

RED DRAGON FRUIT (*Hylocereus costaricensis* Britt. Et R.) PEEL EXTRACT AS A NATURAL DYE ALTERNATIVE IN MICROSCOPIC OBSERVATION OF PLANT TISSUES: THE PRACTICAL GUIDE IN SENIOR HIGH SCHOOL

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ABSTRACT

Prepared slide of plant tissue needs to be staining to facilitate observations under microscope. Laboratorium activities in schools usually use synthetic dyes which expensive and can be damaged the student. Therefore the exploration of alternative dyes need to be established, such as utilizing of red dragon fruit (*Hylocereus costaricensis* Britt. Et R.). This study aims to (1) find out the best concentration of dragon fruit peel extract for staining plant tissue prepared slide and (2) to develop the practical guide related to plant tissue observation. The qualitative research used different concentration of red dragon fruit peel extract, namely: 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% with 3 repetitions. Data were obtained from observation photos of prepared slide. The result showed that the most contrast prepared slide was used red dragon fruit extract in 60% concentration. The result use to arrange practical guide in observation of plant tissues which is validated by material expert. The validation result showed "very good" criteria (86.01%).

Keywords: Natural dye, practical guide, red dragon fruit, staining prepared slide.

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INTRODUCTION

Biology is natural science that involves study of living organism including molecular level until organism level. In biology student also discusses a lab activity in the laboratory, such as how to observe the anatomy of plant tissue under microscope. Some cells or tissues of plants are translucent, because they have little or no color pigment in their cells. This will not be able to observe under microscope, because these cell components are not able to absorb and refract light. As a result staining process on plant tissue slide needed to facilitate the observation.

Synthetic dye for tissue staining, such as safranin-O and methylene blue (MB), is relatively used to facilitate the observation under microscope. However these dyes have negative effect to the human. Safranin-O, a cationic dye, is one of the harmful contaminant that has been found in pharmaceutical and textile manufacturing waste. It was reported that this contaminant is harmful for the human's health due to its negative effects on the skin (such as skin allergies), digestive system and

respiratory systems (Bayazit, 2014). As well as the MB that causes cardiovascular disorder, dizziness, fever, headache, skin problem and anemia (Patil & Shinde, 2016). The other study reported that MB potentially effect the central nervous system (Vutskits et al., 2008). MB is carcinogenic and very difficult to decompose (Patil & Shinde, 2016; Rafatullah, et al., 2010).

Consequently the use of harmless, cheap, non-toxic and ecofriendly stains has become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic ones. The alternative solution is by using natural dyes which are eco-friendly, non-toxic, non-carcinogenic and biodegradable (Bhuyan & Saikia, 2003; Deepali, et al., 2014). Plants are highly potential source of natural dyes, that primarily they are present in parts of the plant like root, bark, leaf, fruit, wood, seed, and flower (Bhuyan & Saikia, 2003; Egbujo, et al., 2008; Kamel & Najmaddin, 2016; Kumar, et al., 2015; Raheem, et al., 2015; Siva, 2007)

Although plants exhibit a wide range of colors, not all of these pigments can be used as dyes. Some do not dissolve in water, some cannot be adsorbed on to fibers, whereas others

fade when washed or exposed to the air or sunlight (Siva, 2007). Therefore the examination to choose the good dye is needed to be established. One such alternative material is peels of red dragon fruit (*Hylocereus costaricensis* Britt. Et R)

Dragon fruit is one of the cultivated plants in Indonesia. Dragon fruit basically has many benefits for the human body to increase metabolism process due to vitamin C richness and its minerals content (Corleone, 2017). However, people rarely utilize the dragon fruit peel that contains anthocyanin. Anthocyanin are flavonoid commonly found in flower petals, fruits, and leaves and produce orange, red, violet, and blue colors (Jackman, et al., 1987; Kong, et al., 2003). Anthocyanin were used fitfully as histological stain (Al-Tikritti & Walker, 1978; Kumar, et al., 2015; Shehu et al., 2012; Sridhara et al., 2016; Suebkhampet & Naimon, 2014) and plant anatomical stain (Deepak & Omman, 2013). Thus dragon fruit peel extract can be used as natural dye for staining plant tissue. Regarding to that situation, research on utilizing of dragon fruit peel extract as natural dye for plant tissue slide is expected to be used as practical guide in the laboratory. The practical guide can be used as a guide or reference before doing hands-on activity in laboratory.

METHOD

This research used qualitative descriptive experimental method to describe the quality of staining plant tissue slide using dragon fruit peel extract. The qualitative research using different concentration of red dragon fruit peel extract, namely: 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% with 3 repetitions.

Data collected by directly observing the object under microscope with a series of experiments systematically. Its main purpose is to prove the effectiveness of natural dyes and to find the right concentration that has good contrast and high affinity on its result. Data analysis was described by qualitative descriptive from material expert. There are 4 lecturers as material expert who will observe the data and validate the practical guide that developed. Validation was using questionnaire.

Criteria of slide clarity and the color contrast that has been stained are presented in Table 1 and Table 2.

Table 1. Criteria of slide clarity

| No. | Criteria | Indicator |
|-----|------------|---|
| 1 | Very clear | If the parts of tissue can be distinguished very clearly. |
| 2 | Clear | If the parts of tissue can be clearly distinguished. |
| 3 | Not clear | If the parts of tissue cannot be clearly distinguished. |

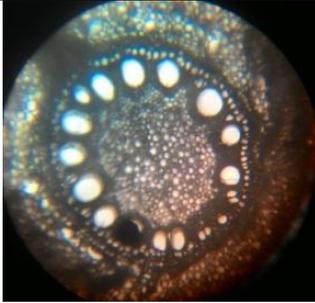
Table 2. Criteria of color contrast

| No. | Criteria | Indicator |
|-----|-----------------|---|
| 1 | Highly contrast | If the dye is only strongly bonded to a particular part of the tissue (not coloring all tissues). |
| 2 | Contrast | If the dye is only bonded to a particular part of the tissue (not coloring all tissues). |
| 3 | Lowly contrast | If the dye is tied to all the tissues (coloring all the tissues). |

RESULTS AND DISCUSSION

The study was conducted using natural dye from a dragon fruit peel extract to replace the safranin-O in observation of stem and root tissue of *Zea mays* L. under microscope at 100x magnification. The data of clarity slide and color contrast on *Zea mays* L. stem and root that has been stained with dragon fruit peel extract and safranin is presented in Table 3 and 4.

Table 3. Data of clarity slide and color contrast on *Zea mays* L. stem and root

| No. | Photos under microscope | Description |
|-----|--|--|
| 1. |  | Slide of <i>Zea mays</i> L. root with dragon fruit peel extract 30% staining |
| 2. |  | Slide of <i>Zea mays</i> L. root with dragon fruit peel extract 40% staining |

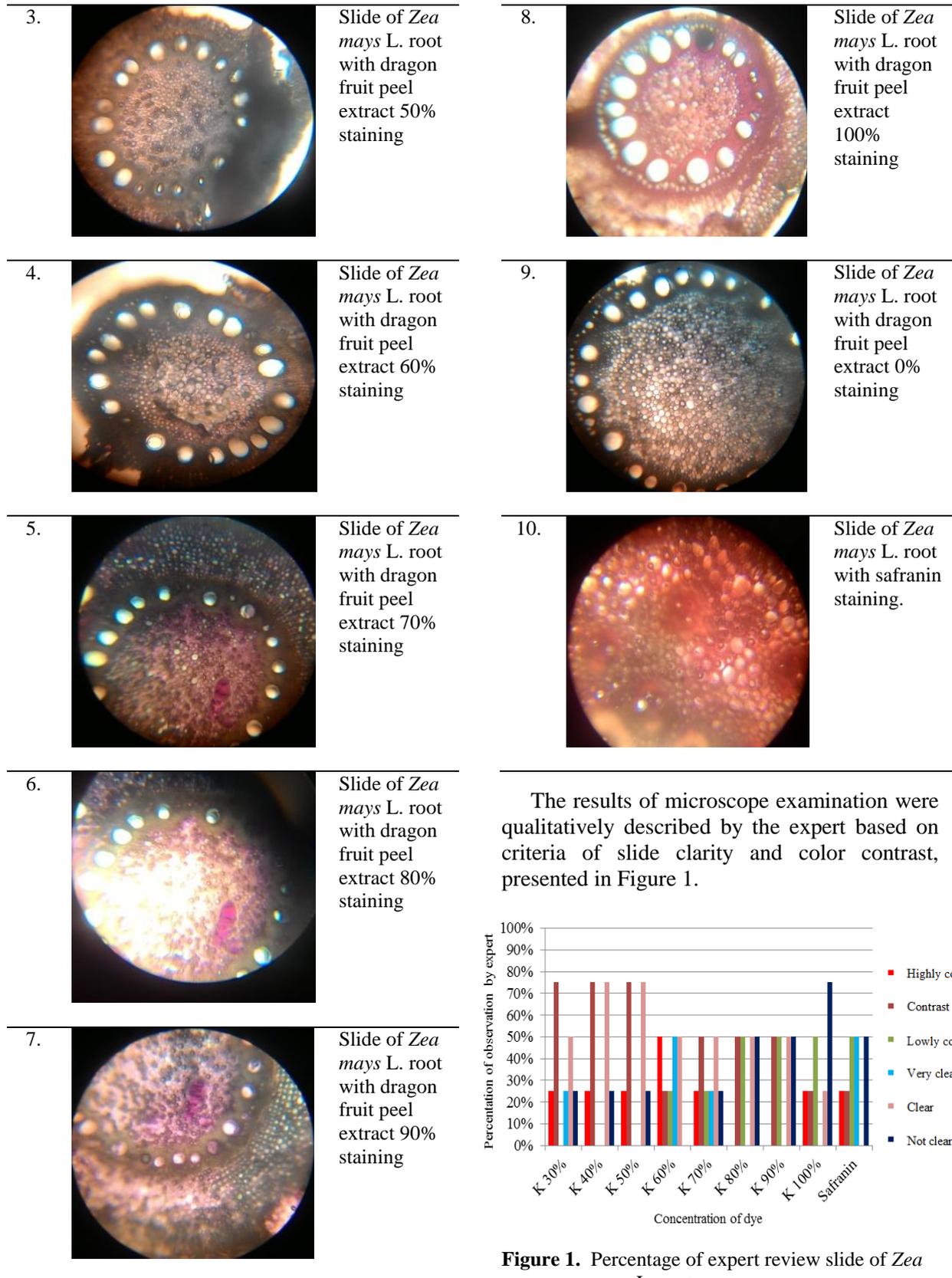


Figure 1. Percentage of expert review slide of *Zea mays* L. root

Based on the results dragon fruit peel extract can be used as staining in observation slide of plant tissue under microscope. The most effective concentration of dragon fruit peel extract is 60% which have higher percentage than the other concentrations. Utilizing dragon fruit peel extract as natural dye can distinguish the each part of root; include epidermal tissue, phloem, xylem, and cambium, presented in Figure 2.

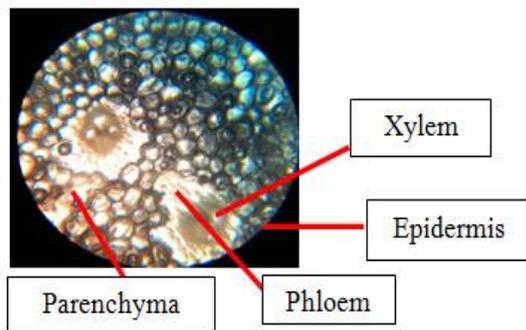


Figure 2. Slide of *Zea mays* L. stem with dragon fruit peel extract 60% staining

Additionally, dragon fruit peel extract staining of *Zea mays* L. root showed that the color can be absorbed and distinguish the endodermic tissue, stele, and transport tissue (xylem and phloem), presented in Figure 3.

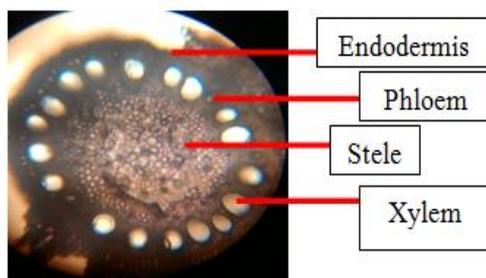


Figure 3. Slide of *Zea mays* L. root with dragon fruit peel extract 60% staining

Staining by using safranin make the part of tissue visible but cannot be distinguish because of the thickness. Staining of plant slide using safranin showed that stem and root tissue did not show any contrasting color because all parts of the tissue looked red with the same intensity of color absorption. It is making difficult to distinguish parts of the tissue. Whereas staining by using dragon fruit peel extract in the root and stem tissue facilitate the observation easily.

The questionnaire result of color contrast showed that the safranin make the cells more clearly than using of dragon fruit peel extract.

However, the result of staining with red dragon fruit peel extract is not much different from the dye using safranin as synthesis dyes. Staining will facilitate the observation of cells or tissues under a microscope, because the dyestuff (dyestuff) has a selective affinity to cell organelle (Gresby, 2013; Kumar, et al., 2015).

The staining process of plant tissue slide with red dragon fruit peel extract may occur due to an electrostatic bond reaction between the dyestuff charge and the different cell parts so that the plant tissue can be stained red. The resulting red dye is derived from the anthocyanin contained in red dragon fruit peel. Anthocyanin has an acidic pH can color cellulose in cell walls that have an alkaline pH. The positive ions in the dye will be released and bind covalently to the negative ions present on the cell wall of tissue (Chukwu et al., 2011; Nurwanti, et al., 2013). Natural dye of red dragon fruit peel extract can make contrast in slide so that the structure of tissue can be distinguished. Hence utilizing plant extract, like dragon fruit, as natural dye beside fitfully distinguished the structure of plant tissue slide, but also eco-friendly and non-hazardous for student (Chukwu et al., 2011; Deepak & Omman, 2013; Kamel & Najmaddin, 2016; Korade, et al., 2014; Raheem, et al., 2015; Shehu et al., 2012; Suebkhampt & Naimon, 2014).

Furthermore, the results obtained will be applied as a practical guide in senior high school. Based on questionnaire result by material expert on learning resources as practical guide showed that “very good” criteria (86.01%). Thus it can be concluded that the practical guide developed is feasible to be use in learning process, especially in structure and function of plant tissue material.

CONCLUSION

Based on the results and discussion it can be concluded that:

1. Red dragon fruit (*Hylocereus costaricensis* Britt. Et R) peel extract, concentration 60%, can be used as alternative dye in plant tissue slide preparation.
2. The research results can be used as a learning source as practical guide in senior high school, especially in structure and function of plant tissue material with “very good” criteria (86.01%).

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