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Development of a natural eosinophil stain based on cordyline fruticosa leaves extract and selenicereus monacanthus (dragon fruit peel extracts) for hematology diagnostic applications

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ARTICLE INFO

Article history:

Received Jan 31, 2026
Revised Feb 2, 2026
Accepted Feb 27, 2026

Keywords:

Cordyline Fructiosa
Eosinophil
Hematology
Hylocereus Polyrhizus
Natural Stain

ABSTRACT

The search for natural alternatives to conventional hematological stains has gained attention due to issues of dependency on imported reagents, cost, and potential environmental impact. This study aimed to explore the potential of Cordyline fruticosa (andong leaves) and Selenicereus monacanthus (dragon fruit peel) extracts as natural eosinophil stains in hematology. Pigment extraction was performed using standard procedures, followed by phytochemical screening for anthocyanins and flavonoids. The total anthocyanin content was higher in C. fruticosa (1.20 mg/L) compared to S. monacanthus (0.30 mg/L), while relative flavonoid absorbance values also indicated greater pigment density in andong leaves. Application of the extracts in the hemocytometer method demonstrated staining of the background and cellular components, although with weaker intensity than conventional eosin. In peripheral blood smear preparations, Giemsa stain produced the most distinct results, clearly differentiating eosinophil nuclei, cytoplasm, and characteristic granules. In contrast, the natural extracts yielded paler orange cream staining, with less clarity and limited granule visualization, and some smears exhibited detachment due to imperfect fixation. These findings suggest that while andong leaves and dragon fruit peel contain bioactive pigments with staining potential, their application in hematology requires further optimization, particularly in fixation methods, extract concentration, and stability testing over longer storage periods.

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INTRODUCTION

Staining techniques play a crucial role in hematology, allowing us to see and distinguish the shapes and features of blood cells, including specific types of white blood cells like eosinophils (Kay, 2015). Traditional stains such as eosin and Giemsa are widely used because they produce clear, sharp contrasts that make identification straightforward. However, these stains depend on synthetic dyes, which can be costly, sometimes hard to obtain, and may carry potential health or environmental concerns (de Haan et al., 2021). This has prompted a growing interest in finding safer, locally available, and more affordable alternatives that can be used reliably in routine laboratory practice (Masuda et al., 2022).

Plant derived pigments, especially anthocyanins and flavonoids, are increasingly attracting attention as promising natural colorants. They are not only biocompatible and environmentally friendly but also offer a range of colors that can change depending on the pH, making them versatile for various applications (Masuda et al., 2022). In histological applications, anthocyanins have been explored as non toxic staining agents showing adequate binding to tissue structures with reasonable diagnostic clarity (Chan, 2014). Theoretically, this study contributes to the understanding of staining mechanisms of plant derived pigments in hematology, particularly regarding how anthocyanin and flavonoid based compounds interact with eosinophil cellular structures under different preparation conditions.

In hematology, the use of natural dyes has been relatively underexplored. However, recent studies indicate that they could be a feasible alternative. For example, extracts from Syzygium cumini peel have been tested on peripheral blood smears and compared with Giemsa. While these natural extracts show potential, challenges remain, particularly in achieving clear and consistent staining of leukocytes (Kass, 1981). Another study on alternative to eosin using Curcuma longa extract demonstrated biodegradability and lower toxicity while maintaining staining capability (Suryawanshi et al., 2017)

In recent years, the exploration of natural dyes as safer and more environmentally friendly alternatives has gained momentum, particularly in biomedical applications (Ramadhani et al., 2025). Conventional hematological stains, such as eosin and Giemsa, although effective, are associated with potential toxicity and environmental concerns due to their synthetic chemical composition (Wick, 2019). Plant derived pigments, especially anthocyanins and flavonoids, offer promising advantages because of their natural origin, wide availability, and reported bioactive properties (Al-Alwani et al., 2017). Extracts from *Cordyline fruticosa* (andong leaves) and *Selenicereus monacanthus* (dragon fruit peel) have been identified as rich sources of these compounds, yet their potential use as hematological stains has been scarcely investigated. This study addresses this gap by evaluating their staining ability on eosinophils in both hemocytometer and peripheral blood smear preparations (Sabban & Wahyuni, 2024).

The selection of Cordyline fruticosa (andong leaves) and dragon fruit peel as sources of natural pigments was based on both scientific and practical considerations. Cordyline fruticosa is a plant that is widely distributed, easily cultivated, and well adapted to tropical environments. Its leaves exhibit a distinct and intense coloration, indicating a relatively high content of bioactive pigments, particularly anthocyanins and flavonoids. These pigment groups are known for their chromatic properties and potential interactions with cellular components, making the plant a relevant candidate for exploration as a natural staining agent. In addition to pigment richness, the accessibility and sustainability of C. fruticosa were important factors. The plant grows abundantly with minimal cultivation requirements, allowing it to serve as a locally available and renewable resource. Moreover, fallen or unused leaves can be collected without damaging the plant, thereby supporting the concept of low-impact raw material utilization.

Dragon fruit peel was selected primarily from a sustainability and waste valorization perspective. The peel constitutes a significant proportion of the fruit mass and is typically discarded as agricultural or household waste. However, it contains substantial levels of

anthocyanins and other phenolic compounds with recognized coloring potential. Utilizing dragon fruit peel as a pigment source aligns with environmentally responsible laboratory practices by converting organic waste into a value-added product.

Therefore, exploring natural extracts as alternative hematological stains is valuable not only from an academic perspective but also for practical laboratory applications. When optimized, stains derived from *Cordyline fruticosa* and *Selenicereus monacanthus* could offer laboratories especially those in resource limited settings an affordable, accessible, and environmentally friendly substitute for synthetic dyes. This approach aligns with the global push toward sustainable biomedical practices while reducing dependence on imported staining reagents. In addition, it highlights the potential of local biodiversity, demonstrating how indigenous plant resources can contribute to innovative solutions in hematology diagnostics (Al-Alwani et al., 2017).

RESEARCH METHOD

This study was an experimental laboratory research conducted at the Hematology Laboratory, Universitas Bakti Tunas Husada, Tasikmalaya, Indonesia. The research aimed to evaluate the staining potential of *Cordyline fruticosa* (andong leaves) and *Selenicereus monacanthus* (dragon fruit peel) extracts as natural eosinophil stains in hematology, compared with conventional eosin and Giemsa stains.

Fresh *C. fruticosa* leaves and *S. monacanthus* peels were collected from local sources in Tasikmalaya, Indonesia. All chemicals used were of analytical grade, including methanol, aquadest, ethanol, and Tween 20, A light microscope (Olympus CX23, Japan) was used for blood smear observation. Absorbance measurements for anthocyanin and flavonoid analysis were performed using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Hemocytometer (Marienfeld, Germany) was used for eosinophil cell counting.

Pigments were extracted using the maceration method with methanol containing 1% HCl. The crude extracts were filtered and concentrated under reduced pressure. Phytochemical analysis included determination of total anthocyanin content using the pH differential method at pH 1.0 and 4.5 (Giusti & Wrolstad, 2001) and total flavonoid content using the aluminum chloride colorimetric method at 430 nm (Chang et al., 2002).

Two approaches were employed to evaluate the staining capacity of natural extracts: (1) Hemocytometer method: eosinophil cells in whole blood samples were stained with the prepared natural extracts and compared with standard eosin solution. (2) Peripheral blood smear method: smears were fixed with methanol for 3–5 minutes and stained with natural extracts for 15 minutes, then rinsed with distilled water. Conventional Giemsa staining was used as the control. Morphological features, including nucleus, cytoplasm, and eosinophilic granules, were observed under 1000× magnification with oil immersion.

The study protocol was reviewed and approved by the Health Research Ethics Committee of Universitas Bakti Tunas Husada, Tasikmalaya, Indonesia (Approval No: [260-01/E.01/KEPEK-BTH/VII/2025]) Blood samples were obtained from allergic volunteers after informed consent.

The analysis was carried out using a semi-quantitative scoring system based on direct microscopic observation of eosinophil cells in both hemocytometer preparations and peripheral blood smears. Scoring was performed by assessing parameters such as the clarity of the nucleus, cytoplasmic staining, visibility of eosinophilic granules, and background contrast. Each parameter was rated using a descriptive scale (poor, fair, good, excellent), adapted from standard hematology staining evaluation methods (Bancroft et al., 2013). Results were presented descriptively as observational scores rather than numerical quantification. Representative microscopic images were documented for each treatment group (natural extracts and conventional stains) to illustrate staining outcomes. Since the data relied on visual scoring, no inferential statistical tests were applied; instead, the comparison was made qualitatively against conventional eosin and Giemsa stains as controls.

RESULTS AND DISCUSSIONS

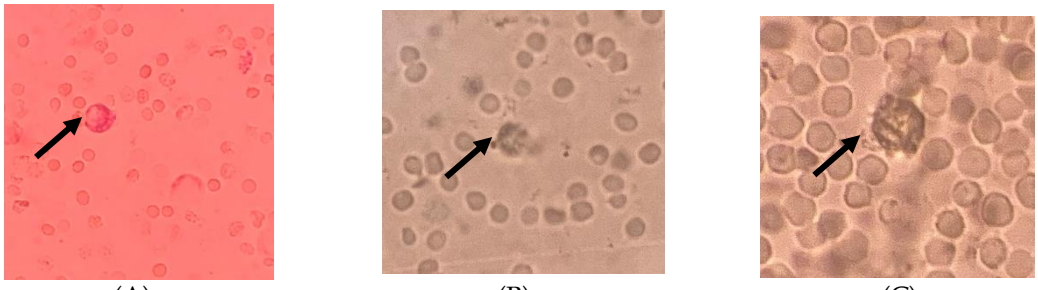
Phytochemical analysis confirmed that both Cordyline fruticosa (andong leaves) and Selenicereus monacanthus (dragon fruit peel) extracts contain anthocyanins and flavonoids. Using the pH differential method, the total anthocyanin content was measured at 1.20 mg/L for C. fruticosa and 0.30 mg/L for S. monacanthus. Flavonoid content, assessed via the aluminum chloride method, showed higher absorbance for C. fruticosa (Abs = 0.560 at 20 ppm) compared to H. polyrhizus (Abs = 0.349 at 20 ppm), suggesting a greater pigment density in the andong leaves extract.

Observations of eosinophils using a hemocytometer showed that both natural extracts were capable of providing background staining and faint cellular contrast, although the intensity was noticeably lower than that achieved with standard eosin. In peripheral blood smear preparations, Giemsa consistently produced optimal contrast, allowing clear visualization of nuclei, cytoplasm, and eosinophilic granules. By comparison, smears stained with the natural extracts appeared paler, displaying an orange-cream coloration in the cytoplasm and less distinct granule definition. Some preparations also exhibited partial detachment from the slide, likely resulting from incomplete fixation.

Table 1. Semi-quantitative scoring of eosinophil staining with natural extracts compared to conventional stains

Stain / Extract	Nucleus clarity	Cytoplasm Contrast	Granule visibility	Background cleanliness	Overall score*
Eosin (control)	3 (Good)	3 (Good)	2 (Fair)	3 (Good)	11
Giemsa (control)	3 (Good)	3 (Good)	3 (Good)	3 (Good)	12
Andong leaf extract 10%	2 (fair)	2 (fair)	1 (poor)	2 (fair)	7
Dragon fruit peel extract 3%	2 (fair)	2 (fair)	1 (poor)	2 (fair)	7

Overall score = sum of all parameters (maximum score = 12).
Scorig scale: 0 = very poor 1 = poor; 2 = fair; 3 = Good



(A) (B) (C)

Figure 1. (A) Eosin Standard in Haemocytometer; (B) *S. monacanthus Extract 3%* in Haemocytometer (C) *C. fruticose 10%* in Haemocytometer

(A) (B) (C)

Figure 2. (A) Giemsa Standard in Blood Smear; (B) *S. monacanthus Extract 3%* in Blood Smear (C) *C. fruticose Extract 10%* in Blood Smear

Discussion

The present study showed that natural extracts from Cordyline fruticosa (andong leaves) and Selenicereus monacanthus (dragon fruit peel) have a modest yet noticeable ability to stain eosinophils when compared with conventional stains. Using a semi-quantitative scoring system (Table 1), both extracts received an overall score of 7 out of 12, whereas eosin and Giemsa achieved higher scores of 11 and 12, respectively. These findings reaffirm that traditional stains still provide superior contrast, offering clearer visualization of nuclei, cytoplasm, and eosinophilic granules.

The semi quantitative scores presented in Table 1 were based on the best-performing concentration out of seven tested levels, ranging from 3% to 10%. For Selenicereus monacanthus (dragon fruit peel), the 3% extract yielded the most favorable results, showing good color uptake by eosinophils while keeping background staining minimal. Higher concentrations, however, produced excessive background coloration, which reduced the contrast between the cells and their surrounding matrix. In contrast, Cordyline fruticosa (andong leaves) showed optimal staining at the 10% concentration, providing slightly better cellular color retention and clarity compared with lower concentrations.

These observations indicate that each plant extract has a distinct optimal concentration range, likely influenced by the pigment content, solubility, and affinity for cellular components. The concentration dependent variation in staining efficiency underscores the importance of careful titration in the development of natural hematological stains. It also emphasizes that a higher concentration does not necessarily correspond to better staining quality, as excessive pigment may compromise clarity by increasing background intensity.

The extracts, at their respective optimal concentrations, were then applied to peripheral blood smear preparations. These smears were re-evaluated using the same semi quantitative scoring system. This approach ensured that only the best performing extracts were carried forward for further microscopic observation, allowing a more reliable comparison with conventional stains. The results confirmed that, although the natural extracts were able to partially highlight eosinophil granules, conventional stains still offered superior contrast for both nuclei and cytoplasm.

Microscopic observations further supported these findings. In the hemocytometer method, extracts provided faint background staining that allowed partial visualization of eosinophil cells, but with markedly lower intensity than standard eosin. In peripheral blood smear preparations, Giemsa showed excellent staining quality, with distinct differentiation of the nucleus, cytoplasm, and characteristic eosinophilic granules (Arwie et al., 2024). In contrast, smears stained with natural extracts exhibited pale orange-cream coloration, with poorly defined granules and less cytoplasmic clarity. Some slides also showed detachment of the smear layer, which may be related to incomplete fixation.

Several factors may explain the suboptimal performance of natural extracts. Anthocyanins and flavonoids, the main pigments in both plants, are known to be unstable and highly sensitive to pH, temperature, and light exposure (Wittekind, 2003). In this study, fixation with methanol was applied as in standard smear preparation, however it is hypothesized that methanol may interfere with pigment binding to cellular components, thereby reducing staining efficiency. Previous reports suggested that anthocyanins exhibit greater stability in buffer systems such as citrate phosphate (Rezende et al., 2019) and alternative fixation or solvent systems may improve pigment retention in hematology smears.

Synthetic eosin is a xanthene based acidic dye characterized by high chemical stability, consistent molecular structure, and strong affinity toward basic cellular components. Its compatibility with alcohol-based fixation methods further enhances staining uniformity and reproducibility (Enaru et al., 2021). In contrast, anthocyanins undergo structural transformations depending on pH and solvent conditions, shifting between flavylium cation and quinoidal base forms. These transformations can significantly alter color intensity and binding behavior during staining procedures (Yusuf et al., 2017).

In the present study, methanol fixation, which is optimal for eosin and Giemsa staining, may have contributed to pigment instability in the natural extracts. The reduced staining contrast observed with *C. fruticosa* and *S. monacanthus* extracts therefore reflects not only lower pigment affinity but also the inherent chemical variability of natural dyes (Wahyuni & Sabban, 2022). These findings reinforce that natural pigments cannot be directly substituted for eosin without modification of staining protocols, particularly in terms of solvent systems and stabilization strategies (Enaru et al., 2021).

The staining behavior observed in this study can be explained by the fundamental chemical differences between plant derived pigments and conventional synthetic dyes. Anthocyanins and flavonoids, which constitute the primary pigments in *Cordyline fruticosa* leaves and *Selenicereus monacanthus* peel, are polyphenolic compounds that are highly polar and sensitive to environmental conditions such as pH and solvent polarity (Sachdev et al., 2021). These compounds tend to interact weakly with cellular proteins, relying mainly on hydrogen bonding and electrostatic interactions.

Eosinophil granules are known to be rich in strongly basic proteins, particularly major basic protein (MBP), which exhibit high affinity for acidic synthetic dyes such as eosin. In contrast, anthocyanins do not possess the same degree of ionic interaction strength, resulting in limited penetration and weaker binding to eosinophilic granules (Wadephul et al., 2024). This explains why, in the present study, cytoplasmic coloration was detectable, while granule definition remained indistinct. Similar observations have been reported in previous investigations using anthocyanin based natural stains, where color uptake was adequate but lacked diagnostic sharpness when compared to conventional eosin based staining (Sachdev et al., 2021).

The detachment of smears observed in some preparations also highlights technical limitations. Proper fixation is essential to ensure strong cell adhesion and uniform staining. Literature indicates that modifications in fixation time or the use of ethanol-based fixatives may help preserve smear integrity while facilitating pigment absorption (Wadephul et al., 2024).

Fixation plays a critical role in determining staining quality, especially when using non synthetic dyes. Methanol fixation, while widely accepted for peripheral blood smears, may disrupt the interaction between anthocyanins and cellular structures due to its dehydrating and protein-denaturing effects. This may partially explain the smear detachment and uneven staining observed in some preparations (Wadephul et al., 2024).

Recent studies suggest that ethanol based fixatives or buffered fixation systems may improve pigment retention and cellular adhesion when working with natural dyes (Sudarmi et al., 2015). Buffer systems such as citrate phosphate have also been shown to stabilize anthocyanin structures, potentially enhancing color consistency and longevity. Therefore, future optimization of fixation protocols represents a key step in improving the performance of plant based hematological stains (Negi, 2025).

Despite these limitations, the study provides valuable preliminary evidence that plant-derived pigments can be applied in hematology. Compared with other studies that trialed natural stains such as Syzygium cumini peel (Sari et al., 2021) or *Curcuma longa* extract (Suryawanshi et al., 2017) our results are consistent in showing that natural pigments can impart coloration but still lack the diagnostic clarity of conventional synthetic stains. Therefore, further optimization in extraction, formulation, and fixation conditions is required before natural stains can be considered feasible substitutes in diagnostic hematology.

From a clinical perspective, the natural extracts evaluated in this study do not yet achieve diagnostic equivalence with standard eosin or Giemsa staining. The reduced clarity of eosinophilic granules limits their immediate applicability in routine diagnostic hematology, where precise cell identification is essential. However, the ability of the extracts to provide consistent background and cytoplasmic staining suggests potential utility in non diagnostic contexts (Yusuf et al., 2017).

These natural stains may be suitable for educational laboratories, preliminary screening, or training settings, particularly in resource limited environments where access to imported synthetic dyes is constrained (Vu et al., 2021) With further optimization, natural stains could serve as complementary tools rather than complete replacements for conventional reagents, supporting broader accessibility to basic hematological visualization techniques.

Beyond technical considerations, the use of plant derived pigments aligns with global efforts toward sustainable and environmentally responsible laboratory practices. Synthetic dyes used in hematology often involve complex chemical synthesis, generate hazardous waste, and rely on imported raw materials. In contrast, *Cordyline fruticosa* and *Selenicereus monacanthus* are locally available plants with renewable pigment sources.

Utilizing these plants as staining agents highlights the potential of local biodiversity to contribute to biomedical innovation. This approach not only reduces environmental impact but also supports cost effective laboratory practices, particularly in developing regions. By integrating local natural resources into diagnostic research, this study contributes to the growing movement toward greener and more sustainable laboratory methodologies.

Future research should focus on several optimization strategies to improve staining performance. These include: (1) testing alternative fixation methods such as ethanol based or buffer stabilized fixatives to enhance pigment penetration; (2) adjusting extract concentration and pH stabilization with citrate phosphate buffers to maintain pigment integrity; (3) conducting longer term stability studies (2–4 weeks) to evaluate color retention; and (4) applying digital image analysis tools (e.g., ImageJ) for quantitative assessment of staining intensity. Implementation of these steps is expected to maximize the staining potential of C. fruticosa and S. monacanthus extracts and bring natural stains closer to practical application in hematology diagnostics.

CONCLUSION

This study demonstrated that Cordyline fruticosa (andong leaves) and dragon fruit peel extracts contain anthocyanins and flavonoids with measurable pigment concentrations. Both extracts were capable of producing observable background and cytoplasmic staining effects on eosinophils using hemocytometer and peripheral blood smear methods. However, the staining performance remained inferior to conventional eosin and Giemsa, particularly in terms of granule definition and staining intensity.

The main contribution of this research lies in the experimental exploration of locally available plant materials as potential natural alternatives to synthetic hematological dyes. The findings provide preliminary evidence that pigments derived from underutilized botanical resources and organic waste materials can interact with eosinophil cellular structures. Additionally, this study highlights important technical considerations, including optimal extract concentration, fixation compatibility, and pigment stability, which are essential for future stain development.

Although these natural extracts have not yet achieved diagnostic equivalence with standard stains, they demonstrate promising potential as sustainable, low cost, and environmentally friendly staining agents. With further optimization, plant-based dyes derived from local resources may serve as complementary tools in educational laboratories and resource limited settings.

The study now emphasizes that the primary priority for achieving diagnostic equivalence is the optimization of pigment stabilization and fixation compatibility. The study findings indicate that the current limitations in staining performance are strongly associated with the physicochemical instability of anthocyanins and their interaction with alcohol based fixation methods. Therefore, future investigations should focus on developing stabilization strategies, including pH-controlled buffer systems, co-pigmentation approaches, and modified solvent formulations, alongside the evaluation of alternative fixation protocols. This clarification has been

incorporated to provide a more focused and scientifically grounded recommendation for subsequent research.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Ministry of Education, Culture, Research, and Technology, Indonesia, for funding this research through the Hibah Penelitian Dosen Pemula scheme (Grant Number: [0070/C3/AL.04/2025]). We also thank the Health Research Ethics Committee of Universitas Bakti Tunas Husada, Tasikmalaya for providing ethical approval. Special appreciation is extended to the laboratory technicians who supported the preparation of equipment and reagents, and to the students who assisted with sample preparation and data collection. The authors also acknowledge the constructive feedback provided by reviewers and colleagues, which helped improve the quality of this manuscript.

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