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Phytochemical profiling and dose-dependent erythrocyte membrane-protective, haematological and antioxidant effects of aqueous *Ocimum gratissimum* (Scent Leaf) leaf extract in Wistar rats

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Abstract

Ocimum gratissimum (scent leaf) is a widely consumed African medicinal herb whose rich phytochemical composition has been linked to diverse pharmacological activities, yet its direct in vivo effect on red blood cell (RBC) membrane integrity remains poorly characterized. This study profiled the phytochemical composition of an aqueous leaf extract of *O. gratissimum* and evaluated its dose-dependent effects on RBC membrane stability, haematological indices, and oxidative stress markers in Wistar rats. Twelve adult Wistar rats were randomly allocated into four groups (n = 3): a distilled-water control and three extract-treated groups receiving 300, 400, or 500 mg/kg body weight orally for 14 days. Qualitative and quantitative phytochemical screening, full haematological profiling, osmotic fragility testing, and biochemical assays for total protein, reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) were performed using standard methods, and data were analyzed by one-way ANOVA with Tukey-Kramer post-hoc comparison ($P < 0.05$). Eleven of twelve screened phytoconstituents were detected; only anthraquinone derivatives were absent. Osmotic fragility curves showed a rightward shift at 400 and 500 mg/kg, indicating improved membrane resistance. Haematocrit and RBC count rose significantly at these two doses relative to control ($P < 0.05$), with stable mean corpuscular volume confirming normocytic erythropoiesis. The 300 mg/kg dose was associated with significantly reduced MCH and MCHC and elevated MDA ($P < 0.05–0.01$), consistent with a transient hypochromic, pro-oxidant low-dose effect, whereas 500 mg/kg fully normalized MDA and total protein to control-equivalent levels. These findings indicate that aqueous *O. gratissimum* leaf extract confers dose-dependent erythrocyte membrane-protective and erythropoietic benefits at 400–500 mg/kg body weight, while identifying a narrower safety margin at lower doses that warrants further toxicological characterization before therapeutic application is considered.

Keywords: *Ocimum gratissimum*; Erythrocyte Membrane Integrity; Osmotic Fragility; Oxidative Stress; Phytochemical Screening; Wistar Rats

1. Introduction

Medicinal plants remain central to primary healthcare across much of the developing world, where herbal remedies are favoured for their accessibility, affordability, and cultural acceptance (Yuan et al., 2016; Ehiremen et al., 2025). Among the medicinal plants widely utilized in West Africa is *Ocimum gratissimum* L. (Lamiaceae), popularly known as scent leaf, which is used both as a culinary spice and as a traditional remedy for gastrointestinal, respiratory, and reproductive health conditions, and is frequently incorporated into postpartum restorative soups in Nigerian ethnomedicine (Ugbogu et al., 2021; Bamisaye et al., 2025).

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Phytochemical profiling has consistently identified a broad spectrum of bioactive secondary metabolites in *O. gratissimum* leaves, including alkaloids, flavonoids, phenolic acids, tannins, saponins, and terpenoids (Akinmoladun et al., 2007; Ikokide et al., 2025), many of which possess marked free-radical scavenging activity (Okeke et al., 2023; Moneme et al., 2025). Because oxidative stress is a principal driver of red blood cell (RBC) membrane damage — promoting lipid peroxidation, reduced deformability, and increased haemolysis — antioxidant-rich plant extracts have attracted interest as potential erythrocyte membrane-stabilizing agents (Risinger and Kalfa, 2020; Spinelli et al., 2024). In vitro work has shown that hydroethanolic extracts of *O. gratissimum* reduce heat- and hypotonicity-induced erythrocyte destabilisation (Arthur et al., 2025), while in vivo studies report dose-dependent increases in red cell count and haemoglobin concentration (Arthur et al., 2025) and amelioration of phenylhydrazine-induced anaemia (Akara et al., 2021).

Despite these findings, few studies have directly linked the phytochemical composition of *O. gratissimum* to functional, in vivo measures of RBC membrane integrity; most existing work addresses general haematological indices or antioxidant capacity in isolation (Onyema-Iloh et al., 2019). This gap limits both the mechanistic understanding of the plant's haematological pharmacology and the rational selection of an effective and safe dose range. The present study therefore combined qualitative and quantitative phytochemical screening with osmotic fragility testing, full haematological profiling, and a multi-parameter oxidative stress panel to evaluate the dose-dependent effect of aqueous *O. gratissimum* leaf extract on RBC membrane integrity in Wistar rats, with the aim of identifying the dose range at which membrane-protective and erythropoietic benefits are maximized while characterizing any low-dose safety concerns.

2. Materials and methods

2.1. Study Design, Area and Ethical Approval

A controlled, in vivo experimental design was used. The study was conducted at the Experimental Animal House, Department of Animal Science, School of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria (7°29′00″N, 2°53′00″E), over a three-month period (January–March 2026). Ethical approval was obtained from the Babcock University Health Research Ethics Committee (BUHREC) prior to commencement, and all procedures conformed to the Regulations and Standards for Animal Care of the National Research Council (2011) and the Institute for Laboratory Animal Research (ILAR, 1996).

2.2. Plant Material and Extract Preparation

Fresh *O. gratissimum* leaves were procured from Ilishan Market, Ogun State, Nigeria, and botanically authenticated. Leaves were washed, shade-dried at room temperature, and pulverised to a fine powder. The powder was aqueous-extracted, filtered, concentrated by rotary evaporation at 60°C, and dried to a stable powder under vacuum, which was stored in an airtight container at low temperature until use (Enenebeaku et al., 2024).

2.3. Experimental Animals and Grouping

Twelve adult Wistar rats (150 ± 50 g) of comparable age were obtained from the University of Ibadan animal facility, Oyo State, Nigeria, examined for clinical health on arrival, and acclimatized for two weeks under standard husbandry conditions (25 ± 2°C; 45–50% relative humidity) with free access to a standard rodent diet and water. Apparently healthy adult rats were included; underweight, juvenile, or symptomatic animals were excluded. After acclimatization, animals were randomly assigned to four groups of three rats each: Group 1 (control, distilled water), Group 2 (300 mg/kg extract), Group 3 (400 mg/kg extract), and Group 4 (500 mg/kg extract). Body weight was recorded before, during, and after the administration period using an electronic analytical balance.

2.4. Sub-Chronic Administration Protocol

Plant extract was administered by oral gavage twice daily for 14 consecutive days at the assigned dose, prepared by dissolving the dried extract in distilled water to the required working concentration. Animals were monitored daily for signs of toxicity, and body weight and general health were recorded throughout. On day 15, animals were euthanized by diethyl ether, and blood was collected by cardiac puncture into EDTA bottles for analysis. Carcasses and specimens were disposed of in accordance with standard biosafety procedures.

2.5. Qualitative Phytochemical Screening

Qualitative screening for twelve phytoconstituent classes — saponins, reducing sugars, carbohydrates, flavonoids, tannins, phenolics, terpenoids, alkaloids, deoxysugars, phytosterols, anthraquinone derivatives, and proteins — was

performed in triplicate using standard colour and precipitation reactions, following the methods of Borokini and Omotayo (2012) and Njoku and Obi (2009), as adapted by Enenebeaku et al. (2024).

2.6. Quantitative Phytochemical Determination

Total flavonoid content was determined by the aluminium chloride colorimetric method of Ebrahimzadeh et al. (2008), with absorbance read at 415 nm against a quercetin standard curve and results expressed as mg quercetin equivalents (QE)/g extract. Total phenolic content was determined by the Folin–Ciocalteu method of Kim et al. (2023), with absorbance read at 750 nm against a gallic acid standard curve and results expressed as mg gallic acid equivalents (GAE)/g extract. All absorbance readings were obtained using a UV/Vis spectrophotometer (model T80).

2.7. Haematological Analysis

Whole blood was analyzed within 2–3 hours of collection using an automated veterinary haematology analyser (Biobase) based on laser flow cytometry, generating white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB), haematocrit (HCT), platelet (PLT) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and the leucocyte differential (lymphocytes, neutrophils, and mixed monocyte/eosinophil/basophil populations).

2.8. Biochemical Assays

Reduced glutathione (GSH) was assayed by the DTNB method, based on the reaction of GSH with 5,5′-dithiobis-(2-nitrobenzoic acid) to yield a chromogen measured at 412 nm. Malondialdehyde (MDA) was assayed by the thiobarbituric acid reactive substances (TBARS) method, with the MDA–TBA chromogen measured at 532 nm. Catalase (CAT) activity was determined by the Sinha method, based on the reaction of residual hydrogen peroxide with ammonium molybdate, measured at 410 nm. Superoxide dismutase (SOD) activity was determined by the adrenaline auto-oxidation inhibition method, with absorbance monitored at 480 nm over two minutes. Total protein was determined alongside the above panel using standard spectrophotometric procedures.

2.9. Osmotic Fragility Testing

Erythrocyte osmotic fragility was assessed by exposing whole blood to a graded series of hypotonic buffered saline solutions (0.1–0.7% NaCl, pH 7.4) and recording the degree of haemolysis at each concentration to generate a fragility curve for each group.

2.10. Statistical Analysis

Data were analyzed using SPSS version 22.0. One-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparisons post-hoc test was used to compare group means, with statistical significance set at $P < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

3. Results

Qualitative screening confirmed the presence of eleven of the twelve phytoconstituent classes tested in the aqueous extract of *O. gratissimum*; anthraquinone derivatives were the only class not detected (Table 1).

Table 1 Qualitative phytochemical screening of aqueous *Ocimum gratissimum* leaf extract

S/N	Phytoconstituent	Result
1	Alkaloids	+
2	Carbohydrates	+
3	Reducing sugars	+
4	Deoxysugars (Keller test)	+
5	Saponins	+
6	Phytosterols	+
7	Tannins	+

8	Phenolics	+
9	Flavonoids	+
10	Anthraquinone derivatives	–
11	Terpenoids	+
12	Proteins	+

Note: + = present; – = absent.

Quantitative spectrophotometric analysis confirmed substantial flavonoid and phenolic content in the extract, providing a measurable biochemical basis for its antioxidant and membrane-protective activity.

Osmotic fragility curves were sigmoidal across all groups, consistent with the classical pattern of progressive haemolysis under increasing hypotonic stress. Groups 3 (400 mg/kg) and 4 (500 mg/kg) showed a rightward shift relative to control, indicating that erythrocytes from these animals required greater osmotic stress to haemolyze to an equivalent degree — the defining signature of improved membrane resistance — whereas the curve for Group 2 (300 mg/kg) did not differ materially from control.

Table 2 Haematological parameters of Wistar rats following 14-day administration of aqueous *Ocimum gratissimum* leaf extract (mean ± SEM)

Parameter	**Group 1 (Control)**	**Group 2 (300 mg/kg)**	**Group 3 (400 mg/kg)**	**Group 4 (500 mg/kg)**
WBC ($\times 10^9$/L)	7.07 ± 1.15	10.90 ± 1.60	14.04 ± 2.00	16.99 ± 2.26[1]
HCT (%)	49.03 ± 2.36	58.50 ± 5.22	65.77 ± 2.02[1]	66.37 ± 3.11[1]
HGB (g/L)	152.00 ± 7.09	128.30 ± 28.69	170.00 ± 5.13	165.33 ± 3.71
RBC ($\times 10^{12}$/L)	9.40 ± 0.44	9.86 ± 0.92	11.35 ± 0.19[1]	11.76 ± 0.69[1]
PLT ($\times 10^9$/L)	1103.6 ± 78.87	1773.3 ± 242.08	1564.3 ± 97.83	1432.0 ± 259.08
MCV (fL)	58.40 ± 0.51	59.33 ± 0.26	57.90 ± 0.95	56.53 ± 1.05
MCH (pg)	18.13 ± 0.21	12.67 ± 1.98[2]	14.96 ± 0.21	14.13 ± 0.59
MCHC (g/L)	310.67 ± 0.88	213.67 ± 34.04[2]	258.67 ± 2.84	250.00 ± 8.62
Lymphocytes (%)	61.53 ± 3.08	52.70 ± 9.11	40.86 ± 6.00	55.46 ± 8.14
MID (%)	7.90 ± 0.97	12.13 ± 2.48	8.33 ± 0.48	8.46 ± 0.89
Neutrophils (%)	30.56 ± 3.93	35.16 ± 11.50	50.80 ± 0.58	36.00 ± 8.13

[1]$P < 0.05$ vs Group 1 (control); [2]$P < 0.05$ vs Group 1 (control), indicating significant reduction.

WBC count rose progressively with dose, reaching significance only between control and Group 4 ($P < 0.05$). HCT and RBC count were significantly elevated in Groups 3 and 4 relative to control ($P < 0.05$ for each), while MCV remained statistically unchanged across groups ($P = 0.1533$), indicating that the increases reflected genuine normocytic erythropoiesis rather than haemoconcentration. HGB ($P = 0.2802$) and PLT ($P = 0.1623$) did not differ significantly overall, although HGB was numerically lowest in Group 2 and PLT was numerically elevated across all treated groups relative to control. MCH and MCHC were both significantly reduced in Group 2 relative to control ($P < 0.05$ for each), with partial, non-significant numerical recovery at higher doses. Leucocyte differential parameters (lymphocytes, MID, neutrophils) did not differ significantly across groups, although Group 3 showed a numerically reduced lymphocyte percentage alongside a reciprocally elevated, tightly clustered neutrophil percentage.

Body weight gain over the 14-day period was significantly suppressed in all three extract-treated groups relative to control ($P < 0.05$ for Groups 2 and 3; $P < 0.01$ for Group 4), against a background of a pre-existing, statistically significant baseline weight difference between Group 1 and Group 4 ($P < 0.05$) that warrants cautious interpretation of the Group 4 weight-change data.

Table 3 Oxidative stress and protein biomarkers of Wistar rats following 14-day administration of aqueous *Ocimum gratissimum* leaf extract (mean ± SEM)

Parameter	Group 1 (Control)	Group 2 (300 mg/kg)	Group 3 (400 mg/kg)	Group 4 (500 mg/kg)
Total protein (mg/mL)	50.00 ± 7.56	21.63 ± 3.61[3]	26.00 ± 3.83[3]	48.73 ± 1.68
GSH (μmol/mg protein)	0.00023 ± 0.0001	0.00072 ± 0.00017[4]	0.00043 ± 0.0005	0.00034 ± 0.0003
MDA (nmol/mg protein)	0.26 ± 0.03	0.79 ± 0.14[5]	0.53 ± 0.08	0.27 ± 0.03
CAT (U/mg protein)	0.044 ± 0.014	0.352 ± 0.159	4.518 ± 4.356	0.162 ± 0.013

[3]P < 0.05 vs control (Group 1); [4]P < 0.05 vs control; [5]P < 0.01 vs control. Overall ANOVA: total protein P = 0.0043; GSH P = 0.0283; MDA P = 0.0052; CAT P = 0.4446 (not significant).

Total protein showed a significant overall, U-shaped dose response (P = 0.0043), being significantly reduced at 300 and 400 mg/kg relative to control (P < 0.05 each) but fully restored to control-equivalent levels at 500 mg/kg, which was also significantly higher than both lower extract doses (P < 0.05 each). GSH differed significantly overall (P = 0.0283), with a significant elevation confined to the 300 mg/kg group relative to control (P < 0.05), and a descending, non-significant numerical trend through the higher doses. MDA showed a highly significant overall pattern (P = 0.0052): the 300 mg/kg dose produced a marked elevation relative to control (P < 0.01), whereas the 500 mg/kg dose normalised MDA to a level statistically indistinguishable from control and significantly lower than the 300 mg/kg group (P < 0.01), with the 400 mg/kg group occupying an intermediate, non-significant position. CAT activity showed a numerically large, near-stepwise increase with dose but did not reach statistical significance (P = 0.4446), reflecting high inter-animal variability, particularly at 400 mg/kg.

4. Discussion

Qualitative screening confirmed that aqueous *O. gratissimum* leaf extract contains eleven of twelve assayed phytoconstituent classes, a profile consistent with previously reported West African accessions of the plant (Ugbogu et al., 2021). Of these, flavonoids and phenolics are of greatest mechanistic relevance to the erythrocyte-protective outcomes observed here: these compounds intercalate into the erythrocyte lipid bilayer, donate hydrogen atoms to neutralize reactive oxygen species before peroxidative chain propagation, and chelate transition metal ions that catalyze Fenton-type oxidative damage, thereby preserving both the membrane lipid matrix and the underlying cytoskeletal protein network (Atanasov et al., 2021). Saponins are known to interact with membrane cholesterol (Orrico et al., 2023), while alkaloids are plausibly linked to the leucocytosis observed at the highest dose (Michael, 2023; Arthur et al., 2025).

The osmotic fragility data provide the most direct functional evidence for membrane protection: the rightward shift of the fragility curve at 400 and 500 mg/kg indicates that erythrocytes from these groups withstood greater hypotonic stress before lysis, the classical signature of improved osmotic resistance (Lippi and Mattiuzzi, 2020; Obeagu et al., 2024). This is mechanistically coherent with reduced lipid peroxidation of membrane phospholipids and protection of cytoskeletal proteins such as spectrin and ankyrin from oxidative cross-linking (Sobreira et al., 2020; Męczarska et al., 2025), and extends earlier in vitro reports of membrane-stabilising activity of *O. gratissimum* extracts (Arthur et al., 2025) into a controlled in vivo dosing context.

The concurrent, significant elevation of haematocrit and RBC count at 400 and 500 mg/kg, in the presence of an unchanged MCV, indicates that the extract stimulated production of morphologically normal, normocytic erythrocytes rather than producing a haemoconcentration artefact (Perrone et al., 2018). This is consistent with the capacity of flavonoids and phenolics to enhance iron utilization and protect erythroid precursors from oxidative attrition (Ezeorba et al., 2024; Moneme et al., 2025), and with the statistically equivalent haematocrit achieved at 400 and 500 mg/kg, which together suggest a therapeutic ceiling near 400 mg/kg beyond which further dose escalation confers no additional erythropoietic benefit.

A distinct, dose-specific concern emerged at 300 mg/kg, where MCH and MCHC were both significantly reduced relative to control — the defining haematological signature of hypochromia — despite RBC count and haematocrit remaining statistically unchanged from control at this dose (Fakoya et al., 2025). This pattern, together with the significant elevation of MDA at the same dose, is consistent with a transient pro-oxidant, iron-chelating effect of tannins and phenolics at sub-threshold phytochemical concentrations that may transiently impair haem synthesis without reducing

overall erythropoietic output (Sobreira et al., 2020; Cotoraci et al., 2021). The progressive, though not statistically significant, numerical recovery of MCHC at higher doses suggests that the erythropoietic and iron-mobilising activity of higher phytochemical concentrations eventually outweighs this low-dose chelating effect.

The biochemical oxidative stress panel reinforces this dose-dependent interpretation. The hermetic, U-shaped total protein response — significantly reduced at 300 and 400 mg/kg but fully restored at 500 mg/kg — is consistent with a transient hepatic protein-synthetic burden at sub-optimal phytochemical concentrations that is overcome by the hepatoprotective, antioxidant activity of higher doses, plausibly mediated through Nrf2 pathway activation (Atanasov et al., 2021; Alabi et al., 2021). The significant elevation of MDA at 300 mg/kg, normalising to control-equivalent levels at 500 mg/kg, directly parallels and corroborates the osmotic fragility findings: the doses at which lipid peroxidation was most effectively suppressed are precisely those at which the greatest membrane-protective shift was observed, providing convergent functional and biochemical evidence for a genuine, dose-dependent erythroprotective effect (Spinelli et al., 2024). The transient elevation of GSH at 300 mg/kg may reflect a compensatory antioxidant response to the heightened oxidative burden at this dose (Cotoraci et al., 2021), while the numerically large but statistically unconfirmed rise in catalase activity across treated groups is consistent with flavonoid-mediated upregulation of antioxidant enzyme expression (Ezeorba et al., 2024), tempered by the limited statistical power of the small group sizes used in this study.

The consistent suppression of body weight gain across all extract-treated groups is plausibly attributable to the antihyperglycaemic and lipogenesis-inhibiting properties of flavonoids and terpenoids, which may reduce glucose absorption and enhance energy expenditure (Ikokide et al., 2025); a contribution from mild gastrointestinal effects on feed efficiency cannot be excluded and warrants direct assessment of feed and water intake in future work. Collectively, these findings position 400–500 mg/kg as the dose range at which the membrane-protective, erythropoietic, and antioxidant properties of aqueous *O. gratissimum* leaf extract are most reliably expressed, while identifying 300 mg/kg as a dose at which low-grade pro-oxidant and hypochromic effects emerge and should be characterised further before any therapeutic recommendation.

Limitations

The principal limitation of this study is the small group size (n = 3), which limited statistical power for parameters showing biologically plausible but statistically unconfirmed trends (haemoglobin, platelet count, neutrophil shift, and catalase activity). The 14-day treatment duration did not include a recovery or washout phase, and toxicological endpoints (liver and kidney function markers, histopathology) were not assessed, limiting safety inference. Individual phytochemical constituents were not identified by HPLC, GC–MS, or LC–MS, and osmotic fragility was interpreted by curve positioning rather than median corpuscular fragility values.

5. Conclusion

Aqueous *Ocimum gratissimum* leaf extract, characterized by a broad phytochemical profile dominated by flavonoids, phenolics, alkaloids, and saponins, exerted significant, dose-dependent effects on erythrocyte membrane integrity, erythropoiesis, and oxidative stress in Wistar rats. Osmotic fragility testing demonstrated improved membrane resistance at 400 and 500 mg/kg, corroborated by significant, normocytic increases in haematocrit and RBC count and by normalisation of lipid peroxidation and total protein at the highest dose. A narrower safety margin was identified at 300 mg/kg, marked by hypochromic red cell indices and elevated malondialdehyde. These findings support the traditional haematological use of scent leaf while indicating that dose selection, within the range of 400–500 mg/kg, is critical to maximising erythroprotective benefit and should be confirmed in larger, adequately powered studies incorporating toxicological and pharmacokinetic endpoints before clinical extrapolation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

This study was approved by the Babcock University Health Research Ethics Committee (BUHREC). All animal procedures complied with the National Research Council (2011) Regulations and Standards for Animal Care and the Institute for Laboratory Animal Research (ILAR, 1996) guidelines.

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Author Contributions

Kabirah Sarumi Oyinade, Emmanuel Olusegun Ileoma, and Chinenye Mary Okorochi conceived and designed the study; Stephen Sunday Udofia , and Kabirah Sarumi Oyinade conducted the experimental procedures and laboratory analyses; Akintunde Akintayo Akinjinmi performed the statistical analysis; Enoch Chibuike Okorochi and Akinyo Adeyinka Samson contributed to data interpretation and literature review; Chinenye Mary Okorochi supervised the study and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.,

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI Use

During manuscript preparation, the authors used an AI-assisted writing tool to support language editing and formatting of the text derived from the authors' original research and data. All scientific content, data, and interpretations are the authors' own, and the authors reviewed and take full responsibility for the final content of this publication.

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