

Evaluation of Selected Natural Plant-Derived Dyes for Histological Staining Of Plant Tissues in Practical Biology Instruction

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ABSTRACT

This research work is concerned with Exploring the Potential of Zobo (*Hibiscus sabdariffa*), Henna (*Lawsonia inermis*) and Teak (*Tectona grandis*) as Natural plant dyes as a sustainable alternative to Synthetic dyes in teaching and learning practical Biology in Kogi State College of Education, Ankpa. Experimental and survey design was used. Three basic research questions were formulated and answered for the purpose of this research work. The population of the study comprises of all the academic staff of Biology Education department making a total of 14 respondents. Extract from the plants were used for the experiment and questionnaire was used to test the perception of lecturers on the use of plant based dye for practical Biology instructions. Data analyzed showed that that extracts can be used as a dye in place of synthetic dyes. Synthetic dye stains faster than the plants extracts when compared. The knowledge of using plant based dyes as an alternative to synthetic dye is limited as it has never been used in conducting plant histological practical in the department. It was recommended that the plant extracts should be used as a dye in place of synthetic dyes and experiment with different extraction methods to maximize colour yield and minimize waste should be carried out.

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INTRODUCTION

Histological staining is an essential practice in plant biology that facilitates the observation of structural features under the microscope. Staining helps in differentiating between various tissues such as xylem, phloem, parenchyma, and sclerenchyma by enhancing contrast and visibility (Abdullahi et al, 2022). Traditionally, synthetic dyes like safranin and fast green have been used in laboratory settings for this purpose. However, these synthetic chemicals often pose health risks, involve complex disposal procedures, and incur higher costs—particularly in resource-limited educational environments (Ajayi, 2020)

Given the growing awareness of sustainability and environmental protection, there is a renewed interest in utilizing naturally derived substances for scientific applications. In recent years, plant-derived dyes have shown potential as effective alternatives to synthetic counterparts. These natural dyes are not only biodegradable and non-toxic but also offer an excellent opportunity for contextual learning. Students can actively participate in the extraction and application processes, thereby deepening their understanding of both plant biology and scientific methodology (Alam and Rahman, 2021).

This study explores the effectiveness of selected natural plant-derived dyes for histological staining in practical biology instructions

The general purpose of this study is to explore the potential of some natural plant, Zobo (*Hibiscus sabdariffa*), Henna (*Lawsonia inermis*) and Teak (*Tectona grandis*) dye as a sustainable alternative to synthetic dye in teaching plant histology in Kogi State College of Education Ankpa. Specifically, the study intends to; Investigate the ability of Zobo (*Hibiscus sabdariffa*), Henna (*Lawsonia inermis*) and Teak (*Tectona grandis*) for plant histological staining,

compare synthetic dye to that of natural plants Zobo (*Hibiscus sabdariffa*), Henna (*Lawsonia inermis*) and Teak (*Tectona grandis*) and investigate if plant based dye have been explored in teaching and learning practical Biology.

Before the widespread use of synthetic dyes in the late 19th century, natural plant-based pigments were commonly employed for various staining purposes. Ancient cultures utilized dyes from indigo, turmeric, and beetroot for cosmetic, textile, and sometimes rudimentary scientific uses (Kadam and Salunkhe, 2019). Early biologists often relied on these natural pigments to observe plant and animal tissues under microscopes, although the inconsistency of results and lack of standardization limited their broader application. Synthetic dyes such as safranin, fast green, eosin, and methylene blue dominate histological practices due to their consistent results and well-established protocols

However, these dyes are typically petroleum-based and contain chemical compounds that are harmful if ingested, inhaled, or if they come into prolonged contact with skin (Egbonuand Nwafor, 2020). In addition to posing health hazards, synthetic dyes require specialized disposal methods to prevent environmental contamination. These limitations are particularly problematic in schools and colleges with limited access to safety infrastructure (Ogunlana and Okonkwo, 2021). Recent studies have revisited natural dyes as sustainable alternatives. For instance, curcumin, extracted from *Curcuma longa* (turmeric), is known for its bright yellow pigment and has shown effectiveness in staining xylem vessels (Sharma and Gupta, 2022)

Similarly, anthocyanins from *Hibiscus sabdariffa* yield vibrant red to purple hues depending on the pH of the medium, making them suitable for parenchyma staining. These natural dyes are renewable, easily extractable using

simple methods, and safe for student use (Yusuf and Bello, 2022)

Lawsonia inermis, commonly known as henna, is a perennial flowering shrub or small tree, widely distributed in tropical and subtropical regions. It grows up to 2–6 meters in height, bearing small, fragrant white or pink flowers, and elliptical leaves. Its leaves contain lawsone (2-hydroxy-1,4-naphthoquinone), a natural dye molecule widely used for cosmetic, textile, and medicinal purposes. Beyond dyeing, henna has antimicrobial, antioxidant, anti-inflammatory, and anticancer potential. Recent pharmacological research supports its use in wound healing, dermatological treatments, and as a natural colorant in biomedical applications (Khalid et al., 2022; Gupta et al., 2023 and Salomi et al., 2024).

Tectona grandis is a deciduous tree species closely related to the well-known teak (*Tectona grandis*). It is native to Myanmar, northern Thailand, and parts of India. The tree is valued for its durable, fine-grained timber used in furniture, shipbuilding, and construction. Ecologically, it thrives in moist deciduous forests and contributes to biodiversity by providing habitats and food for wildlife. Studies emphasize its importance in sustainable forestry and conservation efforts since overexploitation threatens natural populations. Phytochemical investigations have revealed bioactive compounds with antioxidant and antimicrobial activities, highlighting potential medicinal and economic value (Nirmal et al., 2021; Forest Research Institute, 2022).

Hibiscus sabdariffa is an annual or perennial shrub cultivated in tropical and subtropical regions. It is best known for its calyces, which are used in beverages, jams, herbal teas, and traditional medicine. The plant is rich in anthocyanins, flavonoids, organic acids, and ascorbic acid, contributing to its vibrant red color and potent bioactivity. Pharmacological research confirms its role in antihypertensive, hepatoprotective, antidiabetic, and anti-obesity activities. Roselle is also gaining attention in functional foods and nutraceutical industries due to its antioxidant and cardioprotective effects (Alhassan et al., 2022; Mohammed et al., 2023).

MATERIALS AND METHODS

The design for the study is an experimental and survey design which aims to explore the potential of some natural plant;

RESULTS AND DISCUSSION



Plate 1: *Hibiscus sabdariffa*



Plate 3: *Tectona grandis*

Neem (Azadiracta indica), *Zobo (Hibiscus sabdariffa)*, *Henna (Lawsonia inermis)* and *Teak (Tectona grandis)* dye as a sustainable alternative to synthetic dye in teaching plant histology in Kogi state college of education Ankpa and to find out to what extent has plants been used in histological staining

Area of the Study

The study was carried out in Ankpa local government area in Kogi State, Nigeria. The Local government headquarter is in Ankpa town, on the A233 highway in the west of the area at 7° 22' 14" 7° 37' 31" E / 7.37056° N 7.62528° E. It covers an area of 1200Km² and a population of 267,353 at the 2006 census. It is bounded on the East by Benue state, on the West by Dekina local government, on the north by Omala local government and on the south by Olamaboro local government in Kogi state.

Collection of Plants

The leaves and seed of the plants were collected within Ankpa metropolis, Teak and Henna leave. The leaves were dried under shade and the dried leaves were pounded into powder and kept in a clean dried bottle.

Method of Extraction

Zobo leaf was boiled and filtered to get the extract.

100g of both powders of Teak and Henna leaves were measured using weighing balance

100g measured was soaked in 250ml of distilled water for 24hours and filtered using whattman filter paper.

The filtered extract was kept in a clean bottle for the experiment

Experimental Procedure

Thin slices of Onion cell was placed in a petridish and stained with the dyes extracted from the plants and left for 3-4munites before it was placed on the slide for microscopic observation

Instrumentation for Data Collection

The instrument used was a questionnaire titled "Exploring natural plant-derived dyes for plant histological staining in practical biology instruction"



Plate 2: *Lawsonia inermis*

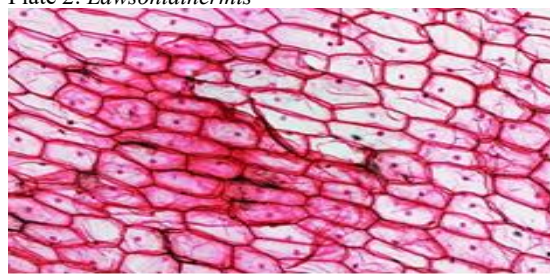


Plate 4: *Safranin dye*

Table 1 show that, the respondents accepted item 3, 7 and 8 and 6 was rejected with mean value of 2.00, 2.14, and 2.40. with mean of 3.28, 3.21 and 2.50 respectively. Item 1, 2, 4, 5 1.78 and 1.90

Table 1: Perception of Lecturers on Plant Based Dye and Synthetic Dye

S/N	ITEMS	SA	A	D	SD	$\bar{X}$	Remark
1.	I am familiar with natural plant-based dyes	2	2	6	4	2.14	Rejected
2.	I have used natural plant-based dyes in plant histology	1	3	7	3	2.14	Rejected
3.	I am aware of the environmental and health impacts of synthetic dyes	5	6	2	2	3.14	Accepted
4	I refer plant-based dyes over synthetic dyes for specific staining applications	4	-	8	2	2.40	Rejected
5	I have stained plant tissues with plant-based dyes	1	2	8	3	1.78	Rejected
6	I have extracted dyes from plants	-	2	8	4	1.90	Rejected
7	There is benefits associated with using natural plant-based dyes	5	6	2	1	3.07	Accepted
8	There is challenges when using natural plant-based dyes	4	3	3	4	2.50	Accepted

In table 2 below, respondents accepted item 1 and 3 with mean value of 2.85 and 3.35. Item 2, 4, 5 and 6 was rejected with mean value of 2.07, 2.40, 1.78 and 1.92 respectively.

Table 2: Perception of Lecturers on Staining Capacity of Plant-Based Dye and Synthetic Dye

S/N	ITEMS	SA	A	D	SD	$\bar{X}$	Remark
1.	Plant-based dyes can be compared to synthetic dyes in terms of staining quality	6	2	4	2	2.85	Accepted
2.	Plant-based dyes can be compared to synthetic dyes in terms of colour consistency	1	10	1	3	2.07	Rejected
3.	Plant-based dyes can be compared to synthetic dyes in terms of colourfastness	5	7	1	1	3.14	Accepted
4	Plant-based dyes can be compared to synthetic dyes in terms of Cost-effectiveness	4	-	8	2	2.40	Rejected
5	Plant-based dyes can be compared to synthetic dyes in terms of Environmental sustainability	1	2	8	3	1.78	Rejected
6	Plant-based dyes is preferred over synthetic dyes for specific staining applications	1	2	6	5	1.92	Rejected

In table 3 below, the respondents accepted item 1, 2 and 3 that synthetic dyes provide better contrast and resolution than natural plant-based dyes, natural dyes are less hazardous than synthetic dyes and that synthetic dyes are more accessed than natural dyes.

Table 3: Perception of Lecturers on Contrast and Resolution of Synthetic Dye and Plant-Based Dyes

S/N	ITEMS	SA	A	D	SD	$\bar{X}$	Remark
1.	synthetic dyes provide better contrast and resolution than natural plant-based dyes	4	8	1	1	3.07	Accepted
2.	Natural dyes are less hazardous than synthetic dyes	5	5	4	-	3.07	Accepted
3.	Synthetic dyes are more accessed than natural dyes	1	6	6	1	2.50	Accepted

Discussion

From research question one, it shows *Lawsonia inermis*, *Tectona grandis* and *Hibiscus sabdariffa* can be used as an alternative to synthetic dye in plant histological staining.

Colourfastness was observed in the plants dyes and the contrast was visibly seen. *Lawsonia inermis* dye retained colour longer than the dye from *Tectona grandis* and *Hibiscus sabdariffa*. *Lawsonia inermis* was observed to retain colour for 72 hours while *Tectona grandis* and *Hibiscus sabdariffa* retained colour for 24 hours. This work is in line with the work of Ihuma et al, (2017) who did methanolic Extracts from *Hibiscus Sabdariffa* line as a Biological Staining Agent and found to be effective.

In research question two, comparing plant-based dye to synthetic dye, it was found out that both synthetic and plant-based dye stained the cell showing clearly all the structures of the cell. The differences observed are the faster rate of absorption of the synthetic dye onto the cell. This is in alignment with the work of Hastuti and Haryatmi (2021) work on *Tectona grandis* as pharmacological stain found it to be effective for helminths.

Research question three is the perception of lecturers on the use of plant based dye in providing better contrast and resolution than synthetic dyes

Table 1 shows that respondents were not familiar with natural plant-based dyes, have not used natural plant-based dyes in plant histology, did not refer plant-based dyes over synthetic

dyes for specific, have not stained plant tissues with plant-based dyes and have extracted dyes from plants. This rejection can be attributed to the limited knowledge technical knowhow on the use of plant based dyes as an alternative to synthetic dye which are harmful to human and environment. This work complies with the work of Saxena and Raja (2014) and stated that hosts of plants can be used to replace synthetic dye both in textile, microbiological and medical specimens. Acceptance of environmental and health impacts of synthetic dyes, benefits associated with using natural plant-based dyes and challenges when using natural plant-based dyes might be a knowledge gotten from books or journals but not having practical experiences about. In this computer age many have head knowledge about so many things but not experientially. This work agreed with that of Cordon (2016) who pointed that hazardous properties of synthetic dyes are well documented in scientific research publications.

On the basis of staining capacity of plant based dye and synthetic dye, respondents accepted that Plant-based dyes can be compared to synthetic dyes in terms of staining quality and can be compared to synthetic dyes in terms of colourfastness. They rejected that plant-based dyes can be compared to synthetic dyes in terms of colour consistency, can be compared to synthetic dyes in terms of Cost-effectiveness, can be compared to synthetic dyes in terms of Environmental sustainability and plant-based dyes is preferred over synthetic dyes for specific staining applications. This rejection can be

attributed to the fact that they have not used plant based dye in plant histological staining in biology department as their knowledge about it is limited except for few. This agreed with the work of Sanjeeda and Taiyaba (2014) who pointed out that natural plant based dyes is well known in developed countries more than developing nations.

The respondents accepted that synthetic dyes provide better contrast and resolution than natural plant-based dyes, that Natural dyes are less hazardous than synthetic dyes and Synthetic dyes are more accessed than natural dyes. This is very clear indication that they are used to using synthetic dyes whereas the observation gotten from the experimental plants says otherwise. This is in line with the work of (Cordon, 2016 and Daberao, 2016) who described perception of teachers and students needs to be encouraged.

CONCLUSION

The study showed that the plants (Zobo, Henna and Teak leaves) extracts can be used as a dye in place of synthetic dyes. Synthetic dye stains faster than the plants extracts when compared. The knowledge of using plant based dyes as an alternative to synthetic dye is limited as it has never been used in conducting plant histological practical in the department. It was recommended that plant based dyes should be explored in histological plant staining, experiment with different extraction methods to maximize colour yield and minimize waste should be carried out to get more extracts from the plant for better dyeing, researchers should partner with local artisans to preserve traditional dyeing techniques and promote cultural heritage since the artisan are closer to the plants and Government and non-governmental organisations should organize educational and training programs on natural dyeing techniques and sustainability to improve teaching and learning.

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