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Dragon Fruit (Hylocereus polyrhizus) Pulp Extract as an Alternative Natural Dye of Eosin in Staining Hematological Studies

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Abstract

Natural dyes in the histological staining process have been widely used in the field of diagnostics. This study utilized *Hylocereus polyrhizus* pulp as a potential alternative eosin stain for peripheral blood staining. Factors considered include the ratio of the solution and the staining time of *H. polyrhizus* pulp. Acquiring pure extract of the *H. polyrhizus* was done by mashing and physically extracting the *H. polyrhizus* pulp utilizing sterile cloth, gauze pad, and filter paper. The pure extract was diluted three times with 100 mL of distilled water, acquiring different concentrations of 30 mL pure extract (23.1%), 20 mL (16.7%), and 10 mL (9.10%), respectively. The blood smears that were prepared were dipped into a series of solutions from 1, 2, and 3, which contain methanol for fixation, then varying concentrations of the natural stain. The smears were air-dried and read under an oil immersion objective on a compound light microscope. The research aimed to determine if the extracted dye from *H. polarizes* can stain the blood cells and identify the potential solution adjusted in different concentrations for an optimal yield of the morphological details of the blood smear. Our findings imply that a concentration of 9.10% stains leukocytes and causes them to stain pale pink. Leukocyte staining at 16.7% concentration is described as lighter, almost pale. The 23.1% showed good staining potential to white blood cells and made the red blood cells appear light red to pink, revealing their visible cytoplasm and biconcave disc without changing their size or shape.

Keywords: alternative stain, diagnostics, histological staining, morphological details, peripheral blood staining

Introduction

H. polyrhizus, more commonly known as “Dragon Fruit " has grown to have a reputation because of its possible health advantages (Ramil et al., 2021). These fruits are ubiquitous and can be found in most of the local markets. Aside from its health benefits, *H. polyrhizus* has staining characteristics due to the chemical called “anthocyanins," which can be found naturally in the fruit. Color yield in Hylocereus spp. (Dragon fruit) came from the accumulation of specific chemicals found in its pulp, the anthocyanins, particularly the *betalanins*. Some studies show the presence of anthocyanins in dragon fruit and the potential of this fruit to yield color. However, the coloring characteristics of these chemicals, forming color, have not yet been explored (Fan et al., 2020). Anthocyanins are one of the commonly used natural colorants for beverages, confectionery, fruit preparation, and ice cream. The strong antioxidant activity of these anthocyanins has attracted great interest in the food industry. The instability of anthocyanins through factors such as light, pH, temperature, and oxygen has caused problems during the processing and storage of these compounds (Khoo et al., 2017).

The number one most common laboratory test performed in the Clinical Laboratory is the complete blood count (CBC). This test evaluates the different cellular components in the blood, which aids in the presumptive diagnosis of various diseases. Staining blood films for hematological studies has been going on since 1878. Dr. Paul Erlich, having a background in developing dyes to stain animal tissues, classified the types of dyes depending on their pH, whether it is basic, acid, or neutral, having variations on the effect on staining different components of blood cells such as granules, nucleic acid, and cytoplasm which lead to the advancement in hematology (*Paul Ehrlich – Biographical - NobelPrize.org*, n.d.). In recent times, the practice of staining has been improving the evaluation and differentiation of the different types of blood cells, particularly the leukocytes. Manual techniques in the hematology laboratory serve as a quality assurance protocol to confirm the results of uncalibrated or questionable results yielded by the machines.

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The study Dragon fruit (*H. polyrhizus*) pulp extract as an alternative natural fye for staining hematological studies rind extract poses a safer alternative for staining in hematological studies. Due to the rising expense of synthetic dyes, researchers sought to develop a natural stain that would be cheaper and safer and produce results that were comparable to those of better synthetic dyes. The study will be able to identify the staining characteristics of Dragon fruit (*H. polyrhizus*) on hematological samples as an alternative to eosin stain for hematological studies. Synthetic dyes are widely used in present applications for laboratory procedures and methods; natural dyes are utilized as an alternative option for safer and outstanding visualization of the morphological characteristics of peripheral blood smears. Staining of histological samples is crucial in the tissue processing procedure, particularly in the field of pathology. In the clinical laboratory, there are various stains available in the market. The available stains are mostly priced highly.

The study aimed to use the phenolic compound property "anthocyanins" present on the pulp of Dragon fruit (*H. polyrhizus*) exhibited more profoundly in its extract as an alternative natural dye for staining hematological studies as a safer alternative stain.

The general objective of this research is to determine the staining capacity of dragon fruit (*H. polyrhizus*) pulp extract as an alternative for Eosin staining in hematological studies. Due to the rise in the cost of synthetic dyes, the researchers aimed to provide a natural stain where it is safer and more effective than synthetic dyes. The study was able to identify the potential of staining characteristics of Dragon fruit (*H. polyrhizus*) on blood smear samples as an alternative stain for hematological studies. Briefly describe the optimal concentration of the solute-to-solvent ratio adjusted in different concentrations for an optimal yield of the morphological details of the blood smear.

The findings of this study redounded the benefit of society, in particular to the field of histology. Histological staining is a process wherein the demonstration of cell and tissue is highlighted for

identification and determination of morphology and structure with the assistance of coloring - the stain (Shanthi, n.d.).

The significance of the study of Dragon fruit pulp extract is intended to determine the quality of this natural dye used to stain and give color to hematological studies. This indicates that one of many natural dyes has antioxidant properties, with an addition that extracts produce natural colors and are less harmful. The study will highlight that the naturality of plant extracts is an advantage in identifying different blood cells and morphological studies of blood smears.

The individuals randomly chosen for blood extraction have not undergone multiple screening tests for blood disorders that manifest abnormal morphologies of blood cells (RBCs, platelets, and WBCs). This is due to the lack of the researcher's capabilities to do qualitative or quantitative tests that will show modifications of blood cell morphologic disorders due to the cost, time, and expertise of the researchers. The research utilized the material of dragon fruit that has a seasonal harvest. The pericarp of the fruit was washed with running tap water and was removed and discarded. The pulp of the dragon fruit was used as the main source of the stain extract as a substitute for the eosin stain. The pulp, including the seeds, was mixed and was not separated intentionally. The researchers had not subjected the extracting process to varying temperatures for comparison. The mashing of the dragon fruit's pulp was done in a steel pan and a steel masher provided by the laboratory of the Medical Technology department. The researcher has not been able to run the experiment with varying temperatures and extracting reagents that will determine a different course of the research. The researchers chose hematological samples since it is difficult to acquire histological samples, and the processing of these histological samples is time-consuming and labor-intensive due to the lack of automated machines. Hematological samples are much easier to acquire since the researchers have already undergone training in phlebotomy, and the resources are already available since it is a requirement in their enrolled program, Medical Technology or Medical Laboratory Science.

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Materials and Methods

Research Design

The study was conducted in the Medical Technology Laboratory at Misamis University, where multiple experiments were done. Extraction, mashing, and dilution of *H. polyrhizus* were done to obtain pigmenting properties from its pulp to be applied in a smear hematological study. Experimental research establishes the existence of a cause-and-effect relationship between two variables. Through an experimental design, the researcher would be able to describe the potential of the pulp stain extract as an alternative for eosin stain through a constructed rate of observation based on the cytoplasm, shape, and size of the blood cells in a smear on which concentration gives off better staining ability without altering the integrity of the cell in the peripheral blood.

There were five volunteer patients served as participants of the study where 2-5 ml of blood samples were collected, depending on the anticoagulant to blood ratio of the EDTA (Ethylenediaminetetraacetic acid) or Citrated tube, each with their full consent. Checking for a hemolyzed sample before being utilized in the experiment was done for every blood specimen extracted from the patients. Moreover, the age, gender, patients' history, nationality, and status were not taken into consideration. Thus, a random collection of blood convenient for the researchers was prioritized.

Ethical Considerations

Considering the blood sample donor identity, confidentiality was practiced since the researchers are dealing with blood, which can contain infectious and communicable diseases, in fulfillment of the OSHA standards (Chao, 2003). The practice of patient confidentiality has been stated in the Board of Medical Technology Code of Ethics (Rabor, 2016).

Sample collection

The researchers utilized a variety of related studies to back up the observed results acquired during the duration of the experiment. The granules of the leukocytes yielded a color from the stain utilizing the *H. polyrhizus* extract with a pH of 4.0 (*Figure 13*). This is backed up by the study of Shanthi (n.d.), which states that the optimal and most common pH used in staining with Romanowsky stains is 6.8. It shows the acidic component of the leukocytes, specifically the granules. The components of Eosin are enhanced at the expense of Azure B if the pH is acidic; however, if the pH of the stain is on the basic side of the scale, which makes it more alkaline, the Azure components are enhanced at the expense of the Eosin.

The morphological characteristics of the component of the blood, which was stained with the control stain (Wright's Stain), were also compared to the stain with *H. polyrhizus* extract. Researchers observed some of the leukocytes containing unstained nucleated lobes. The granules of the granulocytes yielded a color using the *H. polyrhizus* extract. It was observed that these cellular components are leukocytes, specifically granulocytes. The morphological structures, particularly the hallmarks of the granulocytes, which are the granules contained in the cytoplasm, yielded a pinkish-red color since the *H. polyrhizus* extract stain is acidic (Otto et al., 2019).

Preparation of Peripheral Blood Smear

Necessary materials for the extraction (glass slides, preferably frosted slides, a spreader slide, and Pasteur pipettes) and collection of blood samples were prepared. Pre-extracted 2mL of EDTA-anticoagulated blood from the co-members was also used. Two-slide or Wedge method, which is the simplest and the most popular method for the preparation of peripheral blood smear (Sadang, 2015) was used in the research.

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A a drop of blood on the far end near the frosted part of a clean glass slide. After the drop, the researchers positioned the spreader slide before the drop of blood at an angle of about 30-45 degrees, allowing the blood to spread evenly on the vertex. After that, the spreader slide was smoothly and rapidly over the length of the slide at a constant angle, making sure to keep the spreader slide firm on the smear. Proceed to air-dry after spreading.

Collection and Extraction of H. polyrhizus extract

One to two kilograms of *H. polyrhizus* with the price range of 100-200 pesos per kilogram was used in each test trial. *H. polyrhizus* varies depending on where these fruits were bought or the season of the fruit. The fruit was subjected to experimental processing to satisfy the said objectives. The extraction of dye from the fruit of *H. polyrhizus was* conducted at the Misamis University Medical Technology Laboratory and Natural Science Building (NatSci) department.

The Dragon fruit was peeled off, and the pericarp was discarded. The pulp of the fruit was specifically used and mashed in a steel pan. The mashed pulp of fruit was filtered in a 3-step filtration process. First, the mashed pulp was filtered using a sterile cloth. Next, the filtered mashed pulp was refiltered using a gauze pad to carefully remove the excess seeds and tiny gel-like pulp that were unable to be filtered by the cloth in the first step. Lastly, the filtered juice from the gauze pad was finally filtered using filter paper to ensure that no particulate gel-like substances passed through the final step of the extraction. The extracted juice from the 3-step process was used as the solute to stain the peripheral blood samples (Figure 1) (Martín et al., 2017).

Staining of the Peripheral Blood Smear

The extracted fluid from the pulp was divided into three volumes (30 mL, 20 mL, 10 mL) diluted with 100 ml water that would yield 23.1% v/v, 16.7% v/v, and 9.10% v/v concentrations, respectively.

The prepared blood smear was fixed using methanol, then stained using the fruit extract (23.1%, 16.7%, 9.10%) for five minutes, dipped in methylene blue, washed with running tap water, and then dried, as shown in Figure 2. This process was repeated with different prepared concentrations from the fruit extract to determine the standard percentage of volume needed to stain blood smears. The dried blood smears stained with the fruit extract were read and interpreted in an oil immersion objective under a compound light microscope.

Histological Preparation and Microscopic Viewing

The researcher prepared three slides in each trial that corresponded to 23.1%, 16.7%, and 9.10% concentrations. These slides were read and interpreted using an oil immersion objective under a compound light microscope, differentiating the capability of *H. polyrhizus* pulp extract to determine which of these varying concentrations yielded a better staining outcome. The researchers considered the color, size, and shape of the different blood cells (Otto et al., 2019).

Results and Discussion

Figure 1. A. shows that 23.1% stained the granules and cytoplasm of the leukocytes darker pink, making it more visible; however, the type of leukocyte identified was partially distinguishable. There were no alterations in the size of the leukocytes in contrast to the control. The red blood cells under the 23.1% stain appeared light red - pale pink, showing a visible cytoplasm and biconcave disc with no alteration of size and shape. In the field, the platelets remained unstained—figure 1. B shows a microscopic field of a Romanowsky-stained blood film. The

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differentiation of leukocytes is evident and can be differentiated easily by the medical technologist.

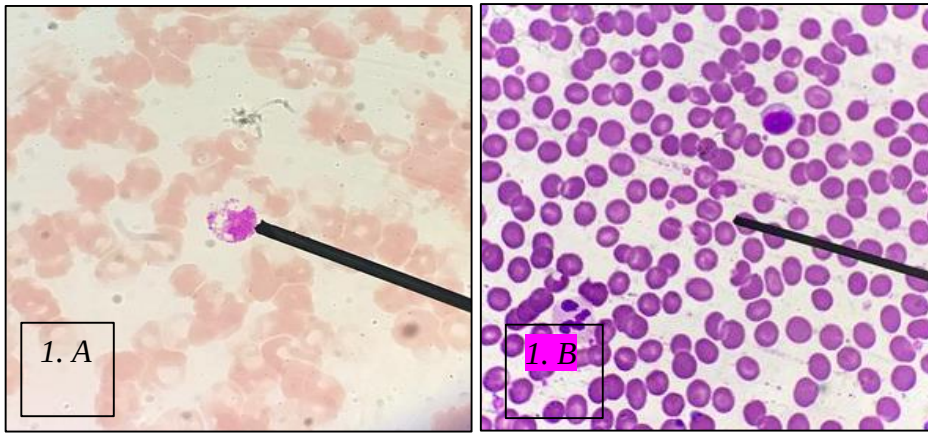

Figure 1. A, A field of WBC and RBC appearance in 23.1% stain extract 1000x objective in the trial. Figure 1. B, A field of WBC and RBC appearance in Romanowsky stain (control).

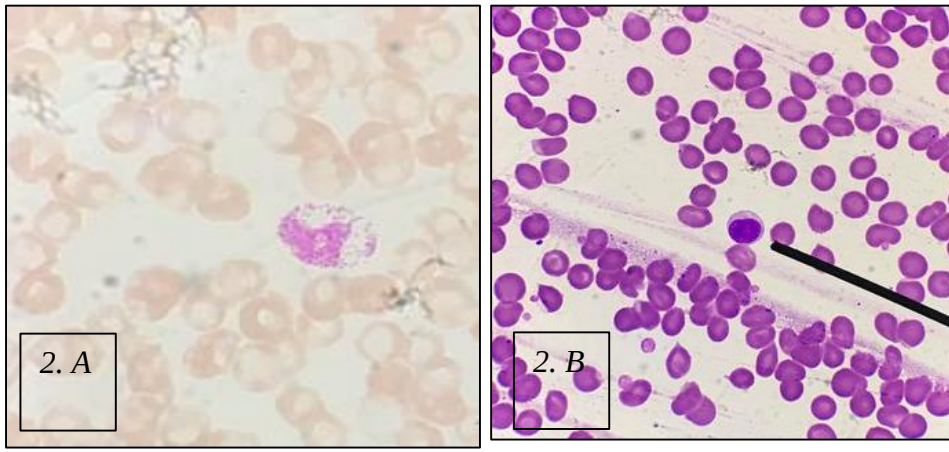

Figure 2. A, A field of WBC and RBC appearance in 16.7% stain extract 1000x objective in trial 1. Figure 2.B., A field of WBC and RBC appearance in Romanowsky stain (control).

Figure 2. A shows the 16.7% stain concentration where the granules and cytoplasm of the leukocytes appeared, with pinkish granules paler than 23.1%. There were no alterations in the size of the leukocytes; however, the type of leukocytes was hardly distinguishable. The red blood cells' cytoplasm, size, and shape remained normal. However, the color was paler and still can be identified. The platelets remained unstained. Figure 2. B shows a microscopic field of Romanowsky-stained blood films. The differentiation of leukocytes is evident and can be differentiated easily by the medical technologist.

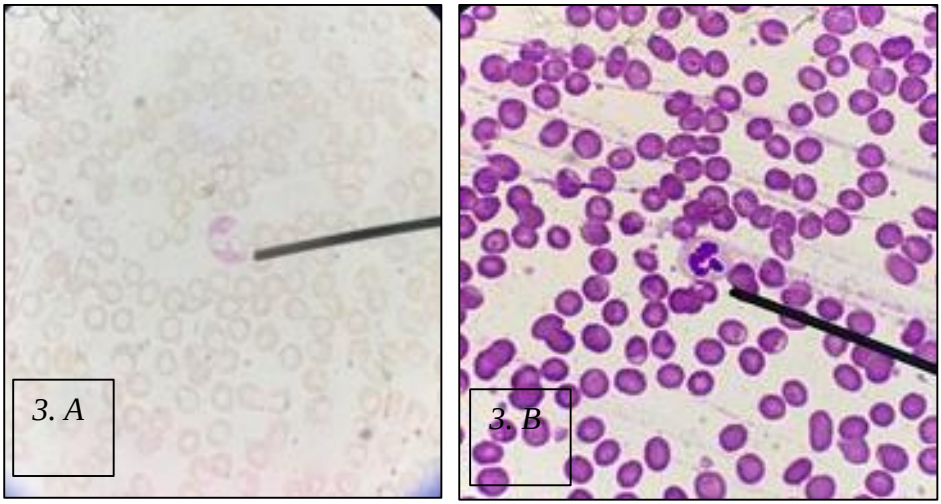

Figure 3. A, A field of WBC and RBC appearance in 9.10% stain extract at 1000x objective in trial 1. Figure 3. B, A field of WBC and RBC appearance in Romanowsky stain (control).

Figure 3. A demonstrates cells using a 9.10% concentration of stain. The appearance of the leukocytes was reported to be pale to unstained. The granules of the WBCs manifested a lighter pink in comparison to the higher concentrations. The RBCs are normal in size, and the concavity of the RBCs is still seen, yet the cytoplasm is poorly stained (Otto et al., 2019). No platelets were seen in the field—figure 3.

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B shows a microscopic field of Romanowsky-stained blood films. The differentiation of leukocytes is evident and can be differentiated easily by the medical technologist.

Trials 2 and 3 show the same characteristics that manifested in trial 1. The granules, cytoplasm, shape, size, and color in each concentration show consistency based on the results of WBC, RBC, and platelets.

Table 1. Different volumes and concentrations of the pulp extract of *Hylocereus pol*arize with its solvent.

Pulp Extract Of H. polyrhizus	Solvent (Distilled Water)	Concentration (V/V%)
30 mL	100 mL	23.1%
20 mL	100 mL	16.7%
10 mL	100 mL	9.10%

Results indicated that, 30 mL volume that yields 23.1% concentration stains better than 20 mL (19.7%) and 10 mL (9.10%). In research from Harivainda et al. (2008), the use of distilled water as solvent yielded a comparable high content of betacyanin to its juice counterpart, resulting in resuspended pigments having high pigment retention and was stable for up to 7 days as a natural colorant. The use of a higher concentration of stain extract, which contains higher anthocyanins, explained why 23.1% yielded better staining capabilities, which gave off a clearer view of WBCs, RBCs, and platelets at 1000x objective.

Table 2. The normal appearances of RBC are based on Romanowsky stains.

	Normal
Cytoplasm	Visibly stained with no morphological alterations
Shape	Normal Biconcave disc
Size	7-8 um (normal)
Color	Red-or pink

Table 2 shows the summary of the normal appearances of Red Blood Cells using the Romanowsky stain (Otto et al., 2019)**.** The table was used to evaluate the staining effectiveness of the *H. polyrhizus* pulp extract to RBCs.

Table 3. The normal appearances of platelets are based on Romanowsky stains.

	Normal
Cytoplasm	Visibly stained with no morphological alterations.
Size	2-4 um (normal)
Color	Remained unstained since Methylene blue stains platelet to light blue colored to colorless red-violet granules.

Table 3 shows the summary of the normal appearances of platelets using the Romanowsky stain (Otto et al., 2019)**.** The table was used to evaluate the staining effectiveness of the *H. polyrhizus* pulp extract to platelets.

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Table 4. The normal appearances of white blood cells are based on Romanowsky stains.

	GENERAL MORPHOLOGY
Cytoplasm	Visibly Stained with no morphological alterations.
Neutrophils	●All cytoplasm is visible with a pale purplish-pink color. ●All lobes are visible with deep blue-violet color. ●All granules are visible with a light pink or bluish-purple color.
Eosinophils	●All cytoplasm is visible with a colorless or light blue color. ● All lobes are visible with deep blue-violet color. ●All granules are visible with orange to pink color.
Basophils	●All the granules are visible with deep blue-violet color.
Monocyte	●All cytoplasm is visible with a pale gray-blue color. ●All lobes are visible with deep blue-violet color.
Lymphocyte	●All cytoplasm is visible with a light blue color. ●All lobes are visible with deep blue-violet color.

Table 4 shows the summary of the normal appearances of white blood cells using the Romanowsky stain (Otto et al., 2019)**.** The table was used to evaluate the staining effectiveness of the *H. polyrhizus* pulp extract to WBCs.

Conclusion and Recommendations

With the natural staining property present in *H. polyrhizus or* dragon fruit, it is observable that it can exhibit its staining capacity when exposed to clothes or when eaten. Hence, when applied to hematological studies, it is concluded that it has the potential to be utilized as an alternative for solution two or the Eosin Y in peripheral blood smear, emphasizing the cytoplasm in different tissue samples where granules are identified explicitly. 23.1% showed better staining of the cytoplasm and granules of the cells. The acidic nature of Eosin Y causes it to stain the cytoplasm and other fundamental cell components pink and orange, making it a good counterstain for methylene blue.

Hematological studies are easily drawn in research cases because, when compared to histological studies, they do not require much time, labor, or the availability of automated machines. In this study, for future researchers, it is recommended to further adjust the staining time, which is in comparison between varying times in staining the smear may yield a better staining outcome. The study was specific to 30 mL, 20 mL, and 10 mL volume of pure extract diluted with distilled water. For future studies, it is recommended to increase the volume of pure extract diluted with a solvent to yield a higher concentration of solution since 23.1% showed better staining than 16.7% and 9.10%. For future research, the types of extracting reagents or solvents are highly recommended for comparison on which solvent yields a better stain on peripheral blood smear. In addition, future researchers may venture to different methods, such as drying the pulp or maceration used to examine which method best extracts stain as an alternative stain for eosin Y. Since distilled water is only utilized, the researchers of this study suggest the usage of other options of different extracting reagents to be used as contrasting reagents because its staining capacity does not satisfy the standard Romanowsky stain due to the reason that the staining capacity of the fruit depends solely on the pulp extract. Another factor to be considered is the temperature when processing the pulp because it is not covered in the process of this study.

Considering all of these factors, further development and enhancement of this study by future researchers to develop a more efficient stain.

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