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The use of alternative coloring variations to mitotic observations of shallots using the squash method

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ABSTRACT

Practicum cannot be carried out due to limited facilities and infrastructure such as dyes for observation of mitotic cell chromosomes in onions. Alternative dyes that can be obtained from nature can be explored. The purpose of this study was to obtain preparation of the mitotic division process in various onion root cells using alternative dyes using dyes from dragon fruit peel and turi flower. In obtaining the right preparation using the squash method. Data were obtained using observation sheets that observed each variation of the material. The instrument used in this study is a valid observation sheet. The results showed that the coloring of the preparation using dragon fruit skin and turi flowers was successful and clear coloring results were obtained. Coloring in practicum activities using turi flowers is better than dragon fruit coloring. Natural dyes from dragon fruit peel and turi flower can be used as alternative dyes in the squash method to observe onion root mitosis, replacing synthetic dyes.

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INTRODUCTION

Observation preparations for practicum are often needed to support Biology learning. Quality preparations require good staining to sharpen and clarify the picture of tissues and cells so that they are easily observed under a microscope. In order, for learning and practicum activities to be realized and run well, diligence, skills, and supporting infrastructure in the laboratory and equipment are needed. One of them is making observations in the form of preparations or specimens in the form of fresh preparations or specimens of both animals and plants and preserved specimens of both plant and animal anatomy However, many Biology lessons cannot be carried out due to limited facilities and infrastructure, especially in coloring preparations in practicum material for observing mitotic cell chromosomes in onions (Latifa, 2015).

The material in the observation of mitotic cell chromosomes in onions is a concrete activity that can be understood by observing cell division directly. Practicum activities with direct

observation can train skills and improve understanding. Mitosis is a cell division in which daughter cells have the same properties as the parent cell. The phases in mitosis consist of prophase, metaphase, anaphase, and telophase (McIntosh, 2016). The main material for making mitotic preparations is cells that carry out mitotic division. Cells that undergo division are found in the parts of plants that are actively undergoing growth (meristematis) most easily found at the tip of the root (Perilli et al., 2012). A commonly used method of making mitotic preparations is by squash. The squash method is a method of obtaining a preparation by tissue squeezing a piece or an organism as a whole so that a thin preparation can be observed on a microscope (Yang, 2009).

Previous research related to onion mitotic index studies for making learning media for mitotic preparations concluded that IM A. sativum, A. cepa and A. fistulosum were different even though in one genus IM A. sativum the highest occurred at 09.00 WIB with a value of 11.410%; The highest IM A. cepa occurred at 12.00 WIB with a value of 11.326%; while the highest IM A. fistulosum occurred at 06.00 WIB with a value of 12.617%. Squash mitotic preparation media about the mitotic index of Allium root tip meristem with hematoxylin dye, suitable for use as a learning medium for observing cell mitotic division (Abidin et al., 2014).

The problem that is often encountered in mitotic observations in onions is the difficulty of adding coloring to clarify the appearance or appearance of onion cells and also get the right time during the mitotic division process in onion cells. Widely used dyes are synthetic dyes with certain dyes that can be attached to tissues. Usually, the dye is safranin which gives the preparation its red color. Safrantiin dye is preferred because it is practical and its coloring properties are stable and diverse (Aryadini et al., 2003). Virgianti & Luciana, 2017 state that in addition to being used as food and textile dyes, natural dyes from natural materials can also be used as dyes in the process of staining microorganisms.

However, artificial dyes have drawbacks that when used long-term have carcinogenic properties. Waste from artificial dyes will also have an impact on the environment, therefore environmentally friendly alternative dyes are needed. The limitations of the use of dyes can be anticipated with natural alternative dyes from plant extracts. Natural dye pigments are safer to use even though the level of stability to heat, light, and acidity is erratic. Natural dyes that have not been widely explored for use as dyes for onion root mitotic preparations are extracts of dragon fruit peel and turi flower. Therefore, this study aims to explore the extraction of dragon fruit peel and turi flower, as well as its use as a dye in the observation of mitotic preparations of onions, garlic, onions, and leeks. The novelty of this study is the use of dyes from dragon fruit peel extract and turi flower flowers by considering the soaking process.

RESEARCH METHODS

Research Design

Observation of the results of the study was carried out descriptively, namely by observing the results of coloring using dragon fruit skin and turi flowers as coloring materials using observation sheets. The coloring results are then compared with the control. Parameters observed include clarity and color contrast.

Population and Samples

The population and samples used in this study were dragon fruit skin waste, turi flowers, and onion variations. Tools and materials used FAA, onion bulbs, garlic bulbs, onion bulbs, onion bulbs, dragon fruit, turi flowers, glacial vinegar acid and hydrochloric acid, analytical balances, microscopes with digital cameras connected to computers/laptops, Erlenmeyer flasks, beakers, measuring flasks, measuring cups, drip pipettes, glass objects and glass decks, Petri dishes, falcon bottle, funnel, electric bath, pH meter or pH indicator, stirring rod, filter paper, mortar and pestle, vacuum pump, blender.

Instruments

Data collection was carried out by making observations using observation sheets from onion preparation. The observations were photographed and then validated by experts based on their expertise.

Procedures

The method used in this study began with the extraction of dragon fruit skin and turi flowers using maceration methods. Furthermore, the extraction of natural dyes is used to color cell mitotic observation preparations in onion roots. Some of the stages carried out in this study include

1. Preliminary stage

At this stage, the tools and materials used in the study are prepared which include root growth on onion bulbs, garlic, onions, and leeks in beakers for a period of ± one week.

2. Extraction stage of dragon fruit peel and turi flower

Next, dragon fruit skin and turi flowers are extracted by cutting the ingredients and then crushing them until smooth with a blender or pestle die. Then, in the mortar, a little water is added and then the extract is filtered with filter paper. Then 10% (w/v) of citric acid is added to the filtrate obtained.

3. Trial phase as a natural dye for preparations

Sprouted onion roots are cut at the ends by 3-5 cm. Then soak into the FAA solution for 15-20 minutes. The soaked onion roots are then rinsed using aquades three times. Onion roots are soaked in natural dyes that have been prepared, namely extracts of dragon fruit peel and turi flower. Each marinade is heated in a water bath to a boil and the dye is firmly absorbed into the onion roots.

4. Observation stage

At the observation stage, microscopes and equipment are prepared along with onion root preparations on glass objects and covered with glass decks. The onion root cells are then observed using a microscope.

Data Analysis

Data analysis in this study is descriptive qualitative to see the quality of preparate as staining mitotic preparations. Data is collected by observing objects under a microscope and then photographed. The indicator used to determine the quality of the preparation consists of color contrast and preparate clarity. The criteria for preparation clarity and color contrast are in Table 1.

Table 1. Criteria of clarity and contrast of preparat

Aspects	Criterion	Indicator	Score
Clarity	Very clear	Fulfilled when parts of the tissue can be distinguished very clearly When the division stage includes prophase, metaphase, anaphase, telophase.	3
	Clear	Fulfilled if the tissue parts can be clearly distinguished when the division stage includes prophase, metaphase.	2
	Not clear	Fulfilled when parts of the tissue can be distinguished very clearly When the division stage includes prophase.	1
Contrast	Highly contrast	Fulfilled if the dye is only firmly bound to certain parts (does not color all tissues).	3
	Contrast	Fulfilled if the dye is only bound to a certain part (does not color all tissues).	2
	Lowly contrast	If the dye is bound to all tissues (dyeing the tissue).	1

The preparation clarity and contrast criteria that have been assessed by experts are then calculated using the percentage technique. The assessment criteria can be seen in Table 2.

Tabel 2. Kriteria penilaian preparat

Percentage (%)	Criterion
$33{,}3 \leq Percentage \leq 46{,}64$	Bad
$46{,}64 < Percentage \leq\leq 59{,}98$	Not Good
$59{,}98 < Percentage \leq \leq 73{,}32$	Good enough
$73{,}32 < Percentage \leq \leq 86{,}66$	Good
$86{,}66 < Percentage \leq \leq 100$	Excellent

RESULTS

The pH and absorbance value of red dragon fruit peel extract and red turi flower crown can be seen in Table 3.

Table 3. pH and absorbance value of red dragon fruit peel extract and red turi flower crown

Types of extracts	pH		Wavelength	Absorbance value
	first	last		
Red dragon fruit skin	5.0	2.0	399	0.570
			425	0.584
			452	0.654
			539	1.997
Red turi flower crown	5.0	2.0	381	2.586
			405	1.097
			533	1.388

The quality results of the preparation using coloring preparations of red dragon fruit peel extract and red turi flower crown can be seen in Table 4.

Table 4. The quality results of the preparation using coloring preparations of red dragon fruit peel extract and red turi flower crown.

Dye	Criterion
Dragon fruit skin	Good
Red turi flower crown extract	Excellent

DISCUSSION

The extraction of natural dyes derived from the skin of red dragon fruit and red turi flowers has been successfully carried out by optimizing the soaking process. The skin of the red dragon fruit (Hylocereus polyrhizus) and the crown of the red turi flower (Sesbania grandiflora L.) have anthocyanin color pigments that are classified as flavonoid compounds and have strong color characters and are soluble in water. Anthocyanins in the skin of red dragon fruit and red turi flowers have the potential to be natural dyes for food and in this study can be used as an alternative to synthetic dyes that are safer for health (Anam, 2016).

The extraction of red dragon fruit peel and red turi flower produces a strong red dye derived from anthocyanin color pigments that can be applied to the coloring of onion root squash preparations. Extraction can be interpreted as a way of pulling out or separating a material from its mixture, generally by using solvents. The nature of anthocyanin pigments is greatly influenced by

pH, where anthocyanin extract if added base then the pigment will turn green, while if reacted with acidic compounds, the extract will turn red. In the extraction process, citric acid is added which is acidic in nature so that a strong dark red pigment is obtained. The solubility of pigments in water solvents and citric acid has a compatibility between solutes and solvents (like dissolve-like). This solvent affects the pH, absorbance, color intensity, content, and yield of extracted pigments. The color intensity values of dragon fruit peel extract and red turi flower are shown in Table 5.

Table 5. The intensity of color and degree of redness of red dragon fruit peel extract and red turi flower crown

Types of extracts	Color intensity	Degree of redness
Red dragon fruit skin	+ +	Red is strong enough
Red turi flower crown	+ + +	Strong red

Based on qualitative observational analysis, visually it is known that there is a difference in the intensity of the red color obtained from the skin of the red dragon fruit and the crown of the red turi flower. For red dragon fruit peel extract, the redness level is quite strong while the color intensity is ++, while the red turi flower crown extract has a strong redness level and +++ color intensity. These results show that the type of plant organ chosen has different pigment levels and affects the extraction results. The results of research on the extraction of red turi flowers using aqueous solvents: citric acid: ethanol produced extracts containing anthocyanin pigments and the solvent affects pH, absorbance, color intensity, and yield (Saati, 2008). The pH and absorbance measurement results of red dragon fruit peel extract and red turi flower are shown in Table 6.

Table 6. pH and absorbance value of red dragon fruit peel extract and red turi flower crown

Types of extracts	pH		Panjang gelombang	Absorbance value
	first	end		
Red dragon fruit skin	5.0	2.0	399	0.570
			425	0.584
			452	0.654
			539	1.997
Red turi flower crown	5.0	2.0	381	2.586
			405	1.097
			533	1.388

The results of the extraction of red dragon fruit peel and red turi flower crown show a deep red color, a characteristic of anthocyanin pigments (Figure 1). The addition of citric acid, the color of anthocyanin pigments that react with acidic compounds shows a strong red color. Anthocyanin properties are strongly influenced by pH and the results of pH measurements on dragon fruit peel extract and red turi flower crown extract after the addition of citric acid show pH 2.0.

Figure (a) represents the appearance of dragon fruit skin before extraction. Dragon fruit peel is used as a base material for dye preparations. Figure (b) shows the appearance of turi flowers before the extraction process. The extracted dragon fruit peel seen in Figure (c) has an initial pH of 5.9 then citric acid pH is added to 2.0. Turi flowers that have gone through the extraction process seen in Figure (d) have an initial pH of 5.0 and then given citric acid pH of 2.0.

The highest absorbance value of red dragon fruit peel extract was found at a wavelength of 1,997 nm, while for the crown of red turi flowers, the highest absorbance value was obtained at a wavelength of 1,388 nm. From these data, the intensity and pH value of pigments affect pigment absorbance. The higher the pH value, the smaller the absorbance. Anthocyanin pigments are more

stable under acidic conditions and their absorbance value depends on the concentration in the extract. In this case, the concentration of anthocyanin pigments in red turi flower crown extract is higher than that of red dragon fruit peel extract. Anthocyanins are a group of pigments that are red to blue in color that are present in plants including in fruits, flowers, roots, and leaves. From the absorbance data, it is proven that anthocyanins from the skin of the red dragon fruit and the crown of the red turi flower contain pelargonidin and cyanidin which are shown the maximum absorbance wavelength of the spectrum at 500-550 nm. The type of anthocyanin read at the 525 nm wavelength is pelargonidin, while the one read at the 535 nm wavelength is cyanidin (Simanjuntak & Sinaga, 2014).

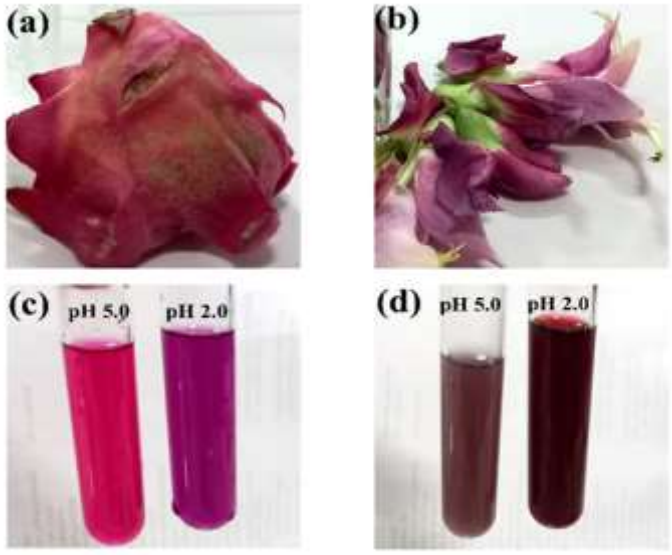

Figure 1. Presentation of red dragon fruit skin (a) and natural dyes from the extraction of red dragon fruit peel (c), red turi flower crown (b), and natural dyes from the extraction of red turi flower crown (d).

The results of red dragon fruit peel extract and red turi flower crown were then applied to the preparation process of onion cell mitotic preparations by the squash method. The results of preparation of preparations and observations of preparations by staining red dragon fruit peel extract are presented in Figure 2a-d and red turi flower crown extract in Figure 2e-h.

This research proves that the use of natural dyes that have the same function as safranin dyes can be done with a simple extraction method. In some plants, natural dyes have several color pigments such as chlorophyll, carotenoids, tannins, and anthocyanins. Natural coloring substances obtained from plants have a great affinity for cellulose and plant fibers. In addition, natural dyes can be absorbed by cell nuclei that contain chromosomes, where chromosomes themselves can absorb color. The behavior of chromosomes at the time of cell division was successfully described as related to the process of mitotic division. Chromosomes are stainable bodies based on the concept of anatomical morphology and chromatin thread arrangement. Chromosomes in the nucleus can absorb dyes well so that they look contrasting with other parts of the cell (Aziz, 2019; Sharma, 2020).

Natural dyes used instead of synthetic dyes in preparations have received evidence from several studies that have been conducted. Roselle petal extract can clarify the picture of the epidermis, parenchyma, cambium, and xylem tissue in large red chili plant section preparations (Bisri, 2014). Research on the use of natural dyes using red dragon fruit skin and telang flower extract has been carried out on chicken femoral bone rubbing preparations (Wahyuni, 2015; Susliana & Wahyuni, 2019).

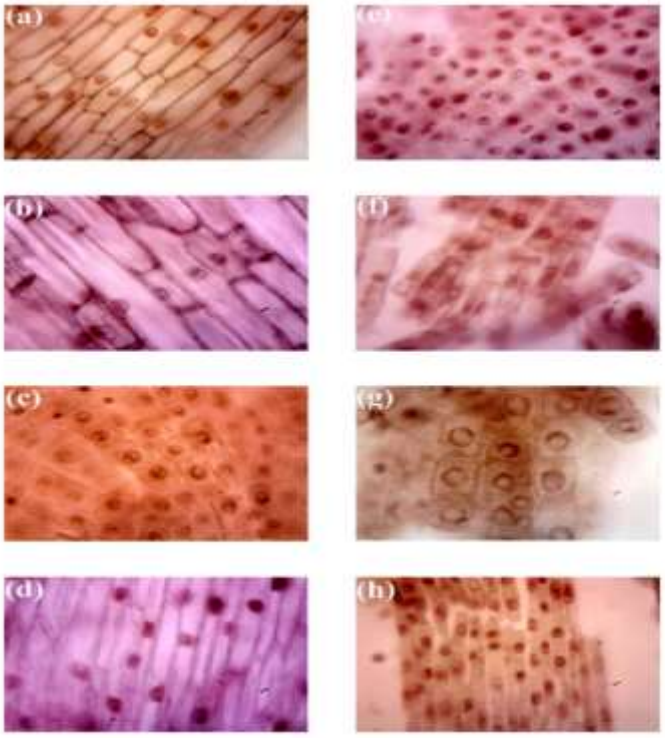

Figure 2. Results of observations of cell mitotic preparations (magnification 400×) by staining red dragon fruit peel extract on onion root preparations (a), garlic (b), onion (c), and onion (d); and coloring of red turi flowers on shallots (e), garlic (f), onions (g), and shallots (h) at the time of cleavage at 09.00 WIB for onions, garlic, and onions, and at 06.00 WIB for shallots.

Making microscopic preparations is an art that requires accuracy, accuracy, high ability, and is supported by the individual interests of the perpetrators. One of the methods of making microscopic preparations is the mitotic division of onion cells. Usually, the sample used is onion. In this study, onions, garlic, onions, and leeks were used as samples for making preparations by the squash method. (I. N. Wahyuni & Sabban (2022) in his research stated that in the skin of dragon fruit, there are two almi dyes, namely anthocyanin, and betacyanin. Betasianin merupakan zat warna yang berperan memberikan warna merah dan merupakan golongan betalain yang berpotensi menjadi pewarna alami (Said et al., 2021) Anthocyanin dyes are more stable at low Ph, therefore the solvent used is citric acid (Muslim & Rosidah, 2023). Citric acid solution used as a solvent functions as a control negative and dye ((Hidayah et al., 2014). In addition, according to other studies, anthocyanin chemicals in dragon fruit peel extract can effectively color soursop fruit preparations (Permatasari & Suriani, 2022).

The importance of research on the use of natural dyes, in this case, uses anthocyanin pigments from the skin of red dragon fruit (Hylocereus polyrhizus) and red turi flower crown (Sesbania grandiflora L.) to answer the development of science, especially in observing cell division and utilizing dragon fruit skin waste and red turi flower crown. The product of coloring preparations using red dragon fruit skin and red turi flowers can be used as a simple Biology learning medium. Biology learning activities are a process that requires student activities, such as learning process activities in practicum at school that use microscopic preparations of plant cells and tissues can be assisted by natural coloring that is easy to prepare and make by teachers and students. This is to reduce costs and limitations that exist in schools because the price of preparations that are quite expensive is also hampered by the high cost of synthetic dyes.

CONCLUSION

Natural dyes from the skin of the red dragon fruit and the crown of the red turi flower were successfully extracted by maceration techniques. Furthermore, the dye can be absorbed by chromosomes and facilitates the coloring of the cell nucleus at the time of mitotic cell division in root preparations of onions, garlic, onions, and leeks. This natural dye can be used as an alternative to synthetic dyes, making it easier to learn Biology practicum in high schools that have limited access to synthetic dyes.

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