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Evaluation of Mulberry (*Morus rubra*) Fruit Extract as a Natural Dye for Leukocytes & Thrombocytes: An Alternative to Conventional Stain

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ABSTRACT

This study evaluated the potential of *Morus rubra* (red mulberry) fruit extracts as natural stains for peripheral blood smear (PBS) analysis, focusing on leukocyte and platelet visualization. Forty blood specimens were processed using ethanolic and aqueous extracts, with and without mordants, and compared to conventional Wright–Giemsa staining. Staining quality was assessed in terms of cytoplasmic coloration, nuclear clarity, platelet visibility, and reproducibility.

Results demonstrated that *Morus rubra* extracts, whether ethanolic or aqueous, produced weak cytoplasmic staining, poor nuclear differentiation, and insufficient platelet visualization. Even with the addition of mordants, the extracts failed to achieve staining quality comparable to Wright–Giemsa. The study concludes that *Morus rubra* extracts are not suitable alternatives for hematological applications in peripheral blood smear analysis. These findings highlight the chemical limitations of anthocyanins in effectively binding and staining blood cells, and suggest that conventional stains remain necessary for accurate laboratory diagnostics.

Keywords: *Morus rubra, natural dye, peripheral blood smear, leukocyte staining, platelet visualization, hematology, anthocyanin, ethanolic extract, aqueous extract, mordant*

INTRODUCTION

The use of natural plant-based dyes has garnered increasing attention in laboratory and biomedical research due to health, environmental, and sustainability concerns associated with conventional synthetic stains. Among these, *Morus rubra* (red mulberry) fruit contains anthocyanin pigments, which exhibit pH-sensitive coloration and potential bioactive properties (Khoo et al., 2017). Despite extensive research into synthetic dyes such as Wright–Giemsa for peripheral blood smears, there is a growing interest in exploring natural alternatives for leukocyte and platelet staining that are non-toxic, biodegradable, and locally accessible.

Peripheral blood smear (PBS) analysis remains a cornerstone of hematology, enabling identification and quantification of white blood cells (WBCs) and thrombocytes (platelets), which are critical for diagnosing infections, hematologic disorders, and immune system abnormalities (Tyrrell et al.,

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2022; Robier, 2020). Conventional staining methods provide optimal visualization of nuclear and cytoplasmic features, but synthetic dyes may pose health and environmental risks. The challenge lies in developing a natural dye capable of producing clear, reproducible staining comparable to standard laboratory reagents while maintaining cellular integrity.

Previous studies have demonstrated the utility of plant extracts in microbial, chromosomal, and histological staining (Laela et al., 2022; Srichantra Thaiudom & Chaemchaiyaporn, 2022). However, few investigations have evaluated their efficacy in hematology for WBC and platelet visualization. Moreover, factors such as pigment stability, extraction methods, and dye–cell interactions can affect staining outcomes (Mulla et al., 2024; Khasim et al., 2024). This study addresses this gap by experimentally evaluating ethanolic and aqueous extracts of *Morus rubra* fruit, with and without mordants, as potential alternatives to conventional Wright–Giemsa stains.

The purpose of this research is to determine whether *Morus rubra* extract can provide sufficient staining quality, reproducibility, and morphological clarity for leukocytes and platelets in peripheral blood smears. The study aims to contribute to the ongoing search for eco-friendly, cost-effective, and safe laboratory reagents, while providing practical insights for clinical laboratory applications in resource-limited or environmentally conscious settings

Review of Related Literature

Plant-Based Natural Dyes in Laboratory Applications

Natural dyes derived from plants have been increasingly studied for their potential in scientific and clinical applications due to their non-toxic, biodegradable, and sustainable characteristics. Anthocyanins, flavonoids, and other bioactive pigments extracted from fruits and flowers are capable of producing vivid colors and can act as pH-sensitive indicators (Khoo et al., 2017; Laela et al., 2022). Recent studies have demonstrated the effectiveness of plant-based dyes for microbial staining, cytogenetic analysis, and histological preparations, offering environmentally friendly alternatives to traditional synthetic dyes (Srichantra Thaiudom & Chaemchaiyaporn, 2022; Mulla et al., 2024).

The extraction method and solvent choice are critical factors influencing dye stability, intensity, and binding affinity to cellular components. Ethanolic and aqueous extractions are commonly employed to optimize pigment yield while preserving biological activity (Khasim et al., 2024). Additionally, the use of mordants, such as aluminum or iron salts, has been shown to enhance color retention and improve staining quality, particularly in histological and hematological applications (Laela et al., 2022).

Peripheral Blood Smear Analysis

Peripheral blood smear (PBS) examination is a fundamental hematological procedure used to evaluate the morphology and count of white blood cells (WBCs), red blood cells (RBCs), and platelets (Tyrrell et al., 2022). Accurate visualization of leukocytes and thrombocytes is essential for diagnosing infections, hematologic disorders, and immune system abnormalities. Traditional staining methods, such as Wright, Wright–Giemsa, and May-Grünwald stains, provide high contrast between nuclear and cytoplasmic features, enabling precise cellular identification and differentiation (Robier, 2020). Despite their effectiveness, conventional synthetic stains pose several disadvantages. They are often toxic, require hazardous solvents, and generate chemical waste that can harm the environment. In addition, their cost and

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limited local availability may restrict use in resource-limited settings. Consequently, there is a growing demand for natural alternatives that are safe, sustainable, and readily accessible.

Anthocyanins as Bioactive Staining Agents

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue hues observed in various fruits, including *Morus rubra*. These pigments exhibit pH-sensitive color changes, which have been exploited in histology and microbiology for differentiating cell structures (Khoo et al., 2017). Studies suggest that anthocyanins can bind to cellular components, providing distinct coloration of nuclei and cytoplasm, and can be enhanced using mordants to improve durability and intensity (Srichantra Thaiudom & Chaemchaiyaporn, 2022).

Furthermore, anthocyanins possess antioxidant and antimicrobial properties, which may protect cellular integrity during staining procedures (Mulla et al., 2024). These characteristics make anthocyanin-based dyes suitable candidates for laboratory applications, especially in peripheral blood smear analysis where preservation of cell morphology is crucial.

Natural Dyes in Hematology

Recent investigations have explored the use of natural dyes in hematology to replace or supplement synthetic reagents. Studies using beetroot, hibiscus, and pomegranate extracts demonstrated that plant-derived pigments could successfully stain leukocytes and platelets while maintaining cell morphology and contrast comparable to Wright–Giemsa stains (Laela et al., 2022; Khasim et al., 2024). These studies highlight the potential of natural dyes to provide cost-effective, non-toxic, and environmentally friendly alternatives for clinical laboratories.

However, challenges remain, including standardization of extraction protocols, determination of optimal concentrations, and evaluation of long-term stability. Additionally, limited research has focused specifically on *Morus rubra* fruit extracts in PBS staining, leaving a gap in knowledge regarding their efficacy and practicality for routine hematology applications.

Research Gap

Although plant-based dyes have been applied successfully in microbiology and histology, their use in hematology—particularly for leukocyte and platelet visualization in peripheral blood smears—remains underexplored. Specifically, *Morus rubra* anthocyanins have not been systematically evaluated for staining efficacy, reproducibility, and morphological clarity in comparison with Wright–Giemsa stains. This study addresses this gap by investigating the potential of *Morus rubra* fruit extracts, with and without mordants, to provide effective, eco-friendly alternatives for routine PBS analysis in clinical laboratories.

METHODOLOGY

Research Design

This study employed a descriptive-experimental research design to evaluate the staining efficacy of *Morus rubra* (red mulberry) fruit extracts on peripheral blood smears (PBS). The descriptive component

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documented the quality of leukocyte and platelet visualization using natural dyes, while the experimental component assessed differences in staining outcomes across varying extraction methods (ethanolic and aqueous) and the use of mordants. This design enabled the researchers to systematically compare natural extract staining to the conventional Wright–Giemsa method and to evaluate the reproducibility, clarity, and intensity of staining for clinical laboratory purposes.

Research Locale

The study was conducted at the Clinical Laboratory Department of Calamba Medical Center, Laguna, Philippines. This location was selected due to its capacity for controlled handling and storage of human blood specimens and its adherence to standard laboratory procedures. Ethical and administrative approval was obtained from the Ancillary Director, Chief Medical Technologist, and Head of the Blood Bank, granting permission to collect, process, and analyze human-derived blood samples. All donor information remained confidential, PRBC units were assigned unique identification codes, and all data were anonymized. Blood collection, handling, and PBS preparation strictly followed institutional protocols, ensuring safety for both laboratory personnel and the integrity of the specimens.

Respondents/Samples

The study utilized forty peripheral blood specimens collected from voluntary, screened donors. The inclusion criteria ensured that donors were healthy and free from hematologic disorders to maintain baseline consistency in red and white blood cell morphology. Only samples collected specifically for research purposes were included, with no additional risk to donors. Each specimen was divided into groups to test different extraction methods (ethanolic and aqueous) and the presence or absence of mordants, allowing for comparative evaluation against the standard Wright–Giemsa stain.

Research Instrument

Staining performance was assessed using laboratory-prepared ethanolic and aqueous extracts of Morus rubra, with optional mordants to enhance dye binding and color stability. Standard Wright–Giemsa stains were used as a control to benchmark staining quality. Peripheral blood smears were prepared on clean glass slides, fixed with methanol, and stained following optimized protocols. Observations were conducted using bright-field microscopy at 1000× magnification with oil immersion. Staining quality was evaluated in terms of cytoplasmic color, nuclear clarity, platelet visibility, and overall reproducibility. A structured evaluation checklist was employed for scoring, and observations were performed in triplicate to ensure reliability.

Data Gathering Procedure

Blood specimens were collected according to standard venipuncture procedures under aseptic conditions. Each sample was divided for staining with ethanolic extract, aqueous extract, extract with mordant, and Wright–Giemsa as control. Smears were prepared and stained under controlled laboratory conditions. Observers documented staining intensity, cell morphology, and reproducibility using the pre-designed checklist. Data collection occurred over four consecutive weeks to account for consistency across batches. All slides were anonymized to prevent observer bias.

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Statistical Treatment of Data

Data were analyzed using descriptive statistics, including mean, standard deviation, and frequency counts, to summarize staining quality across treatment groups. Comparative analyses employed one-way ANOVA to identify significant differences in staining performance between natural extracts and the conventional Wright–Giemsa method. Post-hoc Tukey tests were applied to determine pairwise differences between groups. Statistical significance was set at $p < 0.05$. This approach allowed the researchers to quantify the effectiveness, reproducibility, and clarity of *Morus rubra* extracts for PBS staining.

Ethical Considerations

The study was conducted in accordance with ethical standards for research involving human-derived biological materials. Permission was obtained from the Ancillary Director, Chief Medical Technologist, and Head of the Blood Bank to handle blood specimens, store PRBC units within the blood bank, and collect minimal samples for laboratory examination. Donor confidentiality was strictly maintained by assigning unique codes to each specimen and anonymizing all collected data. No additional risk was posed to donors, as the study exclusively utilized blood units collected specifically for research purposes, and all handling followed institutional safety protocols.

RESULTS AND DISCUSSION

This study assessed the staining efficacy of *Morus rubra* fruit extracts in peripheral blood smears, focusing on leukocyte and platelet visualization. Forty specimens were analyzed using ethanolic and aqueous extracts, both with and without mordants, and compared to conventional Wright–Giemsa staining. Evaluation criteria included cytoplasmic color, nuclear clarity, platelet visibility, and reproducibility of staining.

Staining Quality of Ethanolic Extract

Ethanolic extracts of *Morus rubra* demonstrated weak cytoplasmic coloration and inadequate nuclear differentiation. Leukocyte nuclei appeared poorly contrasted, and platelet visualization was inconsistent across specimens. Although the addition of mordants slightly enhanced color intensity, the overall staining quality remained insufficient for reliable hematological analysis. Mean scores for cytoplasmic color and nuclear clarity were 2.8 and 2.5 out of 5, respectively, indicating suboptimal performance. The observed limitations likely stem from the chemical characteristics of anthocyanins, including limited binding affinity to cellular proteins and instability under laboratory conditions.

Staining Quality of Aqueous Extract

Aqueous extracts showed even poorer performance, producing faint cytoplasmic staining and indistinct nuclear structures. Platelets were difficult to identify, and the addition of mordants only marginally improved visualization. Mean scores were 2.4 for cytoplasmic color and 2.2 for nuclear clarity, reflecting inadequate staining. These results suggest that water-based extraction does not effectively solubilize anthocyanins to achieve functional hematological staining, corroborating the chemical constraints observed with ethanolic extracts.

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Comparison to Wright–Giemsa Staining

In contrast, Wright–Giemsa stains provided clear cytoplasmic and nuclear contrast, with consistently high visibility of platelets. Statistical analysis using one-way ANOVA confirmed significant differences between *Morus rubra* extracts and Wright–Giemsa ($p < 0.05$). This indicates that the plant-based extracts, in their current form, are unable to replicate the staining quality necessary for diagnostic hematology.

Discussion of Findings

The results demonstrate that *Morus rubra* fruit extracts are not suitable for staining peripheral blood smears. The poor performance of both ethanolic and aqueous extracts is attributable to the chemical limitations of anthocyanins, including low pigment stability and insufficient binding to cellular components. Although mordants provided minor improvement, the extracts failed to achieve adequate nuclear or cytoplasmic differentiation and could not reliably reveal platelet morphology. These findings align with prior research suggesting that not all plant-based anthocyanin dyes are effective in hematological applications (Srichantra Thaiudom & Chaemchaiyaporn, 2022; Khasim et al., 2024). The study highlights the necessity of conventional synthetic stains such as Wright–Giemsa for accurate, reproducible, and clinically reliable PBS analysis.

Conclusion

This study concludes that *Morus rubra* (red mulberry) fruit extracts, whether ethanolic or aqueous and with or without mordants, are not suitable for peripheral blood smear staining in hematological applications. Both extracts produced weak cytoplasmic staining, unclear nuclear differentiation, and inadequate platelet visualization, failing to provide results comparable to Wright–Giemsa staining. The chemical limitations of anthocyanins, including low binding affinity and pigment instability, are the primary factors limiting staining effectiveness.

Implications of the Study

The findings emphasize that, despite growing interest in plant-based natural dyes, *Morus rubra* extracts cannot replace conventional synthetic stains for accurate hematological analysis. Laboratories should continue to rely on established stains for peripheral blood smear examination to ensure diagnostic accuracy. This study also underscores the need for careful evaluation of natural dyes' chemical properties before adoption in clinical settings.

Recommendations

Future research should investigate alternative plant-based pigments with greater chemical stability and binding affinity for cellular components. Optimization of extraction methods, pigment concentration, and mordant selection may improve staining potential, but current results indicate that *Morus rubra* extracts are inadequate for routine hematology. Laboratories and educators are advised to use synthetic dyes for accurate leukocyte and platelet visualization while continuing to explore safe, eco-friendly alternatives.

Future Research Directions

Further studies could explore a wider range of anthocyanin-rich plants and evaluate combinations with modern staining enhancers or stabilizers to improve efficacy. Investigations into novel extraction techniques, pigment concentration optimization, or hybrid natural-synthetic stains may provide practical solutions. Additionally, research may assess the feasibility of plant-based dyes in other biological applications such as microbial, histological, or cytogenetic staining, where structural requirements differ from hematology.

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