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Extraction, characterization, and workability of natural biological dye from local berries: A laboratory protocol for rural schools

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Key words: Characterization, Local berries, Natural biological dye, Protocol, Workability

Abstract

The application of natural biological dyes finds its use as an alternative to synthetic stains in temporary and permanent mounts. Extract of natural stains from peels of Lubeg (*Syzygium lineatum*), Bignay (*Antidesma bunius*), and Duhat (*Syzygium cumini*) fruits were made to test their staining potential on plant and animal tissues. The study utilized the Completely Randomized Design to compare the staining property of the peels. It used the Analysis of Variance (ANOVA) and Least Significant Differences (LSD) to ascertain the differences between the treatments. The natural biological dyes, in comparison with the synthetic dye, stained the cross-section of the Wiregrass and Narra stems, longitudinal section of an Autumn crocus, and hepatic tissues of a frog with remarkably deeper color. They are more effective in highlighting structures of a cross-section of a wiregrass stem and longitudinal section of an Autumn crocus, and they have a higher bulk density, which is an essential requirement of container volume. However, the synthetic dye shows higher absorbing capacity and solubility than the natural biological dyes. The success of producing a low-cost biological stain is achieved using Lubeg, Bignay, and Duhat fruits. The natural biological dyes used in this study could serve as a useful alternative to synthetic dyes.

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Introduction

The global demand for the application of natural biological dyes is increasing in the international market (Samanta & Agarwal, 2009) due to environmental issues related to the use of most of the synthetic dyes (Yusuf *et al.,* 2017). Research into new sources of natural dyes from plants has dramatically aided in widening the scope of biological dyes in science laboratories. The purpose of this is to develop useful, non-toxic, eco-friendly, cost-efficient dyes, and cleaner process technologies for use in plant and animal histology. Akinloye *et al.* (2010), for instance, examined the efficacies of some indigenous herbal dyes for use in staining plant materials.

The plant materials used for extraction of dyes in their work were the matured seeds of Annatto (Bixa Orellana), dried heartwood of Camwood *(Pterocarpus osun*), the rhizome of Turmeric (*Curcuma domestica*), and the young leaves of African indigo (Lonchocarpus cyanescens). Adeyemo *et al.* (2017) explored the staining potentials of four Nigerian plant extracts, which include Praying-hands (*Harungana madagascariensis*), African yellow wood (*Enantia chlorantha*), Ivory coast *(Sphenocentrum jollyanum*), and African Peach (*Sacrocephalus latifolius*) using selected bacteria. Deepali *et al.* (2014) studied six local plants, such as Henna (*Lowsonia inarmis*), Hibiscus (*Hibiscus rosa-sinensis*), Madder (Rubia tictorium L.), Fire flame bush (*Butea monosperma*), Rose (*Rosa indica*), and Bougainvillea *(Bougainvillea glabra*). They used their aqueous extracts for histological, fungal, and Paramecium staining.

Science laboratories heavily depend on chemical companies or foreign laboratories for adequate supply and are spending a lot on the purchase of biological dyes. Biological dyes are used to facilitate the microscopic examination of tissues, microorganisms, or other cells (Adeyemo *et al.,* 2017; Lyon, 2000). Dyes do not only provide for the higher resolution of internal structures of organisms, but they become more defined and render tissue and cellular constituents more visible through the color variations when viewed under a microscope (Sy & Wei, 2007).

While a staggering number of dyes and other compounds are available to chemical companies, in laboratories, particularly of rural high schools, dyes are difficult to secure and are expensive. Specific dye formulations and reagents are generally harmful and cause allergies to humans on exposure and produce toxic waste (Deepali *et al.,* 2014). Fortunately, there is now an increasing awareness among people towards natural dyes. Extracts obtained from natural sources such as animal and vegetable sources, plants, insects, and soil hold promise as a potential source of cheaper stains (Deepali *et al.,* 2014; Krizova, 2015). They are clinically safer than their synthetic analogs in handling and use because they are non-carcinogenic and have lower toxicity and better biodegradability than the synthetic ones (Saravanan *et al.,* 2013; Mitra & Das, 2015).

In the Philippines, particularly in Cagayan Valley, there are a large number of plants that yield stains. These plant species, which are also common in the locality, are found growing throughout the province. Among the plant species that have received much attention as a source of natural color for the food is Bignay (*Antidesma bunius*) fruit. Bignay fruit has been reported to contain anthocyanins and is accounted for the red coloration of the seed coat (Amelia *et al.,* 2013). Similarly, Lubeg (*Syzygium lineatum*) produces red to violet fruits as they ripen (Ocampo & Usita, 2015). The presence of flavonoids in the fruit is responsible for its vivid color (Panche, *et al*., 2016). Black plum (*Syzygium cumini*), which is known in the country as Duhat, is a source of natural dye. The fruits, which are edible and oblong berries, produce deep violet or bluish color with pinkish pulp (Suabjakyong *et al.,* 2011). These fruits are highly perishable. Converting a perishable substance into powder, before further utilization, increases its shelf life and enables it to be stored for an extended period without substantial loss of quality, even at ambient temperatures (Sharma, *et al*., 2012).

This study, therefore, explored the possibility of using the aforesaid local plants that are readily available, environmentally sustainable, and risk-free to the amateur microscopist, or ordinary students, or school teacher, as an essential alternative to potentially used synthetic dyes for temporary and permanent mounts. Specifically, it determined the acceptability of the synthetic and natural biological dyes as to color intensity and effectiveness in the clear tracing of plant and animal tissues, physical characteristics, and preliminary product cost.

Materials and methods

Experimental Design

The study made use of a single factor experiment in a completely randomized design wherein the control and three other treatments with three replicates and five samples per treatment were manipulated.

Statistical Analysis

Data on acceptability and physical characteristics were analyzed using analysis of variance (ANOVA) to determine whether the treatments show a significant difference from each other. Least significant difference (LSD) was used to ascertain differences between treatments at.01 and .05 levels of significance.

Evaluators of the Study

Twenty-five students from the Master of Science in Teaching Biology program and 25 from the Bachelor of Secondary Education who have taken Biotechniques subject served as evaluators of the study. They were purposely chosen due to their exposure and availability. They evaluated the finished product in terms of the variables of the study.

Extraction of Natural Biological Dyes

The powdered Lubeg, Bignay, and Duhat fruit pericarps were dissolved in 70 percent ethyl alcohol using a magnetic stirrer until the color of the stain diffused into the alcohol. Then the mixture was strained using a filter paper. The filtrate was stored in a glass bottle.

Standardization of Natural Biological Dyes

Various concentrations of dye powder (0.5g, 1.0g, 1.5g, and 2.0g) were weighed using an electronic digital weighing scale to standardize the natural biological dyes. The dye powder was dissolved in 10 ml of 70 percent ethyl alcohol. The concentration which provided the best result was used for staining tissue sections.

Preparation of a Leaf/Stem Cross Section

A leaf/stem was preserved in a 90 percent formaldehyde for 24 hours. A single cut across the leaf/stem in a smooth continuous sliding motion was made. The section was mounted after getting a good look at the specimen.

Preparation of Longitudinal Section of a Root

A root tip was preserved in the same way as the leaf/stem. The root was cut, making sure that the cut is thin, perpendicular to the length of the root beginning at the root tip, and moving towards the base of the root. The best sections were those in which all the cell walls were visible in focus, and no part of the section was crumpled or damaged.

Harvest of Muscle Tissue of a Frog

A frog was etherized. Then the muscle of interest (triceps femoris in this case) was carefully separated from the bones. The etherized muscle was excised to produce sufficiently thin slices of samples. In doing so, the detail of the microstructure of the tissue can be observed using a compound microscope.

Harvest of Visceral Tissue of a Frog

Histological investigation of the hepatic frog tissue was prepared for histology. The frog's liver was etherized. After which, it was sectioned thinly until the detail of the microstructure of the hepatic tissue can be examined under a compound microscope.

Preparation of Microscope Slide

A glass slide was placed on a flat surface. Then the specimen was positioned at the center of the glass slide. A drop of dye was added to the sample and left to dry to increase contrast. The sides of the coverslip were held. Then the bottom edge of the slide was laid on the slide close to the specimen. Keeping the coverslip at a 45^{o} angle will help. Slowly the coverslip was lowered so that it spreads the stain out.

If there were air bubbles (looking like little black doughnuts), the coverslip was pressed gently to move them to the edge, but if there were dry areas under the coverslip, a bit more stain was added at the side of the coverslip. Too much dye was dabbed off with a piece of paper towel. The specimen was cemented between the coverslip and the glass slide using clear glue.

Data Gathering Procedure

Color Intensity and Effectiveness

The color intensity and the effectiveness of the dyes in the clear tracing the plant and animal tissues were determined using weighted mean. They were interpreted using a nine-point Hedonic scale.

Physical Test

Physical test such as flowability, bulk density, solubility, and absorbency to tissues was conducted for the different biological dyes.

Flowability

One gram of each sample was poured in a funnel. The flow characteristic of the sample was observed. It was rated free-flowing if the powder drained quickly from the funnel in one second or less. It was rated cohesive if the powder poured moderately from the funnel without assistance. It was rated nonflowing if the powder was not able to drain entirely from the funnel in more than four seconds.

Bulk Density

Ten grams of the sample was weighed and placed in a 25-ml graduated cylinder. The graduated cylinder was tapped 100 times. The resulting volume that was occupied by the sample after tapping was noted. Bulk density was determined by taking the ratio of the weight of the volume of the sample remaining after tapping.

Solubility

One gram of powdered dye was dissolved in 10 ml 70% ethyl alcohol using a magnetic stirrer. Then the mixture was strained using a filter paper. The undissolved material was sun-dried, and the amount was weighed in an electronic digital weighing scale. Materials are treated as freely soluble if 1g of the content requires 1 to 10ml of solute to dissolve. The result was interpreted using the nine-point Hedonic scale.

Absorbency to Tissues

Absorbency to tissues is determined by the rate (in seconds) at which the tissues take in the dye.

Product Costing

The specific measurements of the materials in the study were the basis for the product cost. The calculation includes the amount of electric power that was consumed in producing the powder and the cost of labor.

Results and discussion

Comparison of the Mean Score on the Acceptability of Color Intensity of the Synthetic and Natural Biological Dyes

Cross-Section of a Monocot (Wiregrass, Aristida stricta) Stem

The statistical result of the differences in the acceptability of the color intensity of the four treatments shows that they vary significantly. Bignay dye, in comparison with the other natural dyes, obtained the highest mean of 8.88, which means that Bignay dye is versatile because it penetrates the cell structures of a Wiregrass stem. The synthetic dye, having the lowest mean among the treatments, produced a shade that is least acceptable to the raters. The natural biological dyes made the internal structures of Wiregrass stem more defined and rendered its cellular constituents with remarkably deep color suggesting its extreme affinity to the cell parts. Colors derived from natural sources tend to be bolder. The finding is in agreement with the statement of Cowley (2019) that synthetic dyes often look stark and garish. Some natural dyes and pigments may compete with synthetic dyes for quality and stability (Krisova, 2015).

Cross-Section of a Dicot (Narra, Pterocarpus indicus) Stem

Multiple comparisons among the treatment means reveal that there is a significant difference in the rates given.

Duhat dye was assessed as "extremely deep, while Bignay and Lubeg dyes were both assessed as "very deep." The synthetic dye, with the lowest mean but has the same descriptive value as the Bignay and Lubeg dyes, differs significantly from the other treatments. The color tint of Duhat surpassed the other stains in clear tracing the cross-section of a Narra stem. It can be deduced that Duhat dye is acidic because it stained the cytoplasm of the stem tissues well with deep blue-violet shade. This deduction on the strong attraction of Duhat for the cytoplasm is corroborated by the phytochemical analysis made by (Aqil *et al.*, 2012; Ayyanar & Subash-Babu, 2012) on *Syzygium cumini* fruit. Their findings reveal that the fruit contains different phytochemicals, including ellagic acid and anthocyanins, which are stable in acidic conditions.

Longitudinal section of a root (Autumn crocus, Colchicum autumnale)

Results reveal that Bignay dye has the highest affinity to the longitudinal section of the root of Autumn crocus. It surpassed the synthetic dye and the other natural biological dyes in elucidating the cellular components of the root. The result agrees with the finding of (Barcelo 2014) that the cyanidin-based anthocyanins in Bignay (*Antidesma bunius*), which give red pigments, are chromophoric and exhibit stability in acidic solutions. The Bigay powder, which is dissolved in ethyl alcohol, produces a weak acid solution. The use of weak organic acids may minimize the degradation of the anthocyanin pigments (Amelia *et al.*, 2013).

Muscle Tissue of a Frog

The utilization of synthetic and Bignay dye had no significant difference as regards their staining qualities. Both have comparable color intensity. The finding means that the color tint of Bignay dye is as good as the synthetic dye in the clear tracing the muscle tissues of a frog. In the study of Ajileye *et al.* (2015) on *Zingiber officinale* (Ginger) extract as a histological dye for muscle fibers and cytoplasm, they established that the staining reaction was observed to be similar to the synthetic dye, except for its greenish-yellow color. Furthermore, Bignay dye had a significantly higher affinity to the frog's muscle tissues than Lubeg and Duhat, which produced the same rating. Consideration may be due to the interaction or bonding between the Bignay dye molecule and the muscle tissues. Hence, the staining color of the muscle tissues is affected by the tissue medium.

Hepatic Tissue of a Frog

Synthetic and various natural dyes stain histological structures of the liver. The evaluation shows that Bignay dye stained the hepatic tissues with remarkably deep color as assessed by the raters, followed by Duhat dye but with the same descriptive value of "extremely deep." The synthetic and the Lubeg dyes received lower ratings. Yet, they are equally assessed as "very deep" In this study, the natural dyes were extracted by a weak-acid (neutral) solvent, and the pH of the medium did not affect the stain of Bignay and Duhat dyes making them very efficient in histological staining. The result is consistent with the study of Suabjakyong *et al.* (2011), which favored the optimal staining condition of the nucleus and cytoplasm of rat hepatic cells of the dried black plum dye extract over the fresh black plum extract and the synthetic dye.

The lower rating given to Lubeg dye coincides with the result of the phytochemical screening conducted by Manicad (2016) on the Lubeg species, mainly in the leaves and fruit extracts. Her findings showed that the fruits contain flavonoids and tannins. But tannins are only present in a small amount. Tannin, also called tannic acid, can cause a light yellow to light brown cast in water (Ashok & Upadhyaya, 2012). Thus, Lubeg dye creates weaker tint than the other dyes.

Table 1. Mean score on the color intensity of the synthetic and natural biological dyes.

Treatment	X-section of Monocot Stem	X-section of Dicot Stem	L-section of a Root	Muscle Tissue of a Frog	Liver Tissue of a Frog
1	6.80^{d}	7.50^{c}	7.92^{b}	8.30^{a}	8.10^{c}
2	7.96^{c}	8.06^{b}	7.86^{b}	7.66^{b}	7.92^{c}
3	8.88^{a}	8.08^{b}	8.38^{a}	8.38^{a}	8.74^{a}
4	8.38^{b}	8.66^{a}	8.10^{b}	7.78^{b}	8.58^{b}

Means followed by the same letter are not significantly different from each other at P≤0.05.

Treatments: 1 - Control (synthetic dye), 2 - Lubeg powder, 3 - Bignay powder, 4 – Duhat powder

Range of scales: Color intensity: 1 – extremely light, 9 – extremely deep

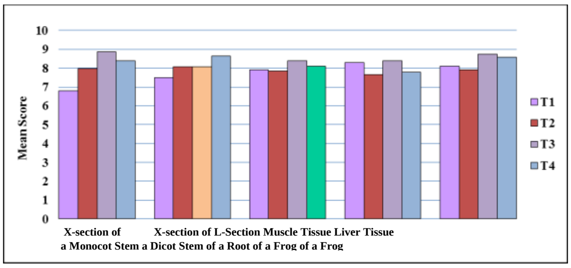

Fig. 1. Comparison of the mean scores of the four treatments on color intensity.

Fig. 2. A cross-section of a Wiregrass *(Aristida stricta*) stem stained with synthetic and natural dyes.

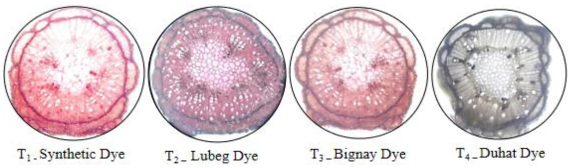

Fig. 3. A cross-section of a Narra (*Pterocarpus indicus)* stem stained with synthetic and natural dyes.

Fig. 4. A longitudinal-section of an Autumn crocus (*Colchicum autumnale*) root stained with synthetic and natural dyes.

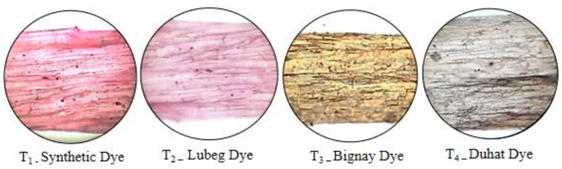

Fig. 5. Muscle tissue of a frog stained with synthetic and natural dyes.

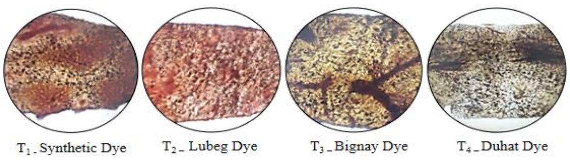

Fig. 6. Hepatic tissue of a frog stained with synthetic and natural dyes.

Comparison of the Mean Score on the Acceptability of the Effectiveness of the Synthetic and Natural Biological Dyes in Clear Tracing of Plant and Animal Tissues

Cross-Section of a Monocot (Wiregrass, Aristida stricta) Stem

It can be gleaned from Table 2 that the treatments gained different rates in terms of the effectiveness of biological stains on a cross-section of a Wiregrass stem. Bignay and Duhat dyes were the best in highlighting the structures of a stem. The Lubeg dye could also enhance contrast in the microscopic image, yet Bignay and Duhat dyes could sustain the image more as assessed by the raters. The synthetic dye was least preferred among the treatments. The finding justifies the report of Del Mundo *et al.* (2007) that natural stains are best known for their distinct property - the permanence of coloration. The stability of coloration is especially crucial for preparations that need considerable handling over some time. Besides, natural stains used in the study are more superior to synthetic dyes because they keep with and give a better glow to the specimen.

Cross-Section of a Dicot (Narra, Pterocarpus indicus) Stem

The difference of the treatment means reveals that an insignificant difference exists as regards the efficacy of the stains in the clear tracing the cross-section of a Narra stem despite the distinctions of the treatment means. The result is proven by the F value, which is less than .05 and .01 levels of significance. The result further implies that the synthetic dye is as useful as the natural biological dyes in creating contrast in the microscopic image of the cross-section of the Narra stem.

Longitudinal section of a root (Autumn crocus, Colchicum autumnale)

The trend of the effectiveness of the dyes in the clear tracing of the longitudinal root section was similar to the monocot stem cross-section. Bignay and Duhat dyes were typically efficient in examining the longitudinal section of the Autumn crocus root because they penetrated the cell structures and permanently bond with them. The color from the dyes, as mentioned earlier, tends to stay vibrant.

The more visible the color, the more effective it is. Practitioners of the craft of natural staining (i.e., using naturally occurring sources of dye) maintain that natural stains have a far superior aesthetic quality (Siva, 2007). The color produced by natural stains usually is soft, lustrous, and soothing to the human eye (Krizova, 2015).

Muscle Tissue of a Frog

The acceptability of the raters as regards the efficacy of the synthetic and natural biological dyes in clear tracing the muscle tissue of a frog does not significantly vary ($F < 0.05$ and 0.01). The result means that their perception along this parameter is not influenced by the dye used. Irrespective of the dye used, the result depicts that the efficacy of the dye remains the same.

Hepatic Tissue of a Frog

A comparison among the treatment means reveals that there is no significant difference in the rates given as $F < 0.05$ and 0.01 levels of significance. The staining ability of the dyes does not vary, which suggests that there is no chance for any inconsistencies in the efficacy of the stains in marking out the hepatic tissues of a frog.

Table 2. Mean score on the acceptability of the effectiveness of the synthetic and natural biological dyes in the clear tracing of plant and animal tissues.

Treatment	X-section of Monocot Stem	X-section of Dicot Stem	L-section of a Root	Muscle Tissue of a Frog	Liver Tissue of a Frog
1	6.86^{c}	7.90	7.56^{c}	8.40	8.10
2	8.12^{b}	8.10	8.06^{b}	8.08	8.08
3	8.82^{a}	8.18	8.44^{a}	8.48	8.38
4	8.56^{a}	8.28	8.40^{a}	8.26	8.28

Means followed by the same letter are not significantly different from each other at $P \leq 0.05$.

Treatments: 1 - Control (synthetic dye), 2 - Lubeg powder, 3 - Bignay powder, 4 - Duhat powder

Range of scales: Color intensity: 1 – extremely effective, 9 – extremely ineffective

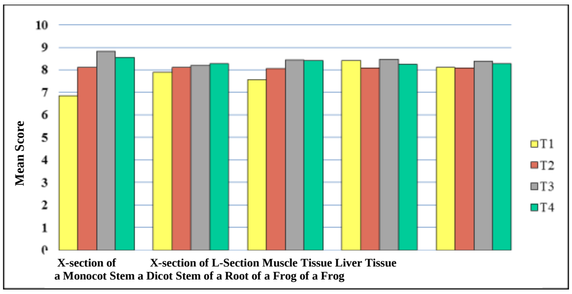

Fig. 2. Comparison of the mean scores of the four treatments on effectiveness in the clear tracing of plant and animal tissues.

Physical Test of the Natural Biological Dye

Flowability

The flowability of the dye powders was compared and evaluated to establish the relationships between them. The result of the testing method displays that T_2 (Lubeg powder) obtained the lowest mean, which means that it drained the quickest among the other dye powders because it has the most massive powder particle. Powders with larger particles have better flowability than powders with small particles. Powder flowability is dependent on particle size. It increases with an increase in particle size. Xu *et al.* (2018) find the same result as the present study. Hart (2015) likewise confirm that smaller size powder particles have higher tensile strength than larger particles, thus, making the powder more compact and showing lower flowability. However, a test on the equality of the treatment means reveals that the treatments do not significantly vary and can be surmised that there is no chance for any discrepancies. The insignificant relationship means that the flow characteristics of the dye powders are comparable.

Bulk Density

Result reveals that the bulk density of the treatments significantly varies. T_1 (synthetic dye), which has the smallest powder particle has the lowest bulk density, while T_2 (Lubeg powder), has the highest bulk density. Bulk density is primarily affected by the size and distribution of the powder particles. As could be expected, the bulk cohesion decreases as the particle size increases (up to 150 μm), owing to the decrease of inter-particle cohesion (Shi, 2018). Low bulk density, as affected by agglomeration, is an essential characteristic of instant powders (Barbosa-Cánovas & Juliano, 2005). But when powders are transported over long distances, they must have a high bulk density to reduce the volume. A high bulk density saves packaging material. It also decides the container volume, requirement of packaging materials, and selection of machinery for handling. Hence, it is more important both economically, and functionally.

Solubility

One of the most important properties that a biological dye must possess is solubility. As reflected in the table below, T_1 (synthetic dye) is "freely soluble." It significantly differs from natural dyes. It, being freely soluble, depends mainly on the nature of the surface of the particle. Multiple comparisons of the treatment mean of the natural dyes show that T_2 (Lubeg powder), T_3 (Bignay powder), and T_4 (Duhat powder) are equally soluble. The lower solubility level of the natural dyes is due to the insoluble fiber present in the peel powder. As reported by Wanlapa, (2015), the peels of many tropical fruits are a valuable source of dietary fiber. Dietary fiber is the insoluble non-starch polysaccharides (NSP) in plant foods (Englyst & Cummings (1988).

Absorbency to Tissues

The ability of the dyes to be absorbed varies. Results confirm that T_1 (synthetic dye) interacts with all the specimen very quickly in 9.33 seconds, making them stand out against the background. Results suggest that the synthetic dye is extraordinarily permeable and has an extreme affinity to the tissue sections because the synthetic dye typically serves as a positive dye. A positive dye directly binds with the tissue component to produce contrast (Staining Microscopic Specimens, n.d.). T_2 (Lubeg powder), T_3 (Bignay powder), and T_4 (Duhat powder) have the same response time of about three minutes to all the specimens. They are absorbed more slowly than synthetic dye. The result agrees with the finding of Kafle (2014) that a synthetic dye shows a higher absorbing capacity than a natural dye. The penetrability of the synthetic dye made it pretty well for staining tissues.

Table 3. Physical test of the Natural Biological Dye.

Treatment	Flowability (sec)	Bulk Density (g/ml)	Solubility (g/ml)	Absorbency to Tissues (sec)
I	1.77^{a}	0.48^{d}	0.000^{a}	9.33^{a}
2	1.23^{a}	0.68^{a}	0.030^{b}	170.00^{b}
3	1.27^{a}	0.54^{b}	0.029^{b}	173.33^{b}
4	1.33^{a}	053^{c}	0.025^{b}	181.67^{b}

Means followed by the same letter are not significantly different from each other at P≤0.05.

Treatments: 1 - Control (synthetic dye), 2 - Lubeg powder, 3 – Bignay powder, 4 - Duhat powder

Range of scales: Flowability: 1 – nonflowing, 5 – free-flowing

Solubility: 1 – insoluble, 5 – freely soluble

Relative Cost Estimation of the Natural Biological Dyes

The cost of the natural biological stains per treatment was computed to find out the reduction in the price of a 20 ml solution per treatment when compared with the synthetic dye.

Table 4. Cost estimation of biological dye

	Treatment 1 (control)	Treatment 2 (Lubeg fruit peel extract)	Treatment 3 (Bignay fruit peel extract)	Treatment 4 (Duhat Fruit peel extract)
Cost of 100 g stain powder				
Electric consumption	-	Php 1.00	Php 1.00	Php 1.00
Labor	-	1.88	6.25	6.25
Total	Php 1,000.00	Php 2.88	Php 7.25	Php 7.25
Cost of 10 ml stain solution				
Ethyl alcohol	Php 4.00	Php 4.00	Php 4.00	Php 4.00
Stain powder	1.00	0.03	0.03	0.03
Total	Php 5.00	Php 4.03	Php 4.03	Php 4.03

The cost of 100 grams of the synthetic dye is Php 1,000.00. In contrast, each preparation of 100 grams of natural stain powder of T_2 (Lubeg powder), T_3 (Bignay powder), and T_4 (Duhat fruit powder) worked out to be Php 2.88 each. The computation includes the labor cost of Php 1.88. The fruit peels are the discarded byproducts of the small-scale wine processing industry in Sanchez Mira, Cagayan Valley, Philippines. The cost difference of the natural biological dyes from the synthetic stain is Php 997.12.

In formulating a 10 ml stain solution, T_1 (synthetic dye) costs Php 5.00 since only 0.1 g is dissolved in 10 ml ethyl alcohol. The cost of each solution of T_2 (Bignay powder), T_3 (Lubeg powder), and T_4 (Dragon fruit powder) is around Php 4.03 centavos with one gram of natural powder dissolved in 10 ml ethyl alcohol.

Based on the cost analysis, the cost difference between the synthetic and the natural biological stain solutions is Php 0.97. Utilizing the natural biological stains would, therefore, reduce the cost of a synthetic dye by 20 percent.

Conclusion

Extracts obtained from Lubeg, Bignay, and Duhat fruit powder hold promise as a functional alternative to synthetic dyes, and they could serve as potential sources of cheaper biological dyes. They also have higher bulk density than the synthetic dye which is an essential criterion for selecting packaging material. However, the synthetic dye used in the study has superior fastness dyeing properties and is more soluble.

Recommendation

Due to the increasing demand in the application of natural materials for environmental sustainability across the globe, Lubeg (*Syzygium lineatum*), Bignay (*Antidesma bunius*), and Duhat (*Syzygium cumini*) powders are strongly recommended for use in biological and other laboratories. Further studies may be performed to increase the stability of the pigments present in the fruits. Scientific conservation, propagation, and cultivation of the species is recommended for sustainable use.

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