±0.18 DE mg/g and 18.59±0.42 DE mg/g; p=0.001 respectively in each case). The mean level of the saponins was also significantly higher in the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) subjects (21.11±0.18 DE mg/g) compared to the group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (18.59±0.42 DE mg/g) (p=0.016).

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Table 2 shows the activities of the different antioxidants in the *Phyllantus amarus* extracts. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) had the highest concentration in the aqueous extract (54.77 ± 0.12 µM/g), and the lowest concentration in the ethanolic extract of *Phyllantus amarus* (42.03 ± 0.24 µM/g). The mean level of the ABTS was significantly higher in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (54.77 ± 0.12 µM/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (45.02 ± 0.29 µM/g and 42.03 ± 0.24 µM/g; $p<0.001$ respectively in each case). The mean level of the ABTS was also significantly higher in the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) subjects (45.02 ± 0.29 µM/g) compared to the group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (42.03 ± 0.24 µM/g) ($p=0.04$).
For the Diphenyl picryl hydrazine (DPPH), the highest concentration was observed in the ethanol extract (7.08±0.06 SC_{50} µg/g), and the lowest concentration in the aqueous extract of *Phyllantus amarus* (3.11±0.03 SC_{50} µg/g). The mean level of the DPPH was also significantly lower in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (3.11±0.03 SC_{50} µg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (5.71±0.05 SC_{50} µg/g and 7.08±0.06 SC_{50} µg/g; $p<0.001$ respectively in each case). The mean level of the DPPH was also significantly reduced in the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) subjects (5.71±0.05 SC_{50} µg/g) compared to the group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (7.08±0.06 SC_{50} µg/g) ($p=0.001$).
The mean level of the Reducing power was highest in the aqueous extract (2.33 ± 0.15 mg/g), and lowest in the ethanolic extract of *Phyllantus amarus* (0.84 ± 0.02 mg/g). The mean level of the Reducing power was also significantly greater in the group C (aqueous extract of *Phyllantus amarus* prepared by room drying) subjects (2.33 ± 0.15 mg/g) compared to the group D (methanol extract of *Phyllantus amarus* prepared by sun drying) and group E (ethanol extract of *Phyllantus amarus* prepared by oven drying) subjects (1.11 ± 0.05 mg/g and 0.84 ± 0.02 mg/g; $p=0.003$ and $p<0.001$ respectively in each case). The mean level of the Reducing power in group D subjects (1.11 ± 0.05 mg/g) was significantly higher when compared to that of group E subjects (0.84 ± 0.02 mg/g) ($p=0.001$).

Table 3 shows the mean differential WBC count of groups A, B, C, D, and E. This study found the mean value of TWBC to vary among groups. No significant difference was observed when the absolute lymphocyte count, absolute neutrophil count and absolute basophil count in animals treated with the aqueous, methanolic and ethanolic extracts were compared with the control group ($p=0.241$; $p=0.151$ and $p=0.278$ respectively).
A significant increase was observed in the total WBC count in animals treated with the ethanolic extract of *Phyllantus amarus* when compared with the control at $p<0.05$, while the absolute eosinophil count and absolute monocyte count was reduced in animals administered with the aqueous extract of *Phyllantus amarus* when compared with the control at $p<0.05$. Conversely, the

absolute monocyte count was increased in animals administered with the methanolic and ethanolic extracts of *Phyllantus amarus* when compared with the control at p<0.05.

Table 4 shows the mean red blood cell parameters of groups A, B, C, D, and E. After 21 days of treatment, no significant difference was observed when the RBC count in animals treated with the aqueous and ethanolic extracts were compared with the control group at p<0.05.
A significant increase was observed in the RBC count, Hb and PCV in animals treated with the methanolic extract of *Phyllantus amarus* when compared with the control at p<0.05, while the positive control comprising animals fed with Benzene and feed increased the RBC count, Hb and PCV of these animals when compared with the control at p<0.05. No significant changes were observed in the MCV, MCH and MCHC of animals in the treatment groups when compared with the control at p<0.05.

Table 5 shows the mean haemostatic parameters of groups A, B, C, D, and E. After 21 days of treatment, no significant difference was observed when the platelet count, mean platelet volume and Platelet larger cell ratio in animals treated with the aqueous, methanolic and ethanolic extracts were compared with the control group at p<0.05.
A significant increase was observed in the Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT) in animals treated with the aqueous, methanolic and ethanolic extracts of *Phyllantus amarus* when compared with the control at p<0.05, while the positive control comprising animals fed with Benzene and feed increased these parameters of these animals when compared with the control at p<0.05. The Platelet distribution width (PDW) of animals treated with the ethanolic extract was significantly reduced when compared with the control at p<0.05.

Table 1: Phytochemical constituents of *Phyllantus amarus leaf extracts*

Parameters	**Group C: Aqueous extract of *Phyllantus amarus***	**Group D: Methanol extract of *Phyllantus amarus***	**Group E: Ethanol extract of *Phyllantus amarus***	**P-value**
Total phenolics (GAE mg/g)	27.46±0.75	21.36±0.28	18.15±0.08	0.001*
Tannins (TAE mg/g)	35.12±0.98	28.79±0.29	23.26±0.15	0.001*
Total flavonoids (QE mg/g)	10.82±0.21	7.98±0.18	7.47±0.04	0.001*

Total saponins (DE mg/g)	27.50±0.18	21.11±0.18	18.59±0.42	0.001*
POST-HOC	**GROUP C/D**	**GROUP C/E**	**GROUP D/E**	
Total phenolics (GAE mg/g)	0.001*	0.001*	0.004*	
Tannins (TAE mg/g)	0.001*	0.001*	0.001*	
Total flavonoids (QE mg/g)	0.005*	0.004*	0.058	
Total saponins (DE mg/g)	0.001*	0.001*	0.016*	

* Statistically significant at *P* value < 0.05.
GAE: gallic acid equivalent; Tannic Acid Equivalent; QE: quercetin equivalent; TAE: DE: diosgenin equivalent
Group C: aqueous extract of *Phyllantus amarus* prepared by room drying
Group D: ethanol extract of *Phyllantus amarus* prepared by oven drying
Group E: methanol extract of *Phyllantus amarus* prepared by sun drying

Table 2: *In vitro* antioxidant activities of different leaf extracts of *Phyllantus amarus* prepared by different drying methods in Wistar rats

Parameters	**Group C: Aqueous extract of *Phyllantus amarus***	**Group D: Methanol extract of *Phyllantus amarus***	**Group E: Ethanol extract of *Phyllantus amarus***	**P-value**
ABTS (µM/g)	54.77 ± 0.12	45.02 ± 0.29	42.03 ± 0.24	0.04*

DPPH (SC_{50} µg/g)	3.11±0.03	5.71±0.05	7.08±0.06	0.001*
Reducing power (mg/g)	2.33 ± 0.15	1.11 ± 0.05	0.84 ± 0.02	0.003*
POST-HOC	**GROUP C/D**	**GROUP C/E**	**GROUP D/E**	
ABTS (µM/g)	<0.001*	<0.001*	<0.001*	
DPPH (SC_{50} µg/g)	0.001*	0.001*	0.001*	
Reducing power (mg/g)	0.003*	<0.001*	0.001*	

* Statistically significant at *P* value < 0.05.
ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH: Diphenyl picryl hydrazine
Group C: aqueous extract of *Phyllantus amarus* prepared by room drying
Group D: ethanol extract of *Phyllantus amarus* prepared by oven drying
Group E: methanol extract of *Phyllantus amarus* prepared by sun drying

Table 3: Mean distribution and comparison of total and differential white blood cell counts across the experimental groups

Parameters	Group A	Group B	Group C	Group D	Group E	P-value
Total WBC count ($X10^9$/L)	13.64±3.60	15.50±3.59	11.23±4.60	16.47±1.50	20.34±8.11*	0.05*
Absolute Lymphocyte count ($X10^9$/L)	9.67±3.18	9.07±1.91	5.89±1.83	8.21±1.83	11.38±5.53	0.241
Absolute Neutrophil count ($X10^9$/L)	2.34±0.63	4.23±1.56	2.52±1.42	3.12±0.63	6.23±3.01	0.151
Absolute Eosinophil count ($X10^9$/L)	0.05±0.12	0.04±0.03	0.02±0.01*	0.03±0.01	0.01±0.02	<0.001*
Absolute Basophil count ($X10^9$/L)	0.03±0.06	0.02±0.01	0.01±0.00	0.02±0.01	0.01±0.01	0.278
Absolute Monocyte count ($X10^9$/L)	0.47±0.43	0.51±0.26	0.19±0.31*	0.61±0.45*	0.81±0.60*	0.037*

Values are presented as Mean±S.D, where N = 25. Values with * are statistically significant at p-value ≤ 0.05when compared with the control.
Key:

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Group A: Negative control (water + feed)
Group B: Positive control (Benzene + feed)
Group C: Benzene + aqueous extract of *Phyllantus amarus* prepared by room drying
Group D: Benzene + ethanol extract of *Phyllantus amarus* prepared by oven drying
Group E: Benzene + methanol extract of *Phyllantus amarus* prepared by sun drying

Reference ranges:

WBC (Total white blood cell count) = 9.1-28.7 x 10^9/L
Absolute Lymphocyte count = 6.7-15.7 x 10^9/L
Absolute Neutrophil count =1.9-11.5 x 10^9/L
Absolute Eosinophil count = 0.05-0.51 x 10^9/L
Absolute Basophil count = 0.00-0.02 x 10^9/L
Absolute Monocyte count = 0.3-1.4 x 10^9/L

Table 4: Mean distribution and comparison of the Red Blood Cell (RBC) count, Haemoglobin concentration, Packed Cells Volume (PCV), and RBC indices

Parameters	Group A n=5	Group B n=5	Group C n=5	Group D n=5	Group E n=5
RBC Count (10^{12}/L)	6.26±0.46	9.29±0.74[a]	6.31±1.82	9.41±0.32[a]	7.01±1.77
Hb (g/dL)	9.64±0.79	13.20±0.99[a]	9.40±2.55	14.27±0.38[a]	10.62±2.60
PCV (%)	34.86±2.79	39.20±3.89[a]	34.00±10.15	40.33±2.08[a]	37.80±8.41
MCV (fL)	55.57±4.12	53.60±2.70	53.33±1.53	54.33±4.04	54.60±3.13
MCH (pg)	15.43±0.98	15.40±1.14	14.67±0.58	15.33±0.58	15.40±0.89
MCHC (g/dL)	28.00±2.00	27.60±2.70	28.00±1.73	28.00±1.00	27.80±1.79

Values are given as mean ± S.D
Those with superscript ([a]) showed significant difference with Group A (negative control) at $p<0.05$ (*Duncan's Multiple Range Test*)
Key:
Group A: Negative control (water + feed)
Group B: Positive control (Benzene + feed)
Group C: Benzene + aqueous extract of *Phyllantus amarus* prepared by room drying
Group D: Benzene + ethanol extract of *Phyllantus amarus* prepared by oven drying
Group E: Benzene + methanol extract of *Phyllantus amarus* prepared by sun drying

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Reference ranges:
RBC (Red blood cell count) = 8.48-15.15 x 10^{12}/L
Haemoglobin concentration (Hb) = 14.8-18.3 x 10^{9}/L
Packed cells volume (PCV) =39.7-71.8 %
Mean Cell Volume (MCV) = 47.4-54.4 fL
Mean Cell Haemoglobin (MCH) = 15.3-19.3 pg
Mean Cell Haemoglobin Concentration (MCHC) = 28.7-34.3 g/dL

Table 5: Mean distribution and comparison of haemostatic parameters across the experimental groups

Parameters	Group A	Group B	Group C	Group D	Group E	P-value
PT(Sec)	19.20 ± 4.66	36.86 ± 10.19*	32.00 ± 6.00*	42.33 ±5.86*	43.20 ± 7.36*	0.04*
APTT(Sec)	44.80 ± 7.33	122.43 ±24.83*	76.67 ± 22.03*	91.00 ±20.30*	73.80 ± 15.75*	0.02*
Platelet count ($X10^{9}$/L)	428.8±146.6	468.0±211.35	331.3±283.15	341.33±104.88	379.69±190.74	0.278
PDW (%)	13.27±2.68	10.4±0.73	10.1±0.00	12.2±1.1	7.35±0.17*	<0.001*
MPV (fL)	7.8±0.4	7.4±0.4	7.5±0.0	7.7±0.57	7.35±0.17	0.538
P-LCR (%)	13.27±2.68	11.0±2.6	9.9±0.00	12.95±5.45	10.23±0.83	0.526

* Statistically significant at *P* value < 0.05.
Key:
Group A: Negative control (water + feed)
Group B: Positive control (Benzene + feed)
Group C: Benzene + aqueous extract of *Phyllantus amarus* prepared by room drying
Group D: Benzene + ethanol extract of *Phyllantus amarus* prepared by oven drying
Group E: Benzene + methanol extract of *Phyllantus amarus* prepared by sun drying
Reference ranges:
Prothrombin time (PT): (25-50 secs)
Activated Partial Thromboplastin Time (APTT): (50-80 secs)
Platelet count: 668-1543 x 10^{9}/L
Platelet Distribution Width (PDW): 12-18%
Mean platelet volume (MPV): 6-10 fL
Platelet larger cell ratio (P-LCR): 15-35%

Table 6: Summary of blood cell picture among experimental groups

RBC	WBC	Platelet

Group A	- **Normal**	- **Normal**	- **Normal**
Group B	- Microcytic hypochromic cells	- Presence of Immature granulocytes - Blast cells, immature lymphocytes(lymphoblast). -	
Group C	- Pallor cells - Microcytic hypochromic cells	- Few immature lymphocytes.	- Mild Aggregate
Group D	- **Normal**	- Mature lymphocytes (Normal)	- Platelets clumps - Giant platelets seen
Group E	- Normal	- Normal	- Platelet aggregates

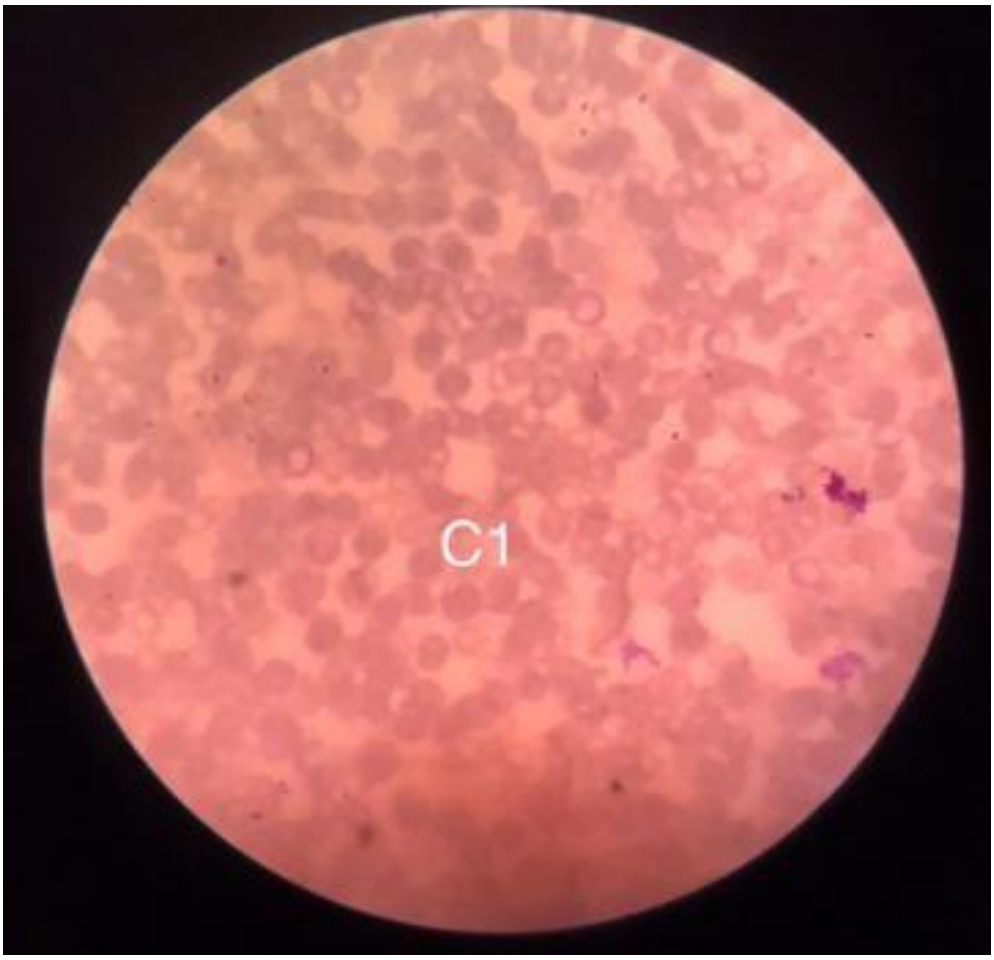

Plate 1: Pallor cells, Microcytic hypochromic cells. (X 100 magnification).

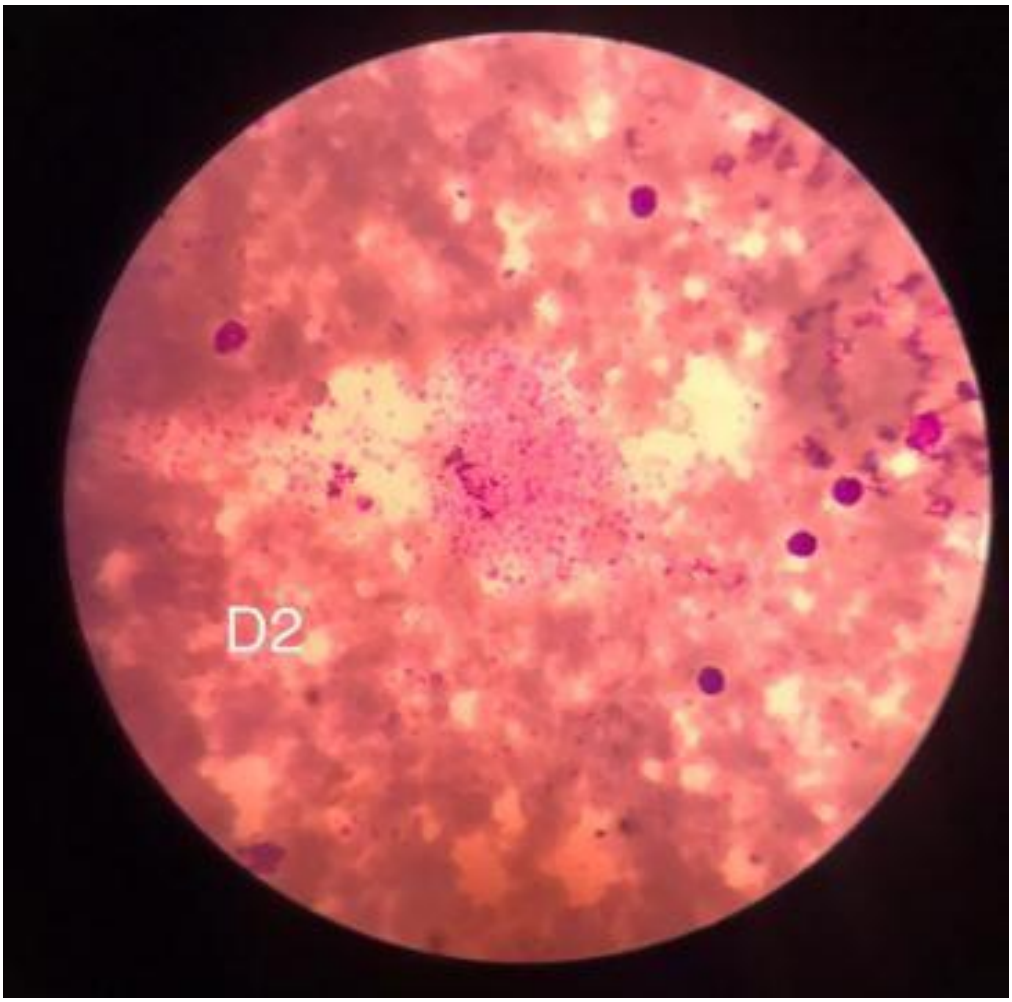

Plate 2: Platelets Clumps, Giant platelets. (X 100 magnification).

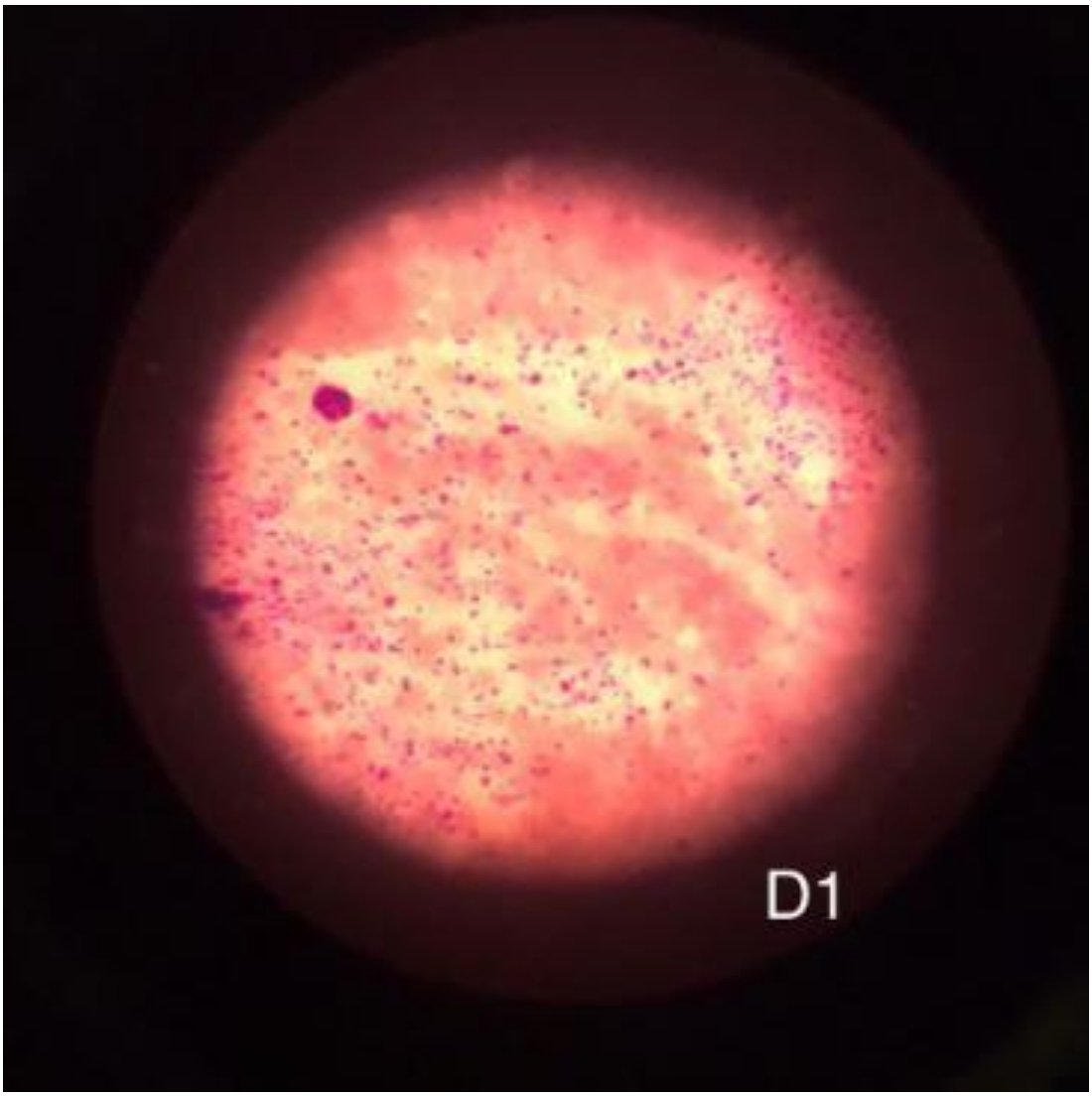

Plate 3: Platelet Aggregates (X 100 magnification).

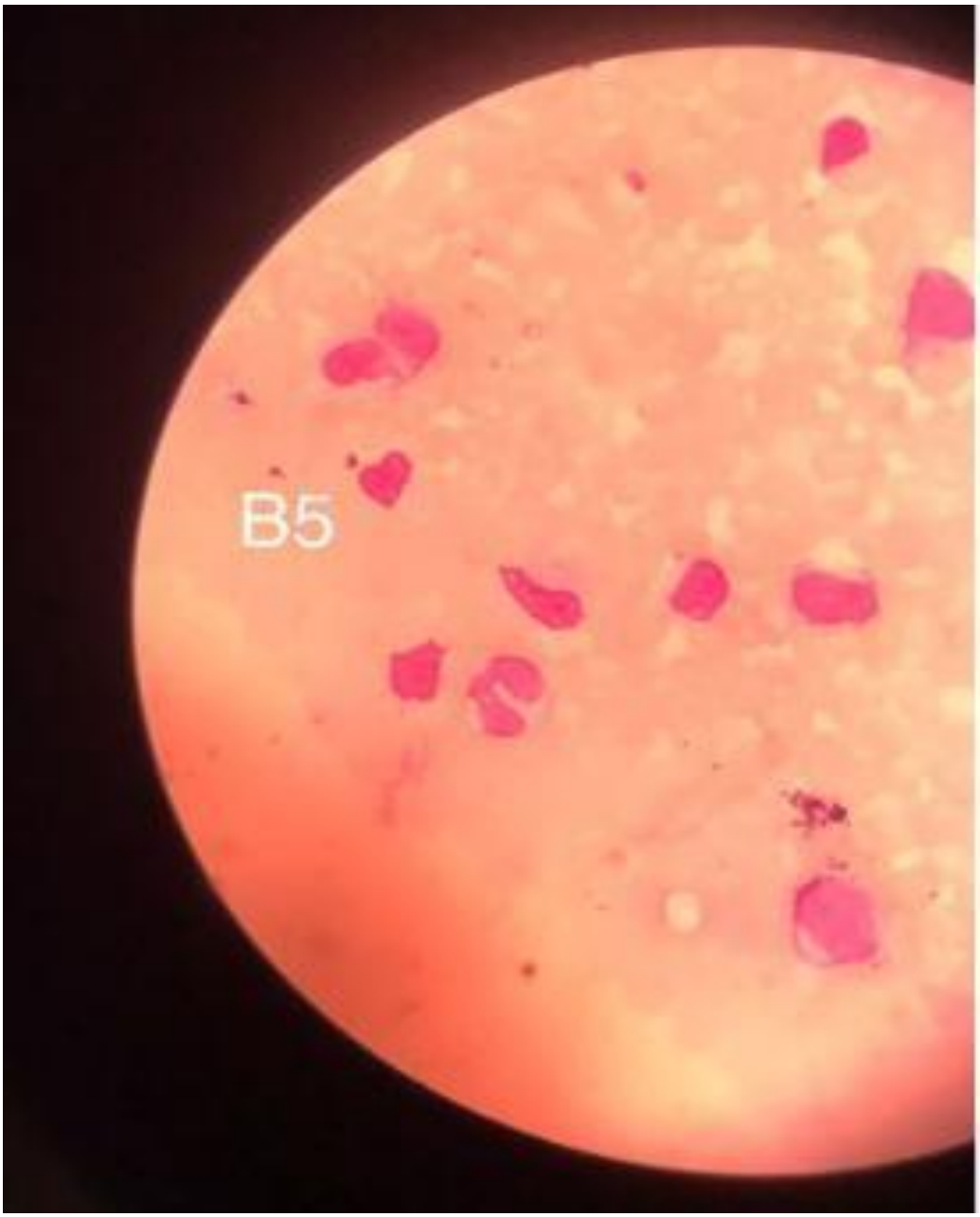

Plate 4: Presence of immature granulocytes, Blast cells. (X 100 magnification).

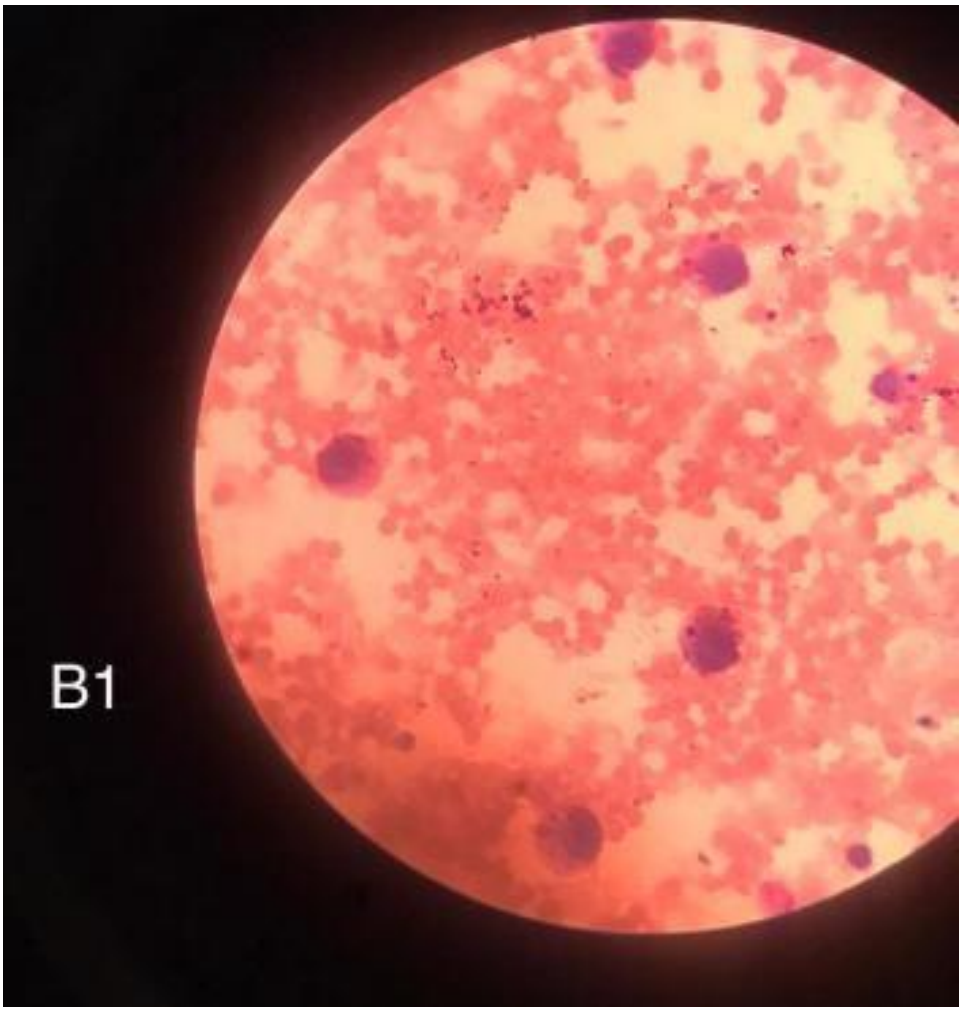

Plate 5: Microcytic, hypochromic cells. (X 100 magnification).

Discussion

Since ancient times, plant materials have played a significant role in the preservation of human health as a source of medicinal chemicals. Over half of all current chemical medications are derived from natural plant products, which is critical in pharmaceutical industry drug development programs. The study investigated the effects of different extracts from *Phyllantus amarus* prepared through different drying methods on haematological and haemostatic parameters in benzene-induced leukemia in Wistar rats. Results revealed variations in the concentrations of phytochemical constituents such as total phenolics, tannins, flavonoids, and saponins among the extracts. These findings agree with those of (21-25) and indicate the potential of the herb as a source of natural antioxidants and bioactive compounds. The aqueous extract consistently

exhibited higher concentrations of these constituents compared to methanol and ethanol extracts, with the highest antioxidant activities observed in the aqueous extract which exhibited significant free radical scavenging activity, as demonstrated by its ability to inhibit DPPH, reducing power, and ABTS radicals. This suggests that the aqueous extract of *Phyllantus amarus* leaves have strong antioxidant properties. These findings agree with that of (21). These findings suggest that the drying method significantly affects the phytochemical profile and antioxidant potential of *Phyllantus amarus* extracts.

Furthermore, the study assessed the effects of these extracts on haematological parameters and haemostatic functions in benzene-induced leukemia in rats. While all extracts showed some influence on blood cell counts and clotting parameters, notable differences were observed. The methanolic extract demonstrated significant improvements in red blood cell count, hemoglobin levels, and packed cell volume, indicating potential therapeutic benefits in treating anemia associated with leukemia. These findings agree with those of (26, 27). Conversely, the ethanol extract exhibited significant alterations in certain white blood cell counts, suggesting immune-modulating effects. This finding contradicts the research findings of (28) which demonstrated significant immunomodulatory changes with the methanolic extract. However, all extracts prolonged prothrombin time and activated partial thromboplastin time, indicating potential risks for bleeding complications.

Conclusion

The results from this study shed light on the diverse effects of *Phyllantus amarus* extracts prepared through different drying methods on both phytochemical composition and biological activity. The variations in concentrations of phytochemical constituents such as total phenolics, tannins, flavonoids, and saponins among the extracts underscore the importance of drying techniques in preserving the therapeutic properties of the herb. Moreover, the observed antioxidant activities of the extracts, with the aqueous extract demonstrating the highest potency, suggest their potential as natural antioxidants for combating oxidative stress-related conditions. In terms of their effects on haematological parameters and haemostatic functions in benzene-induced leukemia in rats, the extracts exhibited diverse influences. While the methanolic extract showed promising improvements in red blood cell count, haemoglobin levels, and packed cell volume, the ethanol extract demonstrated notable alterations in certain white blood cell counts, indicating potential immunomodulatory effects. However, it's crucial to note that all extracts prolonged prothrombin time and activated partial thromboplastin time, which may pose risks for bleeding complications.

In general, these findings contribute to our understanding of the therapeutic potential of *Phyllantus amarus* extracts in leukemia treatment. Nevertheless, further research is warranted to elucidate the underlying mechanisms of action, optimize dosing regimens, and evaluate their safety and efficacy in clinical settings.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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