



Effect of *Dalium guineense* and *Annona muricata* Leaf Extracts on Haematological profiles, Lipid Metabolism and Antioxidant Capacity in Wistar Rats

*IBEZUTE, AC; AWANA, E

Department of Environmental Management and Toxicology, College of Sciences, Federal University of Petroleum Resources, P.M.B. 1221, Effurun, Delta State, Nigeria.

*Corresponding author Email: emekaibezute@gmail.com

*ORCID: <https://orcid.org/0000-0001-9051-1020>

Tel: +2348039188873

Co-Author Email: awana.edirin@fupre.edu.ng

ABSTRACT: The objective of this paper was to investigate the effects of *Dalium guineense* and *Annona muricata* leaf extracts on haematological profiles, lipid metabolism, and antioxidant capacity in Wistar rats. Rats underwent a 30-day treatment and were divided into four groups: a control group and three experimental groups receiving either *Dalium guineense* (100 mg/kg b.w.), *Annona muricata* (100 mg/kg b.w.), or a combination of both (50 mg/kg b.w.). Haematological parameters were evaluated, along with lipid profiles and total antioxidant capacity using standard methods. Results indicated a non-significant decrease in red blood cell count, haemoglobin, and haematocrit across all treated groups compared to the control, with the highest values in the combination treatment group. Platelet counts significantly increased in all treated groups, particularly in the combined treatment group. Lipid analysis revealed increased glucose and total cholesterol in the treated groups, with *Annona muricata* showing the highest levels. Total triglycerides and C-reactive protein decreased, except in the *Annona muricata* group. Total antioxidant capacity was reduced in all treated groups, with the lowest levels observed in the combination group. These findings suggest that *Dalium guineense* and *Annona muricata* have variable effects on haematological profiles and lipid metabolism. The combination of these extracts may impact antioxidant capacity. The study highlights the potential benefits and limitations of these plant extracts, indicating the need for further research to understand their mechanisms and therapeutic potential.

DOI: <https://dx.doi.org/10.4314/jasem.v28i11.33>

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Cite this Article as: IBEZUTE, A. C; AWANA, E (2024). Effect of *Dalium guineense* and *Annona muricata* Leaf Extracts on Haematological profiles, Lipid Metabolism and Antioxidant Capacity in Wistar Rats. *J. Appl. Sci. Environ. Manage.* 28 (11B Supplementary) 3745-3753

Dates: Received: 21 September 2024; Revised: 27 October 2024; Accepted: 04 November 2024 Published: 15 November 2024

Keywords: *Annona muricata*; Antioxidant Capacity; *Dalium guineense*; Haematological Profiles; Lipid Metabolism.

The exploration of medicinal plants for their potential therapeutic benefits has gained considerable momentum in recent years. *Dalium guineense* (black velvet tree) and *Annona muricata* (soursop) are two prominent examples of such plants, recognized for their rich phytochemical profiles and traditional uses in various health conditions. *Dalium guineense*, commonly used in West African medicine, is noted for

its anti-inflammatory, antimicrobial, and antioxidant properties. The plant's diverse medicinal applications include treatment for fever, gastrointestinal disorders, and skin conditions (Ali *et al.*, 2024). *Annona muricata*, widely known for its fruit, leaves, and seeds, is utilized in traditional medicine systems across tropical regions. It is reputed for its antioxidant, anti-inflammatory, and anticancer effects, which are

*Corresponding author Email: emekaibezute@gmail.com

*ORCID: <https://orcid.org/0000-0001-9051-1020>

Tel: +2348039188873

attributed to its rich content of bioactive compounds such as acetogenins and alkaloids (Nwachukwu *et al.*, 2023). Recent studies have begun to elucidate the scientific basis for these traditional uses, particularly in relation to their effects on haematological profiles, lipid metabolism, and antioxidant capacity. Haematological parameters are critical indicators of systemic health, providing insights into blood cell production, function, and overall physiological status. Variations in these parameters can reflect the impact of disease or therapeutic interventions. *Dalium guineense* has been observed to influence haematological parameters, potentially offering protective benefits against anaemia and other blood disorders due to its antioxidant and anti-inflammatory effects (Johnson *et al.*, 2023).

Lipid metabolism, a key component of metabolic health, plays a crucial role in preventing cardiovascular diseases. Dyslipidemia, characterized by abnormal levels of lipids in the blood, is a significant risk factor for atherosclerosis, heart disease, and stroke. *Annona muricata* has shown promise in modulating lipid profiles, with studies indicating reductions in total cholesterol and triglyceride levels, which may contribute to cardiovascular health (Kumar *et al.*, 2022). The plant's ability to positively influence lipid metabolism is of considerable interest for developing dietary or therapeutic strategies to manage dyslipidaemia. Antioxidant capacity is another vital area of research, as oxidative stress is implicated in the pathogenesis of various chronic diseases, including cancer, diabetes, and neurodegenerative disorders. Antioxidants neutralize free radicals and protect cells from oxidative damage, thereby contributing to overall health and disease prevention (Kumar *et al.*, 2022). Both *Dalium guineense* and *Annona muricata* are known for their antioxidant properties, which may enhance the body's ability to combat oxidative stress and inflammation (Smith *et al.*, 2022). This study aims to investigate the effects of leaf extracts from *Dalium guineense* and *Annona muricata* on haematological profiles, lipid metabolism, and antioxidant capacity in Wistar rats. By examining changes in these parameters, the research seeks to provide a deeper understanding of the potential health benefits of these medicinal plants and their efficacy in modulating key physiological processes.

MATERIALS AND METHODS

Collection, Identification and Laboratory analysis of plant: Leaves of *Dalium guineense* (black velvet) and *Annona muricata* (soursop) were collected from two locations: the junior staff quarters at the University of Benin and Upper Sakponba in Benin City. The plants'

taxonomic identities were authenticated by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. Phytochemical analysis of the powdered leaves was conducted to determine the presence of various compounds using established procedures outlined by Sofowora (1982), Trease and Evans (1989), Kokate *et al.* (2008), and Harborne (1988). Furthermore, proximate analysis was carried out to assess parameters such as ash, crude fibre, crude protein, fat, dry matter, moisture content, and nitrogen-free extract, as described by Olomu (2011).

Preparation of Abatements: In this research, we used leaf extracts from *Dalium guineense* (black velvet tree) and *Annona muricata* (soursop). The collected leaves were air-dried to a crisp state in a laboratory environment maintained at around $30 \pm 2^\circ\text{C}$ for duration of two weeks. Once dried, the leaves were crushed into a coarse powder using a pestle and mortar, and then finely ground using a Viking Exclusive Joncod pulveriser (Model: YLH2M2-4). Thirty grams of powdered leaves from each species were extracted with 200 mL of water for 48 hours. After extraction, the mixture was filtered through sterile Whatman paper No. 1, and the resulting liquid was evaporated to dryness using a freeze dryer. The residue was then reconstituted in distilled water to the appropriate concentrations for the study. Studies by Akinmoladun *et al.* (2007) and Ibrahim *et al.* (2011) have reported that the LD50 values of *Dalium guineense* and *Annona muricata* leaf extracts exceed 2000 mg/kg in rats, indicating relatively low acute toxicity. These findings were used to determine a suitable dose for this research.

Experimental setup: All experimental animals were conducted in accordance with standard guidelines (Council for International Organizations of Medical Sciences (CIOMS), 2008) on use of animals for experimental toxicology study. Male and female Wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. They were acclimatized for 2 weeks until they were 8-9 weeks and their weights taken. The animals were housed (males and females) in wooden cages with wire mesh covers. The animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water *ad libitum*. After acclimatization, the rats were distributed randomly into four groups and labelled A-D. The rats were administered different treatment protocol as stated below.

Group A – Control (C)

Group B – *Dalium guineensis* (100mg/kg b/w) (AA)

Group C – *Annona muricata* (100mg/kg b/w) (AB)
 Group D – *Dalium guineensis* (50mg/kg b/w) +
Annona muricata (50mg/kg b/w) (AC)

The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*. The different protocols were given for 30 consecutive days (once every 48 hour). At the end of exposure period, survivors were fasted overnight and sacrificed under slight Anaesthesia; then blood samples and organs were collected.

Laboratory analysis: Serum biochemical markers such as electrolytes (such as sodium, chloride and pH), uric acid, magnesium, calcium was measured as functional marker for nephrotoxicity.

These biomarkers were determined colourimetrically by employing the standard ready-to-use kits and methods of Human. Total cholesterol and triglyceride were determined using AGAPPE diagnostic assay kit (AGAPPE diagnostics, Switzerland GmbH); Glucose was determined using RANDOX assay kits (RANDOX laboratories Limited, UK); C-reactive protein and total antioxidant capacity were determined using CALBIOTECH Elisa kit (CALBIOTECH Inc, CA). The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigations.

The absorbance of the tests was measured spectrophotometrically using OPTIMA, SP-300 (Japan).

Blood was collected from the inferior vena cava of the rats with plain 5ml sterilized syringe into a vial containing 0.5 m EDTA for haematological analysis. Haematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation

kobe, Japan) following the manufacturer's instructions. Briefly the sample was mixed and placed in contact with the sample probe for aspiration, when the buzzer sounds twice "beep, beep" and when the LCD screen displays ANALYZING, the sample was removed. Following this, the unit executed automatic analysis, and the result was displayed on the LCD screen and printed out.

Data Analysis: All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean \pm SE (n=5/sex). One-way ANOVA was used to determine the differences among various groups.

RESULTS AND DISCUSSION

The results of the findings from this study are shown below. Table 1 illustrates the changes in some biochemical parameters of Wistar rats exposed to different plant extract. It was seen that glucose and total cholesterol increased in treated group when compared to control. Amongst the treated group, glucose and total cholesterol was highest in *Annona muricata* treated rats (168.75 \pm 19.56mg/dL and 95.23 \pm 13.43mg/dL respectively).

Total triglyceride and C-reactive protein decreased in all treated group except in rats treated with *Annona muricata* (148.32 \pm 28.99mg/dL and 2.31 \pm 0.24mg/dL respectively) which higher than the control. Total antioxidant capacity decreased in all treated groups with the rats treated with a combination of *Dalium guineensis* and *Annona muricata* showing the most decrease (309.17 \pm 56.88mg/dL) and rats treated with *Dalium guineensis* showing the least decrease (479.10 \pm 111.22mg/dL).

Table 1: Changes in general systemic function parameter of Wistar rats exposed to different plant extract

	Experimental setups				P-value
	C	AA	AB	AC	
Glucose (mg/d)	95.25 \pm 20.72	147.50 \pm 16.82	168.75 \pm 19.56	168.25 \pm 21.25	P<0.05
Total cholesterol (mg/dl)	70.70 \pm 15.61	80.33 \pm 9.71	95.23 \pm 13.43	78.93 \pm 15.02	P>0.05
Triglyceride (mg/dl)	123.95 \pm 16.10	97.90 \pm 5.83	148.32 \pm 28.99	106.45 \pm 9.26	P>0.05
C-RP (mg/l)	2.25 \pm 0.25	2.19 \pm 0.21	2.31 \pm 0.24	2.19 \pm 0.19	P>0.05
TAC (mg/dl)	505.25 \pm 166.92	479.10 \pm 111.22	446.30 \pm 155.99	309.17 \pm 56.88	P<0.05

NB: All values are expressed as Mean \pm SE; P<0.05 = significant difference; P>0.05 = non-significant difference; C-RP = C - reactive protein; TAC = Total Antioxidant Capacity.

The Changes in white blood cell count and differentials of Wistar rats exposed to different plant extract is shown in table 2. It was observed that white blood cell increased non-significantly across the treated group (13.73 \pm 1.66 \times 10³/ μ L, 15.73 \pm 1.01 \times 10³/ μ L and 13.20 \pm 1.53 \times 10³/ μ L for rats treated with *Dalium guineensis*, *Annona muricata* and

a combination of *Dalium guineensis* and *Annona muricata* respectively) when compared to the control. Amongst the white blood cells seen, percentage lymphocyte, mid-sized cells and granulocyte was higher in all treated group. Again, lymphocyte, mid-size cells and granulocyte increased non-significantly in all treated groups when compared to the control.

Table 2: Changes in white blood cell count and differentials of Wistar rats exposed to different plant extract

	Experimental setups				P-Value
	C	AA	AB	AC	
White blood cell ($\times 10^3/\mu\text{L}$)	12.73 \pm 0.74	13.73 \pm 1.66	15.73 \pm 1.01	13.20 \pm 1.53	P>0.05
Lymphocyte (%)	68.48 \pm 4.94	67.90 \pm 1.68	69.05 \pm 0.65	72.60 \pm 1.29	P>0.05
Mid-Sized cells (%)	8.48 \pm 0.68	8.23 \pm 0.34	9.08 \pm 0.51	9.30 \pm 0.43	P>0.05
Granulocyte (%)	23.05 \pm 4.98	24.88 \pm 1.44	21.88 \pm 0.77	18.10 \pm 1.61	P<0.05
Lymphocyte ($\times 10^3/\mu\text{L}$)	8.75 \pm 0.91	9.33 \pm 1.19	10.85 \pm 0.61	9.53 \pm 0.97	P>0.05
Mid-Sized cells ($\times 10^3/\mu\text{L}$)	1.10 \pm 0.07	1.10 \pm 0.15	1.43 \pm 0.10	1.23 \pm 0.15	P>0.05
Granulocyte ($\times 10^3/\mu\text{L}$)	2.88 \pm 0.55	3.30 \pm 0.39	3.45 \pm 0.33	2.45 \pm 0.46	P>0.05

NB: All values are expressed as Mean \pm SE; P<0.05 = significant difference; P>0.05 = non-significant difference

The Changes in red blood cell count and indices of Wistar rats exposed to different plant extract is reported in table 3. There was a non-significant decrease in red blood cell count, haemoglobin and haematocrit in all treated groups when compared to the control. However, amongst the treated group, red blood cell count, Haemoglobin and Haematocrit was highest in rats treated with a combination of *Dalium guineensis* and *Annona muricata* (6.57 \pm 0.21%, 16.90 \pm 0.84% and 48.75 \pm 3.33% respectively) while the lowest Values was reported in rats treated with *Annona muricata* (5.63 \pm 0.26%, 14.83 \pm 0.30%,

43.75 \pm 1.35% respectively). Mean corpuscular volume and mean corpuscular haemoglobin increased non-significantly in all treated groups when compared to the control. Mean corpuscular haemoglobin concentration slightly increased in rats treated with *Dalium guineensis* (36.03 \pm 1.52g/dL) while almost constant in other treated groups when compared to control. Red blood cell distribution width coefficient of variation was higher in rats treated with *Annona muricata* (14.78 \pm 0.69fL) while the reverse was the case in other treated groups.

Table 3: Changes in red blood cell count and indices of Wistar rats exposed to different plant extract

	Experimental setup				P-Value
	C	AA	AB	AC	
Red blood cell ($\times 10^6/\mu\text{L}$)	6.78 \pm 0.38	6.00 \pm 0.20	5.63 \pm 0.26	6.57 \pm 0.21	P>0.05
Haemoglobin (g/dL)	17.05 \pm 0.85	15.80 \pm 0.29	14.83 \pm 0.30	16.90 \pm 0.84	P>0.05
Haematocrit (%)	48.93 \pm 2.40	44.03 \pm 1.96	43.75 \pm 1.35	48.75 \pm 3.33	P>0.05
MCV (fL)	72.43 \pm 2.82	73.40 \pm 1.82	77.98 \pm 1.53	74.18 \pm 3.50	P>0.05
MCH (pg)	25.15 \pm 0.78	26.35 \pm 0.67	26.38 \pm 0.92	25.68 \pm 0.78	P>0.05
MCHC (g/dL)	34.98 \pm 2.07	36.03 \pm 1.52	33.88 \pm 0.55	34.75 \pm 0.69	P>0.05
RDW-CV (fL)	14.33 \pm 1.17	14.20 \pm 1.18	14.78 \pm 0.69	14.10 \pm 1.05	P>0.05
RDW-SD (%)	44.08 \pm 1.70	45.28 \pm 0.88	47.33 \pm 0.47	45.58 \pm 1.97	P>0.05

NB: All values are expressed as Mean \pm SE; P<0.05 = significant difference; P>0.05 = non-significant difference; MCV= Mean corpuscular volume; MCH = Mean corpuscular Haemoglobin; MCHC = Mean corpuscular Haemoglobin concentration; RDW-CV = Red blood distribution width coefficient of variation; RDW-SD = Red blood distribution width standard deviation

Changes in blood platelet count and indices of Wistar rats exposed to different plant extract is shown in table 4. From the table, it was seen that there was a significant increase in platelet count in all treated group, with the highest count seen in rats treated with a combination of *Dalium guineensis* and *Annona muricata* (602.75 \pm 60.12 $\times 10^3/\mu\text{L}$). Mean platelet

volume and platelet distribution width was seen to higher in rats treated with *Annona muricata* (10.30 \pm 0.44fL and 0.47 \pm 0.02% respectively). Plateletocrit and P-LCR was higher in all treated groups when compared to the control, however, P-LCR was highest in rats treated with *Annona muricata* (6.68 \pm 0.72%) amongst the treatment group.

Table 4: Changes in blood platelet count and indices of Wistar rats exposed to different plant extract

	Experimental setup				P-value
	C	AA	AB	AC	
Platelet ($\times 10^3/\mu\text{L}$)	373.00 \pm 101.43	591.75 \pm 30.97	560.00 \pm 27.32	602.75 \pm 60.12	P<0.05
MPV (fL)	7.90 \pm 0.22	7.83 \pm 0.18	8.48 \pm 0.15	7.95 \pm 0.21	P>0.05
PDW (%)	9.43 \pm 0.57	8.78 \pm 0.36	10.30 \pm 0.44	9.35 \pm 0.49	P>0.05
PCT (%)	0.22 \pm 0.06	0.46 \pm 0.03	0.47 \pm 0.02	0.47 \pm 0.04	P>0.05
P-LCR (%)	2.73 \pm 1.50	4.10 \pm 0.89	6.68 \pm 0.72	3.45 \pm 1.35	P<0.05

NB: All values are expressed as Mean \pm SE; P<0.05 = significant difference; P>0.05 = non-significant difference; MPV= Mean platelet volume; PDW = Platelet distribution width; PCT = plateletocrit; P-LCR = Platelet large cell ratio

The lipid profile, glucose levels, C-reactive protein (CRP) and total antioxidant capacity (TAC) parameters are critical indicators of overall health and

are widely used in both clinical and research settings to assess metabolic and cardiovascular risk, inflammatory status, oxidative stress, and blood

function. These biomarkers collectively provide a comprehensive overview of an individual's metabolic, cardiovascular and inflammatory health, making them indispensable tools in both clinical diagnostics and research. The findings from the study reveal several key effects of *Dalium guineense* and *Annona muricata* leaf extracts on the biochemical parameters of Wistar rats. The observed changes in glucose, total cholesterol, total triglycerides, C-reactive protein, and total antioxidant capacity reflect the impact of these plant extracts on metabolic and inflammatory processes in the rats. The study shows that glucose and total cholesterol levels were elevated in the treated groups compared to the control, with the highest levels observed in the *Annona muricata* treated rats. This suggests that *Annona muricata* may have a significant effect on glucose metabolism and cholesterol synthesis or absorption. This finding aligns with some studies that have reported potential hyperglycemic effects associated with certain phytochemicals in *Annona muricata*, which could explain the elevated glucose levels (Moghadamtousi *et al.*, 2015). The increase in total cholesterol might be due to the modulation of lipid metabolism pathways by the active compounds in *Annona muricata*, possibly leading to enhanced cholesterol synthesis or reduced cholesterol clearance.

Total triglycerides and C-reactive protein (CRP) decreased in all treated groups except for the *Annona muricata* group, which showed higher levels compared to the control. The reduction in triglycerides in the *Dalium guineense* group might indicate an effect of the extract on lipid metabolism, possibly promoting the utilization or breakdown of triglycerides. However, the higher triglyceride levels in the *Annona muricata* group suggest that this extract might affect triglyceride metabolism differently, potentially through the inhibition of triglyceride breakdown or increased synthesis. CRP, a marker of inflammation, was lower in the *Dalium guineense* and combination-treated groups, which could suggest an anti-inflammatory effect of *Dalium guineense*. Conversely, the elevated CRP in the *Annona muricata* group indicates a possible pro-inflammatory response, which contrasts with some studies that have reported anti-inflammatory properties of *Annona muricata* (de Sousa *et al.*, 2016). This discrepancy could be due to differences in dosage, duration of treatment, or specific experimental conditions.

The total antioxidant capacity decreased across all treated groups, with the combination of *Dalium guineense* and *Annona muricata* showing the most significant reduction. This decline suggests that the combination treatment might have led to oxidative

stress, possibly by depleting antioxidant reserves or by generating reactive oxygen species (ROS). On the other hand, the least decrease observed in the *Dalium guineense* group may indicate a relatively better preservation of antioxidant capacity, suggesting a potential protective effect of *Dalium guineense* against oxidative stress. The reduction in antioxidant capacity in these treatments could contradict findings in the literature where *Dalium guineense* and *Annona muricata* have been reported to possess strong antioxidant properties (Akinmoladun *et al.*, 2010; Moghadamtousi *et al.*, 2015). The observed reduction might be explained by a high oxidative challenge or an adaptive response of the rats to prolonged exposure to the extracts.

White blood cells (WBCs), also known as leukocytes, are essential components of the immune system, playing a pivotal role in defending the body against infections, foreign invaders, and abnormal cells. WBCs are categorized into different types, each with specific functions, and their differential count provides critical insights into various health conditions. The total WBC count measures the overall number of leukocytes in the blood. An elevated WBC count, known as leucocytosis, can indicate the presence of infections, inflammation, stress, or malignancies such as leukaemia. Conversely, a low WBC count, or leukopenia, may suggest bone marrow suppression, autoimmune diseases, or severe infections (Hoffbrand *et al.*, 2016). The differential WBC count breaks down the total WBC count into its five main types: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Each type of cell has a unique role in the immune response (Kumar and Clark, 2016). The differential WBC count is therefore a powerful diagnostic tool that helps clinicians identify and monitor a wide range of health conditions, including infections, immune disorders, allergic reactions, and haematological malignancies.

The observed non-significant increase in WBC count across the treated groups suggests a mild immune response induced by the plant extracts. WBCs are key players in the immune system, and an increase in their count often indicates an activation of the immune system, possibly in response to a perceived threat or as part of a general enhancement of immune function. The lack of statistical significance might indicate that while there was an upward trend, the effect was not strong enough to be considered a definitive immune response. Lymphocytes, which include B cells, T cells, and natural killer cells, play a crucial role in adaptive immunity. The higher percentage of lymphocytes observed in all treated groups suggests that the extracts may have stimulated the adaptive immune system.

This aligns with some studies that have shown that certain plant extracts can modulate lymphocyte activity, potentially enhancing the body's ability to respond to infections or other immune challenges (Oladipo *et al.*, 2016). However, the non-significant increase implies that this effect might be subtle or require a longer duration or higher dose to become more pronounced. The increase in mid-sized cells (which could include monocytes) and granulocytes (such as neutrophils, eosinophils, and basophils) in all treated groups might indicate a broad stimulation of the immune system, encompassing both innate and adaptive responses. Granulocytes are often involved in the initial, non-specific defense against pathogens, while monocytes can differentiate into macrophages and dendritic cells, key players in both immune response and inflammation (Rogers *et al.*, 2020). The non-significant increase suggests that while the extracts may support immune function, the effect is not robust enough to induce a significant change in these cell populations under the conditions of this study.

Red blood cells (RBCs), or erythrocytes, are crucial components of the blood, primarily responsible for transporting oxygen from the lungs to the tissues and facilitating the removal of carbon dioxide from the body (Hoffbrand and Moss, 2016; McMullin, 2016). The primary function of RBCs is facilitated by haemoglobin, an iron-containing protein that binds oxygen molecules. The number, shape, and size of RBCs, along with various indices, are vital indicators of an individual's overall health and can reveal underlying pathological conditions. Haemoglobin (Hb) is the protein in RBCs that binds oxygen. Measuring haemoglobin concentration provides a direct assessment of the oxygen-carrying capacity of the blood (Rodak *et al.*, 2016). Haematocrit (HCT) measures the proportion of blood volume occupied by RBCs. It is typically expressed as a percentage and provides an estimate of the blood's oxygen-carrying capacity (Wintrobe *et al.*, 2017). Mean corpuscular volume (MCV) is the average volume of individual red blood cells and is used to classify anaemia's (Hoffbrand and Moss, 2016). Mean corpuscular haemoglobin (MCH) measures the average amount of haemoglobin per red blood cell. It is closely related to MCV and provides additional information for classifying anaemia's (Rodak *et al.*, 2016). Mean corpuscular haemoglobin concentration (MCHC) is the average concentration of haemoglobin in a given volume of packed red blood cells (Wintrobe *et al.*, 2017). Red cell distribution width (RDW) measures the variation in size (anisocytosis) of red blood cells (Hoffbrand and Moss, 2016).

The lower values of RBC count, haemoglobin, and haematocrit in the *Annona muricata* treated group could suggest that this extract alone might not be as effective in supporting erythropoiesis or could potentially have a mild inhibitory effect. This result contrasts with the findings of other studies where *Annona muricata* has been reported to have positive effects on various health parameters (Moghadamtousi *et al.*, 2015). The discrepancy might be due to differences in dosage, extract preparation, or the specific biological model used. The non-significant increase in MCV and MCH across treated groups indicates a possible mild increase in the average size and haemoglobin content of red blood cells, which might reflect an adaptive response to maintain oxygen-carrying capacity. The slight increase in MCHC in rats treated with *Dalium guineense* suggests a higher concentration of hemoglobin within red blood cells, which might be a compensatory mechanism (Aldredge *et al.*, 2018). The higher RDW-CV in *Annona muricata* treated rats suggests greater variability in red blood cell size. This could indicate that *Annona muricata* might affect red blood cell production or maturation, leading to a wider range of cell sizes.

The findings extend the existing body of knowledge by providing insights into how *Dalium guineense* and *Annona muricata* influence red blood cell parameters in Wistar rats. Previous research has shown that *Annona muricata* has various biological activities, but its effect on erythropoiesis has been less studied (Moghadamtousi *et al.*, 2015). This study contributes to understanding the potential haematological effects of these plant extracts, suggesting that while they may not cause significant changes individually, their combination might offer more pronounced benefits. The non-significant decrease in RBC parameters and the variability in RDW-CV observed in this study suggest that while the extracts have some effect on haematological parameters, these effects are subtle and might depend on specific conditions or doses. These results align with some studies that report varied effects of plant extracts on blood parameters depending on the model and extract preparation (Yagura *et al.*, 2018).

Platelets, also known as thrombocytes, are small, anucleate cell fragments derived from megakaryocytes in the bone marrow. They play a crucial role in haemostasis, the process that prevents excessive bleeding when blood vessels are injured. Upon vascular injury, platelets rapidly adhere to the exposed endothelium, aggregate to form a platelet plug, and release granules that promote coagulation, thereby stabilizing the initial platelet plug with a fibrin clot (Michelson, 2012). While platelet indices such as count, Mean platelet volume (MPV), platelet

distribution width (PDW), plateletcrit (PCT), and platelet large cell ratio (P-LCR) are valuable tools in diagnosing and managing a range of haematological and cardiovascular conditions. They provide insights into platelet production, function, and turnover, helping clinicians to better understand and treat disorders related to haemostasis and thrombosis.

The significant increase in platelet count across all treated groups suggests that the plant extracts may stimulate thrombopoiesis or affect platelet production and survival. Platelets play a crucial role in haemostasis and wound repair, and an increase in their count could be indicative of a compensatory mechanism to enhance blood clotting and tissue repair processes. The highest platelet count in the combination treatment group suggests a potential synergistic effect of *Dalium guineense* and *Annona muricata* on platelet production or regulation. The higher MPV and PDW observed in *Annona muricata* treated rats indicate that this extract might induce changes in platelet size and distribution. MPV is often used as an indicator of platelet activation or turnover, with larger platelets generally being younger and more reactive (Sahni *et al.*, 2017). The increased PDW suggests a broader variability in platelet size, which might reflect increased platelet production or release of immature platelets from the bone marrow (Kopp *et al.*, 2018). Elevated PCT and P-LCR in all treated groups indicate an increase in the overall volume of platelets and the proportion of large platelets, respectively. PCT is the product of platelet count and MPV, thus an increase suggests either a higher platelet count or increased platelet size, or both. The highest P-LCR in the *Annona muricata* treated group suggests that this extract may particularly enhance the proportion of large platelets, which could be associated with more active or functional platelets (Hirsh *et al.*, 2012).

The results of this study extend our understanding of the effects of plant extracts on platelet dynamics. The increase in platelet count and associated indices aligns with findings from other studies that report hematopoietic effects of plant extracts. For instance, *Annona muricata* has been reported to exhibit various biological activities, including potential effects on blood parameters (Moghadamtousi *et al.*, 2015). The observed increase in platelet count and indices suggests that *Annona muricata* and its combination with *Dalium guineense* might have a stimulatory effect on platelet production or release, which aligns with research on other medicinal plants that enhance haematological parameters (Sahni *et al.*, 2017; Kopp *et al.*, 2018). The highest platelet count and P-LCR in the combination treatment group might indicate a

synergistic effect of the plant extracts, which supports the idea that combining different botanical extracts can sometimes yield enhanced therapeutic effects (Kumar *et al.*, 2016). Conversely, the specific increase in MPV and PDW in *Annona muricata* treated rats might be attributed to unique phytochemical components of the extract that influence platelet physiology differently than other treatments.

Conclusion: Overall, this research highlights the differential effects of *Dalium guineense* and *Annona muricata* leaf extracts on haematological parameters, lipid metabolism, and antioxidant capacity. The findings suggest that while these extracts may offer some benefits, particularly in enhancing platelet count and modulating lipid profiles, their combined effect might influence antioxidant defences and immune cell distribution. Further research is needed to elucidate the mechanisms underlying these effects and to determine the potential therapeutic applications of these plant extracts.

Declaration of conflict of interest: The authors declare no conflict of interest.

Data availability statement: Data are available upon request from the corresponding author.

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