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Original Article

Mangosteen (*Garcinia mangostana*) Pericarp Extract as a Promising Natural Stain for Erythrocyte Morphology in Thalassemia Patients: A Comparative Evaluation

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Abstract

Erythrocyte morphology evaluation via peripheral blood smear is essential for thalassemia patients. While Wright and Giemsa stains remain the established standard, eco-friendly natural dyes are gaining research interest. Mangosteen (*Garcinia mangostana*) pericarp contains natural pigments with potential biological staining applications. This study comparatively evaluated erythrocyte staining quality using Wright stain, Giemsa stain, and mangosteen pericarp extract as a natural alternative. This laboratory experimental study used 25 peripheral blood samples from thalassemia patients. Three peripheral blood smears were prepared per sample and stained using the three methods. Staining quality was assessed semi-quantitatively based on color intensity, morphological clarity, and background contrast by two observers. Statistical analysis (Friedman test, Wilcoxon signed-rank test) revealed significant differences among methods ($p < 0.001$). Wright stain yielded the highest quality, followed by Giemsa, while mangosteen pericarp extract showed lower performance but still allowed identification of basic erythrocyte morphology. These findings confirm the novelty of mangosteen peel extract as an environmentally friendly natural stain in hematology. Practically, this extract may support basic erythrocyte morphological observation in laboratory settings, though optimization of extract concentration and fixation methods is needed to improve staining quality. Further development could yield a more sustainable alternative staining agent for erythrocyte morphology examination. In conclusion, mangosteen pericarp extract shows potential as a natural alternative stain for erythrocyte morphology analysis in thalassemia patients. However, achieving effectiveness comparable to standard stains requires further optimization, particularly regarding extract concentration and fixation methods.

Keywords: Erythrocyte Morphology, Mangosteen Pericarp Extract, Natural Stain, Peripheral Blood Smear, Thalassemia.

1. Introduction

Peripheral blood smear (PBS) examination remains a cornerstone of hematological diagnostics, critical for evaluating erythrocyte morphology. Accurate assessment of erythrocyte morphology is vital for identifying and differentiating various erythrocyte-related disorders, most notably thalassemia. This condition is characterized by distinct abnormalities such as microcytosis, hypochromia, and anisopoikilocytosis, which are crucial for distinguishing it from other anemias and monitoring disease progression (Bain et al., 2017; Chase et al., 2023). Consequently, precise and reliable staining techniques are indispensable for clear microscopic visualization of erythrocyte structures.

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Romanowsky-based staining methods, primarily Wright and Giemsa stains, are the established standards for PBS analysis, offering excellent cellular contrast and detailed morphological insights. These stains utilize acidic and basic dyes to differentially interact with cellular components, producing characteristic colorizations of blood cells. However, their reliance on synthetic chemical compounds raises environmental and safety concerns related to their composition and laboratory waste disposal (Sari et al., 2022; Huang et al., 2023). This has driven significant interest in exploring alternative staining agents derived from natural, eco-friendly sources within biomedical research.

Mangosteen (*Garcinia mangostana*) pericarp has emerged as a promising candidate for natural staining due to its rich content of bioactive pigments, particularly anthocyanins and xanthones. Mangosteen peel is widely available in tropical regions and is often regarded as agricultural waste, making it a potential source of inexpensive and sustainable natural pigments. Its use as a coloring agent could provide a more affordable alternative to commercial synthetic dyes, particularly for laboratories with limited budgets and access to coloring materials. As well as reducing agricultural waste, the use of mangosteen peel can also support more economical and environmentally friendly laboratory practices. These compounds are known to interact with cellular proteins and membrane components, theoretically facilitating the visualization of erythrocyte morphology during microscopy (Kurniawan et al., 2020; Saristiana et al., 2024). While plant-derived pigments have been explored for histological and cytological staining, their application in hematological staining, particularly for detailed erythrocyte morphology, remains limited. Recent investigations have identified other pigment-rich plants like *Lawsonia inermis* and *Hibiscus sabdariffa* as potential natural alternatives (Deepthi et al., 2025; Karlina et al., 2025; Mulla et al., 2024).

Despite these advancements, studies specifically evaluating natural plant-derived pigments for hematological preparations, especially for the nuanced assessment of erythrocyte morphology in peripheral blood smears, are scarce. Existing research has predominantly focused on histological staining or general cytological applications. While a few studies have explored *Garcinia mangostana* pericarp extract for blood smear staining, their focus has primarily been on leukocyte visualization, leaving its efficacy for erythrocyte morphology evaluation largely unexplored. This gap is particularly critical in thalassemia, where the peripheral blood smear displays complex variations like microcytosis, anisopoikilocytosis, and target cells, demanding highly sensitive staining. Therefore, this study was designed to comprehensively evaluate the potential of mangosteen (*Garcinia mangostana*) peel extract as a natural staining agent for erythrocyte morphology in peripheral blood smears from thalassemia patients, and to quantitatively compare its staining performance with that of the conventional Wright and Giemsa methods. The novelty of this study lies in the expansion of the use of plant-based stains from merely serving as natural alternatives to an approach that supports sustainable laboratory practice through the utilization of natural materials and a reduction in reliance on synthetic stains. Thus, this study contributes to the development of alternative erythrocyte staining methods that are not only assessed based on their ability to visualize cell morphology but are also relevant to the needs of more sustainable laboratory practice.

2. Literature Review

2.1. Thalassemia and Erythrocyte Morphology

Thalassemia is a group of hereditary hematological disorders characterized by reduced or absent synthesis of globin chains in hemoglobin, leading to chronic hemolytic anemia and ineffective erythropoiesis (Sadiq et al., 2024). This pathogenesis results in significant morphological abnormalities in erythrocytes, which are readily observable through peripheral blood smear (PBS) examination. Key erythrocyte features in thalassemia include microcytosis, hypochromia, anisocytosis, poikilocytosis, and the presence of target cells (Musallam et al., 2024). These morphological characteristics play an important role in the laboratory diagnosis and differentiation of thalassemia from other forms of anemia, particularly iron deficiency anemia (Chokchaipermpoonphol et al., 2025). PBS examination remains a widely utilized diagnostic tool in hematology due to its ability to provide direct visualization of blood cell morphology under a microscope, making accurate assessment of erythrocyte morphology essential for identifying pathological changes, evaluating disease severity, and monitoring treatment response (El-Beshlawy et al., 2024).

2.2. Peripheral Blood Smear Staining: Wright-Giemsa Method

Romanowsky-type stains, predominantly Wright and Giemsa stains, are the established standard for peripheral blood smear analysis in hematological laboratories. These stains function through a combination of acidic dyes (e.g., eosin) and basic dyes (e.g., methylene blue and its derivatives). Their interaction with cellular components leads to characteristic color differentiation, enabling the identification of various blood cells and their morphological features (Kalinin et al., 2024). Eosin stains basic cellular structures like hemoglobin in erythrocytes, yielding pink to orange hues, while methylene blue and azure derivatives stain acidic components such as nucleic acids in leukocytes, resulting in blue to purple coloration (Cheng et al., 2024). This differential staining mechanism facilitates clear differentiation between erythrocytes, leukocytes, and platelets, establishing Wright-Giemsa staining as a widely accepted diagnostic standard (Kalinin et al., 2024). However, the reliance on synthetic dye reagents and the multi-step washing protocols involved in Wright-Giemsa staining generate chemical waste, posing environmental and occupational health concerns in laboratory settings (Baliga et al., 2025). Consequently, the exploration of alternative staining agents derived from natural sources has emerged as a significant area of interest in biomedical research.

2.3. Natural Pigments as Potential Biological Staining Agents

Natural pigments extracted from plants are increasingly being investigated as viable alternatives to synthetic dyes in biological staining applications. Various plant extracts contain pigment compounds such as anthocyanins, flavonoids, tannins, and polyphenols, which possess the capability to form colored reactions upon interacting with cellular structures (Jasphin et al., 2023; Mulla et al., 2024). Studies have demonstrated that plant-derived pigments can stain different biological tissues, although their effectiveness varies based on pigment composition, extraction methods, and staining conditions (Mulla et al., 2024). For instance, research has highlighted pigment-rich plants like *Lawsonia inermis* and *Hibiscus sabdariffa* as potential natural staining agents for biological microscopy, offering alternative colorations and staining characteristics (Deepthi et al., 2025; Karlina et al., 2025). Although plant-based stains show potential as an alternative to synthetic stains, their effectiveness still varies and they have not yet consistently provided the diagnostic accuracy required to identify subtle morphological changes in erythrocytes. Variations in pigment composition, extraction

methods and staining conditions are factors that can affect the quality of staining results; therefore, more specific validation is required for hematological applications.

2.4. Mangosteen Pericarp as a Source of Natural Pigments

Mangosteen (*Garcinia mangostana*) a tropical fruit indigenous to Southeast Asia, is well-known for its pericarp, which is abundant in natural pigments and bioactive compounds, notably anthocyanins and xanthones, responsible for its characteristic dark purple color. These compounds exhibit potent antioxidant properties and have been studied for various biological and chemical applications (Tocmo et al., 2026; Yuvanatemiya et al., 2022). Anthocyanins, in particular, are water-soluble pigments that can produce a spectrum of colors (red, purple, blue) contingent on pH, and critically, possess the potential to interact with cellular proteins and membrane components. This interaction is theorized to facilitate the staining of biological structures (Saristiana et al., 2024). The unique chemical properties of these pigments make mangosteen pericarp an attractive candidate for developing natural staining agents for biological specimens.

However, research specifically investigating the application of mangosteen pericarp extract as a staining agent for hematological preparations, particularly for the detailed evaluation of erythrocyte morphology in peripheral blood smears, remains notably limited. While some studies have explored its potential, their focus has often been on leukocyte visualization or general cytological applications, leaving the efficacy for nuanced erythrocyte morphology assessment in conditions like thalassemia largely unaddressed. This discrepancy highlights the need for a more targeted evaluation of the staining characteristics of mangosteen peel extract, particularly with regard to maintaining the clarity of erythrocyte morphology under specific hematological conditions. This study provides the first comparative evaluation of mangosteen peel extract as a natural stain for the morphological identification of erythrocytes in thalassemia patients, by comparing its staining results with those of the Wright and Giemsa methods.

3. Methods

This study employed a laboratory experimental design to comparatively evaluate the staining quality of erythrocytes using Wright stain, Giemsa stain, and mangosteen (*Garcinia mangostana*) pericarp extract as a natural staining alternative.

3.1. Study Samples

Peripheral blood samples were prospectively collected from 25 patients diagnosed with thalassemia. Patients had a clinically confirmed thalassemia diagnosis by a qualified physician based on standard hematological evaluation, which included complete blood count (CBC), peripheral blood smear morphology, and hemoglobin electrophoresis. Patients with severe complications that could significantly affect erythrocyte morphology, such as severe infection or chronic liver and kidney disease, were excluded from the study. Informed consent was obtained from all participants. Each blood sample was used to prepare three peripheral blood smears, resulting in a total of 75 smear preparations. Each smear was randomly designated for staining using one of the three methods: Wright stain, Giemsa stain, or mangosteen (*Garcinia mangostana*) pericarp extract. Sample collection was conducted at a tertiary referral hospital in Surabaya, Indonesia. Extraction procedures and staining preparation were performed at the Toxicology Laboratory, Faculty of Health Sciences, Universitas Maarif Hasyim Latif, Sidoarjo. Peripheral blood smear preparation, staining, microscopic observation, and data analysis were conducted at the Hematology Laboratory of the same

faculty. The study was carried out over a period of five months, from October 2025 to February 2026.

3.2. Preparation of Mangosteen Pericarp Extract

Fresh mangosteen (*Garcinia mangostana*) pericarps were collected, washed thoroughly to remove debris, and dried under shade at room temperature until a constant weight was achieved. The dried pericarps were then ground into a coarse powder using a mechanical grinder. Extraction was performed using the maceration method. Approximately 1:10 w/v of powdered pericarp was macerated with 70% ethanol as the solvent. The mixture was stirred periodically using a magnetic stirrer and allowed to stand at room temperature for 24–48 hours. Following maceration, the mixture was filtered through Whatman No. 1 filter paper to remove solid plant material. The filtrate was then concentrated using a rotary evaporator at 40°C to obtain a thick, viscous, dark purple extract.

The concentrated extract was subsequently diluted with distilled water to obtain a working staining solution. The pH of the working solution was adjusted to an optimal range of approximately 5.5–6.0 using dilute HCl to enhance staining performance and ensure consistent color development. The optimal working concentration of the diluted extract was determined through preliminary trials. Various dilutions of the concentrated extract in distilled water 1:5, 1:10, 1:20 v/v were tested on blood smears, and the concentration yielding the best erythrocyte clarity, color intensity, and overall staining quality was selected for the main study. The final working solution used was 1:10 v/v.

3.3. Staining Procedure

Peripheral blood smears were prepared using standard wedge techniques and air-dried completely before staining.

A. Wright-Giemsa Staining: Standard Wright and Giemsa staining procedures were performed according to established laboratory protocols. For Wright staining, smears were stained for 3-5 minutes with Wright's stain, followed by the addition of buffer solution (pH 6.8) and mixing for 1 minute, then diluted with buffer and incubated for 5-10 minutes. For Giemsa staining, smears were stained with 5% Giemsa solution in buffer (pH 7.2) for 15-20 minutes. Following staining, slides were rinsed with distilled water and air-dried.

B. Mangosteen Pericarp Extract Staining: Prepared blood smears were stained with the optimized mangosteen pericarp extract solution (dilution 1:10 v/v). Slides were fixed in absolute methanol for 30 seconds prior to staining. Subsequently, the slide was immersed in the staining solution for 10 minutes. Following staining, the slide was gently rinsed with distilled water for 15 seconds and then air-dried.

3.4. Quality Assessment of Staining

The quality of erythrocyte staining was assessed using a semi-quantitative scoring system developed on the basis of criteria for evaluating erythrocyte staining quality that had been used in previous studies. To minimize observer bias, each slide was assessed by two independent observers who were unaware of the staining method used. The assessment results were then compared to ensure consistency between observers. The scoring system evaluated the following parameters on a scale of 1 to 4:

A. Color Intensity: (1 = Very weak, 4 = Very intense) - assessed for the overall color saturation of erythrocytes.

B. Morphological Clarity: (1 = Poorly defined, 4 = Excellent clarity) - assessed for the distinctness of erythrocyte outlines, internal structures (if visible), and the presence of inclusions.
C. Background Contrast: (1 = Poor contrast, 4 = Excellent contrast) - assessed for the clarity of the background against the stained cells, crucial for differentiating subtle morphological features.

A consensus score was obtained for each parameter by discussion between the two observers if initial scores differed by more than one point.

3.5. Statistical Analysis

Data were analyzed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (mean, standard deviation, median, interquartile range) were used to summarize the staining quality scores for each method. Inferential statistical analysis was performed using the Friedman test to determine significant differences in staining quality among the three staining methods. When significant differences were observed, post hoc analysis was conducted using the Wilcoxon signed-rank test to perform pairwise comparisons between the methods. The level of statistical significance was set at $p < 0.05$.

4. Results and Discussion

4.1. Research Results

4.1.1. Characteristics and Phytochemical Profile of Mangosteen Pericarp Extract

The mangosteen (*Garcinia mangostana*) pericarp extract obtained in this study exhibited a thick consistency with a dark reddish-purple to brownish coloration. This color is characteristic of anthocyanin-rich plant extracts and indicates the presence of phenolic compounds. Phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, tannins, polyphenols, and xanthones. These compounds are known to contribute to staining properties due to their ability to interact with cellular proteins and membrane components, thereby facilitating color binding to erythrocytes.

4.1.2. Staining Characteristics of Erythrocytes

Microscopic examination of peripheral blood smears revealed distinct differences in erythrocyte staining quality among the three methods evaluated. Under Wright stain, erythrocytes exhibited the highest staining quality among the three methods. This was characterized by optimal color intensity, excellent clarity of erythrocyte morphology, and strong contrast against a clear background. Consequently, morphological features crucial for thalassemia diagnosis, such as microcytosis, hypochromia, anisopoikilocytosis, and target cells, were clearly visible (Figure 1).

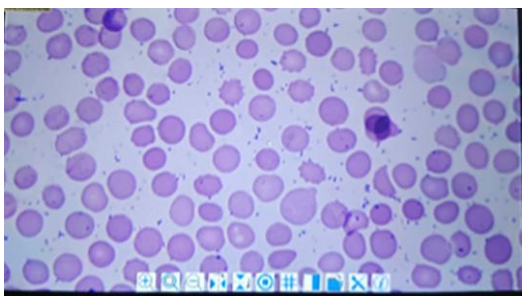


Figure 1. Wright Staining of Peripheral Blood Smear

Giemsa stain also produced satisfactory staining results, although the intensity and contrast were slightly lower compared to Wright stain. Erythrocyte morphology was observable, but the staining appeared relatively less intense and with slightly lower contrast compared to Wright staining. Variations in erythrocyte morphology were identifiable (Figure 2).

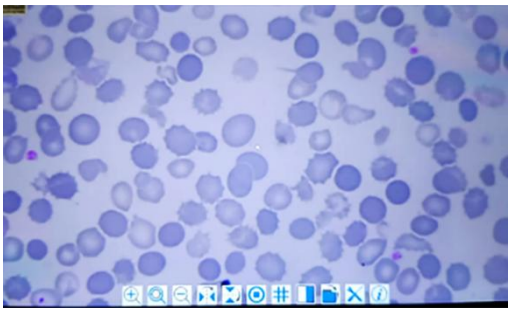


Figure 2. Giemsa Staining of Peripheral Blood Smear

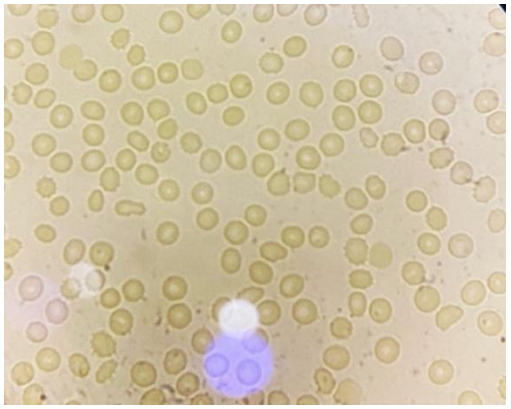


Figure 3. Mangosteen (*Garcinia mangostana*) Pericarp Extract Staining

In contrast, mangosteen (*Garcinia mangostana*) pericarp extract produced a visible staining effect on erythrocytes, characterized by a brownish coloration. While the extract enabled the visualization of erythrocyte morphology and identification of variations in size and shape, the overall clarity and contrast were lower compared to conventional staining methods. Nevertheless, differences in erythrocyte morphology, including variations in size and shape, remained observable (Figure 3).

4.1.3. Statistical Analysis of Staining Quality

Statistical analysis was performed to quantitatively compare the subjective scoring of staining quality. The Friedman test indicated a significant difference in staining quality among the three methods ($p < 0.001$), confirming that the staining method significantly impacts erythrocyte visualization. The mean ranks for each method were: Wright stain (3.00), Giemsa stain (1.76), and mangosteen pericarp extract (1.24). The detailed results of the Friedman test are presented in Table 1.

Table 1. Friedman Test Results for Erythrocyte Staining Quality

Staining Method	Mean Rank
Wright	3.00
Giemsa	1.76
Mangosteen pericarp extract	1.24
Statistical Parameter	Value

Staining Method	Mean Rank
Sample size (N)	25
Chi-Square	46.455
Degrees of freedom (df)	2
p-value	< 0.001

The results of the Friedman test showed that the Wright stain produced the highest quality erythrocyte staining, followed by the Giemsa stain, whilst mangosteen *(Garcinia mangostana)* peel extract produced the lowest quality staining. These differences in ranking indicate variations in the quality of erythrocyte staining amongst the three methods, and these quantitative results are consistent with microscopic observations showing differences in the staining characteristics of each method.

4.1.4. Post Hoc Analysis of Pairwise Comparisons

Post hoc analysis using the Wilcoxon signed-rank test was conducted to evaluate pairwise differences between the staining methods. The results, presented in Table 2, demonstrated statistically significant differences between all pairs of staining methods ($p < 0.001$).

Table 2. Wilcoxon Signed-Rank Test Results for Pairwise Erythrocyte Staining Quality Comparisons

Comparison	*Z*-value	*p*-value
Wright vs Mangosteen pericarp extract	-4.507	< 0.001
Giemsa vs Mangosteen pericarp extract	-3.606	< 0.001
Wright vs Giemsa	-5,000	< 0.001

The test results showed significant differences in the quality of erythrocyte staining across all comparisons. Wright's stain produced a better quality of staining than both Giemsa stain and mangosteen *(Garcinia mangostana)* peel extract. Giemsa stain also produced a better quality of staining than mangosteen peel extract. These findings indicate that both conventional stains produce better erythrocyte staining results than mangosteen peel extract.

4.2. Discussion

This study aimed to comparatively evaluate the potential of mangosteen (*Garcinia mangostana*) pericarp extract as a natural stain for erythrocyte morphology in peripheral blood smears of thalassemia patients, against conventional Wright and Giemsa stains. Our findings indicate that while Wright stain provided the highest quality of staining, followed closely by Giemsa stain, the mangosteen pericarp extract demonstrated a visible but lower performance in terms of color intensity, morphological clarity, and background contrast.

The superior performance of Wright and Giemsa stains is well-established and attributed to their optimized chemical composition of acidic and basic dyes that effectively interact with cellular components (Kalinin et al., 2024). Eosin, an acidic dye, stains hemoglobin and other basic cellular structures in erythrocytes, producing the characteristic pinkish-red hue, while methylene blue and its derivatives, basic dyes, stain acidic components like nucleic acids in leukocytes, yielding blue to purple colors (Cheng et al., 2024). The strong contrast and clear visualization of erythrocyte morphology, including subtle changes like microcytosis and hypochromia crucial for thalassemia diagnosis, observed with Wright stain, underscore its role as the gold standard in hematological laboratory diagnostics (Bain et al., 2017). Giemsa stain, while also effective, often shows slightly less intensity and contrast, as observed in our study.

The mangosteen pericarp extract, rich in anthocyanins and xanthones (Tocmo et al., 2026; Yuvanatemiya et al., 2022), exhibited a brownish coloration and allowed for the visualization of basic erythrocyte morphology. This suggests that the phenolic compounds present in the extract, such as anthocyanins, can indeed interact with erythrocyte components (Sadowska-Bartosz & Bartosz, 2024). However, the lower staining quality compared to conventional stains highlights several potential limitations. The brownish color may not provide the same level of diagnostic detail for subtle abnormalities as the more distinct red/pink hues from eosin. Furthermore, the lower contrast and clarity might make it challenging to precisely identify critical morphological features of thalassemia, such as the degree of hypochromia or the presence of fine inclusions, which are essential for accurate diagnosis and management (Musallam et al., 2024). This aligns with the general observation that while plant-derived pigments can stain biological tissues, their performance often varies and may not fully replicate the diagnostic precision of synthetic stains (Mulla et al., 2024).

The significant statistical differences ($p < 0.001$) observed between all pairwise comparisons, with Wright stain outperforming Giemsa, and both conventional stains significantly outperforming the mangosteen extract, strongly support these microscopic observations. The mean ranks (Wright: 3.00, Giemsa: 1.76, Mangosteen: 1.24) clearly delineate this hierarchy of performance.

The potential of mangosteen peel extract as a natural colorant is demonstrated not only by its ability to visualize erythrocytes, but also by the opportunity to utilize mangosteen peel, which is generally discarded as waste following fruit consumption or processing. Utilizing this waste can add value to biomass that was previously underutilized, whilst supporting a more waste-reduction-oriented approach. From a cost perspective, the availability of local raw materials and the possibility of using relatively simple extraction processes may be of interest to laboratories with limited access to synthetic dyes. However, these benefits must be weighed against the costs of extraction, raw material standardization, extract stability, storage, and quality testing requirements before the method can be assessed as a truly economical alternative. Consequently, the ecological advantages and potential cost savings need to be substantiated through further evaluation of process efficiency and the consistency of coloring results (Sari et al., 2022; Huang et al., 2023).

From a practical perspective, the use of mangosteen peel extract has the potential to be relevant for laboratories with limited resources, provided that the raw material is readily available and the preparation process can be carried out using simple equipment. This is particularly important in settings facing constraints in obtaining or maintaining a supply of synthetic dyes. Nevertheless, its application still requires standardization of concentration, pH, staining time, fixation methods, and storage conditions to ensure consistent results across samples. Therefore, the feasibility of using mangosteen peel extract in laboratories with limited resources is best viewed as an opportunity requiring further validation, rather than as a direct substitute for Wright's or Giemsa's stains.

However, the currently lower performance of mangosteen peel extract highlights the need to optimize pigment characteristics and staining conditions. The pH-sensitive nature of anthocyanins must be taken into account, as changes in pH can affect the stability and color of the pigments; consequently, regulating the pH of the extract and working solutions has the potential to enhance color intensity and stability. Meanwhile, the xanthone compounds present in mangosteen peel could be considered in a fractionation process to obtain components with more consistent staining characteristics. Optimization of the solvent, extraction time and temperature is also required to increase the yield of active compounds without causing pigment degradation. Furthermore, the use of appropriate extract

concentrations, fixation methods and mordant agents can be evaluated to enhance pigment interaction with cellular components and strengthen the contrast between erythrocytes and the background. Thus, improvements in staining quality depend not only on increasing the extract concentration, but also on the chemical stability of the pigments and the conditions under which they interact with the cellular structure.

The findings of this study are consistent with previous, albeit limited, research on the use of natural stains in hematological applications, which generally show lower intensity or contrast compared to conventional stains (Deepthi et al., 2025; Karlina et al., 2025). The brownish staining produced by mangosteen peel extract still allows for the visualization of the erythrocyte nucleus, but differs from the reddish-pink color of erythrocytes produced by eosin in Wright-Giemsa staining, which may limit the assessment of characteristics such as hypochromia. Nevertheless, the use of mangosteen peel as a staining agent offers ecological benefits as it utilizes agricultural waste that could otherwise add to the environmental burden if not properly managed. From a cost perspective, the use of materials derived from agricultural waste also has the potential to reduce dependence on synthetic dyes and provide an alternative raw material that is more readily available. Thus, the use of mangosteen peel extract has the potential to be a more sustainable and economical alternative, although formulation optimization is required to improve the quality of the staining.

The main limitation of this study is the subjectivity inherent in the semi-quantitative assessment system, although inter-observer reliability was sought through blinding and consensus. The limited sample size is also a constraint, as it may reduce the study's ability to identify smaller differences and limit the generalization of results to a more diverse thalassemia population. Furthermore, the pigments responsible for producing the coloration were not specifically quantified, as the phytochemical profile was based solely on general screening. Differences in coloration results indicate a gap in diagnostic detail compared with conventional methods. Another limitation is the absence of a direct comparison with other plant-based hematological dyes that have demonstrated success.

While statistically significant, the differences in mean ranks illustrate the practical gap in diagnostic detail compared to conventional methods. Furthermore, the phytochemical profile was based on general screening, and the specific active compounds responsible for the observed staining were not quantified. The sample size, while sufficient for preliminary evaluation, might be limited for detecting subtle differences or for generalizing findings to diverse thalassemia populations. The absence of a direct comparison with other successful plant-based hematological stains also represents a gap.

In conclusion, mangosteen pericarp extract shows promise as a natural source of erythrocyte stain, capable of visualizing basic morphology, and could potentially serve as an eco-friendly alternative in certain contexts. However, its current performance does not yet match the diagnostic clarity and detail provided by Wright and Giemsa stains, particularly for conditions like thalassemia where subtle morphological variations are critical. Future research should focus on optimizing extraction and staining protocols to enhance its effectiveness and diagnostic utility.

5. Conclusion

This study successfully evaluated the potential of mangosteen *(Garcinia mangostana)* pericarp extract as a natural staining agent for the morphological analysis of erythrocytes in peripheral blood samples from thalassemia patients. Comparison with Wright's and Giemsa's stains showed that mangosteen pericarp extract produces staining that allows for the

visualization of basic erythrocyte morphology, although its color intensity, clarity and contrast are still lower than those of standard stains, particularly when it comes to revealing subtle morphological changes. These findings suggest that mangosteen pericarp extract cannot yet replace standard stains to support the clinical accuracy of thalassemia diagnosis. However, its use still holds potential as a supplementary staining agent for initial morphological observation, particularly in the context of screening or monitoring that requires further examination and must still be confirmed using standard diagnostic methods.

Further development should focus on optimizing the extraction process and pH conditions to improve staining quality, testing the use of mordants to enhance color intensity and stability, and comparing it with various other natural staining agents to determine the most suitable staining characteristics. Furthermore, a cost-effectiveness evaluation should be carried out, taking into account the availability of materials, the extraction process, the use of additives, and the quality of the resulting staining. Such optimization could clarify the suitability of mangosteen pericarp extract as an auxiliary stain in hematological applications whilst reinforcing its potential as a natural and more environmentally friendly alternative to conventional stains.

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5.2. Competing interests

The authors declare no competing interests.

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