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Parijoto (*Medinilla speciosa*) extract as a natural dye alternative for peripheral blood smears: A comparative study with giemsa stain

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Abstract

Background: Peripheral blood smear staining is an essential hematological procedure used to evaluate blood cell morphology. The commonly used standard stain is Giemsa stain; however, its chemical components may pose potential risks to health and the environment. Therefore, safer natural alternatives are needed. Previous studies have reported that anthocyanin-containing plant extracts, such as *Hibiscus sabdariffa, Tectona grandis, and Garcinia mangostana*, can effectively stain blood *cells. Medinilla speciosa (parijoto)* also contains anthocyanins and has potential as a natural dye. *Objective:* This study aimed to evaluate the staining quality of peripheral blood smears using *Medinilla speciosa* extract at different concentrations and to compare the results with a *10% Giemsa stain control. Methods:* This laboratory-based experimental study used a post-test only control group design. A total of 30 blood smears were stained using *Medinilla speciosa* extract at concentrations of 25%, 50%, and 70%, along with a 10% *Giemsa stain control*. The extract was prepared using maceration with pH adjustment to optimize anthocyanin stability. Staining quality was assessed based on color intensity, background clarity, and erythrocyte morphology using a semi-quantitative scoring system. Data were analyzed using the *Kruskal-Wallis test* and *Mann-Whitney U test. Results:* Increasing extract concentration was generally associated with improved staining quality. A significant difference among groups was observed ($p = 0.002$). Pairwise analysis showed that the 70% extract was not significantly different from the 10% Giemsa stain control ($p = 0.321$), while lower concentrations differed significantly. *Conclusion:* The 70% *Medinilla speciosa* extract showed the best performance among extract groups and demonstrated comparable staining quality in certain parameters. However, Giemsa stain remains the more consistent standard. Further optimization is required before routine application.

Keywords

Erythrocyte morphology, Giemsa, Natural dyes, Parijoto, Peripheral blood smear

1. Introduction

Blood cell morphology examination is an important parameter in hematology testing. Blood disorders such as anemia are generally detected through blood morphology using the peripheral blood smear method (Khasanah et al., 2023). Hematology examination using the peripheral blood smear method is carried out by making a thin layer of blood on a glass object, then fixing it and coloring it with a certain substance, then viewing it using a microscope (Alawiyah, 2023). The addition of dye to the peripheral blood smear preparation aims to clarify and provide contrasting color to the blood cell structure, making it easier to observe using a microscope (Varghese et al., 2023).

Giemsa is a commonly used to stain for staining blood smears (Alawiyah, 2023). Although it produces sharp and high-contrast results, this type of stain has negative impacts, particularly on the environment. This is due to its main component, methanol, which is toxic and therefore hazardous to aquatic organisms and human health. Additionally, synthetic stains such as eosin and methylene blue are persistent, difficult to degrade, and have the potential to cause toxicity in aquatic microorganisms. If Giemsa waste is disposed of directly without treatment, the accumulation of these chemical compounds can disrupt ecosystem balance, degrade water quality, and contribute to environmental pollution (N. A. Putri et al., 2024).

Growing awareness of the environmental impact of synthetic dyes has spurred the exploration of natural and eco-friendly materials as potential alternatives (Agustin et al., 2022). Previous research using related natural materials has shown that natural dyes derived from plant extracts have the potential to be used as stains for peripheral blood smears. In this study, rosella flower extract (*Hibiscus sabdariffa*), with its anthocyanin content, can impart a reddish-purple color to erythrocytes. A study using teak leaf extract (*Tectona grandis*) on blood cells produced a reddish-purple color. In another study, mangosteen peel (*Garcinia mangostana*), with its anthocyanin content, also provided good staining on blood cells (Ghofur et al., 2022). Based on this research, the materials used contain natural pigments that can bind to blood cell components through electrostatic bonds and chemical interactions without damaging the cell structure (Nuraini & Tianto, 2023).

The parijoto plant (*Medinilla speciosa*) is an endemic species that grows in highland areas such as mountain slopes and forests in Indonesia. According to research conducted by

Sholikhati et al, (2024), parijoto fruit has been shown to possess pharmacological activities such as antidiabetic, antioxidant, antibacterial, and anticancer properties. Its various chemical constituents include saponins, flavonoid glycosides, and tannins. Flavonoid compounds are divided into several groups: chalcones, anthocyanins, anthocyanidins, isoflavones, flavanones, flavonols, and flavones. These pigment compounds produce the colors found in plants, such as anthocyanins, which produce blue, purple, and red colors (Ifadah et al., 2021).

The anthocyanin pigments in parijoto are deeply colored and water-soluble. These pigments are found in the leaves, flowers, and fruits of higher plants. Structurally, the hydroxyl and methoxyl groups in anthocyanins influence the intensity of the color produced by these compounds (Garcia et al., 2022). An increase in hydroxyl groups can produce a strong blue color, while an increase in methoxyl groups produces a reddish color.

The parijoto plant (*Medinilla speciosa*) is generally used only as a food ingredient, such as in jams and syrups. The use of parijoto (*Medinilla speciosa*) as a natural dye has not yet been explored. Furthermore, to date, no studies have examined the use of natural parijoto extract (*Medinilla speciosa*) as a natural dye for peripheral blood smears.

This study aimes to determine the staining ability of parijoto extract (*Medinilla speciosa*) in peripheral blood smears compared to the standard Giemsa stain. In addition to identifying a safe and environmentally friendly alternative stain, this study may also contribute to scientific advancement, innovation in medical laboratory technology, and the sustainable use of local natural resources.

2. Materials and Methods

2.1 Study Design

This study employed a laboratory-based experimental design using a control group approach without a pretest. The treatment group consisted of peripheral blood smears stained with *Medinilla speciosa* extract at various concentrations, while the control group was stained using Giemsa stain as the standard reference. The primary variable observed was the quality of blood cell staining produced by each extract concentration when compared to the control group.

2.2 Materials and Equipment

The materials used include parijoto fruit, 70% ethanol, distilled water, venous blood human samples, absolute methanol, 3% citric acid, 10% Giemsa, 25%, 50%, and 70% parijoto extract, immersion oil.

The instruments and equipment used include an oven (Memmert, Germany), Erlenmeyer flask (Iwaki, Tokyo, Japan), Whatman filter paper no. 1, digital analytical balance (Ohaus PA214C, Parsippany, NJ, USA), stirring rod (Iwaki, Tokyo, Japan), aluminum foil, volumetric flask (Pyrex®, Corning, NY, USA), measuring cylinder (Pyrex®, Corning, NY, USA), glass object (Citotest, Jiangsu, China), glass beaker (Iwaki, Tokyo, Japan), staining rack, dropper pipette, binocular microscope (Olympus CX23, Olympus Corporation, Tokyo, Japan), gloves (Sensi, Medisafe, Indonesia), surgical masks (One Care)

2.3 Preparation of Making Parijoto Extract

Fresh parijoto fruits were cleaned to remove impurities, washed under running water, and oven-dried at 50°C for four hours. The dried material was ground into powder and macerated using acidified 70% ethanol (pH 2–3) at a ratio of 1:10 for 24–72 hours under light-protected conditions (Wijaya et al., 2022). The maceration process is carried out at room temperature for 24-72 hours in an airtight container that is tightly closed and protected from light (Garcia et al., 2022). The maceration solution is homogenized periodically 2-3 times. The resulting maceration solution is filtered using filter paper until 100% extract filtrate is obtained. To achieve the desired concentration, mix:

Tabel 1. Concentration of Parijoto Extract

Concentration	100% Concentrated Extract (mL)		Aquadest (mL)
Extract 25%	25	+	75
Extract 50%	50	+	50
Extract 70%	70	+	90

2.4 Preparation and Examination of Peripheral Blood Smear

One drop of venous blood sample obtained in a vacuum tube was placed onto a glass slide. Each sample on the slide was assigned a code for each respondent. Another glass slide was

placed on the drop of blood on the slide at a 30-45° angle until the blood spread and pushed forward quickly. The resulting blood smear was air-dried and fixed with absolute methanol for 3 minutes (Ghofur et al., 2022). The fixed blood smears were immersed in a staining solution (10% Giemsa, 25%, 50%, and 70% parijoto extract) for 10–15 minutes, adjusted for each treatment. The staining solution was discarded, and the smears were rinsed with running water. After the smears were dry, one drop of immersion oil was added for microscopic observation. Observations were made starting at 40x to 100x magnification to observe the morphology of red blood cells (erythrocytes).

2.5 Data Analysis

This study was conducted using a quantitative comparative approach involving univariate and bivariate analysis. Staining quality scores are treated as ordinal data. Staining quality is evaluated using a semi-quantitative scoring system based on color intensity, background clarity, and erythrocyte morphology, with scores ranging from 1 (poor), 2 (fairly clear), 3 (clear), to 4 (very clear).

Univariate analysis was performed using frequency distributions, percentages, medians, and interquartile ranges to describe the data in each group, including the groups treated with 25%, 50%, and 70% *Medinilla speciosa* extracts, as well as the control group stained with Giemsa stain. Bivariate analysis was performed using non-parametric tests. Differences among the four groups were analyzed using the Kruskal–Wallis test, followed by pairwise comparisons using the Mann–Whitney U test. All preparations were evaluated by trained laboratory personnel using standard evaluation criteria to ensure consistency and minimize subjectivity.

3. Results and Discussion

3.1 Result

Overall, the study results indicate that an increase in the concentration of *Medinilla speciosa* extract is associated with an improvement in color quality. These findings are consistent with the role of anthocyanins as natural pigments that contribute to coloring ability, although the effect is not uniform across all parameters.

Table 2 shows that a 25% extract showed relatively low staining quality with moderate variation among samples, as reflected by the distribution of scores. As shown in Table 3, an increase in concentration of up to 50% was associated with improved staining quality and a more consistent distribution of scores compared to a 25% extract. Among the extract

group, a concentration of 70% showed the highest overall coloring quality, as presented in Table 4 The distribution of scores shows a tendency towards higher values, although some variability among the samples is still observed. These findings suggest that increased extract concentrations are generally associated with improved coloring quality; however, the effect is not consistent across all parameters evaluated.

The control group stained with Giemsa staining showed consistently high staining quality with a stable distribution of scores, as shown in Table 5 Although the coloring quality of *Medinilla speciosa* extract does not fully correspond to Giemsa coloring in all parameters, the 70% extract concentration shows comparable performance in certain aspects, which shows its potential as an alternative to natural coloring.

Table 2. Univariate Analysis Results of 25% Extract

Parameter	Mean	Median	Mode	SD
Color Intensity	1.67	2	1	0.707
Background Clarity	1.56	1	1	0.726
Erythrocyte Morphology Clarity	1	1	1	0

Table 3. Univariate Analysis Results of 50% Extract

Parameter	Mean	Median	Mode	SD
Color Intensity	1.78	2	2	0.667
Background Clarity	2.44	2	2	0.527
Erythrocyte Morphology Clarity	2.22	2	2	0.441

Table 4. Univariate Analysis Results of 70% Extract

Parameter	Mean	Median	Mode	SD
Color Intensity	2.56	3	3	0.882
Background Clarity	2	2	1	1
Erythrocyte Morphology Clarity	2.78	3	3	0.441

Table 5. 10% Giemsa Univariate Analysis Results

Parameter	Mean	Median	Mode	SD
Color Intensity	3	3	3	0
Background Clarity	3	3	3	0
Erythrocyte Morphology Clarity	2.67	3	3	0.577

Table 6. Results of the *Shapiro-Wilk* Normality Test

Concentration	N	Sig
Extract 25%	9	0.080
Extract 50%	9	0.019
Extract 70%	9	0.012
Extract 10%	3	0.000

Table 7. Results of the *Kruskal-Wallis* Intergroup Difference Test

	Result
Sig	0.002
Sd	3

Table 8. Results of the - *Mann Whitney* Advanced Test for Differences Between Treatment Groups

Group Comparison	N	Sig
Extract 25% vs Giemsa 10%	9	0.011
Extract 50% vs Giemsa 10%	9	0.030
Extract 70% vs Giemsa 10%	9	0.321

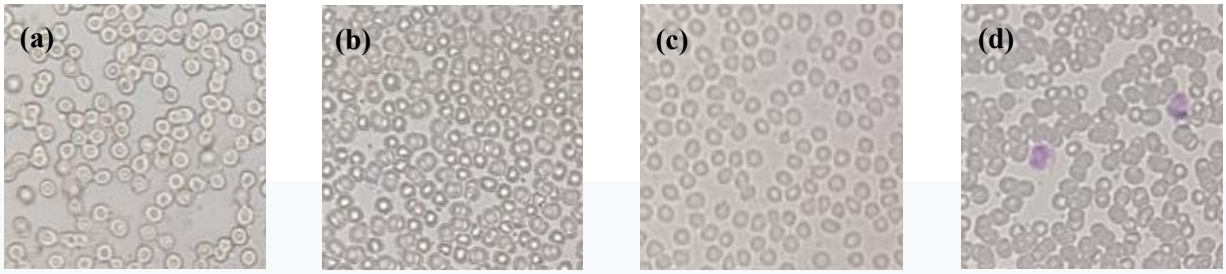

Figure 1. Microscopic Observation 100x of Red Blood Cells with Parijoto Extract Staining at Concentrations of 25% (a), 50% (b), 70% (c) and 10% Giemsa as Control (d)

3.2 Discussion

A comparison between the treatment and control groups of 10% Giemsa staining using the Mann-Whitney U test, as shown in Table 8, showed that the concentrations of the 25% and 50% extracts differed significantly from those of the control group ($p < 0.05$). In contrast, the 70% extract concentration showed no statistically significant difference compared to the control group ($p > 0.05$). These findings suggest that a 70% *Medinilla speciosa* extract showed comparable staining performance to controls; however, these results should be interpreted with caution, as insignificant differences do not imply equivalence.

The flavonoid and tannin compounds in parijoto extract can bind to erythrocyte membrane proteins, producing a stable and even color at certain concentrations. This mechanism is similar to the principle of synthetic dyes, although the colors are obtained from natural materials. Staining can identify the condition of erythrocytes, including the degree of hypochromia or hyperchromia, as well as abnormalities in shape and size (Olyvia Manihiya et al., 2021).

The difference in the intensity of the color of erythrocytes is associated with the concentration of the *Medinilla speciosa* extract used. Statistical analysis using the Kruskal-

Wallis test (table 7) showed significant differences in the treatment group (p = 0.002). A further pairwise comparison using the Mann-Whitney U test (table 8) showed that the concentrations of the 25% and 50% extracts differed significantly from the Giemsa 10% staining control (p < 0.05). In contrast, the 70% extract concentration showed no statistically significant difference compared to the control (p > 0.05). These findings suggest that increased concentrations of *Medinilla speciosa* extract are generally associated with increased erythrocyte color intensity. This effect can be attributed to the presence of anthocyanins, which are known to produce reddish purple pigments. However, this mechanism was not directly evaluated in this study and should be interpreted cautiously.

The high protein content in erythrocytes allows anthocyanin pigments to have an affinity for red blood cell components. Meanwhile, antioxidants can maintain morphological stability during the staining process. Therefore, as the extract concentration increases, the anthocyanin flavonoid pigments bind well to erythrocyte proteins, producing a clear and even color (Pertiwi et al., 2022). The concentration of parijoto extract also affected the clarity of the blood smear background. Statistical tests showed that, compared to other concentrations, the 70% concentration was not significantly different from the 10% Giemsa control. It can be concluded that the 70% extract can produce a clear and clean background.

The chemical properties of anthocyanins significantly influence the staining results, which are related to pH stability, temperature, and environmental conditions. Under acidic conditions, anthocyanin pigments are more stable, producing a purplish-red color. However, at alkaline pH, anthocyanins degrade, producing a pale, unstable color. The amount of anthocyanin pigment is not the only factor contributing to the improved staining quality at a concentration of 70%. Chemical stability can also influence the staining results, allowing the anthocyanin pigment to be active and effective in coloring erythrocytes (Raihani & Wahab, 2024).

The staining results of peripheral blood smears are influenced not only by concentration and chemical stability but also by staining time, preparation method, and fixation conditions. Furthermore, the presence of artifacts or residual pigment in the background can also interfere with the contrast of red blood cells. Other factors that can affect the quality of staining with natural extracts include the extraction method, rinsing process, preparation technique, and observation. To maintain anthocyanin stability, extraction is

carried out using a maceration method using 70% anthocyanin acidified with citric acid (pH 2-3) for 72 hours (S. K. Putri et al., 2025).

The findings of this study are consistent with previous reports of anthocyanin-based natural dyes, which have shown improved coloring quality at higher pigment concentrations. Compared with Roselle and mangosteen peel extracts reported in previous studies, *Medinilla speciosa* extract at 70% concentration showed comparable erythrocyte staining clarity with relatively minimal background artifacts.

The study has several limitations, including a relatively small sample size and reliance on visual assessment performed by a single observer, which can give rise to subjectivity and potential bias. Efforts such as the use of standardized evaluation criteria are implemented to improve consistency; however, further refinement of the methodology is still needed. In addition, the morphology of leukocytes and platelets was not evaluated in the study. Future research should include more objective methods of image analysis and assessing staining performance in different types of blood cells to provide a more comprehensive evaluation.

4. Conclusions

Overall, the findings of this study indicate that *Medinilla speciosa* extract has potential as a natural dye for staining peripheral blood smears. Among the concentrations evaluated, the 70% extract showed a coloring quality most comparable to the Giemsa 10% coloring control. Statistical analysis showed that there was no significant difference between the 70% extract and the control group, although this does not imply equivalence.

Nevertheless, Giemsa tinting remains the standard tinting with more consistent and superior tinting performance. Therefore, further optimization of *Medinilla speciosa* extract, including improvements in extraction methods, staining protocols, and color stability, is required before it can be considered as an alternative for routine laboratory use. In addition, while *Medinilla speciosa* extract may offer potential advantages in terms of natural origin and environmental considerations, its broader application, including use in resource-limited settings, requires further investigation. This study provides a basis for future research focusing on standardization of concentration, staining reproducibility, and evaluation across a broader range of hematologic parameters.

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