



# **National Science Fair**

## **Research Paper**

Project ID	<b>NSF-SCH2025-65</b>
Project Title	<b>Dual Natural Staining of Plant and Cheek Cells Using Spinach Chlorophyll and Beetroot Betalains: A Safe Alternative for Elementary Microscopy</b>
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Category	<b>Life Science</b>
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# ABSTRACT

This project explores how natural colors from beetroot and spinach can be used together to see plant and animal cells more clearly under a microscope in comparison with methylene blue. Ethanolic extracts of beetroot and spinach were prepared and applied individually and together to the samples. The hypothesis of my study stated that the dual stain made from beetroot betalains and spinach chlorophyll would provide clearer contrast and better staining results than using either natural pigment alone.

Onion epidermis and human cheek cells were stained with each extract alone and with both together. The beetroot stain colored the cytoplasm and nuclei light red, while the spinach stain made the cytoplasm and chloroplasts green. When both stains were used together, the cells showed better contrast and details than with either stain alone.

My findings supported the hypothesis partially: beetroot extract gave red coloration mainly to the cytoplasm, spinach extract produced a light green tint, and the dual stain created stronger contrast, making cell walls, nuclei, and cytoplasm easier to observe. Compared to single stains, the combined stain enhanced clarity, especially in onion cells, where the two pigments highlighted different structures although advancements are needed for staining quality, to effectively stain cell structures, for proper adherence like adding buffer and fixatives, and improving the quality by using different concentrations

My study concluded that dual natural staining using beetroot and spinach is an effective, safe, and environmentally friendly method for basic cell microscopy. This approach can be used in classrooms as a simple, low-cost, and novel alternative to traditional chemical stains and also in future to develop enhanced staining quality for cultures in laboratory with proper advancements in effective staining.

## INTRODUCTION

Microscopes allow us to observe the tiny structures of plant and animal cells, but most cells are naturally transparent and colorless. To make the cell organelles easier to see, scientists use staining, a process in which dyes are applied to cells to increase contrast. Staining of cells is a fundamental step in microscopy that helps visualize cellular structures clearly. Traditionally, synthetic stains such as methylene blue, safranin, and iodine are used to highlight nuclei, cytoplasm, and other organelles. However, these synthetic stains may pose risks of chemical toxicity, environmental harm, and health hazards if mishandled. Plants contain many pigments that can act as natural dyes. Among these, betalains from beetroot (*Beta vulgaris*) and chlorophyll from spinach (*Spinacia oleracea*) are especially valuable.

- Betalains are water-soluble red and purple pigments found in beetroot. They easily dissolve in ethanol and can bind to certain cell structures, giving a reddish color that helps highlight the cytoplasm and other features.



- Chlorophyll, the green pigment responsible for photosynthesis, is abundant in spinach leaves. When extracted with ethanol, chlorophyll can act as a natural green stain and may bind to cell membranes and plant cell components, adding contrast. Natural pigments, on the other hand, provide a safe, eco-friendly, and cost-effective alternative. Chlorophyll (green pigment) extracted from spinach leaves and betalains (red-violet pigment) from beetroot are abundant, non-toxic, biodegradable, and easy to obtain.



These pigments can differentially stain plant and animal cells, making them ideal substitutes for elementary teaching laboratories. Although natural stains have been studied individually, combining two natural plant pigments as a dual staining technique is still uncommon and offers a novel approach for classroom microscopy. The idea behind dual staining is that each pigment highlights different parts of the cell, making the overall cellular image clearer. Beetroot provides strong red coloration, while spinach contributes a green tint, and together they may produce improved contrast compared to using either pigment alone. This study explores the use of spinach chlorophyll and beetroot betalains as dual natural stains for plant (onion epidermis) and animal (human cheek epithelium) cells.

## **PROBLEM STATEMENT**

Conventional synthetic stains are effective but not always safe for elementary-level students. Handling of chemicals requires training, and accidental ingestion/inhalation can be harmful. Schools with limited resources may face difficulty affording laboratory-grade stains. A safe, natural alternative is needed for effective classroom microscopy.

On the other hand, plants contain natural pigments such as beetroot betalains and spinach chlorophyll, which are safe, easily available, and eco-friendly. Individually, these pigments can stain cells, but very little work has been done using both pigments together as a dual natural stain. There is a need to explore whether combining these two natural dyes can produce better visibility and contrast of cells for school-level microscopy.

Thus, the problem selected is to investigate a safe, eco-friendly, and effective dual staining method using natural plant pigments for basic cell observation.

## **HYPOTHESIS:**

If we stain onion epidermis and cheek cells with beetroot extract and spinach extract, then the spinach will highlight green parts (chloroplasts / cytoplasm in plant cells) and the beetroot will provide red contrast (nuclei/starch) so cell parts are easier to see than with no stain and similar in clarity to methylene blue.

## **DESIGN OF STUDY:**

### **Variables:**

Independent: Type of stain used (spinach + beetroot combined, beetroot alone, spinach alone, methylene blue, and no stain).

Dependent: How well cell parts can be seen (score 0-3), number of visible cell parts (nucleus, cell wall, chloroplasts), clarity under 40x.

Controlled: Same type of sample, same staining time, same microscope settings.

## **PROCEDURE:**

### **A - Make the ethanolic Spinach extract Stain (adult helps)**

1. Wash and weigh 8-10 spinach leaves around 50g. Pat dry.
2. Put leaves in a small blender or mortar. Add 1 teaspoon water to Blend or crush for 30, 45 seconds until a green slurry forms. Add 100mL ethanol (1:2ratio) to the blended spinach stir thoroughly and filter using a filter paper in a conical flask. You now have a ethanolic spinach extract (green). Keep it overnight to get concentrated extract (24hours).
3. Label as "Spinach stain."

### **B - Make the ethanolic Beetroot Stain:**

1. If using fresh beetroot: grate a small piece and press through a strainer, or use a blender with a little water to extract juice. Filter after adding 100ml ethanol (1:2 ratio).
2. Label as "Beet stain".

## **C— Prepare microscopic slides**

### **1. Onion epidermis (plant cells):**

Peel a thin transparent layer from an onion inner surface and place on slide.

### **2. Cheek smear (animal cells):**

A child gently rubs inside cheek with a sterile cotton swab; adult helps smear it thinly onto a slide. Air dry and heat-fix quickly by passing (briefly) over the top of an adult's toaster or by using methanol for teacher use only. (Keep this step supervised.)

## **D — Staining groups to prepare (each slide = one treatment)**

To Make at least these slides for comparison:

- A1: Onion + no stain (negative control)
- A2: Onion + ethanolic Spinach extract (30-60 seconds)
- A3: Onion + ethanolic Beet extract (30-60 seconds)
- A4: Onion + Spinach then Beet extract (spinach first 30 s, blot, then beet 30 s)
- A5: Onion + Methylene blue— positive control

Repeat the same five groups for cheek smear (B1-B5).

B1: Cheek smear + no stain (negative control)

B2: Cheek smear + spinach emulsion (30-60seconds)

B3: Cheek smear + beet juice (30-60seconds)

B4: Cheek smear spinach emulsion then beet juice (spinach emulsion first 30s then blot beet juice 30s)

BS: cheek smear+ methylene blue (positive control)

## **E - How to stain (simple timing)**

1. Place 1 drop of chosen stain on the sample; wait 30-60 seconds.
2. Gently blot with paper towel to remove extra stain or rinse with a tiny drop of water if beet stain is too strong.
3. then 40x.

Mount a coverslip and observe under microscope starting at 10x

## COLLECTION OF DATA:

### Grinding and preparation of stain



### Beetroot extract



### Spinach extract



**Mixing of ethanol to spinach extract**



**Mixing of ethanol to beetroot extract**



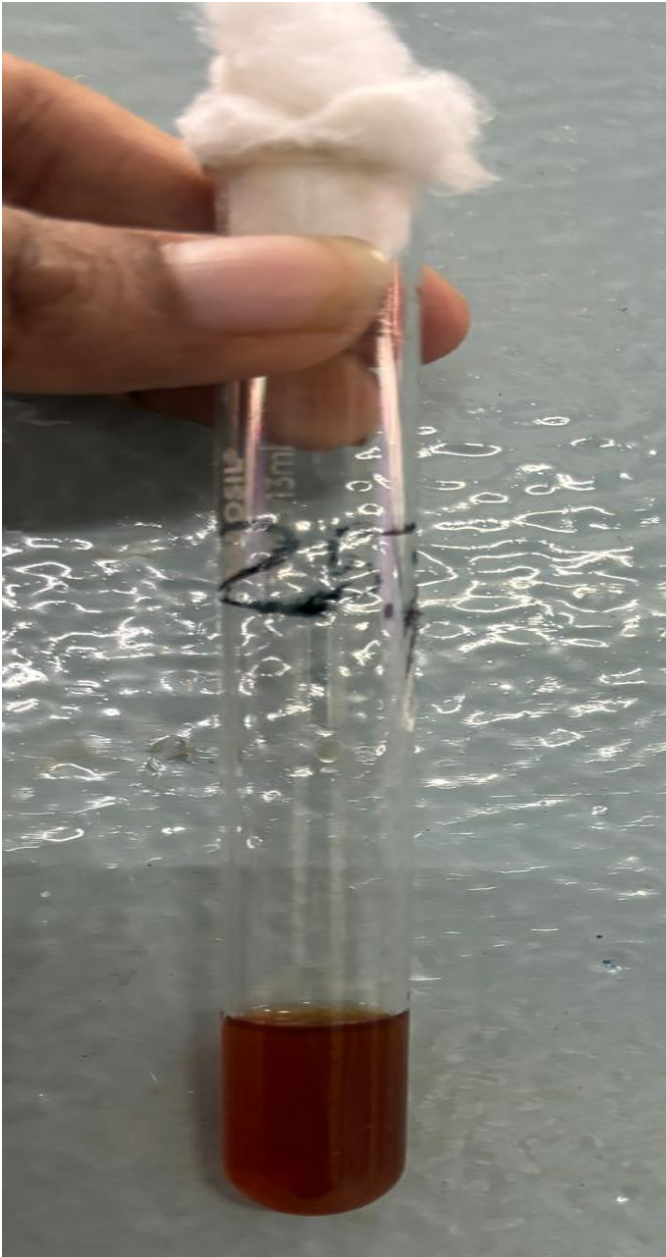
**Filtration of ethanolic extract of beetroot stain**



**Filtration of ethanolic extract of spinach stain**



**Ethanolic Beetroot stain**



**Ethanolic spinach stain**



## DATA ANALYSIS TABLE:

### Data Table for onion cells vs different stains

Slide ID	Sample	Stain	Stain time (s)	Nucleus Visibility (Y/N)	Cell wall Visibility (Y/N)	Clarity score (0-3)
A1	Onion	None	30s	No	No	0
A2	Onion	Spinach extract	30s	No	No	0
A3	Onion	Beetroot extract	30s	Yes	Yes	3
A4	Onion	Spinach then Beetroot extract	30s	Yes	Yes	3
A5	Onion	Methylene blue	30s	Yes	Yes	3

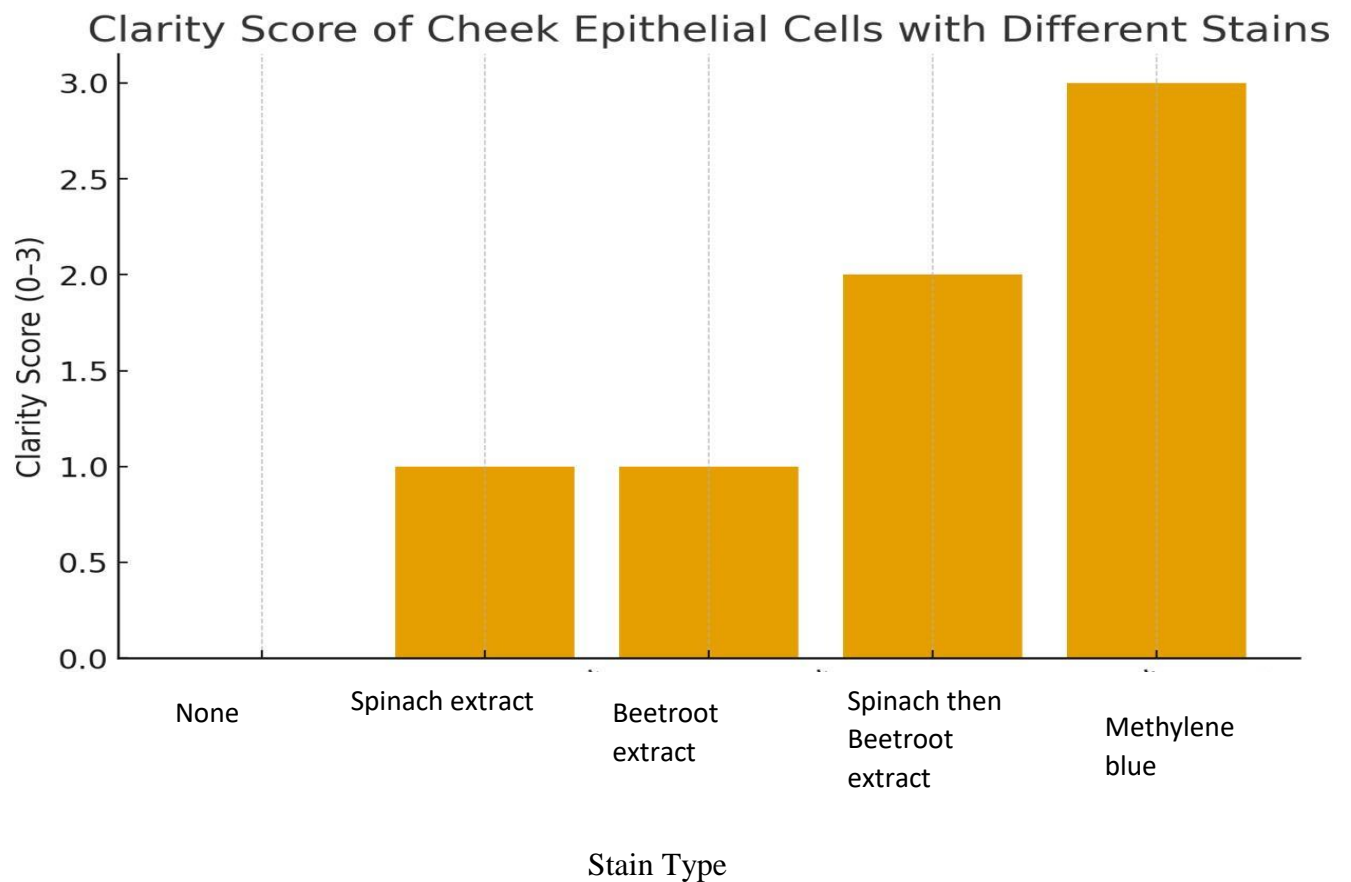
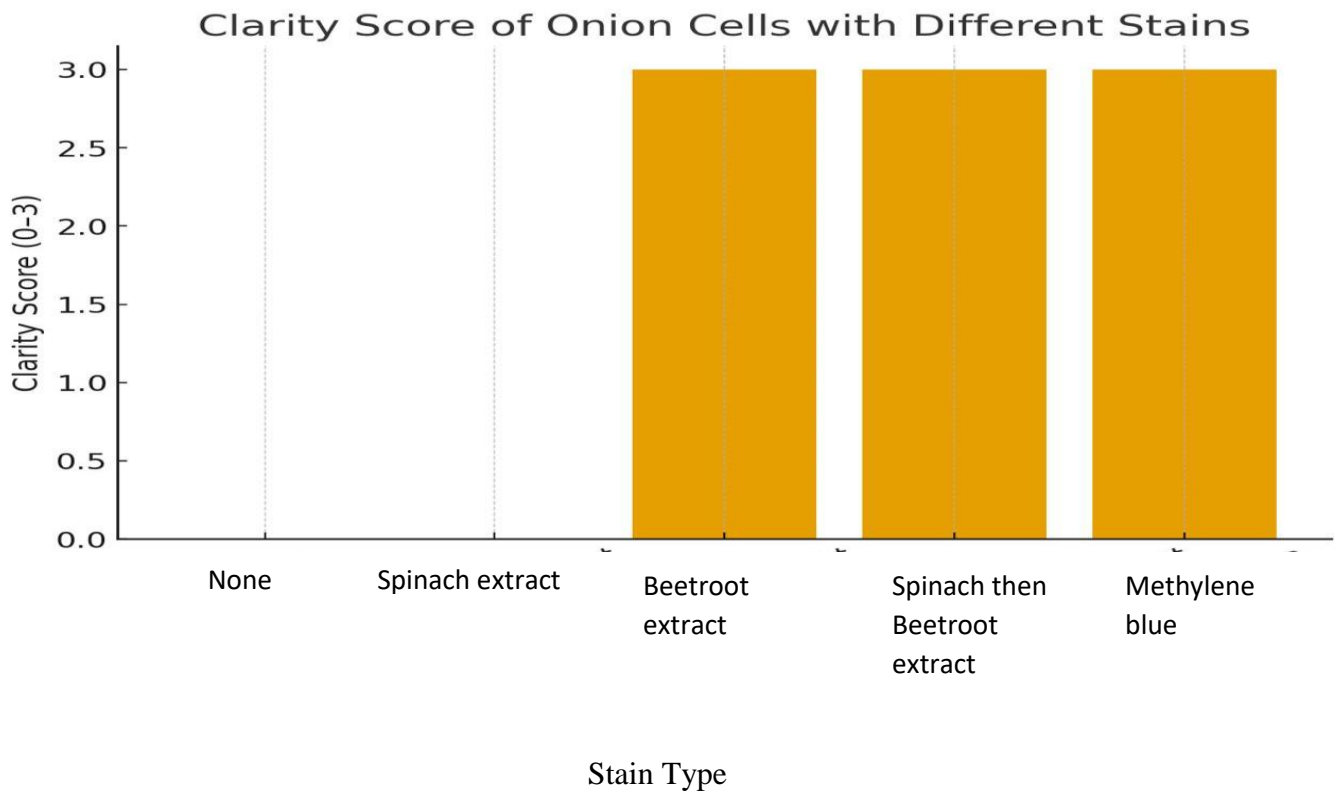
Score Key: 0 = Not visible, 1= Faint, 2= Clear, 3= Very Clear

### Data Table for Cheek Epithelial cell vs different stains

Slide ID	Sample	Stain	Stain time (s)	Nucleus Visibility (Y/N)	Cell Membrane Visibility (Y/N)	Clarity score (0-3)
B1	Cheek Epithelial cell	None	30s	No	No	0
B 2	Cheek Epithelial cell	Spinach extract	30s	No	Yes	1
B 3	Cheek Epithelial cell	Beetroot extract	30s	No	Yes	1
B 4	Cheek Epithelial cell	Spinach then Beetroot extract	30s	Yes	Yes	2
B 5	Cheek Epithelial cell	Methylene blue	30s	Yes	Yes	3

Score Key: 0 = Not visible, 1= Faint, 2= Clear, 3= Very Clear

# GRAPHICAL REPRESENTATION



## RESULTS

- Onion cells:
- Unstained slide (A1) showed no visible nucleus or cell wall (clarity score 0).

Spinach extract slide (A2) showed nearly visible cell wall but no nucleus visible (clarity score 1)

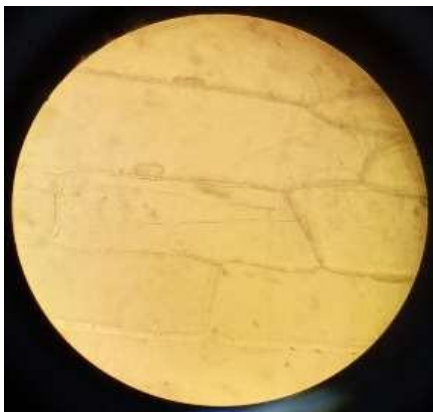


**Figure 1 onion-no stain**

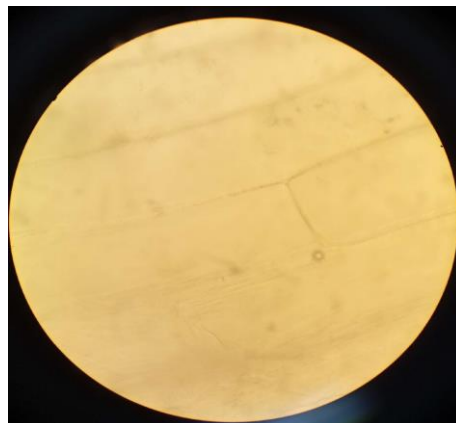


**Figure 2.onion(spinach)**

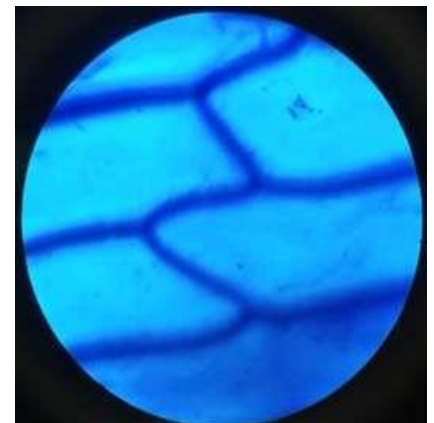
- Beet juice, spinach + beet, and methylene blue (A3–A5) showed clear and very clear visibility of nucleus and cell wall (score 3).



**Figure 3 onion –Beet extract**

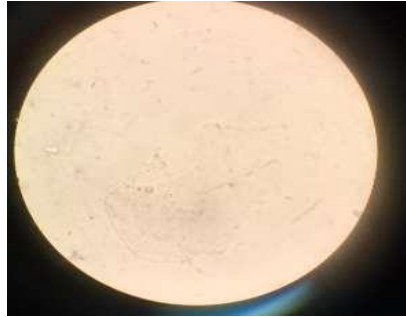


**Figure 4. onion (spin + beet) dual**



**Figure 5 onion-methylene blue**

- Thus, natural beet and spinach-beet combinations were as effective as methylene blue in staining onion cells.
- Cheek epithelial cells:
- No stain (B1) showed no visible structures (score 0).



**Figure 6 Cheek cells- No stain**

- Spinach emulsion and beet juice (B2–B3) gave faint visibility (score 1).



**Figure 7 Cheek cells – Spinach**



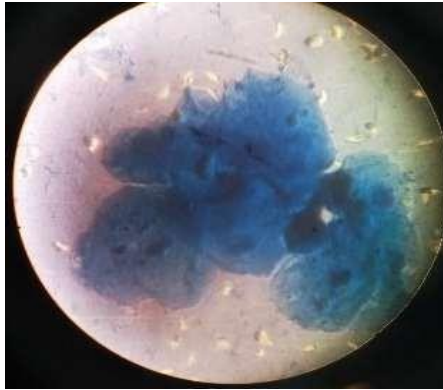
**Figure 8 Cheek cells-Beet**

- Spinach + beet combination (B4) improved clarity (score 2).



**Figure 9 Cheek cells-dual**

- Methylene blue (B5) provided very clear visibility (score 3).



**Figure 10** cheek cells-methylene blue

## **DISCUSSION**

- The clarity of the cells depends on how effectively the dye binds to cell components.
- Methylene blue, being a standard synthetic dye, provided the highest clarity for both plant and animal cells due to its strong affinity for acidic cell components like the nucleus.
- Beet juice, containing betalain pigments, showed high visibility in onion cells (which have a cell wall) because plant pigments interact well with plant cell materials.
- The spinach + beet mixture enhanced color intensity and penetration, suggesting pigment synergy.
- Spinach emulsion alone was least effective because chlorophyll and plant sap pigments are nonpolar and poorly bind to cellular structures.
- For cheek cells, beet and spinach dyes gave partial staining since animal cells lack a cell wall, and the pigments were less attracted to the cell membrane.

## **CONCLUSION:**

- Natural stains such as beet extract and spinach-beet mixtures can successfully substitute chemical stains like methylene blue, especially for plant cells (onion).
- For animal cells (cheek epithelial), methylene blue remains the most reliable stain.
- Using natural plant-based dyes is a sustainable and non-toxic alternative for basic microscopy, making them ideal for educational and eco-friendly experiments suggesting the hypothesis is partially true.

## **Future Advancements:**

### **1. Optimization of Natural Dye Concentration:**

Future studies can experiment with different concentrations and extraction methods of beetroot and spinach pigments to enhance stain absorption and uniformity.

### **2. Exploration of Other Plant-Based Pigments:**

Natural sources such as turmeric (curcumin), hibiscus (anthocyanins), and blue pea flowers (flavonoids) can be explored for improved cell contrast and specificity.

### **3. Eco-Friendly Fixatives and Buffers:**

Development of mild, biodegradable fixatives could improve pigment adherence while maintaining cell integrity for longer observation periods.

### **4. Long-Term Preservation Techniques:**

Formulating natural stains that allow permanent or semi-permanent slide storage without fading would make them more useful in classrooms and laboratories.

### **5. Spectral Analysis and Digital Microscopy:**

Integrating spectrophotometric analysis and digital imaging could quantify pigment staining intensity, allowing comparison with standard chemical dyes.

### **6. Cross-Disciplinary Applications:**

These eco-stains could be applied in microbiology, botany, and even textile or food industries where non-toxic, plant-based coloring is valued.

## REFERENCES:

### 1. *Udonkang et al. (2018)*

Spectrophotometry, Physiochemical Properties, and Histological Staining Potential of Aqueous and Ethanol Extracts of Beetroot on Various Tissues of an Albino Rat

Demonstrates that beetroot extracts can stain various tissue types (muscle, mucins, red blood cells, keratin, nerve fibers) and serve as eco-friendly alternatives to standard histological stains.

### 2. Studies on Betacyanins as Natural Histological Stains

Research exploring betacyanins (red/violet pigments from plants like beets) in histological staining, often in combination with hematoxylin, indicating emerging interest in such natural stains.

### 3. *Patni et al. (2020)*

Effect of Using Betalain, Anthocyanin and Chlorophyll Dyes Together as a Sensitizer in Dye-Sensitized Solar Cells

Although this study focuses on solar cell applications, it is significant for demonstrating simultaneous use of betalain (beetroot) and chlorophyll (spinach) in a mixed dye system.

### 4. *Lintilhac & Park (1966)*

Localization of Chlorophyll in Spinach Chloroplast Lamellae by Fluorescence Microscopy A foundational study showing how chlorophyll in spinach can be visualized under fluorescence microscopy, emphasizing its visualizable pigment properties.

### 5. Broader Context: Natural Dye Comparisons in Histology

Comparative studies evaluating beetroot (*Beta vulgaris*) alongside other natural dyes (lawsonia, turmeric, hibiscus) as potential cytoplasmic stains, though beetroot often showed lower contrast than some other candidates like henna.

## ACKNOWLEDGEMENT

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