BioEdu
Berkala Ilmiah Pendidikan Biologi
Vol.6 No.2 Mei 2017
ISSN: 2302-9528
http://ejournal.unesa.ac.id/index.php/bioedu

The Utilization of Betel Chewing Ingredients (*Nginang*) As an Alternative Dye in Microscopic Slide of Stem Tissues

Dinar Sukma Nirmala
Bachelor of International of Biology Education 2010, The Faculty of Science and Mathematics, Universitas Negeri Surabaya
Gedung C3 Lt.2 Jalan Ketintang, Surabaya 60231
Email: crush.91.girl@gmail.com

Rinie Pratiwi Puspitasari
Department of Biology, The Faculty of Science and Mathematics, Universitas Negeri Surabaya
Gedung C3 Lt.2 Jalan Ketintang, Surabaya 60231

Abstract

Biological concepts not only delivered theoretically, but also practically. One of them is microscopic slide. The damaged of microscopic slide is often caused by the lack of dye visibility. This damage can be solved by making new microscopic slide to support the biological learning using coloring materials which are easier to search, for example the dyes of chewing material. The study aimed to make microscopic slide with alternative dyes from chewing materials and to describe the feasibility of plant tissue preparations with the alternative dyes of betel chewing ingredients. This study involved the development of alternative dyes. There were three stages in this study, namely the manufacture of alternative dyes from betel chewing ingredients, making microscopic slide with paraffin method. Validation of the slide involved the relevancy between slide and the subject material, effectivity and absorption of the slides. The results of this research produced 12 of preparation incision transverse of the stem from 4 kinds of specimen (sirih belanda/*Scindapsus aureus,* keladi dua warna/*Caladium bicolor,* kamboja merah/*Adenium obesum,* kembang sepatu/*Hibiscus rosa-sinensis*). This study revealed that betel chewing ingredients is worth used to support observation criteria very reasonable with the percentage 88%-99%.

Keywords: Development of Dyes, Microscopic Slide, Paraffin Methods, Alternative Dye, Betel Chewing Ingredients

INTRODUCTION

Biology is a kind of science knowledge that studies about living things from the molecular to the organism level. The concepts that exist in biology are not only delivered theoretically, but also practically. The practicum activities can support students' understanding of a biology concept. The practicum activities can give students direct experience of the material, so that students will gain the optimum understanding and more meaningful (Rahmad, *et al*., 2011).

One of the materials that require practicum in biology learning is the structure and tissue of the plants material that contained in basic competence 4.3, that is "observing the structure of root, stem and leaf tissues of monocotyledon and dicotyledon using microscope and making relevancy between its structure and function." (Kemendikbud, 2013). Basic competence of observing the tissue structure using a microscope will be achieved when students do the observation directly. The observations of tissue are often carried out using tissues in dicotyledonae (dicots) and monocots. The observation of this tissue cannot be separated from the use of dye that aims to show gradations, differences and contrast of color on each tissue.

One of the ingredients needed in the media manufacture of this plant tissue preparation is the

dye. The dye has selective affinity ability for cells. The differences of the cell components also give the influences for the dye enters into the cell, so that each different tissue within a plant organ can be identified more easily (Budiono, 1992). Generally, the dyes that used in the manufacture of plant tissue preparation are fast green and safranin. The use of these synthetic dyes has several disadvantages. The synthesis dyes are not safe for students because in its manufacture used chemicals, and then its price is quite expensive and to get it is also quite difficult. The price of safranin in 25 grams' packaging is Rp 3.018.000,- (Edumedia, 2016) and we must order it first. This is one of the barriers for the school to make and update the preparations and their coloring substances. Basically the natural dyes have some advantages, such as much cheaper, easy to obtain, no need to use chemicals too excessive, environmentally friendly, easy to decompose, and easy to use and its application mainly in coloring plant tissue (Saidi, 2011).

The natural materials that can be used as alternative dye is filtrate of water mixture with betel leaf (*Piper betle* L.), areca nut (*Areca catechu* L.), gambier (*Uncaria gambir* R.) and lime betel ($Ca(OH)_2$). Generally, these three ingredients are used to be the betel chewing ingredients. The kind of this tradition has long been done by the people from Thailand by mixing these ingredients wrapped in betel leaves, then chewed for a few minutes so that it is in contact with the oral mucosa. The mixture of these ingredients will produce a reddish brown color after chewing and mixed with saliva. The color can be used to color the wood surface (Haerudin in Bogoriani, et al., 2009). The use of dyes from these ingredients mixture can be applied. Not only easy and cheap to obtain, these ingredients also safe because of its organic compound.

METHOD

This study was a development research to develop the slide of stem using betel chewing ingredients dyes as the learning media. The target in this study were stem slides with alternative dyes from the betel chewing ingredients such as betel leaf (*Piper betle* L.), gambier (*Uncaria gambir* R.), areca nut (*Areca catechu*) and lime water ($Ca(OH)_2$).

There were three stage of procedures in this study. The first stage was making alternative dye from betel chewing ingredients by taking the filtrate. The second stage was preparation step using paraffin method by making the cross section of stem. The third stage was the validation stage by the lecture of microtechnique, the lecture of plant anatomy and a biology teacher of high school. The criteria for the validation stage included the relevancy of the stem slide and subject matter, its effectiveness and safety, its ease of manufacture process, its availability of stem slide, the appearance of the stem slide, and the absorption of color on the plant tissue of stem slide.

The stem slide declared as feasible for the minimum score of validation reached for 61% (Riduwan, 2007)

RESULT AND DISCUSSIONS

This research produced microscopic slide from 4 kind of plant specimens. They were Sirih Belanda/*Scindapsus aureus,* Keladi Dua Warna/*Caladium bicolor,* Kamboja Merah/*Adenium obesum,* Kembang Sepatu/*Hibiscus rosa-sinensis*). Anatomical structure slide produced in this study were cross section of the stems which showed they difference of monocotyledons and dicotyledons.

The observed appearance of tissues were cell walls, parenchyme, schlerenchyme, cambium, xylem, phloem, and cytoplasm for dicotyledons. Whereas data for monocotyledons included the appearance of tissues observed were cell wall (epidermis), cortex parenchyme, pericycle, xylem, phloem, and cytoplas

The obtain data including appearance figures and validation results

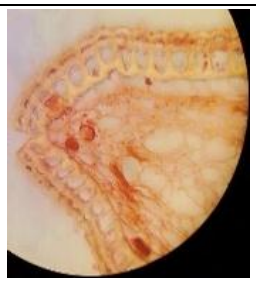
	Monocotyledons		Dicotyledons	
No.	***Scindapsus aureus* (Sirih Belanda)**	***Caladium bicolor* (Keladi dua warna)**	***Adenium obesum* (Kamboja merah)**	***Hibiscus rosa-sinensis*(kembang sepatu)**
1.				
	Color absorbency of betel chewing ingredients applied in *Scindapsus aureus* plant resulted in reddish orange color with 1000x magnification	Color absorbency of betel chewing ingredients applied in *Caladium bicolor* plant resulted in reddish orange color 1000x magnification	Color absorbency of betel chewing ingredients applied in *Adenium obesum* plant resulted in pale orange color with 1000x magnification	Color absorbency of betel chewing ingredients applied in *Hibiscus rosasinensis* resulted in reddish orange color with 1000x magnification

Table 1. Shows the microscopic appearance of four specimens observed

All of the criterias, namely the relevancy of the stem slide and subject matter, its effectiveness, safety and the ease of manufacture process, categorized as valid and completed. As in the first criteria, the observed stem slide for all specimens can be used by student to compare and identified its plant tissues. As in the second criteria, the observed stem slide was easy to carry, easy to store and safe because it did not contain harmful elements. As in the third criteria, the materials used were easy to be found from surrounding environment.

According to the data obtained from this study, all points showed that the results were varied. Most of the characteristics were valid and completed, but there were some incomplete characteristics as shown in Table 2;

Table 2 The Characteristics of Microscopic Slide when the proportion score < 1

Invalid Characteristic	Sirih Belanda	Keladi Dua Warna	Kamboja Merah	Kembang Sepatu
Physical appearance of stem slide	• **Characteristic 4.1**: 4.1.1(identity of slide, scientific name of plant used was present) to 4.1.5 (identity of slide, collector's name of plant used was present) showed that the characteristic was valid and completed • **Characteristic 4.3**: 4.3.2(No part of the tissue was shrunk in one field of view) showed that the condition of the slide was invalid and incomplete • **Characteristic 4.4**: 4.4.2 (No overlap of two layers of cell in the object) showed that the condition of the slide was invalid and incomplete	• **Characteristic 4.1**: 4.1.1(identity of slide, scientific name of plant used was present) to 4.1.5 (identity of slide, collector's name of plant used was present) showed that the characteristic was valid and completed • **Characteristic 4.3**: 4.3.2(No part of the tissue was shrunk in one field of view) and 4.3.3 (object appeared clear under microscope) showed that the condition of the slide was invalid and incomplete • **Characteristic 4.4 :** 4.4.1 (Every part of the tissue was clear) 4.4.2(No overlap of two layers of cell in the object) 4.4.3 (No overlapping tissue observed under microscope) showed that the condition of the slide was invalid and incomplete	• **Characteristic 4.1**: 4.1.1(identity of slide, scientific name of plant used was present) to 4.1.5 (identity of slide, collector's name of plant used was present) showed that the characteristic was valid and completed • **Characteristic 4.2 :** 4.2.3 (No bubbles formed around the sides of the object or disturbed the observation in one field of view) 4.2.4 (No bubbles formed on the outer part of the object or disturbed the observation in one field of view) showed that the condition of the slide was invalid and incomplete. • **Characteristic 4.3 : 4.3.2** (No part of the tissue was shrunk in one field of view) showed that the condition of the slide was invalid and incomplete. • **Characteristic 4.4** 4.4.2 (No overlap of two layers of cell in the object) 4.4.3 (No overlapping tissue observed under microscope) showed that the condition of the slide was invalid and incomplete.	• **Characteristic 4.1**: 4.1.1(identity of slide, scientific name of plant used was present) to 4.1.5 (identity of slide, collector's name of plant used was present) showed that the characteristic was valid and completed • **Characteristic 4.2 :** 4.2.3 (No bubbles formed around the sides of the object or disturbed the observation in one field of view) 4.2.4 (No bubbles formed on the outer part of the object or disturbed the observation in one field of view.) showed that the condition of the slide was invalid and incomplete. • **Characteristic 4.4** 4.4.2 (No overlap of two layers of cell in the object)
Color absorption of stem slide	**Characteristic 5.6** (Color was absorbed	**Characteristic 5.1** (Color was absorbed in	**Characteristic 5.4** (Color was absorbed in	**Characteristic 5.4** (Color was absorbed in

	in cytoplasm) showed that the condition of the slide was invalid and incomplete.	cell wall(epidermis)) to **5.6** (Color was absorbed in cytoplasm) showed that the condition of the slide was invalid and incomplete.	cambium) dan 5.7 (Color was absorbed in cytoplasm) showed that the condition of the slide was invalid and incomplete.	cambium) dan 5.7 (Color was absorbed in cytoplasm) showed that the condition of the slide was invalid and incomplete.

As seen in Table 2, some characteristics of the stem slide has proportion score < 1 were the physical appearance and color absorption of the observed stem slide. In physical appearance characteristic, which has proportion score < 1 included identity, air bubbles, condition of object in observation, and incision thickness, the average validation on all kind of plant tissue slide preparations scored less than 1. The data indicated that slide preparation of sirih belanda, keladi dua warna and kamboja merah experienced tissue shrinkage. This happened because almost all of the slide preparations of both monocotyledons and dicoltyledons plants in the observed specimen have wrinkled tissue. The wrinkled tissue could be caused by imperfect fixation process. Budiono (1992) stated that the purpose of the fixation was to keep cells resistant to solutions of different osmotic pressures. When given alcohol or xylol, there was difference in osmotic pressure that caused plasmolysis and tissue damage due to cell fluids coming out. If the fixation process did not carried out properly, the slide preparation might experience shrinkage. From the next criteria, which was thickness of the incision, average validation of both monocotyledons and dicotyledons scored less than 1 as shown in Table 4.3. The slide preparations made from all four plant specimen experienced overlap of cell layers. This was due to different techniques performed by practitioners so there were still cells that overlapped but this did not disturb the observation under microscope.

In the characteristic of color absorption, there were slides with average validation which scored less than 1 as shown in Table 2 above, those were devil's ivy, red adenium, and hibiscus. This was because in sirih belanda slide, the coloring agent was partially absorbed in the cytoplasm, then on red adenium and hibiscus plant, the color absorption was still difficult to be seen and identified because it was related to the cell structure and growth phase of the specimen. However, in other color absorption characteristics, the proportion of achievement scored 1, which meant that the slide preparations were able to help students to identify and compare several stem tissue organs contained in the observational specimen from color contrast absorbed in each tissue. This was indicated by the color difference in each tissue in the specimen. Differences in the absorption of colors in each plant are caused by the structure of each of the tissues and the effect of the coloring agent used. The absorption of color using the betel chewing ingredients could be absorbed into the cell wall of each tissue with different color contrast.

Coloring process involved several chemical bonds that caused the binding of color to the cell containing cellulose or lignin. For example, the presence of a bond on a hydroxyl group called a hydrogen bond. The binding of the hydroxyl group arose because of the attraction of the opposite charge because the ion charge in the coloring agent was usually positively charged (anion), whereas cellulose was negatively charged (cation) (Suheryanto, 2010). These caused the appearance of color on the tissue. When compared, safranin had brighter color contrast compared to betel chewing ingredients as coloring agent. This was because safranin was non-organic coloring agent, so the binding of color molecules with membrane and cell walls was stronger, in contrast to natural coloring agent. Suntoro (1983) stated that the coloring would facilitate the observation of cells or tissue under microscope, because coloring agent had selective affinity to cell organelles. Not all cell organelles were capable of reacting or binding completely with the one coloring agent, due to the different components of the structure and the characteristics of each cell organelle.

Live, thin-walled cells are cells whose main components contain cellulose. The tissue only had primary cell wall because it has not experienced lignification process yet (Santoso, et al 2007). Tissues with secondary thickening were consisted of dead cells and experienced lignifications. Cells with secondary thickening (lignifications) has good

ability to absorb coloring agent such as safranin (Budiono, 1992).

All produced stem slides have been observed and reviewed to obtain a high percentage. In addition to its ability to facilitate students in observing, identifying and comparing specimens, teachers could also make this preparation independently. This was indicated by the increase of technological advances and sophistication resulted in innovation by utilizing natural resources as material to make simple slide preparation. These preparations were easy to use as learning media for students. Biology teachers could also make these slide preparations because the coloring agent was easy to be found and natural, and this would lead to achieving learning objectives optimally in teaching and learning process.

All slide preparations in this study have been reviewed and stated valid and completed for its characteristics. The slide preparations could be used as teaching and learning media, in accordance with the demands on the applicable curriculum. Curriculum 2013 requires students to be able to learn actively (student centered activities), so that students are required to be skilled in conducting observation activities with plant tissue slide preparations using betel chewing ingredients as coloring agent.

CONCLUSION

Based on the results and discussion of this study, it could be concluded that filtrate obtained from betel chewing ingredients could be used as alternative coloring agent for microscopic slide of stem tissue. The stem slide developed in this research was feasible to be used in learning activities and could be used as learning media in delivering plant tissue material.

SUGGESTION

Upon the completion of this study, it can be suggested that (1) further study on endurance of the betel chewing ingredients as coloring agent is needed; (2) further study is also needed on knowing the proper procedure of tissue fixation to minimalize tissue shrinking; (3) further research is needed to find solvent for natural coloring agent which can be used for wet slide preparation so that it could be applied in classroom.

ACKNOWLEDGEMENT

We would like to express gratitude to the validators which have given precious critics and suggestions towards the production of this slide preparation media, those are Achmad Bashri, S.pd. M.Si and Herlin Wahyutri, S.Pd.

REFERENCES

Budiono, J.D. 1992. *Pembuatan Preparat Mikroskopis (Teori dan Praktek).* Surabaya : University Press IKIP Surabaya.

Bogoriani, N. W. dan Bawa Putra A.A. 2009. *Perbandingan Massa Optimum Campuran Pewarna Alami Pada Kayu Jenis Akasia (Acacia leucopholea)*. Jurusan Kimia FMIPA Udayana, Bukit Jimbaran.

Edumedia.2016. katalog online harga jual bahan kimia.(online). http://www.phyedumedia.com/2016/02/jual-bahan-kimia-laboratorium-sekolah.html?m=1 diakses tanggal 1 mei 2017

Kemendikbud, 2013. *Kurikulum 2013 Kompetensi Dasar Sekolah Menengah Atas (SMA)/ Madrasah Aliyah (MA). Kepala Badan Penelitian dan Pengembangan.*

Rahmad, T., Nuryani Y. R., Nana S.S dan Anna P. 2011. *Program Pembelajaran Praktikum Berbasis Kemampuan Generik (P3BKG) dan Profil Pencapaiannya.*

Riduwan. 2007. *Skala-skala Pengukuran Variabel-variabel Penelitian*. Bandung: Alfabeta

Saidi, Dzul Effendi Bin Mohd. 2011. *Serbuk akar kunyit (Curcuma domestica val) sebagai zat alternatif pada Histotenik*. Skripsi tidak diterbitkan. Medan: Universitas Sumatera Utara

Sass, J.E. 1958. Botanical Microtechnique. Ames: The lowa State Illege Press.

Suheryanto, Dwi. 2010. *Optimalisasi Celupan Ekstrak Daun Mangga Pada Kain Batik*

Katun Dengan Iring Kapur. Prosding seminar nasional rekayasa kimia proses. Semarang: Universitas Diponegoro

Suntoro, S. H. 1983. Metode Pewarnaan Histologi dan Histokimia. Jakarta: Bharatara Karya Aksara.