

FRUIT PEEL EXTRACT OF *Sygyzium cumini* AS AN ALTERNATIVE STAINING FOR PERIPHERAL BLOOD SMEAR PREPARATIONS

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ABSTRACT

Peripheral Blood Smear is preparations of human blood cells made by smear method and through a staining process in hematological examination to see the elements of peripheral blood cells, namely erythrocytes, leukocytes and platelets. The result of the Peripheral Blood Smear examination is the quality of the staining. This study was conducted with the aim of observing blood cells in blood smear preparations using aqueous extract from the skin of the jamblang fruit (*Sygyzium cumini*) as an alternative dye to replace Giemsa dye. This research was conducted by comparing the results of erythrocytes, leukocytes and platelets with Giemsa staining and using an alternative staining of fruit peel extract. The sample in this study used blood, and then it was made as a thin blood smear. The results of this study showed that only erythrocytes had a slightly good staining quality while platelets and leukocytes were not good because the acid content in *Sygyzium cumini* extract could not bind the cell nucleus in platelets and leukocytes. The morphology of normal erythrocyte cells obtained was round and oval disc-like (biconcave) with a purple center and pale edges.

Keywords: Blood Smear, Erythrocytes, Alternative Staining, *Sygyzium cumini*

PRELIMINARY

Blood is the vehicle for mass transport over long distances in the body for various materials between cells and the external environment among the cells themselves. Blood consists of a complex fluid plasma in which cellular elements include erythrocytes, leukocytes and platelets (Fitryadi, 2016). Peripheral blood smear is a method for examination in the laboratory. It is used as an examination to assess the elements of peripheral blood cells erythrocytes, leukocytes and platelets and to look for parasites (Faradisa, 2016).

Peripheral blood smear is human blood cell preparations that are made by smear and then stained for hematological examination to assess various elements of peripheral blood cells such as erythrocytes, leukocytes and platelets. One guarantee validating the results of the Peripheral blood smear examination is the quality of the stain. There are several staining methods recommended by The International Council for Standardization in Hematology such as Wright's stain, Leshman, May-Grünwald, and Giemsa stain (Nugraha, 2015).

Since ancient times, natural dyes from plants have been widely used. The examples of natural dyes commonly used are chlorophyll, carotenoids and anthocyanins. Apart from being a dye that can be applied to food, natural dyes also have the ability to maintain health, prevent and minimize the rise of several diseases such as diabetes mellitus, hypercholesterolemia, cancer and so on (Nugraheni, 2015).

Giemsa stain is the most commonly used, but Giemsa has a weakness, which is not strong enough to stain the granules of the granulocyte series cells. In addition, the content of blue methylene, eosin, and azur B is not easily biodegradable, and creates toxic and flammable waste. In today's global era, public awareness of organic and eco-friendly materials is higher, so alternative coloring methods using natural materials are needed, such as the use of natural anthocyanin dyes deriving from the skin of *Sygyzium cumini* fruit. Jamblang fruit (*Sygyzium cumini*) is one of the plantation products which is quite abundant but underutilized. People in general consume the jamblang fruits directly

without being processed first. Rosanah (2015) said Jamblang fruits found in Aceh Besar generally live in terrestrial areas, hills and also grows on the coast, but in Kreung Raya and Jantho, they grows in hilly areas. The ripe *Sygyzium cumini* fruit which has a blackish purple skin color is one of the fruits that contain anthocyanins which can be used as natural dyes. Anthocyanins can give violet, red and purple colors. Anthocyanin is a compound that belongs to the antioxidant group (Tamymy, 2017).

RESEARCH METHODS

Tools and materials

The tools used in this research were oven, blender, microscope, slide glass, measuring cup, dropper, lancet needle, sample bottle, cotton and filter paper.

The materials used in this study were *Sygyzium cumini* fruit peel, 70% ethanol, Giemsa, methanol and clean water or aquadest.

Design, place and time

The research method used was the meseration method: observing and comparing the appearance of blood cells on blood smears using Giemsa staining and alternative staining from *Sygyzium cumini* fruit peel extract. This research was conducted at the UIN Ar-Raniry Aceh Laboratory on July 27, 2021.

Research Steps

1. Extract Making

The manufacture of extracts from *Sygyzium cumini* fruit peel according to Nanda (2020) was by the meseration method with 70% ethanol solvent. The fresh samples were in the oven. The use of oven was carried out for ± 2 hours at a temperature of 50° . The dried samples were blended to form simplicia. A total of ± 50 grams of simplasia was dissolved in 200 ml of 70% ethanol, allowed to stand for 8 hours at room temperature in a closed container and protected from direct sunlight. Then, the extract was filtered using filter paper. After meseration, evaporation was carried out at 94°C to produce a dark purple extract.

2. Blood Sampling and Peripheral blood smear making

Blood sampling technique by using a lancet needle was done by relaxing the hand and taking the finger. Clean the surface of the skin to be taken blood with 70% alcohol cotton. The tip of the finger was pierced with a lancet needle. Let the blood out and drip on the slide. Furthermore, slide B was placed at an angle of 45° on the drop of blood, and then pulled straight up to the end of the preparation to form a thin blood smear.

3. Staining of the peripheral blood smear using Giemsa stain

The fixation process was carried out by flowing methanol over the preparations for 5 minutes and then dried. Furthermore, the staining stage was carried out by flowing Giemsa solution for 30 minutes and then dried. The preparations were rinsed with distilled water and then dried.

4. Staining of Peripheral Blood Smears Using *Sygyzium cumini* Extract Stain

The fixation process was carried out by flowing methanol over the preparations for 5 minutes and then dried. Furthermore, the staining stage was carried out by flowing *Sygyzium cumini* extract for 30 minutes and then dried. Furthermore, the preparations that have been made were then observed under a microscope with 1000 times of vision enlargement.

RESULTS AND DISCUSSION

The data of this study were the results that have been carried out with the absorption process of color in cells against alternative staining of Peripheral blood smear. The results of Peripheral blood smear images colored used alternative staining from *Syzygium cumini* fruit peel extract and were compared with Giemsa staining shown in Figures 1 and 2.

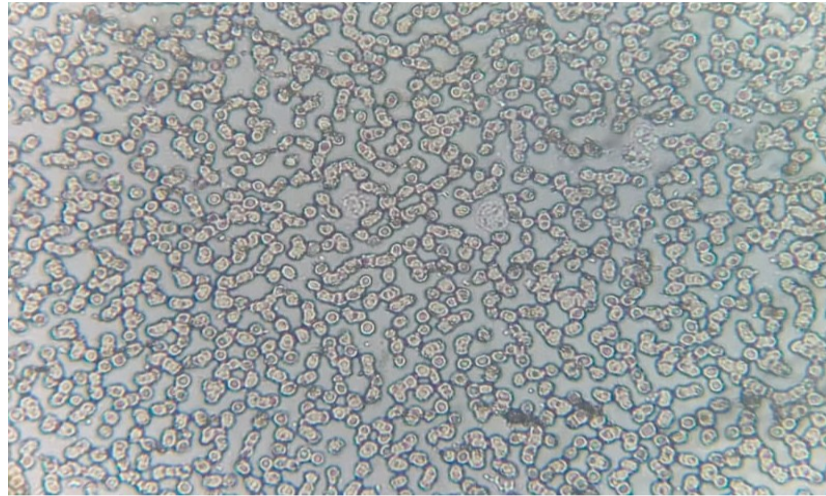


Figure 1: *Syzygium cumini* Extract at 100 x 10 Enlargement.

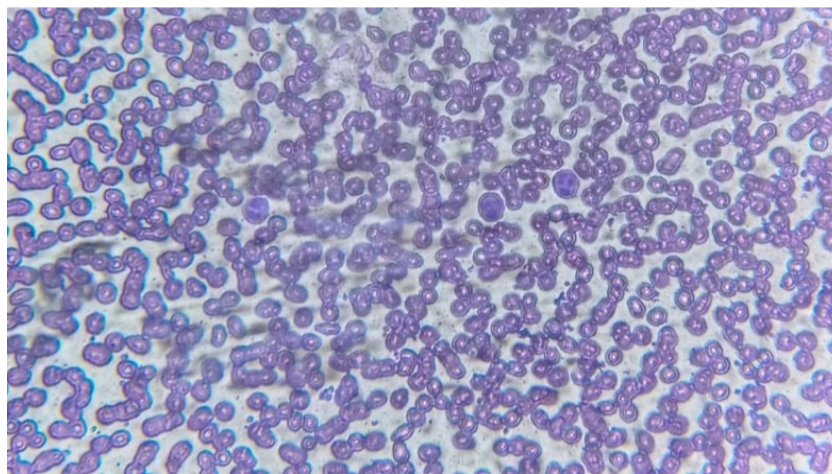


Figure 2: Giemsa Dye at 100 x 10 Enlargement

The study results of the Peripheral blood smear staining included microscopic researches shown in Figures 1 and 2. In the Peripheral blood smear staining, the use of alternative staining extract from the skin of the *Syzygium cumini* fruit only showed the staining of erythrocyte blood cells. This was due to the acidic anthocyanin content that could stain the cytoplasm and alkaline hemoglobin contained in erythrocytes. The quality of the blood cells shown was clear, but the dye was slightly pale and less absorbent. Blood cells seen in erythrocytes looked good where the morphology of the erythrocytes obtained was round or oval and disc (biconcave). While the staining on leukocytes and platelets was less clear, this was because the acidic anthocyanin content found in the skin extracted from *Syzygium cumini* could not color the nucleus of cells in acidic leukocytes and platelets. As a result, nothing can color the acidic nuclei of cells. Therefore, leukocytes and platelets were not visible.

Blood smear staining used the fruit peel extract of *Syzygium cumini*, that was less clear where the staining of leukocytes and platelets was also less clear. This is probably because the timing of the staining was inaccurate, so the staining did not bind well. *Syzygium cumini* contains cyanidin, an anthocyanidin aglycone that can be used to color chromosomes. Cyanidine had a conjugated double bond (benzene nucleus) which was part of chromophore group. Compared with Giemsa staining, the stain results of Peripheral blood smear bound erythrocytes, platelets and

leukocytes due to Giemsa dye having cation and anion dye properties. This was based on the fact that Giemsa dye had the properties of azure B (wet) cation dye, which served to stain platelets and gave a blue-purple color to nucleoproteins, basophil granules and neutrophil granules (McKenzie, 2014).

CONCLUSION

Based on the results of research and observations that have been carried out, it can be concluded that the extract of jamblang fruit peel containing natural anthocyanin dyes showed quite clear results on Peripheral blood smear in showing erythrocyte cells. However, this jamblang peel extract is less clear in showing leukocyte and platelet cells. Thus, the jamblang peel anthocyanin extract has the potential as an alternative dye in the observation of red blood cells (erythrocytes).

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