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Screening of Lipote (*Syzigium currannii*) pure fruit extract for use as cheek cell and onion cell stain

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Abstract

The effectiveness of lipote fruit (*Syzygium currannii*) pure extract for use in staining cheek cells and onion cells were studied to obtain non-toxic, environmentally friendly, and low-cost dyes for use in staining. *Syzygium currannii* is a small to medium-sized tree growing up to 14 meters tall. The fruit extract of Lipote has anthocyanins which are categorized as ternatins. A number of histological techniques have been identified to be used to provide a nuclear stain consisting of natural phenolic compounds, structurally related to anthocyanins. The objective of this study was to determine the effectiveness of lipote fruit extract on cheek cell and onion cell staining. Dye extracts from *Syzygium currannii* were used to stain cheek cells and onion cells using the existing standard staining procedures with little modification. The prepared extracts had an affinity for the cell membrane and nucleus. From the results of the Mann- Whitney U test, the stains had significant results at a P-value of 0.001413 as the highest. Therefore, this study shows that dye extracts from *Syzygium currannii* could be used for cheek cell and plant cell staining. Results of the stain on onion cells have the most significant result compared with the cheek cell stain results.

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Introduction

The diverse forms, sizes, and configurations of the cells reveal the tissue's complexity. The advantage of using stains to examine cells is that they disclose these and other details (Nguyen, 2017). Animal cells are best stained with Methylene blue while plant cells are best stained with iodine. In a normal science laboratory, cheek cells are used to give students a realistic view of an animal cell and onion cells on the other hand represent the plant cells.

Viewing either a cheek cell or an onion cell without any stain could be difficult especially if the microscope is not advanced. Some schools which do not have access to stains like methylene blue or iodine solution resort to viewing the cells without any dye or use. However, inhalation of iodine vapor could lead to eye, skin, nose, and throat irritation and Methylene blue is toxic when ingested and it causes irritation when in contact with the skin and eyes (Chew Weng *et al.*, 2014). Plant and fruit extracts are the potential source of natural dyes which yield different color intensities from yellow to red to black. Moreover, they pose no threat to the users and the environment since they are herbal and organic.

Syzygium currannii (C.B.Rob.) *Merr./Syzygium curani* or Lipote, Jambolan Plum, Baligang (Ilocano), Igot (Waray) (Fig. 1 is a small to medium-sized tree that can reach a height of 14 meters.. This fruit, though eaten as well as also made into wine by local people, is rarely cultivated. It is also not known much outside the Philippines. lipote is found in primary forests in the Philippines and at medium altitudes. The fruit, though somewhat dry, tastes quite pleasant. It is eaten fresh by the local people. Besides eating fresh, it is also preserved as pickle and jam. Lipote is also used for making wine and a beverage. It is known that several histological techniques which are used to provide a nuclear stain consist of natural phenolic compounds, structurally related to anthocyanins (Suebkhampet *et al.*, 2012). Anthocyanins from red cabbage and dahlia were also used as histological stains (Rosemary *et al.*, 2012). The aim of this study was to determine the effectiveness of lipote fruit extract on cheek and onion cells as a stain. Furthermore, this study also aimed to compare what type of cell the aforementioned dye extract is more prominent in staining.

Fig. 1. Lipote Fruit Specimen.

Fig. 2. Stain and stained cells slides set-up.

Material and methods

Study Area

The research was carried out at Chemistry Laboratory of the Science Unit within Leyte Normal University, between the months of April and May 2017.

Collection of Plant Materials

Lipote fruits were collected at the front yard of Isabel Abadies' residence at Brgy. 3, General MacArthur, Easter Samar. Leaves and fruits of lipote were brought to a Botany class of Leyte Normal University for further verification.

Preparation of Fruit Extract

With the aid of mortar and pestle, dye extracts were prepared by crushing and squeezing the fruit pulps of

lipote without using any solvent. At the end of each extraction procedure, the extracts were purified by a double filtration process. Primary filtration is achieved by passing the extract through filter wire mesh and subsequently with No. 2 Whatmann grade filter paper. The supernatant was then transferred into six (3) reagent bottles and replicates were labeled as R 1, R 2, and R 3 for both cheek cell staining and onion cell staining.

Staining Procedure

Cheek cells used in the experiments were obtained from the researchers by scraping the inner buccal area of the cheeks using wooden flat sticks; on the other hand, the source of specimen for the onion cells was from the ¼ kilo of onions bought from the city market. Three (3) replicates of dye extracts were prepared for cheek cell staining and another three (3) for onion cell staining. For cheek cell staining, the stains were administered to the prepared slides following the standard protocol of staining cheek cells.

Observation of all the prepared slides was made and evaluated microscopically using an Electron microscope with 160x and 640x magnification and their staining intensity was identified and the photographs of selected preparations were taken. The same procedures were applied to all specimens. As to the staining procedure applied for the onion cell staining, first, a medium-sized onion was cut laterally into 4 pieces with the help of a knife.

A scale leaf was removed from one of the pieces. With the use of forceps, the inner epidermal peel was then removed, gently in a way so as not to damage the plant tissue. The peel was then placed in a watch glass containing distilled water. In another watch glass, 5ml of lipote fruit extract was placed in it.

With the help of a small brush, the epidermal peel in distilled water was transferred into the lipote fruit extract for staining. The peel was stained for 5 minutes. After then, the epidermal peel was transferred back to the watch glass containing distilled water to remove extra stains sticking to the peel. The peel was transferred to the slide with the help of a small brush.

A coverslip was placed in such a way that no air bubbles enter it. The stained slides were then studied under an Electron microscope with 160x and 640x magnification and their staining intensity was identified and the photographs of selected preparations were taken. The same procedures were applied to all specimens.

Statistical Analysis

The Mann-Whitney U-test was used with the Statistical Package for Social Sciences version 15.0 statistical analysis software (which was first manufactured by SPSS inc. and then acquired by IBM in 2009). Pictures of stained cells were provided and were graded by selected 20 science mentors. Statistical analysis was done by calculating P value.

Grading of each stained cheek cell was done using the following criteria: 1- P (Poor) - Refers to the difficulty in appreciation of a particular tissue structure; 2- G (Good) - Refers to the sufficient appreciation of a particular tissue structure; 3- E (Excellent) - Refers to the fine appreciation of a particular tissue structure.

Results and discussion

After grading and examining the cells under the microscopes, results suggest that the nucleus and cell membrane of the cheek cells became clearer (Fig. 3 and 4). Comparing the control slide to the slides with stain reveals a strong difference in the view of the cells. Statistical analysis (Table 1 and 2) revealed that the overall staining ability of lipote fruit extract is better than the check cells and onion cells without lipote stain.

The critical value of U at $p < .05$ is 2. Therefore, the result is significant at $p < .05$. The p-value varies among the replicates but almost all the slides got significant results. The statistical outcome also reveals that the results of onion cell staining and cheek cell staining are significant from each other.

Table 1. Mann-Whitney U-Test Result on Lipote Pure Extract on Onion Cell.

Stain Replicate	Stained Slide Replicate	Excellent (3)	Good (2)	Poor (1)	P-value
Replicate 1	r1	9	11	0	0.000000
	r2	14	5	1	0.000000
	r3	18	2	0	0.000000
Replicate 2	r1	1	8	11	0.014912
	r2	18	2	0	0.000000
	r3	7	12	1	0.000000
Replicate 3	r1	9	5	6	0.000152
	r2	11	9	0	0.000000
	r3	19	1	0	0.000000

Table 2. Mann-Whitney U-Test Result on Lipote Pure Extract on Cheek Cell.

Stain Replicate	Stained Slide Replicate	Excellent (3)	Good (2)	Poor (1)	P-value
Replicate 1	r1	1	5	14	0.104588*
	r2	1	3	16	0.279251*
	r3	1	1	18	0.588506*
Replicate 2	r1	4	16	0	0.000000
	r2	4	15	1	0.000000
	r3	10	6	4	0.000015
Replicate 3	r1	0	18	2	0.000001
	r2	2	3	15	0.176214*
	r3	7	8	5	0.000050

Table 3. Mann-Whitney U-Test Result on the Comparison of Stained Cheek Cell and Onion Cell.

Stain Replicate	Stained Slide Replicate	P-value
Replicate 1	r1	0.000022
	r2	0.000005
	r3	0.000000
Replicate 2	r1	0.001413
	r2	0.000137
	r3	0.807656*
Replicate 3	r1	0.267407*
	r2	0.000020
	r3	0.000921

*p-value with insignificant result

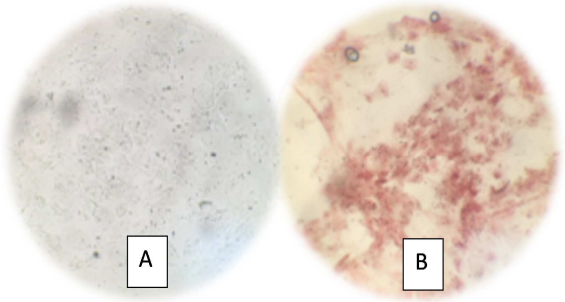

Fig. 3. Comparison of cheek cells with (B) and without (A) stain.

The fruits of *Syzygium currannii* are a rich source of anthocyanins (Santiago *et al.,* 2007) and the anti-microbial effect of the fruit extract of *Syzygium* species was variously tested in a wide range of microorganisms (Chacon, 1995). In staining cells, the concentration of anthocyanins may become so great that the pigment may separate as crystalline or amorphous form causing the anthocyanins to be absorbed by the materials inside the cell (Onslow, 2014). Anthocyanins also are structurally related to several potent intercalators that tend to bind to purines (Webb *et al.,* 2008).

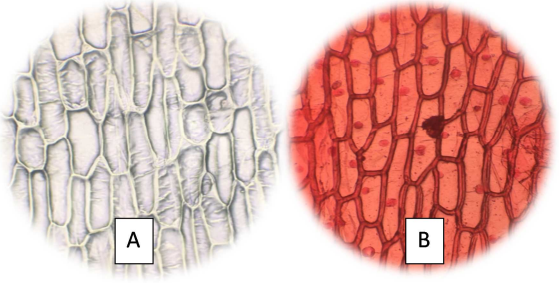

Fig. 4. Comparison of onion cells with (B) and without (A) stain.

Both DNA and RNA can act as strong effective co-pigments for natural anthocyanins (Khoo *et al.,* 2017).

The efficiency of fruits extract as a source of dye for staining plant sections was narrowly identified. The present findings on the competence of dye extract of *Syzygium currannii* recognized the fact that dye extract stain of *Syzygium currannii* could be successfully utilized for cheek cell staining.

In this study, no solvent was used for the extraction and thereby reduced the involvement of any chemicals in the microtechnique procedure. Due to worldwide concern against synthetic hazardous chemicals, a great momentum has been achieved favoring the use of cost-effective, eco-friendly, and biodegradable materials, the use of natural dyes has got much attention and interest among scientists (Macfoy, 2004). The recognition of the exact dye component in *Syzygium currannii* which can stain animal cells will open a way of research feature.

Conclusion and recommendation

The results suggested that fruit extract of lipote could be used to stain cheek cells and onion cells. Furthermore, the dye helped accentuate their cellular structures such as the cytoplasm, cell wall, and nucleus. However, the dye extract was evidently more prominent in onion cell staining. Nuclear staining of both specimens should be refined and enhanced, including adjustments of the staining protocols and staining conditions, especially for the cheek cells where staining was not as effective as with onion cells. Counter-staining with a secondary stain and using a mordant included in the fruit extract before staining may enhance the contrast and color hue of the tissues. Nonetheless, the dye extracts of lipote fruit which are domestically available and easy to prepare are apparently effective for cheek cell and onion cell staining and that it can be adopted in any science classroom that wants to appreciate simple staining procedures. Since the bearing of lipote fruits is seasonal, usually mid-year, preservation of extracts should be further investigated.

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