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Potential Antithrombotic Candidate of *Moringa oleifera* L. Leaf: Study *In Vitro* Microscopic Evaluation of Erythrocyte Morphology

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ABSTRACT

Moringa oleifera leaves are rich in bioactive compounds with purported therapeutic properties, including potential antithrombotic activity. This study evaluated the in vitro effects of *Moringa oleifera* leaf filtrate on erythrocyte morphology and aggregation. Whole blood samples were treated with varying concentrations of the filtrate (25%, 50%, 75%, and 100%), and microscopic examination was performed to assess changes in erythrocyte morphology and aggregation patterns. The results showed distinct, concentration-dependent effects on erythrocytes. At a low concentration of 25%, erythrocytes maintained normal morphology and remained evenly distributed without signs of aggregation. In contrast, increasing the filtrate concentration to 50%–100% induced significant poikilocytosis and anisocytosis, along with increased erythrocyte aggregation characterized by rouleaux formation. These findings suggest that although *Moringa oleifera* L. filtrate may exhibit antithrombotic potential promising antithrombotic potential at low concentrations, higher concentrations may adversely affect erythrocyte integrityerythrocyte integrity and promote aggregation. Further research is needed to elucidate antithrombotic activity through coagulation parameters and their underlying molecular functions and mechanisms and to establish a safe therapeutic range.

Introduction

Indonesia is widely recognized for its high biodiversity, which includes a wide range of plants used for food, cosmetics, and traditional medicine. One such species is *Moringa oleifera* L. (Moringaceae), commonly known as the drumstick tree. Its leaves contain various bioactive compounds, including alkaloids, flavonoids, polyphenols, and saponins. These ingredients offer a range of pharmacological effects, including as cardioprotective, antioxidant, antihypertensive, and antihyperglycemic qualities (Gopalakrishnan et al., 2016; Luh et al., 2025). Recent studies have suggested that M. oleifera leaves possess potential antithrombotic properties based on in vitro anticoagulant and blood clot lysis assays (Ayanti et al., 2025). Plant-derived phytochemicals continue to receive considerable attention because of their diverse biological activities and potential therapeutic applications.

Therapeutic phytochemicals are crucial for accelerating the development of new drugs, leading to continued progress in the research and use of natural substances.

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Many plant-derived phytochemicals, including phenolic acids, flavonoids, tannins, alkaloids, saponins, and glucosinolates, exhibit diverse pharmacological activities that contribute to disease prevention and drug development (Gopalakrishnan et al., 2016; Kashyap et al., 2022).

Among these phytochemicals, flavonoids have been extensively investigated for their potential antithrombotic effects. (Srinivasan et al., 2016), flavonoids have a known mode of action that lowers primary hemostasis and several pathways associated with platelet activation and aggregation. (Ciumărnean et al., 2020; Srinivasan et al., 2016; Subramani & Sathiyarajeswaran, 2022). According to (Mazumder et al., 2022) bioactive substances derived from plants, such as phenolics, polysaccharides, peptides, terpenes, and flavonoids, have demonstrated effectiveness as viable options for creating safe antithrombotic treatments in a variety of in vivo and in vitro models. When a pathological thrombus forms in a blood vessel, it is known as thrombosis. This disease can obstruct blood flow and result in dangerous conditions like myocardial infarction and stroke. Previous studies demonstrated anticoagulant and thrombolytic activities, suggesting potential antithrombotic properties (Ayanti et al., 2022).

However, the use of natural products as potential therapeutic agents requires assessing not only their effectiveness but also their effects on blood cell components, particularly erythrocytes. Red blood cells are often used as models for cytotoxicity and hemocompatibility investigations. The erythrocyte membrane is a useful indicator of cell damage, including hemolysis, oxidative stress, and membrane rupture, as it is highly sensitive to changes in the physical and chemical environment. Changes in erythrocyte morphology and aggregation can affect blood rheology and microcirculation, making erythrocytes an important model for evaluating the hemocompatibility of candidate therapeutic agents (Romo-sáenz et al., 2026). As a result, in vitro testing using erythrocytes is crucial for determining the safety and biocompatibility of bioactive compounds as well as how they affect cell morphology during the pharmaceutical development process. The impact of moringa leaf filtrate on erythrocyte integrity in vitro, especially under a microscope, has not been well studied. Therefore, this study aimed to evaluate the in vitro effects of Moringa oleifera leaf filtrate on erythrocyte morphology and aggregation in anticoagulant-treated blood samples as a preliminary assessment of blood compatibility and to assess the potential effects of antithrombotic drugs on red blood cells in vitro.

Materials and Methods

Material

The materials used in this study were distilled water, Moringa leaves (*Moringa oleifera* L.), 70% alcohol swabs, tubes, syringes, EDTA anticoagulant tube, slide preparations, roller tubes, Giemsa, methanol, and immersion oil. Moringa leaves (*Moringa oleifera* L.) at total of 4 kg, collected from Landasan Ulin, Banjarbaru, South Kalimantan, Indonesia by picking and selecting young leaves (purposive sampling).

Research Location and Time

The study was carried out at the Clinical Pathology of Laboratory Borneo Lestari University July 2025. Plant identifications was conducted at the at Laboratory FMIPA Lambung Mangkurat University Banjarbaru.

Experimental Design

The type of research used a laboratory experimental study with a post-test only control group design where the sample was divided into five groups; one positive

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control group used blood treated with EDTA anticoagulant while four treatment groups used moringa leaf filtrate at concentrations of 25%, 50%, 75%, and 100%. Whole blood was drawn from healthy human volunteers (n = 10) without a history of oral contraception and not using anticoagulant therapy using a protocol approved by the STIKES Suaka Insan Research Ethics Committee No. 208/KEPK-SI/VI/2025. The morphology of blood cell size, red blood cell color, and erythrocyte distribution were assessed using microscopic examination of preparations.

Research Methods and Parameters
Sample Processing and Extraction

A total of 4 kg of Moringa leaves, selected *Moringa oleifera* leaves were washed with distilled water to remove dirt, dust, and potential microbial contaminants. After washing, the leaves were air-dried at room temperature until no moisture remained on the surface. The cleaned leaves were then pulverized using a blender to obtain a homogeneous texture. Finally, the leaf mass was mixed with Phosphate Buffer Saline (PBS) at a ratio of 1:2 (w/v) (Fig.1).

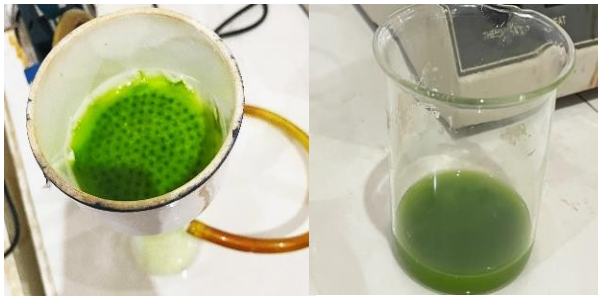

Figure 2. Moringa Leaf Filtration

Eustrek (Microscopic Test)

The Eustrek method was used to measure thin blood smears under a microscope in order to analyze erythrocyte shape. Blood samples with concentrations of 25%, 50%, 75%, and 100% of *Moringa oleifera* L. leaf filtrate were used in the preparation, along with a positive control using EDTA at a magnification of 1000× using immersion oil. The evaluation focused on erythrocyte morphology parameters, cell size, erythrocyte distribution, and the presence of erythrocyte aggregation.The assessment was carried out on a minimum of 15 representative fields of view which were systematically selected to avoid observation bias, then the observation results were averaged. In each field of view, identification of normal erythrocytes (biconcave shape) and abnormal erythrocytes such as poikilocytosis and echinocytes was carried out. Erythrocyte sizes are categorized as normocytic, microcytic, macrocytic, or undergoing anisocytosis. Erythrocyte distribution is evaluated according to the distribution pattern, which includes rouleux, aggregation, and agglutination. Erythrocyte aggregation, including rouleaux formation, was assessed using a semi-quantitative method with a scoring system, Score 0: no aggregation found, score 1: mild aggregation (<25% of the visual field), score 2: moderate aggregation (25–50% of the visual field) and score 3: severe aggregation (>50% of the visual field). The degree of morphological alterations and interactions between erythrocytes brought on by the administration of moringa leaf filtrate at different concentrations can be more objectively assessed thanks to this semi-quantitative method.

Data Analysis

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Data were tabulated according to treatment concentration and evaluated using a semi-quantitative scoring system for erythrocyte morphology, anisocytosis, aggregation, and clot formation. Scores ranged from 0 to 3, where 0 indicated no abnormalities, 1 indicated mild abnormalities (<25% of the visual field), 2 indicated moderate abnormalities (25–50% of the visual field), and 3 indicated severe abnormalities (>50% of the visual field). Clot formation was scored from 0 to 2 based on the presence and extent of clot formation. The scores obtained from each treatment group were presented descriptively in tables and were described to assess concentration-dependent patterns the concentration-dependent effects of *Moringa oleifera* L. leaf filtrate on erythrocyte morphology and aggregation.

Results and Discussion

Results

The treatment group's preparations employed blood that had been supplemented with Moringa leaf filtrate at concentrations of 25%, 50%, 75%, and 100%, while the control group's preparations used EDTA anticoagulant, a common anticoagulant used in blood cell evaluation.

Table 1. Results of Erythrocyte Morphology Evaluation and Cell Distribution at Various Concentrations of Moringa Leaf Filtrate

Code	**Treatment**	Erythrocytes morphological	**Size cell**	**Distribution**	**Aggregation/ Rouleaux**
D1	Moringa Filtrate 25%	Normal dominant, some mild poikilocytosis	Normocytic	Erythrocyte distribution evenly distributed	Aggregation is not visible
D2	Moringa Filtrate 50%	Variations in shape (poikilocytosis) begin to appear.	Normocytic slight anisocytosis	Homogenous erythrocyte distribution	Aggregation mild
D3	Moringa Filtrate 75%	Poikilocytosis increased, some abnormal cells	Poikilocytosis increased, some abnormal cells	Uneven distribution has started to occur	Medium aggregation
D4	Moringa Filtrate 100%	Predominant abnormal erythrocytes, obvious deformation of shape	Anisocytosis indicated	Unequal erythrocyte distribution	Aggregation increases (rouleaux)
K+	Positive Control (+)	Normal Erythrocytes	Normocytic	Erythrocyte distribution evenly distributed	No Aggregation

Table 1, shows that changes in erythrocyte morphology are more apparent as concentration of *Moringa oleifera* leaf filtrate increases. Erythrocytes were still mostly normal in form, distributed evenly, and did not aggregate at a concentration of 25%. Erythrocyte shape changes and slight aggregation started to manifest at a concentration of 50%. At a concentration of 75%, morphological changes were more significant, with enhanced poikilocytosis, mild anisocytosis, and moderate erythrocyte aggregation. Erythrocytes were primarily deformed, unevenly distributed, and more aggregated at a concentration of 100%, showing rouleaux formation. In contrast, normal erythrocyte structure with an even distribution and no aggregation was

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detected in the blood EDTA anticoagulant blood control. These findings were consistent with the microscopic images presented in Figure 2 and result of the erythrocyte morphological observations in Table 1.

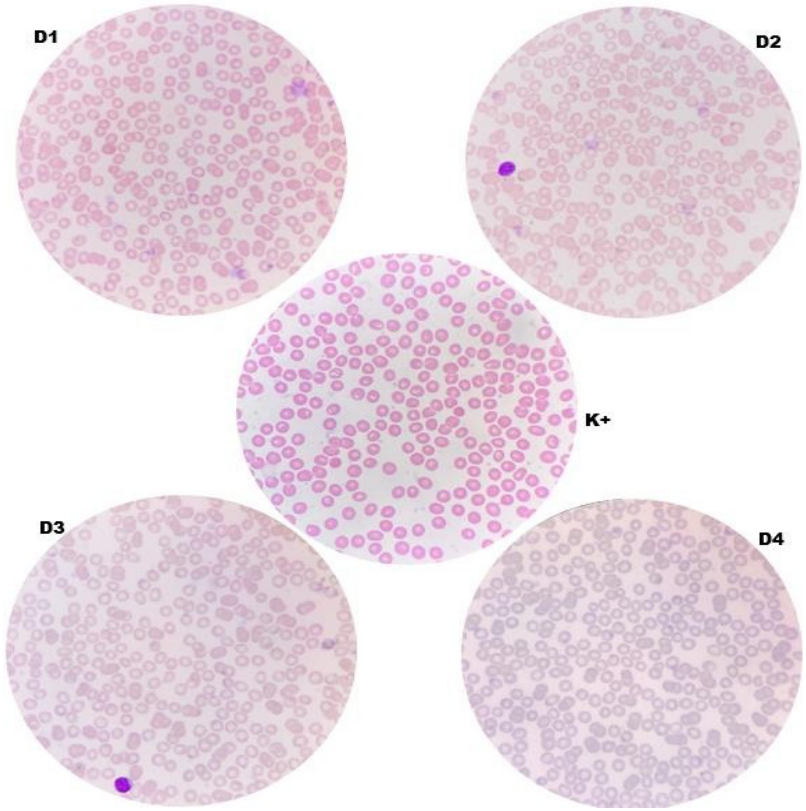

Figure 2. Majority Morphological Test (Eustrek) visualization on thin blood smear preparations D1: 25% concentration of moringa leaf filtrate; D2: 50% concentration; D3: 75% concentration; D4: 100% concentration; and K+: Positive Control (EDTA)

Erythrocytes treated with a 25% concentration of *Moringa oleifera* leaf filtrate showed mostly normal morphology, with biconcave cell morphologies, homogeneous cell size, and even distribution, according to microscopic findings in Figure 2. In the observed field of vision, neither rouleaux development nor erythrocyte aggregation were seen. This suggests that Moringa leaf filtrate did not significantly alter erythrocyte morphology at a 25% concentration while maintaining ideal cell dispersion. The lack of erythrocyte clumping suggests that there were little intercellular contacts. These results imply that Moringa leaf filtrate at this concentration may be able to prevent cell aggregation in vitro, which may influence erythrocyte interactions and blood rheological properties.

Table 1, results that are descriptive indicate the concentration of moringa leaf filtrate causes changes in erythrocyte shape, which are further confirmed in Table 2 is semi-quantitative review of the degree of the resulting alterations.

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Table 2. Evaluation of Erythrocyte Aggregation and Morphological Variations

Code	**Treatment**	Morphology score	**Anisocytosis score**	Aggregation Score	**Clot score**
D1	25%	1	0	0	0
D2	50%	2	1	1	0
D3	75%	2	2	2	1
D4	100%	3	3	3	2
K+	Positive control	0	0	0	0

Notes: (0) no abnormalities found, (1) mild abnormalities (<25% of visual field), (2) moderate abnormalities (25–50% of visual field), (3) severe abnormalities (>50% of visual field).

Table 2 shows a trend of increasing erythrocyte morphological alteration scores with increasing concentrations of *Moringa oleifera* leaf filtrate with an aberrant morphology score of 1 and no anisocytosis or aggregation, morphological alterations are still categorized as mild at a concentration of 25% (D1). Anisocytosis and aggregation scores rise to the moderate category at a concentration of 75% (D3), indicating more significant alterations in erythrocyte distribution and aggregation patterns. At a concentration of 50% (D2), there is an increase in morphological changes along with the appearance of mild anisocytosis and aggregation.

Discussion

The results of this study indicate that *Moringa oleifera* leaf filtrate exerts concentration-dependent effects. At low concentrations (25%), erythrocytes maintain normal morphology without aggregation. Conversely, increasing concentrations (50%–100%) cause changes in erythrocyte morphology in the form of poikilocytosis and anisocytosis. This effect is thought to be related to the content of bioactive compounds, particularly flavonoids and polyphenols, which act as antioxidants (Chiș et al., 2024). At low concentrations, these bioactive compounds can maintain erythrocyte membrane stability by inhibiting lipid peroxidation, reducing hemolysis, and increasing osmotic resistance. This stability is important in maintaining the rheological properties of blood and preventing excessive interactions between erythrocytes that can contribute to thrombus formation (Obohwemu et al., 2025).

Plasma and cellular components, such as erythrocytes, leukocytes, and platelets, make up blood. In order to support metabolic activities, erythrocytes are essential for carrying oxygen from the lungs to every tissue. Erythrocytes are frequently used in preclinical and clinical research as baseline or control values and as biomarkers of physiological conditions as part of a hematology profile (Rodak et al, 2020).

Increasing the concentration of moringa leaf filtrate (50%–100%) showed a tendency for increasingly significant changes in erythrocyte morphology, characterized by increased poikilocytosis, anisocytosis, and aggregation scores. At high concentrations, the bioactive compounds in moringa leaf filtrate are thought to induce changes in erythrocyte membrane structure, manifested in the form of morphological changes such as echinocytosis (Elblbesy & Moustafa, 2017). At high doses, increased erythrocyte

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aggregation also suggests altered intercellular interactions that may impact blood viscosity and flow characteristics. Increased erythrocyte aggregation can physiologically lead to thrombosis by raising blood flow resistance. On the other hand, the lack of aggregation at low concentrations suggests that moringa leaf filtrate may be able to prevent thrombus formation by inhibiting intercellular connections at specific levels (Saputri et al., 2022). The evaluation in this study still focused on microscopic data and morphological factors, the exact mechanism of erythrocyte aggregation cannot be clearly elucidated.

The main limitation of this study is its in vitro methodology, which means the results do not accurately represent in vivo physiological conditions. Furthermore, the interpretation of its antithrombotic potential is limited because this study did not evaluate additional hemostatic parameters such as coagulation factor activity, platelet function, and coagulation time. To validate the mechanism and efficacy of Moringa leaf filtrate as an antithrombotic agent, additional in vivo studies are needed, along with a more thorough assessment of hemostatic measures and molecular analysis. However, further research using an in vivo approach and a more comprehensive analysis of hemostasis parameters is still needed to confirm its mechanism and effectiveness.

Conclusions

Moringa oleifera leaf filtrate has a concentration-dependent effect on erythrocyte aggregation and shape in vitro. Exposure to higher concentrations (50%–100%) was associated with morphological alterations, including poikilocytosis, anisocytosis, and increased erythrocyte aggregation characterized by rouleaux formation.

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