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Utilization of Red Spinach (*Amaranthus tricolor L.*) as a natural dye in the preparation of *Allium cepa* var aggregatum root cell division preparations and its implementation as a learning resource

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Abstract
The use of synthetic dyes such as acetocarmine in cell division preparations has proven effective, but has the potential to cause negative impacts on health and the environment. Therefore, safer and more environmentally friendly natural dye alternatives are needed for biology practicum activities. Red spinach leaves (*Amaranthus tricolor L.*) contain anthocyanin pigments that have the potential to be used as natural dyes. This study aims to determine the effect of variations in the concentration of red spinach leaf extract and determine the optimal concentration in coloring red onion root cell division preparations (*Allium cepa L. var. aggregatum*). This study used an experimental method with a Completely Randomized Design (CRD). The treatments consisted of synthetic dye acetocarmine as a control and red spinach leaf extract with concentrations of 20%, 40%, 60%, 80%, and 100%, each with three repetitions. Data collection was carried out through microscopic observation and digital documentation. Assessment of staining quality was carried out using a questionnaire validated by experts, including indicators of color quality, the ability to distinguish cell nuclei, cell nucleus color, cell membrane color, and color contrast. Data analysis was carried out descriptively qualitatively in the form of percentages. The results showed that red spinach leaf extract was able to color onion root cell division preparations with varying quality at each concentration. Concentrations of 20%–80% produced staining quality with good criteria, with average values ranging from 75.56%–77.78%. A concentration of 100% showed the highest value of 84.44% with very good criteria and approaching the quality of acetocarmine dye. Increasing the extract concentration had a positive effect on color intensity, cell nucleus brightness, and preparation contrast, although in some preparations there was a decrease in the cell brightness limit due to the preparation technique. Red spinach leaf extract, particularly at 100% concentration, has the potential to be used as an alternative natural dye in onion root cell division preparations. Although its effectiveness is still slightly lower than the synthetic dye acetocarmine in highlighting cell nuclei, red spinach leaf extract is safer and more environmentally friendly, making it suitable for use in biology lab activities, especially at the high school level. Further research is needed to optimize the concentration and preparation techniques to improve the staining quality

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1. Introduction

The availability of specimens plays a crucial role in supporting the learning process, particularly in high school (SMA) cell division observation materials, which are conducted using a microscope. To enhance the effectiveness of educational communication in delivering cell division materials, learning can be conducted through joint observation activities between teachers and students of microscopic specimens. Through this approach, students have the opportunity to actively participate in the learning process (Chaeri *et al.*, 2008) [8].

The squash method is a technique used in making cell division preparations, namely by pressing the tissue or cells on a glass slide so that the cell structure can be observed clearly (Wahyuni, 2015) [55]. This method is a method of pressing on a glass object so that cell division can be seen under a microscope (Gunawan *et al*., 2019) [15]. A plant that can be used as a material for making cell division slides is the shallot root. This root has a soft texture, making it suitable for making cell division slides. Furthermore, the roots are readily available and absorb dyes well, ensuring uninterrupted cell observation and clear staining results. To produce a quality slide, the use of high-quality materials is essential, including the dyes used in the slide staining process.

The primary purpose of staining microscopic slides is to enhance the contrast between cellular structures so that cell components such as the nucleus, membrane, and chromosomes can be more clearly observed under the microscope. Without staining, most biological tissues are transparent and difficult to distinguish, especially when observing important processes such as cell division. Dyes also aid in identifying the phases of mitosis and allow for documentation that can be used in teaching and research. Dyes are divided into two types: natural dyes and synthetic dyes. In the practice of staining slides, the most commonly used dyes are synthetic dyes, one of which is safranin (N. Kurniasari *et al*., 2019) [23].

Synthetic dyes are often used in the preparation of cell division slides due to their ability to produce vivid color contrasts, but they have several drawbacks that should be considered. Some dyes, such as eosin, methylene blue, and safranin, are known to contain potentially carcinogenic chemicals and can cause irritation with prolonged exposure (Natsume *et al*., 2019) [30]. Apart from having an impact on health, synthetic dye waste is also difficult to decompose naturally and can pollute the environment if not managed properly, which ultimately endangers the balance of the ecosystem. (Dewi *et al*., 2021) [10] From a sustainability perspective, synthetic dyes are considered less supportive of the concept of environmentally friendly laboratories because they depend on industrial chemicals and are not in accordance with the biology education approach which pays attention to ecological aspects. (Yuliana & Suhartini, 2020) [57]. Therefore, natural dyes are starting to be considered as a safer and more sustainable alternative in educational settings.

Natural dyes derived from plants have several advantages, particularly in the context of educational laboratories, because they are safe for humans and the environment. Unlike synthetic dyes that contain hazardous chemical compounds, natural dyes are extracted from plant parts such as leaves, flowers, or roots, which are generally non-toxic, making them safer for students to use without posing a risk of irritation or health problems (Yuliana & Suhartini, 2020) [57]. Furthermore, natural dyes are readily biodegradable and do not pollute soil or water, making them more aligned with green laboratory principles that prioritize sustainability and the reduction of hazardous waste (Dewi *et al*., 2021) [10].

Another advantage is the abundant availability of local raw materials such as turmeric, pandan leaves, red spinach, and rosella flowers, which are easy to process, thus not only saving costs but also supporting innovation based on local wisdom and environmentally conscious education (Sari & Handayani, 2018) [47].

Another plant that has the potential to be used as an alternative source of natural dye to replace acetocarmine is red spinach. Red spinach (*Amaranthus tricolor L.*) is a vegetable plant belonging to the Amaranthaceae family. Its leaves are known to be rich in antioxidant compounds, particularly anthocyanin pigments. The anthocyanin content in red spinach leaves is reported to reach around 6,350 ppm (C. Pebrianti *et al*., 2015) [35]. The highest anthocyanin content in red spinach is found in the leaves. Red spinach contains anthocyanin pigments not found in green spinach. Anthocyanin is a purplish-red pigment that characterizes red spinach leaves and is also found in other plants such as dragon fruit and sweet potatoes. The presence of these pigments indicates that red spinach has significant potential as a natural dye source. (Saputro, 2014) [46]

Based on research conducted by (Rahmah Ramdhani, 2021) [40], red spinach (*Amaranthus tricolor L.*) leaf extract has the potential to be used as an alternative natural dye in the staining process of mango (*Mangifera indica L.*) stem tissue preparations. The staining results showed that at a concentration of 100%, the staining quality was excellent, with an average value of 98, indicated by the dye's ability to evenly color the entire mango stem tissue. At this concentration, the cell walls and cytoplasm appeared bright and clear. Thus, red spinach leaf extract can be used as an alternative natural dye for plant tissue preparations, particularly mango stem tissue (*Mangifera indica L.*).

The results of this study are expected to provide a solution to practical problems related to the risks of using synthetic dyes, by providing a safe and easy-to-apply alternative dye, making it easier for teachers to carry out practical work. The method used is relatively simple, yet it produces staining quality comparable to acetocarmine and other synthetic dyes. This study is also expected to contribute information regarding the use of natural dyes to clarify observations of the cell division process in shallot roots, thus enabling students to gain a more optimal understanding of the concept of cell division.

Therefore, the results of this study have the potential to be used as a learning resource for biology at the high school level, particularly in the form of a Practical Worksheet (LKP) on cell division using natural dyes.

2. Method

This study used an experimental method. The experimental design in this study used a completely randomized design (CRD). This study aimed to determine the effect of a good concentration of Red Spinach (*Amaranthus tricolor* L) leaf extract as a dye for cell division preparations on shallot roots (A. cepa L var aggregatum) and to determine the most optimal concentration for coloring cell division preparations on shallot roots (A. cepa L var aggregatum).

The independent variable in this study was the source of coloring for shallot root cell division preparations with coloring treatments, namely Acetocarmine dye as a control and red spinach leaf extract at concentrations of 20%, 40%, 60%, 80%, and 100%. The dependent variables included color quality, distinguishing cell nuclei from other cell nuclei, Cell Nucleus Color, Cell Membrane Color, and Color Contrast. Each treatment was repeated 3 times. This research was conducted in the Biotechnology and Biology Study Program Laboratory, Faculty of Science and Technology, Universitas Muhammadiyah Bandung, in December 2025. The equipment used in this study included 1000 ml beakers, microscopes, cover slips, slides, droppers, blenders, glass bottles, filter paper, test tubes, tissue, tweezers, label paper, a digital camera, an analytical balance, a spatula, an oven, and aluminum foil. The materials used were fresh red spinach leaves, shallot roots, mineral water, distilled water, and 96% ethanol.

Data collection methods in this study included direct observation using a microscope and documentation of cell division images using a digital camera. The resulting photographs were then printed and stored in an album for validation through a questionnaire by a validation team selected based on their expertise in microtectics. The questionnaire assessed color quality, distinguishing cell nuclei from other nuclei, cell nucleus color, cell membrane color, and color contrast.

The questionnaire assessed the color quality of the slides, which was assessed by a review team consisting of two lecturers from the Biotechnology Study Program at Universitas Muhammadiyah Bandung. The determination of color quality criteria for the preparations was based on the cell color quality indicators in Kurniasari's (2019) research [23]. The observed indicators included the color of cell division in the shallot root. The assessment criteria for determining color quality included distinguishing the cell nucleus from other cell nuclei, the color of the cell nucleus, the color of the cell membrane, and the color contrast of the cell division preparations, as described in (Figure 5). This study presented the data analysis process using qualitative descriptive methods. The data consisted of descriptive percentages of color quality obtained from a questionnaire completed by the review team.

The results of the study, which explores the use of red spinach leaf extract as a natural dye in shallot root cell division preparations, will be presented in high school biology lessons in the form of a Practical Worksheet (LKP). The Practical Worksheet (LKP) will be validated by two validators, consisting of two lecturers (validator I and II). The results of the two expert validators' assessments will be calculated using the following formula:

Table 1: Variation in Agreement Among Validators.

Validator 2 \ Validator 1	Setuju	Tidak	Total
Setuju	A	B	M1
Tidak	C	D	M2
Total	N1	N2	N

Keterangan:

- A : Jumlah pertanyaan yang mana kedua validator sama-sama setuju
- D : Jumlah pertanyaan yang mana kedua validator sama-sama tidak setuju
- B : Jumlah pertanyaan yang mana validator 1 tidak setuju, validator 2 setuju
- C : Jumlah pertanyaan yang mana validator 1 setuju, validator 2 tidak setuju
- N1 : (a + c)
- N2 : (b + d)
- M1 : (a + b)
- M2 : (c + d)
- N : Total keseluruhan

Po (Proporsi kesepakatan teramati)

$$P_o = \frac{a+b}{N}$$

Pe (Proporsi kesepakatan harapan)

$$P_e = \frac{N_1 x M_1}{N^2} + \frac{N_2 x M_2}{N^2}$$

3. Results and Discussion

Based on observations of red onion (*Allium cepa L.* var. aggregatum) root cell division preparations, staining with red spinach (*Amaranthus tricolor L.*) leaf extract demonstrated the ability to display cell structure, particularly the cell nucleus, allowing for observation of the mitosis process. Microscopic observations at 400× magnification showed varying degrees of color clarity, influenced by the extract concentration and preparation technique. The results are shown in Figure 1

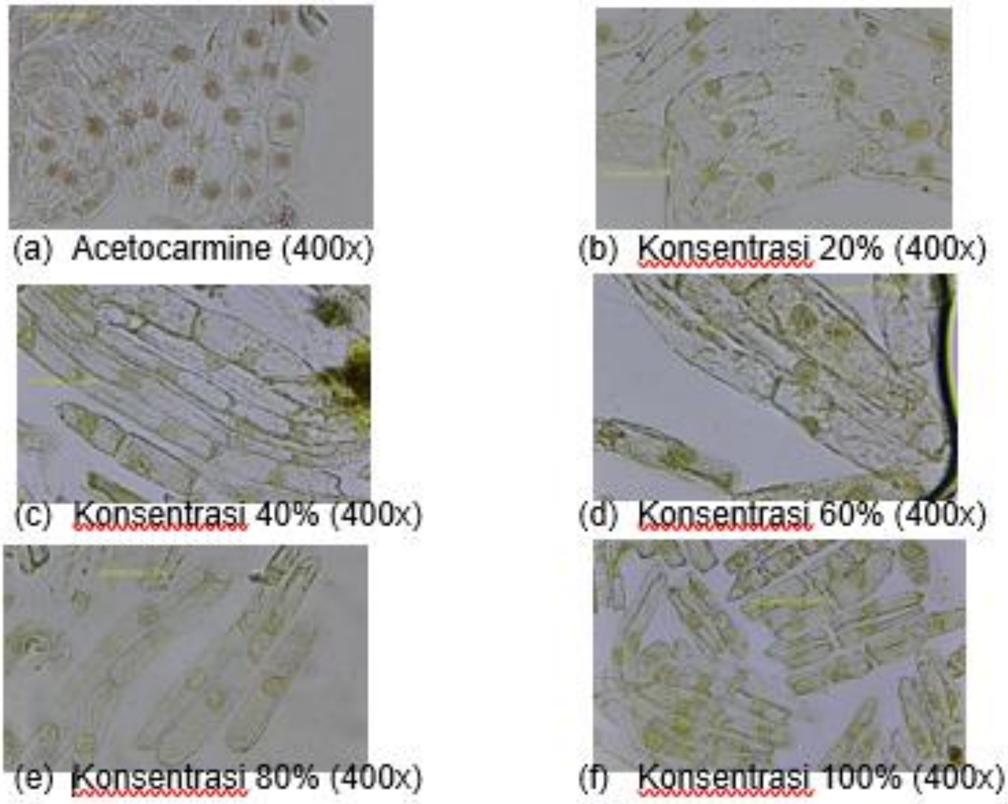

Fig 1: Observations on the Color Quality of Shallot Root Cell Division Preparations Using Natural Dyes at Various Concentrations.

The observations in Figure 3 show that the staining of shallot root cell divisions (*A. cepa L. var. aggregatum*) at each concentration produced different quality results. However, red spinach leaf extract was proven to be effective in staining shallot root cell divisions. A summary of the results of the questionnaire assessing the quality of root cell staining at concentrations of 20%, 40%, 60%, 80%, and 100%, as well as acetocarmine dye as a control, is presented in Table 6

Table 2: Observations on the Color Quality of Shallot Root Cell Division Preparations (*A. cepa L. var. aggregatum*) Using Different Concentrations of Red Spinach Leaf Extract Natural Dye

Indikator yang dinilai	20%	40%	60%	80%	100%	Acetocarmine
Kualitas warna	77,78%	77,78%	77,78%	77,78%	88,89%	77,78%
Dapat membedakan inti sel dengan inti sel yang lain	77,78%	77,78%	77,78%	77,78%	88,89%	77,78%
Warna inti sel	77,78%	77,78%	77,77%	77,78%	77,78%	100%
Warna membran sel	77,78%	77,78%	77,78%	77,78%	88,89%	88,89%
Kekontrasan warna	66,67%	77,78%	77,78%	77,78%	77,78%	77,78%
Total	75,56%	77,78%	77,78%	77,78%	84,44%	84,44%
KPKP	B	B	B	B	SB	SB

Gap: KPKP = Preparation Quality Assessment Criteria, TB = Poor, B = Good, SB = Very Good

Based on Table 1. Based on the results of testing natural dyes from red spinach leaf extract (*Amaranthus tricolor L.*), it is known that the extract is able to provide color to onion root cell division preparations (*A. cepa L var aggregatum*). The results show that the quality of cell division preparations stained using red spinach leaf extract at various concentrations shows differences in the quality of coloration in each observed indicator. In general, concentrations of 20%, 40%, 60%, and 80% produce a total average value ranging from 75.56%–77.78% with the criteria of Good (B), while the concentration of 100% and the synthetic dye acetocarmine show the highest average value, namely 84.44% with the criteria of Very Good (SB). In the color quality indicator, concentrations of 20% to 80% show relatively the same value (77.78%), indicating that the resulting color intensity is quite good but not optimal. The increase in the value at 100% concentration (88.89%) indicates that the anthocyanin pigment content in red spinach extract at high concentrations produces a more intense and clear color in the preparation. The indicator for distinguishing cell nuclei from other nuclei also showed a similar pattern, with 100% concentration producing a higher value than other concentrations. This indicates that increasing the extract concentration affects the visual clarity of cell nuclei, allowing for better observation of the boundaries between them.

For the cell nucleus color indicator, acetocarmine dye achieved the highest value (100%), confirming the superiority of this synthetic dye in providing contrasting and specific cell nucleus color. Nevertheless, red spinach extract at 100% concentration still showed good results, although its color intensity and stability were slightly lower than that of acetocarmine. The cell membrane color indicator showed an improvement in quality at 100% and acetocarmine concentrations, with a value of 88.89%. This indicates that higher extract concentrations can stain cell structures more evenly, allowing for clearer cell membranes than at lower concentrations. Meanwhile, in the color contrast indicator, the 20% concentration obtained the lowest value (66.67%), indicating that staining at low concentrations was not able to produce contrasting color differences between cell parts. Higher values at concentrations of 40% to 100% indicate that increasing the extract concentration has a positive effect on the color contrast of the preparations.

Research findings indicate that red spinach extract has the ability to stain mitotic cell division. This finding is supported by research by Sumatri *et al*. (2021) [49], who found that red spinach contains flavonoid compounds in the form of anthocyanins, which can be used as natural dyes. Anthocyanins can be found in plants in the leaves, fruit, stems, and roots (Fauziah, 2015) [14].

The results of this study showed that the higher the concentration, the better the cells absorbed the dye. Data showed that the overall color quality of red onion (*A. cepa L. var. aggregatum*) root cell divisions was categorized as very good at a concentration of 100%. The results showed that the dye was able to color cell division preparations very well, with bright or light colors. However, some results indicated that cells could not be distinguished from each other because the preparation process used a squashing method (Fauziah, 2015) [14]. The squash method can cause uneven cell distribution, resulting in overlapping and deformed cells. This is supported by strong staining, which can lead to unclear cell boundaries and structural details (Anathawat, 2003) [4].

Observations at 20% and 80% concentrations showed that the dyeing of red onion root cell division preparations met good criteria, as the staining appeared less intense, reducing the contrast between cell structures. This may be due to the low anthocyanin content at 20% and 80% concentrations. These results indicate that if the dilution concentration of red spinach leaf extract as a dye is at the correct and stable level, the resulting preparation can exhibit excellent quality in each cell. This finding aligns with the opinion (Wahyuni, 2009) [54] that the concentration of extract dilution used as a dye has a significant influence on the color quality of the dyed object.

Based on the color quality observations in Figure 3, red spinach leaf extract is capable of staining cell preparations. At good concentrations (20–40%), the resulting color is still pale and lacks contrast, so cell structures, particularly the nucleus and chromosomes, are not yet clearly visible. At medium concentrations (60%), the coloration begins to become more even, but the sharpness of cell details remains limited. High concentrations (80–100%) produce more intense colors, but tend to cause pigment buildup, reducing the clarity of cell boundaries and internal structural details. Low pigment levels result in suboptimal binding between anthocyanin and chromosomes, which can lead to color contrast in cell structures (Dafrita & Sari, 2020).

Compared to the reference dye, acetocarmine, red spinach leaf extract showed lower staining power, particularly in highlighting the cell nucleus and the mitotic division phase. Nevertheless, these results indicate that red spinach leaf extract has potential as an alternative natural dye, particularly in biology learning, due to its natural and environmentally friendly nature. Although optimization of concentration and preparation techniques is still necessary to achieve staining quality close to that of synthetic dyes. This is in line with the results of previous research conducted by Bisri *et al*. (2013) [7], which stated that there are differences in the components and characteristics of each cell organelle. The staining process in plant tissue preparations occurs due to the electrostatic interaction between the positively charged dye ions and acids (Nurwanti *et al*., 2013) [34]. According to Hamid (2005) [16], acidic anthocyanins can color cell walls composed of cellulose and are basic. The positive ions in the dye (H^+) will dissociate and then form covalent bonds with the negatively charged ions present in the cell walls.

Based on observations of red onion root cell preparations, staining using red spinach leaf extract at various concentrations showed that the cell nuclei appeared more faded compared to preparations stained with acetocarmine. The resulting staining tended to be uneven, with only some cells being clearly stained, while others appeared to be underexposed, resulting in more of the color being absorbed by the cell walls. Furthermore, differences in the results of pressing the slides and staining also affect the intensity and clarity of the cells and the resulting color, resulting in variations in cell quality and staining between slides.

Based on the results of the discussion, red spinach leaf extract at 100% concentration demonstrated excellent color quality, nearly equaling, and in some aspects even surpassing, acetocarmine dye. However, observations showed that in some cells, the boundaries between cells were not clearly visible.

5. Conclusion

Based on the research results, the following conclusions can be drawn:

1. Different concentrations of red spinach leaf extract (*Amaranthus tricolor L.*) affect the color quality of the resulting cell division preparations.
2. Cell staining at concentrations of 20-80% can stain the entire shallot root preparation with good criteria, with an average value of 77.78%. The results show that each cell is brightly colored and can distinguish cell nuclei from one another. Red spinach leaf extract at a concentration of 100% showed excellent staining results in some cells, with color intensity nearly similar to acetocarmine. This indicates that the 100% concentration has excellent staining effectiveness.

However, the results showed that some cells could not be distinguished from one another. This was due to uneven squeezing and excessive dye application. Therefore, red spinach leaf extract can be used as an alternative dye for staining plant tissues, especially shallot root cells (*A. cepa L. var. aggregatum*).

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