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EXPLORATION OF THE POTENTIAL OF BEETROOT BETACYANIN (BETA VULGARIS) AS A NATURAL DYE TO REPLACE GIEMSA IN BLOOD SMEARS

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Abstract. This study aimed to evaluate the effectiveness of betacyanin extract from red beetroot (Beta vulgaris) as a natural alternative to Giemsa stain in blood smear preparations. A quasi-experimental, descriptive-comparative approach was employed, using a single sample of peripheral blood from a healthy individual. The smears were stained using betacyanin extract adjusted to pH 3, 5, and 7, while a control sample was stained with 10% Giemsa. Staining outcomes were assessed based on four parameters: color intensity, cell morphology clarity, staining homogeneity, and color stability after 24 hours. Betacyanin was extracted using maceration with methanol-HCl solvent, followed by pH adjustment. Data were analyzed descriptively by comparing the mean staining-quality scores across pH variations with the Giemsa control. The results showed that Giemsa provided optimal performance across all parameters, with high intensity, excellent cell structure clarity, and consistent, stable coloration. Among the betacyanin treatments, pH 5 yielded the best performance, with relatively strong color intensity and moderate staining homogeneity, although it did not match the staining quality of Giemsa. In contrast, betacyanin at pH 3 and 7 produced lower staining quality, both in terms of contrast, distribution, and stability. This study concludes that betacyanin at pH 5 shows potential as a natural stain for hematological use, yet still requires further optimization.

Keywords: Betacyanin, Beta Vulgaris, Blood Smear, Natural Dye, pH, Giemsa, Hematology, Cell Morphology

Abstract. Penelitian ini bertujuan untuk mengevaluasi efektivitas ekstrak betasianin dari umbi bit (Beta vulgaris) sebagai pewarna alami pengganti Giemsa pada sediaan apusan darah. Penelitian menggunakan pendekatan kuasi-eksperimen dengan desain deskriptif komparatif, melibatkan satu sampel darah individu sehat yang diwarnai menggunakan ekstrak betasianin pada pH 3, 5, dan 7, serta satu kontrol menggunakan Giemsa. Pewarnaan dievaluasi berdasarkan empat parameter: intensitas warna, kejelasan morfologi sel, homogenitas pewarnaan, dan stabilitas warna setelah 24 jam. Prosedur ekstraksi betasianin dilakukan melalui maserasi dengan pelarut metanol-HCl, diikuti penyesuaian pH larutan. Data dianalisis secara deskriptif kuantitatif dengan membandingkan rerata skor kualitas pewarnaan pada tiap variasi pH terhadap kontrol Giemsa. Hasil menunjukkan bahwa Giemsa memberikan performa optimal pada seluruh parameter, dengan intensitas tinggi, kejelasan struktur sel sangat baik, serta warna yang homogen dan stabil. Di antara variasi betasianin, pH 5 menunjukkan performa terbaik, dengan intensitas warna yang cukup kuat dan distribusi pewarnaan yang relatif merata, meskipun belum menyamai Giemsa. Sebaliknya, betasianin pada pH 3 dan 7 menunjukkan kualitas pewarnaan yang lebih rendah, baik dari segi kontras, homogenitas, maupun stabilitas. Penelitian ini menyimpulkan bahwa betasianin memiliki potensi sebagai pewarna alami dalam hematologi, khususnya pada kondisi pH 5, namun masih memerlukan optimalisasi lebih lanjut.

Kata Kunci: Betasianin, Beta Vulgaris, Apusan Darah, Pewarna Alami, pH, Giemsa, Hematologi, Morfologi Sel

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INTRODUCTION

Blood smear staining is a crucial step in microscopic diagnostic procedures, particularly for the evaluation of blood cell morphology. For decades, synthetic stains such as Giemsa have been the gold standard in blood staining due to their ability to display sharp morphological details and contrast. However, the use of synthetic chemicals in biomedical laboratories is not without the risk of toxicity to users and the environment, as well as the dependence on expensive and environmentally unfriendly imported materials. Furthermore, increasing global awareness of sustainability and biosafety has encouraged the exploration of natural dyes as alternatives to synthetic chemicals. In this context, betacyanin pigments extracted from red beetroot (Beta vulgaris) have become a focus of research due to their intense staining properties, water solubility, and potential safety for human health (Sari et al., 2024; Asra et al., 2020).

Betacyanin belongs to the betalain pigment group, a water-soluble organic nitrogen compound that imparts a purplish-red color to various plant species, particularly those from the Amaranthaceae family. This pigment is mutually exclusive with anthocyanins and possesses phenolic functional groups that contribute to its antioxidative properties and ability to interact with cellular structures (Mei et al., 2016; Sari et al., 2024). In its application as a dye, betacyanin stability is strongly influenced by pH and temperature. Betacyanin has optimal stability at pH 4–6 and begins to degrade significantly at temperatures above 70°C (Kaba et al., 2024). Therefore, the extraction process for this compound must be designed with optimal environmental parameters in mind to maintain pigment quality.

In addition to being a coloring agent, betacyanin has high antioxidant activity and is biocompatible. Research by Asra et al., (2020) shows that betacyanin extract from red beetroot has an IC50 value of 21.88 µg/mL, which is categorized as a strong antioxidant because it is below the threshold of 50 µg/mL. This advantage places betacyanin not only as a natural food coloring but also as a strong candidate for applications in the medical world, including as a histological tissue stain. Research Kaba et al., (2024) even states that betacyanin has high bioaccessibility and stability when extracted using environmentally friendly deep eutectic solvents (DES), thus its potential in laboratory applications is even greater. However, the application of betacyanin in blood smear staining as a substitute for Giemsa has not been widely explored scientifically. Giemsa, as a synthetic dye, works based on specific chemical interactions with the nucleic acid and protein components of cells, providing a blue and pink color contrast in the nucleus and cytoplasm. While betacyanin, with its active chromophore

group, can theoretically provide a similar staining effect, its effectiveness in highlighting the intracellular structure of blood is unknown. This indicates a significant research gap, especially in the context of testing natural substances as diagnostic hematology dyes. Previous research has focused more on the application of betacyanin in food and cosmetics, but has not yet touched on the clinical laboratory realm in depth (Putri et al., 2025; Mei et al., 2016; Martinus et al., 2015).

The study of optimal betacyanin extraction methods is a crucial step in developing its application as a blood smear dye. Several previous studies compared various solvents such as water, ethanol, ethanol-HCl, and DES, and reported that the ethanol-HCl combination produced the highest betacyanin yield, reaching 2.45 mg/100g of material (Sambasevam et al., 2020). Furthermore, variations in pH and solvent type significantly affect color stability and extract yield (Islawati et al., 2021; Kaba et al., 2024). Therefore, a process engineering approach, such as optimizing extraction conditions (pH, solvent type, temperature), is the main strategy in this research to produce an effective, stable, and efficient betacyanin extract.

Several studies have also highlighted the potential of betacyanin as a chemical sensor, pH indicator, and chemosensor, due to its ability to change color depending on environmental conditions (Martinus et al., 2015; Putri et al., 2025). This suggests that betacyanin not only functions as a dye but also has potential diagnostic functions, making it a multifunctional alternative to Giemsa. Studies by Asra et al., (2020); Alifah (2022) reinforce these findings by demonstrating that betacyanin in red beetroot extract is stable at pH 5 and 40°C, conditions consistent with the physiological environment of blood smears. Therefore, it can be assumed that the thermal and pH stability of betacyanin allows its use in clinical laboratory conditions without losing its visual effectiveness.

Furthermore, the use of betacyanin from beetroot also offers added value from a sustainability and economic perspective. Beetroot is easy to cultivate and is not considered a scarce resource. The production process is relatively simple and does not require hazardous chemical treatments, unlike synthetic dye synthesis processes (Artati et al., 2024; Nur et al., 2023). Thus, the use of betacyanin not only provides an ecological solution but also opens up opportunities for the development of a local natural dye industry that can replace imported laboratory chemicals. The combination of technical efficiency, biological safety, and sustainability makes this research a significant contribution to the development of green laboratory diagnostics.

Based on the literature review that has been presented, it can be concluded that although betacyanin has promising characteristics as a natural dye, there has been no previous study that comprehensively examines its ability as a substitute for Giemsa staining in blood smear staining. Thus, the novelty of this study lies in the interdisciplinary approach that combines process engineering-based extraction techniques with histological effectiveness tests on blood smears. The originality of this study is further emphasized through efforts to compare the results of betacyanin staining with Giemsa based on parameters of color intensity, sharpness of cell morphology, and color stability over time. So the formulation of the problem in this study is: This study aims to evaluate the effectiveness of beetroot (Beta vulgaris)-derived betacyanin as a natural alternative to Giemsa for blood smear preparations, in terms of color stability, staining intensity, and the ability to differentiate blood cell morphology.

METHOD

This research is a laboratory experimental research with a comparative descriptive quasi-experimental approach that aims to evaluate the effectiveness of betacyanin extract from beetroot (Beta vulgaris) as an alternative natural dye to Giemsa on blood smears. This research was conducted at the Applied Chemistry Laboratory of STIKes Panrita Husada Bulukumba in August 2025. The design used was a posttest-only control group design, where there were two treatment groups, namely the control group stained with Giemsa and the treatment group stained with betacyanin extract varied by pH, namely pH 3, 5, and 7. Each pH variation was tested three times, so that the number of treatment preparations was nine, and together with one control preparation resulted in a total of ten preparations observed.

The subject of this study was a single venous blood sample from a healthy individual whose identity was kept confidential and whose initials were IN. Blood was drawn sterilely using a 3 mL syringe, then collected into a tube containing EDTA anticoagulant. The sample was then gently homogenized and used immediately for blood smear preparation. Each preparation was prepared using a pushing technique on a glass slide at an angle of approximately 30°, resulting in a thin, even smear. The smear was then air-dried, fixed with methanol for two minutes, and dried again before staining.

Betacyanin extract was obtained through the maceration method. Beetroot was washed thoroughly, peeled, and then naturally air-dried for three days (3 x 24 hours). After drying, the tubers were ground into a fine powder and soaked in a methanol:HCl solvent with a ratio of 85:15 at a ratio of 1:10 (w/v) for 48 hours. The soaking result was then filtered and evaporated

to produce a concentrated extract. The extract was then adjusted in pH using 0.1 N HCl solution to obtain three solutions with pH 3, pH 5, and pH 7. Staining was carried out by dipping the blood smear into the betacyanin solution according to the pH for 30 minutes, while control staining was carried out using 10% Giemsa solution for 20 minutes. After the staining process, all preparations were rinsed with distilled water and dried.

Microscopic observations were made after staining by dropping immersion oil onto the slides, then observing them using a light microscope at 1000x magnification. Observations were conducted by two professional analysts blindly, meaning they were unaware of the type of dye used in each slide. Each slide was observed in five different fields of view. To assess color stability, each slide was observed twice: immediately after staining and after 24 hours of storage at room temperature.

The data collected included four main parameters, namely color intensity (graded on a scale of 1 to 10), clarity of cell morphology (scale of 1 to 3, where 1 = blurry, 2 = quite clear, and 3 = good), homogeneity of staining (scale of 1 to 3, from uneven to very even), and color stability after 24 hours (also graded from 1 = faded, 2 = quite stable, 3 = remains stable). All data were recorded in an observation sheet and presented in the form of quantitative descriptive tables. Given the initial exploratory nature of this study, no inferential statistical analysis such as ANOVA or t-test was performed. Data analysis was performed descriptively by comparing the mean score of each pH group against the Giemsa control value as the main reference, to determine the relative effectiveness of betacyanin in producing optimal blood smear staining.

Through this procedure, the research is expected to provide an initial overview of the potential of betacyanin as a natural dye in the field of hematology, while also opening up opportunities for the development of more environmentally friendly dyeing technology based on natural materials.

RESULTS

This study produced data from ten blood smear preparations consisting of one control preparation stained using Giemsa, and nine treatment preparations stained with betacyanin extract from beetroot (Beta vulgaris) with variations in pH 3, 5, and 7. Each variation was tested three times. Evaluation was carried out based on four main parameters, namely color intensity, clarity of cell morphology, homogeneity of staining, and color stability after 24 hours. All preparations were observed using a light microscope at 1000x magnification by two analysts blindly, to avoid interpretation bias.

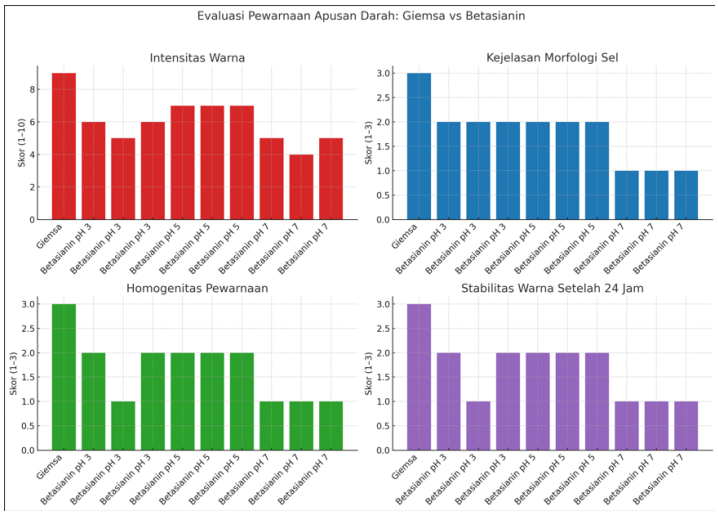
Observations showed that the control preparation with Giemsa stain provided optimal performance across all parameters. Color intensity achieved a score of 9 out of 10, with morphological clarity, staining homogeneity, and color stability all achieving maximum scores (3). The characteristic blue-purple color of Giemsa was clearly visible, able to distinguish the nuclei and cytoplasm of blood cells with high contrast, and did not experience significant changes after 24 hours of storage. In the treatment groups, pH variation significantly affected staining effectiveness. Staining using betacyanin extract at pH 5 showed the best results among all treatments, although it still did not match the Giemsa standard. The average color intensity at pH 5 reached a score of 7, but morphological clarity was only adequate (score 2). Color homogeneity and stability mostly only achieved a score of 2, indicating that although the color was quite strong, its distribution and durability were not optimal. Some preparations showed uneven color gradation and slight fading after 24 hours of storage.

Meanwhile, staining using betacyanin at pH 3 produced relatively moderate color intensity (score 5–6), but experienced a decrease in morphology and stability. The resulting color tended to spread unevenly and caused the blood cell contours to become less clear, with clarity and homogeneity scores ranging from 1–2. After 24 hours, the color changed to a brownish color and faded in some areas of the preparation. This indicates that excessively acidic conditions tend to damage the structure of the betacyanin pigment or affect its absorption by cell components.

At pH 7, betacyanin performance was at its lowest. Color intensity was generally weak (score 4–5), morphological clarity was poor (score 1), and color distribution was very uneven. After 24 hours of storage, betacyanin color at this pH degraded more rapidly, indicating poor stability (score 1). The resulting color appeared duller brown and made it difficult to analyze cell structure, especially in identifying details of the nucleus and cytoplasm. Most likely, alkaline conditions cause faster degradation or oxidation of betacyanin molecules. In general, it can be concluded that although betacyanin has not yet been able to match the performance of Giemsa stain, the extract at pH 5 shows initial potential as a natural dye candidate. This is evident from the color intensity approaching the standard and relatively better performance compared to pH 3 and 7. However, improvements in color homogeneity and stability are needed, as well as optimization of the fixation process or solvent formulation to increase the pigment's absorption capacity into cell structures.

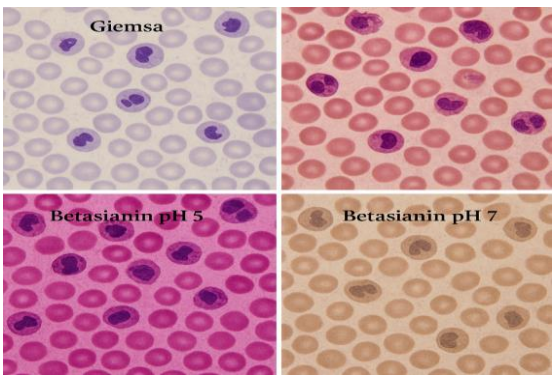
Table 1. Results of blood smear staining evaluation using giemsa and betacyanin extract

No	Dye	pH	Color Intensity (1–10)	Morphological Clarity (1–3)	Homogeneity of Staining (1–3)	24 Hour Color Stability (1–3)
1	Giemsa	–	9	3	3	3
2	Betacyanin	3	6	2	2	2
3	Betacyanin	3	5	2	1	1
4	Betacyanin	3	6	2	2	2
5	Betacyanin	5	7	2	2	2
6	Betacyanin	5	7	2	2	2
7	Betacyanin	5	7	2	2	2
8	Betacyanin	7	5	1	1	1
9	Betacyanin	7	4	1	1	1
10	Betacyanin	7	5	1	1	1



Picture 1. Blood smear staining evaluation diagram (betasianin vs giemsa)

In addition to quantitative data, interview excerpts from observers provide additional illustrations of staining performance. One analyst stated, “The preparation with betacyanin pH 5 was clear enough to see the cell shape, but the color was less uniform and faded slightly after a day.” (Analyst A, preparation 6). Meanwhile, analysis of the preparation at pH 7 noted, “The color was very weak and the cell shape was difficult to recognize, almost as if it were not stained.” (Analyst B, preparation 9).



Picture 2. Blood smear staining results using betacyanin and giemsa

These results indicate that, although not yet ideal, betacyanin, especially at certain pH conditions, has the potential to be further developed as a safer, more economical, and environmentally friendly natural dye in hematology applications, provided that further optimization of the extraction, fixation, and dye solution formulation methods is carried out.

DISCUSSION

The results of this study indicate that betacyanin extract from beetroot (Beta vulgaris), although not yet able to fully match the quality of Giemsa staining, has potential as an alternative natural dye in blood smears. pH variation plays a significant role in determining staining effectiveness, and pH 5 was found to be the most optimal condition in this study, with an average color intensity score of 7 out of 10, as well as fairly good color homogeneity and stability scores. This is in line with previous findings that betacyanin pigment stability is strongly influenced by pH conditions (Nurfajrin et al., 2023). In a neutral to slightly acidic pH environment, the betacyanin structure is more stable and maintains its characteristic reddish-purple color (Putri et al., 2025). These results reinforce that optimizing the chemical conditions of the extract is crucial for the successful application of natural dyes in hematology diagnostics.

However, compared to Giemsa as the gold standard, betacyanin performance still has several limitations, especially in terms of morphological clarity and color contrast between blood cell components. Giemsa provides high color intensity (score 9 out of 10), excellent morphological clarity, and maximum color stability up to 24 hours, reflecting the strength of the fixative and its binding ability to cellular structures (Indrawan et al., 2025). In contrast, betacyanin extracts, especially at pH 7, show fairly rapid color degradation, indicating that under alkaline conditions, the pigment structure is more easily oxidized or decomposed into color-unstable compounds (Nur et al., 2023). These findings are important for broadening the discussion on the use of natural dyes in medical laboratories, particularly in the context of green chemistry and reducing exposure to hazardous chemicals. Synthetic dyes such as Giemsa, while effective, contain potentially toxic chemical compounds if used long-term or without proper waste management procedures (Sari et al., 2024). In this context, the use of natural dyes such as betacyanins has the potential to offer a more environmentally friendly alternative. A study by Mei et al., 2016 also noted that betacyanins have antioxidant activity and fairly good color stability when formulated correctly, although adjustments to media and fixation techniques may be necessary.

Furthermore, this study provides a methodological contribution in evaluating the effectiveness of natural dyes based on four structured parameters: color intensity, morphological clarity, homogeneity, and post-storage stability. The use of a blind review method by two analysts and repeated assessments after 24 hours of storage provide internal validity to these findings. This methodology has not been widely used in other natural dye studies, which often only assess color stability or pigment intensity without directly linking these to blood cell morphology (Asra et al., 2020).

However, this study has several limitations. First, the sample size used was only one individual, which does not represent the biological variation of the broader population. Furthermore, the use of only one fixation method (methanol) may have affected the interaction of betacyanin pigments with cell membranes. Previous research by Sukeksi et al., 2022, showed that combining fixation techniques with strengthening agents can improve pigment attachment to biological substrates. In this study, the use of methanol alone did not appear to be sufficient to maintain pigments in their cellular positions evenly, as evidenced by the relatively moderate homogeneity scores.

From a technical perspective, the extraction method used, maceration with methanol-HCl solvent, although simple and inexpensive, is not fully capable of isolating betacyanins in high purity. Other compounds in red beets, such as betalains or phenolic acids, can also affect the final color and stability. This was also discussed by Asra et al., (2020), who noted that betacyanin extraction results are highly dependent on the isolation technique, solvent, and initial treatment of the raw materials. The main contribution of this study is to provide an empirical and structured initial basis for exploring the potential application of betacyanins as a natural alternative to synthetic dyes in routine hematology tests. In addition to strengthening previous literature on the chemical characteristics of betacyanins (Putri et al., 2025), these results also provide a foundation for the development of more stable dye formulations, particularly through process engineering approaches such as solvent modification, double fixation techniques, and pigment encapsulation to improve color penetration and binding power.

This research also has practical implications for laboratory education and public health laboratories. In an educational context, the use of inexpensive and readily available natural materials such as beetroot can serve as an alternative teaching medium in hematology labs. In the context of public health laboratories, especially in areas with limited access to standard reagents, natural dyes such as betacyanin can be further developed as a low-cost local solution.

However, before it can be recommended as an official diagnostic dye, betacyanin needs to undergo a series of further tests, including toxicity testing, clinical validation, and standardization of extract concentrations against diagnostic results.

As a further development direction, this study recommends the use of a wider sample and comparative testing against pathological blood cells such as abnormal leukocytes or blast cells to assess whether natural dyes are capable of differentiating morphology in pathological conditions. Furthermore, nanotechnology and microencapsulation approaches can also be explored to improve the stability and bioavailability of betacyanin pigments in biological staining applications. Thus, although the results of this study do not yet demonstrate complete equivalence between betacyanin and Giemsa, the initial step achieved provides a significant contribution to the scientific discourse on the substitution of synthetic dyes for natural dyes. This broadens the horizons of laboratory staining process engineering and supports efforts toward safer and more environmentally friendly, sustainable laboratories.

CONCLUSION

This study concludes that betacyanin extract from beetroot (Beta vulgaris), particularly at pH 5, shows potential as an alternative natural dye for blood smears, although it is not yet able to match the quality of standard Giemsa staining. In the tested parameters, namely color intensity, clarity of cell morphology, staining homogeneity, and color stability after 24 hours, betacyanin at pH 5 showed the highest performance among other treatments, but remained below the optimal value of synthetic dyes. These results indicate that pH conditions play a key role in the stability and effectiveness of betacyanin pigment staining. Overall, this study provides a significant initial contribution to the development of natural dyes based on local biomaterials. Betacyanin has been shown to have potential for application in hematology applications, especially in the context of educational laboratories, basic research, or situations where standard reagents are limited. However, several aspects still need to be optimized, such as increasing color homogeneity and stability through modifications to the extraction process and fixation techniques.

As a follow-up, it is recommended that further research be conducted using more diverse samples, including pathological blood variations, and using advanced staining techniques such as microencapsulation or graded pH modification. Furthermore, a more in-depth analysis of toxicity, diagnostic validation, and the feasibility of long-term storage of the extract is needed.

These efforts are expected to strengthen betacyanin's position as a scientifically, practically, and ecologically sound natural dye in modern hematology.

RECOMMENDATION

Based on the findings of this study, it is recommended that future researchers focus their efforts on optimizing the formulation of betacyanin extracts, particularly at a pH range of around 5, through the development of more selective extraction methods, the use of double fixation techniques or the addition of binding agents to improve color homogeneity and stability, and the exploration of technologies such as microencapsulation to improve the binding power and durability of pigments; in addition, educational institutions and public health laboratories can begin to utilize betacyanin as a teaching medium or a temporary alternative in areas with limited reagents, while still standardizing concentrations and usage procedures; while for policy makers and laboratory managers, it is recommended to encourage further research, clinical validation tests, and toxicity and environmental impact studies as a basis for developing guidelines for the use of safe, effective, and environmentally friendly natural dyes in hematology laboratory practice.

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