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Anti-anemic Potential of *Parkia speciosa* Hassk. Peel Extract in Sodium Nitrite-Induced Anemic Wistar Rats: Evaluation of Hematological Parameters

Potensi Anti-Anemia Ekstrak Kulit *Parkia speciosa* Hassk. pada Tikus Wistar Anemia Terinduksi Natrium Nitrit: Evaluasi Parameter Hematologis

Yuliet Susanto[1*], Muhammad Fakhrul Hardani[2], Khildah Khaerati[2], Andi Atirah Masyita[2], Nurul Wahyuni Palangit[2], Nur An-nisa[2], Resmi[2]

[1]*Pharmacist Profession Education, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu 94118, Central Sulawesi, Indonesia*

[2]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu 94118, Central Sulawesi, Indonesia*

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ABSTRACT

Anemia involves reduced hemoglobin, erythrocyte count and hematocrit, often with oxidative damage to erythrocytes. Petai (*Parkia speciosa* Hassk.) peel is underused waste with hematoprotective compounds. This study tested its anti-anemic potential in sodium nitrite-induced rats. Thirty male rats formed six groups of five (three analysed): normal, negative and positive controls (ferrous fumarate), and extract at 300, 400 and 500 mg/kg BW. Sodium nitrite 50 mg/kg BW ran 14 days, the extract orally in the last seven. Hematology was measured on days 0 and 14. Phytochemical screening revealed alkaloids, flavonoids, saponins, tannins and steroids. The extract improved hemoglobin, erythrocyte count and hematocrit at all doses; the highest raised leukocytes markedly (50.14 ± 17.89 $\times 10^3$/µL), possibly an inflammatory or stress response. The 300 mg/kg BW dose was optimal.

ABSTRAK

Anemia ditandai dengan penurunan hemoglobin, jumlah eritrosit, dan hematokrit, sering disertai kerusakan oksidatif pada eritrosit. Kulit petai (*Parkia speciosa* Hassk.) adalah limbah yang mengandung senyawa bioaktif berpotensi hematoprotektif. Penelitian ini mengevaluasi potensi anti-anemianya pada tikus yang diinduksi natrium nitrit. Tiga puluh tikus jantan dibagi menjadi enam kelompok berisi lima ekor, tiga ekor per kelompok dianalisis: kontrol normal, kontrol negatif, kontrol positif (ferrous fumarate), serta ekstrak dosis 300, 400, dan 500 mg/kg BB. Natrium nitrit 50 mg/kg BB diberikan 14 hari, ekstrak oral pada tujuh hari terakhir. Parameter hematologi diukur pada hari ke-0 dan ke-14. Skrining fitokimia menunjukkan alkaloid, flavonoid, saponin, tanin, dan steroid. Ekstrak memperbaiki hemoglobin, eritrosit, dan hematokrit pada semua dosis; dosis tertinggi meningkatkan leukosit secara nyata (50,14 ± 17,89 $\times 10^3$/µL), yang dapat mengindikasikan respons inflamasi atau stres. Dosis 300 mg/kg BB dinilai optimal.

**Corresponding author:*
Yuliet Susanto (yuliet_susanto@yahoo.com)

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1. INTRODUCTION

Anemia remains a global public health problem affecting millions of people worldwide, particularly in developing countries like Indonesia (Priliani et al., 2025). It is a condition in which hemoglobin (Hb) levels or the number of red blood cells fall below normal, reducing the blood's ability to transport oxygen to body tissues, and can result from reduced red blood cell production, increased destruction, or blood loss (Suprapti et al., 2025), with iron deficiency being the most common cause, particularly among women and young girls (Sari et al., 2022). The World Health Organization (WHO) reports that the global prevalence of anemia among women aged 15–49 years is approximately 29.9%, rising to 42% in Southeast Asia (Pulingmuding et al., 2024), while in Indonesia the prevalence among adolescents aged 15–24 remains relatively high at 15.5% (Lutfiasari et al., 2025). Anemia in adolescents, particularly girls, is a serious concern because it can impair physical growth and cognitive development, reduce learning concentration, and cause fatigue and emotional disturbance; if left untreated, it can persist into adulthood and pregnancy, increasing the risk of bleeding, premature birth, low birth weight, and stunting (Septiana et al., 2025). Early treatment of anemia is therefore crucial to prevent these long-term impacts on maternal and child health (Quezada-Pinedo et al., 2021).

Anemia is generally treated with iron supplements or, in certain situations, blood transfusions. However, the continued use of iron supplements often causes side effects such as constipation, gastrointestinal disturbances, and other discomforts that can reduce patient compliance with therapy (Ciont et al., 2025). This situation has prompted the need for safer, more effective, and more readily available alternative treatments. One widely developed approach is the use of natural medicinal plants due to their lower side effects, affordability, and the potential to contain various bioactive compounds that support hematopoiesis and maintain erythrocyte stability (Abuaraki et al., 2024). One plant with potential as an antianemia agent is petai (*Parkia speciosa* Hassk.), a legume widely consumed in Southeast Asia, particularly Indonesia; besides being a food ingredient, it contains active compounds including alkaloids, flavonoids, polyphenols, saponins, tannins, vitamins, minerals, and iron that contribute to red blood cell formation (Chhikara et al., 2018). Petai production in Indonesia is also quite high, making it abundantly available. However, petai peel is generally left as household organic waste. It has not been optimally utilized, even though this part is known to contain bioactive compounds with significant potential as a herbal medicine (Fajrin & Susila, 2019; Saputro et al., 2021).

Several studies have shown that petai peel extract contains phenolic compounds, flavonoids, alkaloids, saponins, and tannins with high antioxidant activity (Nadia et al., 2024), that help protect erythrocytes from oxidative damage and inhibit the formation of Heinz bodies clumps of denatured hemoglobin that can trigger hemolytic anemia (Sheth et al., 2021). Most previous studies, however, have focused on the edible seed rather than the peel; for example, the ethanol extract of petai seeds has been reported to increase hemoglobin levels in white rats induced by sodium nitrite ($NaNO_2$) (Nursucihta et al., 2014). Studies evaluating the hematoprotective potential of *P. speciosa* peel extract remain limited, particularly regarding its effects on a complete hematological profile including hemoglobin, erythrocyte count, hematocrit, leukocyte count, and platelet count in sodium nitrite-induced anemia models, and the underlying antioxidant mechanisms by which petai peel may protect erythrocytes remain unclear.

Sodium nitrite-induced anemia is one experimental model commonly used to study anemia, in which sodium nitrite oxidizes hemoglobin from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) form, leading to methemoglobin formation, impaired oxygen transport, and oxidative stress-related erythrocyte damage, thereby reducing hemoglobin levels, erythrocyte count, and hematocrit (Gluhcheva et al., 2012). This model is therefore relevant for evaluating natural compounds with hematoprotective and antioxidant potential; erythrocyte count and hematocrit serve as key indicators of anemia and of antianemic efficacy, since erythrocytes transport oxygen throughout the body while hematocrit reflects the proportion of red blood cells in the blood, and a decrease in both indicates disrupted erythropoiesis and reduced oxygen-carrying capacity (Helmyati et al., 2023). Based on this background, this study aimed to evaluate the hematoprotective potential of *P. speciosa* peel extract in sodium nitrite induced anemic Wistar rats by assessing hematological parameters, including Hb, RBC, HCT, WBC, and PLT levels. This study also supports the sustainable utilization of petai peel as an underutilized organic waste with potential value as a natural antianemic candidate.

2. METHODS

2.1. Materials

The instruments used in this study included an analytical balance, model AFP-360L (Adam Equipment Co. Ltd., Milton Keynes, United Kingdom), a hot plate and magnetic stirrer, model H4000-HS (Denville Scientific Inc., South Plainfield, New Jersey, USA), a water bath, model WNB 14 (Memmert GmbH + Co. KG, Schwabach, Germany), a rotary evaporator, model OSB-2100 (Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and a hematology analyzer, model Mythic 18 (Orphée S.A., Plan-les-Ouates, Geneva, Switzerland). The materials used in this study were 96% ethanol technical grade (PT Brataco, Jakarta, Indonesia), sodium nitrite ($NaNO_2$) technical grade (Merck KGaA, Darmstadt, Germany), sodium carboxymethyl cellulose (Na-CMC), food grade (PT Brataco, Jakarta, Indonesia), ferrous fumarate (Tablet Tambah Darah Neo, Kimia Farma, Indonesia), diluent solution, lytic solution, and enzymatic cleaner, all original Mythic reagents (Orphée S.A., Plan-les-Ouates, Geneva, Switzerland). All reagents used for phytochemical screening were purchased from CV. Nitrakimia, Yogyakarta, Indonesia.

2.2. Plant Determination and Preparation

Fresh petai peels were collected on 12 May 2025 from Sidera Village, Sigi Regency, Central Sulawesi, Indonesia. Plant identification was conducted at the Plant Systematics Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences, Tadulako University, with identification letter No. 068/UN28.1/PSB/2025. The voucher specimen was deposited under number 068/UN28.1/PSB/2025 at the Plant Systematics Laboratory, Biology Study Program, Tadulako University. The peels were separated from unwanted materials and subjected to wet sorting to remove dirt and damaged parts. Subsequently, the peels were washed thoroughly under running water and dried naturally by air-drying in a shaded area protected from direct sunlight exposure. The drying process was continued until the simplicial reached a sufficiently dry condition, indicated by a brittle texture that could be easily crumbled by hand. The dried simplicial were then weighed and ground using a blender to obtain powdered petai peel simplicial.

2.3. Extraction

The extraction of *P. speciosa* peel was carried out using the maceration method with 96% ethanol as the solvent. A total of 500 g of powdered simplicial was macerated in 96% ethanol at a ratio of 1:5 (w/v) for 72 h (3 × 24 h) at room temperature in a light-protected container, with occasional stirring every 24 h. After maceration, the mixture was filtered using filter paper to obtain the filtrate. The filtrate was then concentrated using a rotary evaporator at 60°C, followed by further concentration using a water bath to obtain a thick extract.

2.4. Phytochemical Screening

Phytochemical screening of the *P. speciosa* peel extract (EPP) was carried out qualitatively on the concentrated extract, each class being identified from a colour change, foam formation or the appearance of a precipitate (Ghasemzadeh et al., 2018; Jamir & Bumo, 2026).

Alkaloids. Extract (0.5 g) was mixed with 5 mL of 2 M HCl in a porcelain dish, heated on a water bath for 5 min and cooled to room temperature. NaCl (0.5 g) was added, and the mixture was stirred and filtered. The filtrate was acidified with 3 drops of 2 M HCl and divided into four portions (A-D). Portion A served as the blank, and portions B, C and D each received 5 drops of Mayer's, Wagner's and Dragendorff's reagent, respectively. Results were read immediately against the blank. A white/cream (Mayer), brown (Wagner) or orange to brick-red (Dragendorff) precipitate indicated alkaloids.

Flavonoids. Extract (0.5 g) was heated in 10 mL of distilled water on a water bath for 10 min and filtered. The filtrate was dissolved in 1 mL of 95% ethanol, and 0.1 g of magnesium powder and 5 drops of concentrated HCl were added. The colour was read immediately. A red-purple colour indicated flavonoids; a yellow colour indicated flavones, chalcones and aurones.

Saponins. Extract (0.5 g) was mixed with 10 mL of hot water in a test tube, cooled and shaken vigorously for about 10 s. A foam 1-10 cm high that remained stable for at least 10 min and persisted after adding 1 drop of 2 N HCl indicated saponins.

Tannins. Extract (0.5 g) was mixed with 20 mL of hot water and 3 drops of 10% NaCl, then filtered. The filtrate was divided into two portions. The first portion received 1-2 drops of 1% $FeCl_3$, and the colour was read immediately; a blue, green or black colour indicated tannins. The second portion was mixed with 15 mL of Stiasny reagent (30% formaldehyde: concentrated HCl, 2:1), heated on a water bath for 15 min and examined immediately. A pink precipitate indicated catechol (condensed) tannins. The mixture was then filtered, and the filtrate was saturated with sodium acetate and treated with 1-2 drops of 1% $FeCl_3$. A blue-black colour appearing immediately indicated gallic (hydrolysable) tannins

Steroids and triterpenoids. The sample (1 g) was extracted with 20 mL of n-hexane for 2 h. The filtrate was evaporated, and the residue was treated with 2 drops of Liebermann-Burchard reagent. The colour was read immediately. A red colour indicated triterpenoids; a bluish-green colour indicated steroids.

The intensity of each reaction was recorded on a three-point scale as weak (+), moderate (++) or strong (+++). The reagents used, the observation recorded for each and the intensity assigned are set out in **Table 1**.

2.5. Ethical Approval and Experimental Animals

All procedures involving animals were approved by the Medical Research Ethics Committee, Faculty of Medicine, Tadulako University, under approval number 10429/UN28.10/KL/2025. The experiment was carried out in the Pharmacology and Biopharmaceutics Laboratory of the Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Tadulako University, and the animals were handled according to the conditions set out in that approval.

This study used 30 male Wistar rats (*Rattus norvegicus*) weighing 200–250 g and aged 2-4 months, obtained from the Pharmacology Laboratory, Department of Pharmacy,Tadulako University, Palu, Indonesia. Rats showing abnormalities, injuries or signs of illness were excluded. Animals were housed in plastic cages (five per cage) at 22–25 °C and 50–60% relative humidity under a 12 h light : 12 h dark cycle, with standard pellet feed and water provided ad libitum, and were acclimatised for 7 days before the experiment. After acclimatisation, the rats were weighed and allocated to six groups (n = 5 per group) by simple randomisation. Baseline haematological parameters haemoglobin (Hb) level, erythrocyte count, leukocyte count, haematocrit, and platelet count were measured as initial data on day 0 (H0). All groups except the normal control group were orally induced with sodium nitrite ($NaNO_2$) at a dose of 50 mg/kg body weight for 14 days, while the normal control group received vehicle only; during the first 7 days the induced groups received sodium nitrite alone, and from day 8 to day 14 sodium nitrite administration continued, with each group receiving its assigned treatment orally 30 minutes after induction the negative control group received vehicle, the positive

control group received ferrous fumarate at 5.4 mg/kg body weight, and the treatment groups received *P. speciosa* peel extract at doses of 300, 400, and 500 mg/kg body weight. Haematological analysis was performed by a technician who was blinded to group allocation. Haematological measurements were obtained from three rats per group; samples from the remaining animals were excluded because the blood haemolysed during collection, and all haematological analyses reported here therefore use n = 3 per group.

2.6. Hematological Analysis

Blood was collected for haematological examination on day 0, before induction, and again on day 14, at the end of the treatment period. Hematological examination was performed using an automated hematology analyzer to determine blood parameters. Blood samples were collected at two time points, each after a 16 h fast with free access to water. On day 0 (H0), approximately 1 mL of blood was collected from the tail without anaesthesia by snipping the distal tip with sterile surgical scissors; the first drop was discarded, and the subsequent blood was collected into a K2-EDTA vacutainer tube. On day 14, after the final dose, rats were anaesthetised with ketamine (100 mg/kg body weight, intraperitoneally), and approximately 1 mL of blood was collected by cardiac puncture into a K2-EDTA vacutainer tube. All samples were analysed within 1 h of collection. Each sample was analysed on a Mythic 18 automated haematology analyser (Orphée S.A., Plan-les-Ouates, Geneva, Switzerland) using the manufacturer's own diluent, lytic solution and enzymatic cleaner, and haemoglobin concentration, erythrocyte count, haematocrit, leukocyte count and platelet count were read from a single run for each animal.

2.7. Statistical Analysis

Data are presented as mean ± SD of the three animals analysed in each group. Homogeneity of variance was checked for every parameter at each time point with Levene's test on median-centred residuals. Haemoglobin, erythrocyte count, haematocrit and platelet count met that assumption and were analysed on the measured scale. Leukocyte counts did not: at day 14 the largest group standard deviation exceeded the smallest by a factor of 55 (Levene, $p = 0.037$), so leukocyte counts were log-transformed before analysis, after which the variances were homogeneous ($p = 0.18$); leukocyte results are therefore reported as fold changes alongside the arithmetic means. Each parameter was then analysed by two-way ANOVA with time (day 0, day 14) and group as fixed factors. Differences between groups within a single day were examined with Tukey's honestly significant difference test and are reported as compact letters, so that groups sharing a letter do not differ; the change from day 0 to day 14 within each group was tested with Šidák's multiple comparisons test. Significance was set at $p < 0.05$ throughout.

3. RESULTS AND DISCUSSION

3.1. Extraction and Phytochemical Screening

Maceration of 500 g of *P. speciosa* peel powder in 96% ethanol gave a yield of 12.10%. Maceration was chosen because it applies no heat and so does not degrade thermolabile secondary metabolites, and 96% ethanol is semi-polar, which allows it to carry over both polar and semi-polar constituents flavonoids, alkaloids, tannins, saponins and steroids alike (Cao et al., 2025; Lee et al., 2024). Yield in a maceration is governed by the simplicia itself, the particle size of the powder, the maceration time, the solvent and its concentration, the simplicia-to-solvent ratio and the evaporation step (Azzahra et al., 2025), so the figure is not comparable across studies unless those conditions are matched; within this experiment it indicates that the peel carries an appreciable extractable fraction and is worth screening further.

Qualitative screening detected all five classes sought (**Table 1**). Alkaloids reacted positively with all three reagents — an orange precipitate with Dragendorff's, a white precipitate with Mayer's and a brown precipitate with Wagner's — at moderate intensity (++), so alkaloids are clearly present but are not the dominant fraction. Flavonoids reacted strongly (+++) with magnesium and hydrochloric acid, giving the characteristic red-orange colour. Saponins produced a stable foam of moderate intensity (++). Tannins gave a dark green colour with ferric chloride and a blue-black colour with sodium acetate followed by ferric chloride, both strong (+++), while the Stiasny reaction was recorded as negative, which places the tannin fraction with the hydrolysable rather than the condensed tannins. Steroids gave a greenish-blue Liebermann-Burchard reaction of moderate intensity (++).

Table 1. Phytochemical screening of *P. speciosa* peel extract

Secondary metabolites	Reagent used	Observation	Intensity
Alkaloids	Dragendorff	Orange precipitate formed	++
	Mayer	White precipitate formed	++
	Wagner	Brownish precipitate formed	++
Flavonoids	Mg-HCl	Red-orange color formed	+++
Saponins	Foam test	Stable foam formed	++
Tannins	$FeCl_3$	Dark green coloration	+++
	Stiasny reagent	White precipitate formed	-
	Sodium acetate + $FeCl_3$	Blue-black coloration formed	+++
Steroids	Liebermann–Burchard	Greenish-blue color	++

Note. Reaction intensity: (+) weak; (++) moderate; (+++) strong; (−) not detected.

Flavonoids and tannins were the two strongest reactions, and both act as antioxidants by donating a hydrogen atom or an electron to the radical species generated during nitrite exposure (Madhikarmi & Murthy, 2014). That composition is what makes the peel a plausible hematoprotective candidate, and it sets the hypothesis the animal experiment was designed to test: if the extract limits oxidative damage to the erythrocyte membrane, then haemoglobin, erythrocyte count and haematocrit should be held near their baseline values in nitrite-induced animals. The remaining three classes were present at moderate intensity and are more likely to act indirectly alkaloids and saponins through immunomodulatory, anti-inflammatory and cytoprotective routes, and steroids through modulation of cellular function rather than by scavenging radicals themselves.

3.2. Effects on Hematological Parameters

Sodium nitrite produced the expected anaemic picture, and it produced it in the negative control alone (**Figure 1**). In that group haemoglobin fell from 12.81 ± 1.90 to 6.07 ± 1.65 g/dL (−52.6%, $p < 0.0001$), the erythrocyte count from 6.92 ± 0.67 to 3.80 ± 1.43 $\times 10^6$/µL (−45.0%, $p < 0.001$) and the haematocrit from 32.20 ± 3.92 to 14.06 ± 1.73% (−56.3%, $p < 0.0001$). At day 0 the six groups did not differ in haemoglobin ($p = 0.82$), erythrocyte count ($p = 0.34$) or haematocrit ($p = 0.34$), so the groups started from a common baseline and the day-14 differences cannot be attributed to allocation. The time × group interaction was significant for haemoglobin ($F(5, 24) = 8.23$, $p = 0.0001$), for the erythrocyte count ($F(5, 24) = 6.27$, $p < 0.001$) and for the haematocrit ($F(5, 24) = 11.18$, $p < 0.0001$), which is the signature of a change confined to one group rather than a change shared by all.

Sodium nitrite is often used as an anemia inducer because excessive nitrite exposure can trigger oxidative stress and methemoglobin formation. Nitrite oxidizes the ferrous iron ion (Fe^{2+}) in hemoglobin to the ferric form (Fe^{3+}), resulting in the formation of methemoglobin, which is unable to bind and transport oxygen effectively. This condition can interfere with oxygen transport to tissues and contribute to erythrocyte membrane damage, accelerated hemolysis, and decreased hemoglobin levels (Ansari et al., 2015).

At day 14 the negative control stood apart from every other group in all three erythroid parameters: haemoglobin 6.07 ± 1.65 g/dL against 13.73 ± 1.05 in the normal control, erythrocyte count 3.80 ± 1.43 against 6.60 ± 0.43 $\times 10^6$/µL, and haematocrit 14.06 ± 1.73 against 32.82 ± 2.27% (Tukey, $p < 0.05$ in each case). All three doses of the extract held these parameters at normal-control level. Haemoglobin reached 14.13 ± 0.65, 15.17 ± 0.65 and 14.80 ± 2.20 g/dL at 300, 400 and 500 mg/kg BW; the erythrocyte count reached 6.46 ± 0.30, 6.91 ± 0.25 and 6.55 ± 0.44 $\times 10^6$/µL; and the haematocrit reached 34.22 ± 1.53, 36.28 ± 1.28 and 33.88 ± 2.10%. None of the three doses differed from one another, from the normal control or from the positive control for any of these parameters (Tukey, $p > 0.05$ throughout), so the erythroid response was already at its maximum at the lowest dose tested.

The three doses were therefore indistinguishable for haemoglobin, erythrocyte count and haematocrit, and 300 mg/kg BW is the lowest dose at which that protection was obtained. The leukocyte and platelet counts did not follow the same pattern, and the equivalence of the doses cannot be carried over to them: at day 14 the 500 mg/kg BW group was separated from the 300 mg/kg BW group for the leukocyte count, and the two higher doses raised the platelet count above the normal control while the lowest did not. Those two parameters are considered separately below.

The effect of petai (*P. speciosa*) ethanol extract on increasing haemoglobin levels in anaemic rats is likely related to its secondary metabolites, such as flavonoids, alkaloids, saponins, tannins, and steroids. These compounds are known to have biological potential to support haematological recovery, particularly through protective mechanisms against oxidative stress (Devi & Barooah, 2025). In this study, anaemia was induced using sodium nitrite, which can cause haematological disorders through the formation of oxidative stress and reduced blood oxygen transport capacity. Therefore, the increase in haemoglobin levels after administration of the extract may be associated with the bioactive compounds' ability to help protect red blood cells from oxidative damage.

Flavonoids are a group of compounds that play an important role as antioxidants. The antioxidant activity of flavonoids can help scavenge free radicals and inhibit lipid peroxidation in erythrocyte membranes. Protection of the erythrocyte membrane is essential for maintaining red blood cell integrity, reducing the risk of hemolysis, and supporting erythrocyte lifespan in circulation. By reducing oxidative damage to erythrocytes, the process of haemoglobin recovery can proceed more effectively. Furthermore, more controlled oxidative conditions may also support a more favourable physiological environment for erythropoiesis (Veiko et al., 2020).

In addition to flavonoids, other metabolites such as alkaloids, saponins, tannins, and steroids are also suspected to contribute to improved haematological profiles. Saponins and alkaloids have been reported to exhibit immunomodulatory, anti-inflammatory, and cytoprotective activities that may indirectly support cellular recovery, including the recovery of hematopoietic tissue from oxidative or inflammatory stress. However, in this study, erythropoietin levels, bone marrow activity, serum iron levels, and molecular markers of erythropoiesis were not evaluated; therefore, the proposed mechanisms require further confirmation (Alarabei et al., 2024).

The leukocyte counts moved in opposite directions between groups, and their variances were so unequal at day 14 — a 55-fold spread between the largest and smallest group standard deviation, Levene $p = 0.037$ — that they were analysed after log transformation. In the negative control the count fell from 13.08 ± 0.86 to 8.40 ± 1.08 $\times 10^3$/µL, a 0.64-fold change over the 14

days ($p < 0.05$). Every extract group ended higher than the negative control: 10.96 ± 0.32, 13.42 ± 0.33 and 50.14 ± 17.89 $\times 10^3$/µL at 300, 400 and 500 mg/kg BW respectively. The 400 mg/kg BW group rose 1.47-fold ($p < 0.05$) and the 500 mg/kg BW group 3.44-fold ($p < 0.0001$) between day 0 and day 14, and at day 14 the 500 mg/kg BW group was the only one separated from all the others (Tukey, $p < 0.05$). A rise of this kind is consistent with a physiological response to the haematological stress imposed by sodium nitrite, since leukocytes both mount the immune response and take part in the regulation of inflammation and tissue repair (Shen et al., 2024).

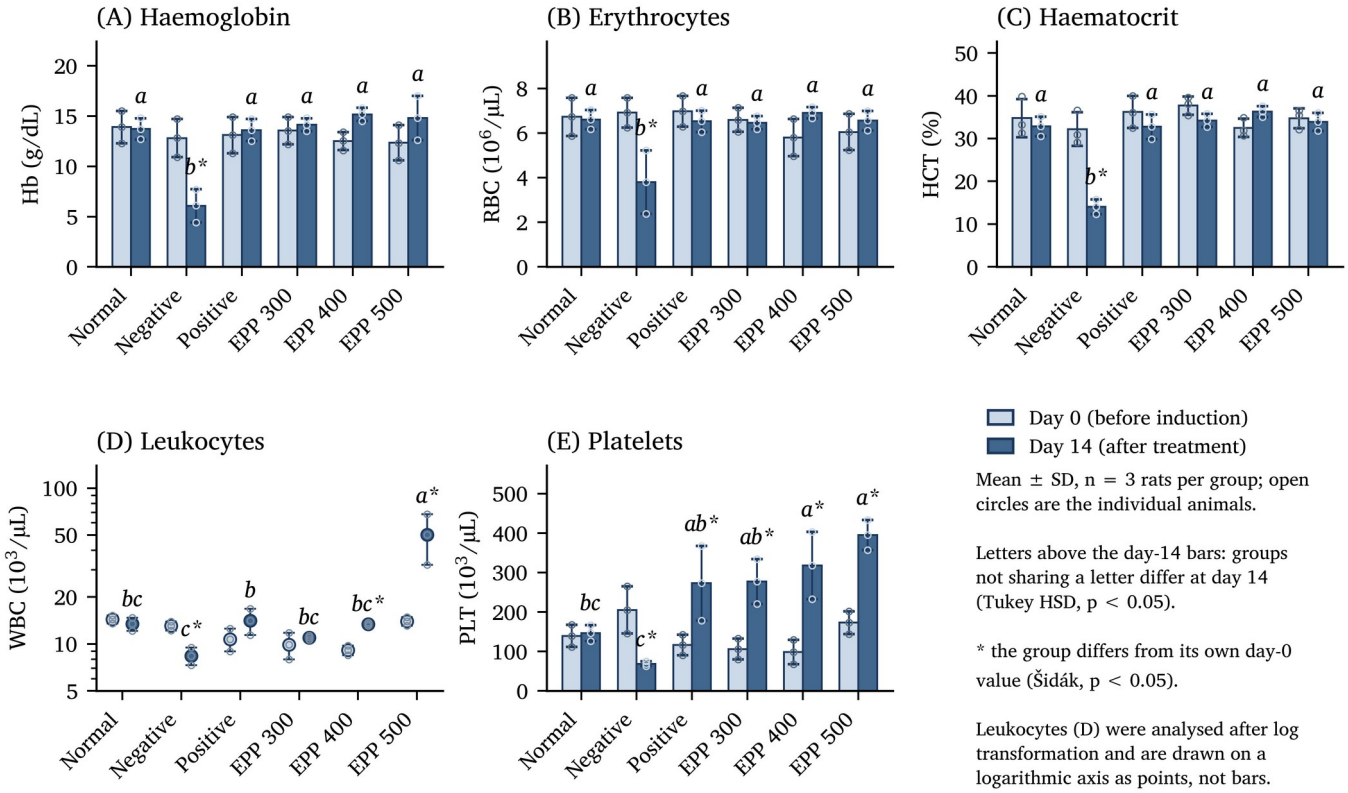

Figure 1. Haematological parameters of sodium nitrite-induced Wistar rats before induction (day 0) and after 14 days of treatment with *P. speciosa* peel extract: (A) haemoglobin, (B) erythrocyte count, (C) haematocrit, (D) leukocyte count and (E) platelet count. Values are mean ± SD of n = 3 rats per group, and the open circles are the three individual animals. Above each day-14 bar, groups that do not share a lowercase letter differ from one another at day 14 (Tukey HSD, $p < 0.05$), and an asterisk marks a group whose day-14 value differs from its own day-0 value (Šidák, $p < 0.05$). The groups did not differ at day 0 in haemoglobin, erythrocyte count or haematocrit ($p = 0.82$, 0.34 and 0.34), but they did differ at day 0 in leukocyte count ($p = 0.001$) and platelet count ($p = 0.018$). Leukocytes (D) were analysed after log transformation and are drawn on a logarithmic axis as points rather than bars. EPP, *P. speciosa* peel extract; HCT, haematocrit; PLT, platelet count; RBC, erythrocyte count; SD, standard deviation; WBC, leukocyte count.

The 500 mg/kg BW result needs particular caution. At 50.14 $\times 10^3$/µL, with individual counts of 32.25, 50.14 and 68.03 $\times 10^3$/µL, it is three to four times the normal control and lies well outside the range usually reported for healthy Wistar rats, so it is as consistent with an inflammatory or stress response, or with a pre-analytical problem, as with recovery. Because this study measured neither the leukocyte differential nor inflammatory cytokines nor tissue histopathology, the increase cannot be attributed to an immunomodulatory action of the extract, and confirming what it represents will require those measurements.

Platelet counts fell in the negative control from 205.20 ± 59.40 to 68.80 ± 5.75 $\times 10^3$/µL (−66.5%, $p < 0.05$) and rose in every treated group: from 106.00 ± 26.43 to 277.20 ± 56.52 $\times 10^3$/µL at 300 mg/kg BW ($p < 0.01$), from 98.20 ± 30.73 to 318.00 ± 85.46 at 400 mg/kg BW ($p < 0.0001$) and from 173.00 ± 28.73 to 394.80 ± 38.22 at 500 mg/kg BW ($p < 0.0001$), with the positive control rising from 116.20 ± 25.86 to 272.80 ± 94.50 ($p < 0.01$). At day 14 all four treated groups exceeded the negative control; the 400 and 500 mg/kg BW groups also exceeded the normal control, whereas the 300 mg/kg BW group and the positive control did not differ from it. Platelets are released from bone-marrow megakaryocytes under haematopoietic and thrombopoietic control, so a change in their number can reflect marrow adaptation to haematological stress (Foy et al., 2022). The three extract doses did not differ from one another, however, so no dose-dependent effect can be claimed, and because megakaryocyte activity, thrombopoietin concentration and marrow histology were not measured, the link between the extract and thrombopoiesis remains an inference rather than a finding.

Taken together, *P. speciosa* peel extract preserved haemoglobin, erythrocyte count and haematocrit in sodium nitrite-induced rats at all three doses tested, and raised the platelet count above the negative control at all three; the leukocyte count rose only at the

two higher doses, and steeply at the highest. The three doses were equivalent for the erythroid parameters, which points to 300 mg/kg BW as sufficient under these conditions rather than to a dose-response relationship. The effect is plausibly linked to the antioxidant secondary metabolites the screening detected, flavonoids and tannins in particular, acting to protect blood cells from oxidative damage. That mechanism remains unverified here: erythropoietin, serum iron status, endogenous antioxidant activity, inflammatory markers and bone-marrow histopathology were all outside the scope of this study, and with three animals per group the experiment is powered to detect the large effect of the induction itself rather than the smaller differences that separate one dose from another.

Previous work on *P. speciosa* also supports its potential antianaemic activity. Nursucihta et al. reported that ethanolic seed extract of *P. speciosa* increased haemoglobin levels in sodium nitrite-induced anaemic Wistar rats. At doses of 400 and 700 mg/kg body weight, the mean changes in haemoglobin were 0.94 ± 0.646 and 0.82 ± 1.941 g/dL, respectively, although an optimum dose could not be established (Nursucihta et al., 2014). The present study reproduces that inability to separate doses and explains it haemoglobin in the peel-extract groups (14.13–15.17 g/dL across 300–500 mg/kg BW) was statistically indistinguishable from the normal control (13.73 ± 1.05 g/dL) and from the positive control, while the negative control fell to 6.07 ± 1.65 g/dL, with erythrocyte count and haematocrit following the same pattern. Because the induction model was shared, the remaining differences are attributable to plant part and study design. The peel was fully protective at 300 mg/kg BW, one-third below the lowest seed dose previously reported, consistent with the high total phenolic content and strong DPPH radical-scavenging activity of ethanolic *P. speciosa* peel extract (Fithri et al., 2019; Nurdyansyah & Widyastuti, 2020).

This distinction constrains comparison with other herbal antianaemic agents tested in $NaNO_2$ models, most of which are curative. Hydroethanolic Anamirta cocculus stem extract raised haemoglobin significantly at 550 and 700 mg/kg BW in rats made anaemic with $NaNO_2$ at 187.5 mg/kg BW for seven days (Muslihin et al., 2025), and a combination of Beta vulgaris and Hibiscus sabdariffa extracts improved haemoglobin, erythrocyte count and haematocrit over 14 days (Herlina et al., 2026). Comparable protection against methaemoglobin formation has been reported for Carica papaya leaf extract in this model (Hermayanti et al., 2021). Those studies measured within-group recovery from established anaemia and are not directly commensurable with the present between-group protection, so the two designs are best read as complementary evidence that antioxidant-rich plant preparations attenuate nitrite-induced haematological injury.

4. CONCLUSION

P. speciosa peel extract showed hematoprotective potential in sodium nitrite-induced Wistar rats, holding haemoglobin, erythrocyte count and haematocrit near normal-control values and raising the platelet count relative to the negative control. The three doses tested 300, 400 and 500 mg/kg BW did not differ from one another in any of the erythroid parameters, so 300 mg/kg BW was the lowest dose that gave the full effect under these conditions; the leukocyte count was the exception, rising markedly at 500 mg/kg BW. These conclusions rest on three animals per group at a single time point, and confirming the mechanism will require measurement of oxidative stress markers, inflammatory biomarkers, serum iron status, erythropoietin and bone-marrow histopathology.

AUTHOR CONTRIBUTIONS

Conceptualization, Y, M.F.H and K.K.; methodology, Y; software, M.F.H.; validation, Y, M.F.H and K.K.; formal analysis, N.W, R and N.A.; investigation, N.W, R and N.A; resources,. N.W, R and N.A; data curation, Y and K.K.; writing-original draft preparation, Y and KK.; writing-review and editing, Y; visualization, MFH; supervision, Y.; project administration, K.K.; funding acquisition, Y. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Medical Research Ethics Committee and the Faculty of Medicine, Tadulako University (Number 10429/UN28.10/KL/2025).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the author(s) used ChatGPT (OpenAI) to assist in improving the clarity, structure, or readability of the

text. After using this tool, the author(s) thoroughly reviewed, edited, and verified the entire content to ensure it accurately represents their own ideas and interpretations. The author(s) take full responsibility for the integrity and originality of the published work.

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