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ANTHOCYANIN UTILIZATION OF MANGSIAN FRUIT EXTRACT(PHYLLANTHUS RETICULATUS) AS AN ALTERNATIVE COLORANT IN BLOOD MEAL PREPARATION (SADT)

Fatma Raihani [1], Irwana Wahab [2]
[1,2]Poltekkes Kemenkes Aceh

**Coressponding email*: *fatmaraihani77@gmail.com*
Irwanawahab1969@gmail.com

ABSTRACT

The peripheral blood smear (SADT) examination is important for medical diagnosis. It involves staining blood preparations to allow clear visualization of peripheral blood cells, which include erythrocytes, leukocytes and platelets. One of the guarantees of the validity of SADT results is the quality of the SADT stain. Giemsa stain has disadvantages related to toxicity, availability of hazardous chemicals, and negative impact on the environment. Anthocyanins, natural pigment compounds found in many fruits, flowers, and vegetables, show great potential as natural colorants in SADT staining. One example is anthocyanins from mangsian fruit (Phyllanthus reticulatus), as a safer and environmentally friendly alternative. This plant contains tannic acid, terpenoids, flavonoids, phenolic compounds, and steroids. This study aims to assess the effectiveness of anthocyanin extract of mangsian fruit (Phyllanthus reticulatus) as an alternative coloring in peripheral blood smear preparations (SADT). The method used is experimental research, which is research that aims to determine the effects that arise as a result of certain treatments on a variable, by testing concentrations of 50%, 40%, 30%, and 20%. The results showed that anthocyanin extract was less effective in staining blood cells, macroscopically and microscopically, erythrocyte and leukocyte cells could not absorb the color of the dye properly.

Keywords: *Mangsian Fruit (Phyllantus Reticulatus), Edge Blood Smear Preparation (SADT)*

INTRODUCTION

A Clinical Laboratory is a healthcare facility that provides clinical specimen examination services to diagnose disease, cure disease, and restore health. It is essential for initial diagnosis, health screening, and medical treatment. One type of service provided by general clinical laboratories is

hematology examination, which is related to the analysis of blood and its components (Supangkat et al., 2022).

Hematology examination is an examination performed to identify and diagnose the patient's condition, as well as provide a basis for treatment by the clinician. One of the hematologic examinations is Tepid Blood Smear (SADT) examination, which must be performed carefully and with good validation for accurate results (Astina, 2020).

SADT examination is a procedure that involves dropping blood on a glass slide preparation which is then stained to assess peripheral blood cell elements. SADT staining is a process performed to sharpen the cell elements and ensure clear contrast. The staining methods suggested by The International Council For Standardization in Hematology are Wright's stain, Lieshman, May-Grünwald, and Giemsa. Among these options, Giemsa staining is the most commonly used (Ardila et al., 2021).

Giemsa stain contains eosin (acidic) and a mixture of methylene blue and methylene azure (basic). This combination forms eosinates that make the stain more stable. Eosin gives a pink color to the cytoplasm, while methylene blue gives a blue-violet color to the cell nucleus. In blood preparations stained with Giemsa, erythrocytes will look pink because they do not have nuclei, while leukocytes, which have nuclei, will be stained mauve blue. Although Giemsa dye is commonly used as a routine dye, it has some drawbacks. Giemsa is toxic, flammable and volatile, and improper storage can reduce the quality of the stain. Direct exposure or inhalation may cause organ damage. In addition, components such as methylene blue, eosin, and azur B are difficult to decompose, creating hazardous and flammable waste (Khasanah et al., 2023).

In today's global era, public awareness of organic and eco-friendly materials is higher, so alternative staining methods using natural materials are needed to minimize the use of Giemsa, such as by utilizing anthocyanin natural dyes (Salnus & Arwie, 2020).

Anthocyanins are flavonoid-derived compounds that are amphoteric, can interact with acids and bases. In acidic environments, anthocyanins are red, while in basic environments they become blue and purple. This compound is polar and more easily soluble in water such as distilled water and tartar acid. Anthocyanins are stable at acidic pH, producing colors varying from red, green, purple blue to black, and are often found in various parts of plants such as flowers, fruits, seeds, and tubers (Riyadi et al., 2021).

Previous research has explored the use of anthocyanins as natural dyes in peripheral blood smear preparations (SADT), such as in a previous study using Senggani Fruit Extract (Melastoma candidum L) as an Alternative to Peripheral Blood Smear Preparations (SADT) by Anita Oktari, M.Si., and Jhoni Hardiansyah. In this study, researchers took the initiative to use mangsian fruit as an alternative natural colorant in SADT (Nuraini & Tianto, 2023).

Mangsian fruit (Phyllanthus reticulatus) is a wild shrub that belongs to the Euphorbiaceae family. Apart from Indonesia, this plant grows in India, and Africa. In Indonesia, this plant is known as mangsi fruit or ink fruit. The natural color substance in mangsi fruit (Phyllanthus reticulatus) is anthocyanin, a pigment that produces orange, pink, red, purple, to blue colors. Research by Hariyani et al. (2013) using spectrophotometry showed that mangsi fruit contains flavonoids with a total of 3.75%. Anthocyanins, which are generally water soluble, provide red, blue, and violet colors in flowers, fruits, and vegetables, and in plants exist as glycosides that form esters with monosaccharides, so mangsi fruit can be used as a natural dye (Wicaksono et al., 2019).

METHODS

This research is experimental research, namely research that aims to determine the effects that arise as a result of certain treatments on a variable, from which treatment is expected to change or influence other variables (Akbar et al, 2023).

The place of research was conducted at the Pathology Laboratory of the Department of Medical Laboratory Technology (TLM) of the Aceh Ministry of Health Polytechnic. This research was conducted on June 12 - June 19, 2024. The population in this study was mangsian fruit (Phyllanthus reticulatus) obtained in Beurawe, Banda Aceh. The samples used in this study were mangsian fruit (Phyllanthus reticulatus) obtained from Gampomg Beurawe and capillary blood samples taken from students of the DIII Medical Laboratory Technology (TLM) Poltekkes Aceh 2024 study program, a total of 3 students with the criteria of being able-bodied and not being sick.

The tools used in this research are erlenmayer, measuring cup, scales, glass container, glass object, filter paper, sterile Blood lancet, Waterbath, aluminum foil, Beaker glass, watch glass, universal indicator, volum pipette, blender and microscope.

The materials used in this study are alcohol swab, blood lancet, filter paper, gauze and cover glass. The reagents used in this study are mangsian fruit extract (Phyllanthus reticullatus), methanol, citric acid (C6H8O7), distilled water, capillary blood, and pH7 buffer. The materials used in this study are alcohol swab, blood lancet, filter paper, gauze and cover glass. The reagents used in this study are mangsian fruit extract (Phyllanthus reticullatus), methanol, citric acid (C6H8O7), distilled water, and pH7 buffer. The specimen is capillary blood

The working procedures are Preparation of Acid Methanol, Preparation of Mangsian Fruit Extract (Phyllanthus reticulatus), Separation of Anthocyanin Extract, Dilution of Anthocyanin Solution, Preparation of Anthocyanin Solution with Concentration of 50%, 40%, 30%, 20%, Working Procedure of Capillary Blood Drawing, Preparation of Edge Blood Smear (SADT), Staining of SADT Preparation Using Mangsian Fruit Extract (Phyllanthus reticulatus), microscopic observation.

Data analysis was carried out descriptively to prove the results of anthocyanin extract from mangsian fruit (Phyllanthus reticulatus) by looking at the absorption of dyes in erythrocyte and leukocyte cells. Data collection in this study was carried out by testing anthocyanin extracts in mangsian fruit (Phyllanthus reticulatus) against Edge Blood Smear Preparations (SADT), the type of data collection used was primary data. Data is presented in the form of images, namely the presentation of research data in the form of images that show all the results of the study.

RESULTS AND DISCUSSION

RESULTS

After the research was carried out, the results of the examination of the utilization of anthocyanin extract from mangsian fruit (Phyllanthus reticulatus) as a natural dye for Edge Blood Smear Preparations (SADT) research results can be seen as follows: on macroscopic examination of peripheral blood smear preparations that have been colored using anthocyanin extract concentrations of 50%, 40%, 30% and 20% look unstained. In microscopic observations on peripheral blood smear preparations (SADT) that have been stained using anthocyanin extract concentrations of 50%, 40%, 30% and 20%, it can be seen that erythrocyte and leukocyte cells do not absorb dye, while in preparations stained with giemsa, erythrocyte and leukocyte cells are clearly colored.

DISCUSSION

Based on research that has been carried out by utilizing anthocyanin extract from mangsian fruit (Phyllanthus reticulatus) as a natural coloring on Blood Edge Smear Preparations (SADT), the results show that anthocyanin extract from mangsian fruit is not effective in staining blood cells. Macroscopically and microscopically, SADT preparations at various concentrations of 50%, 40%, 30%, and 20% indicated that erythrocytes and leukocytes could not absorb the dye.

Giemsa staining produces clear images of blood cells because it contains eosin which is acidic, methylene azur and methylene blue which are basic, which allows erythrocytes and leukocytes to absorb the dye well. Giemsa also helps identify types of leukocytes, parasites and platelets. Research shows that Giemsa gives better results compared to alternative stains such as mangsian fruit extract.

As for several factors that can affect the results of staining peripheral blood smears (SADT) with extracts from mangsian fruit, these are Ph, the type of solvent used, the ratio of solvent types, the effect of heating temperature, the length of heating time, the length of coloring time and light factors. Some of these factors can affect the presence of anthocyanins in the extraction process and its use.

Anthocyanins are natural pigments that are very sensitive to environmental factors such as temperature and light. Temperatures above 70°C and exposure to light can cause degradation (loss of quality or changes in chemical structure) of anthocyanins, so the extraction process must be carried out carefully to maintain its quality. The type and amount of solvent used in the extraction is also influential. The type of solvent used in this study is methanol which is added with citric acid, the use of acids added to the neutral methanol solvent aims to maintain anthocyanin stability, increase solubility, regulate Ph and reduce oxidation. This is also supported by Azmi and Yunianta (2015) which states that the use of acids added to the solvent functions to keep the solvent in acidic conditions so that it will denature the cell membrane of mulberry fruit particles and then dissolve the anthocyanin pigment out of the cell. In this study, the solvent used was 400 ml of acidic methanol.

The color of mangsian fruit extract is also affected by heating temperature. High temperatures can cause anthocyanins to degrade from the aglycone form into chalcones (colorless), and finally into alpha diketones. In addition, the length of heating also affects the coloring results. The longer the heating time, the greater the possibility of anthocyanin decomposition (the process of

anthocyanin molecules undergoing breakdown or changes in chemical structure), which can reduce the effectiveness of staining in blood smears. The results of research on extracting from black glutinous rice katul by Hanum (2000), also showed that heating at 100^0C for 4 hours continuously can reduce the color stability of anthocyanins. Therefore, it is important to control the temperature and heating time during the extraction process and application of anthocyanin extracts (Wicaksono et al., 2019).

CONCLUSION

Based on the results of this study, it can be concluded that the utilization of anthocyanin extract from mangsian fruit (Phyllanthus reticulatus) as a natural dye in the Preparation of Edge Blood Smear (SADT) shows that anthocyanin is not effective in staining blood cells. Macroscopically and microscopically, SADT was seen unable to absorb dye at various concentrations (50%, 40%, 30%, and 20%).

This study can be a reference for future researchers who want to conduct research on coloring peripheral blood smear preparations using natural coloring as an alternative to SADT gun coloring to reduce the use of synthetic materials. For future researchers to consider using different methods and types of solvents with this study.

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