National Science Fair Synopsis

Project ID	**NSF-SCH2025-65**
Project Title	**Dual Natural Staining of Plant and Cheek Cells Using Spinach Chlorophyll and Beetroot Betalains: A Safe Alternative for Elementary Microscopy**
Level	**Primary Level (IV-A)**
Category	**Life Science**
Participant Name	**E MOHAMMED YAHYA**
School	***i*MAX Nursery & Primary School, Royapettah**
City	**Chennai – 600 014**
State	**Tamil Nadu**

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 (alcohol) extracts were made from beetroot, which gives a red color, and spinach, which gives a green color. 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. The results show that using beetroot and spinach together can be a safe, eco-friendly, and effective natural way to stain cells for classroom study.

INTRODUCTION

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. 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.This study explores the use of spinach chlorophyll and beetroot betalains as dualnatural 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.

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)

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:

• Al: Onion + no stain (negative control)

• A2: Onion + Spinach emulsion (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.

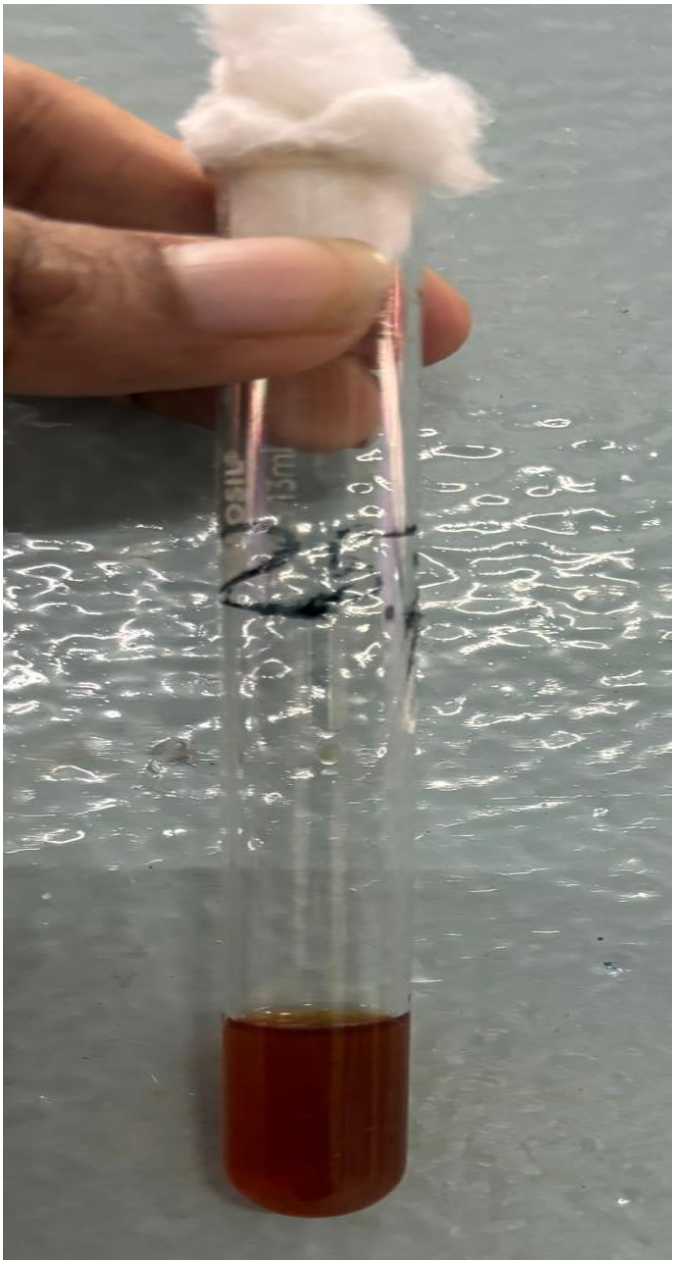
3. then 40x.

Mount a coverslip and observe under microscope starting at 10x

COLLECTION OF DATA:

Grinding and filtration of stain

Ethanolic Beetroot extract



Ethanolic spinach extract

DATA TABLE:

Data Table for onion 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 emulsion	**30s**	**No**	**No**	**0**
A3	Onion	Beet juice	**30s**	**Yes**	**Yes**	**3**
A4	Onion	Spinach then Beet	**30s**	**Yes**	**Yes**	**3**
A5	Onion	Methylene blue	**30s**	**Yes**	**Yes**	**3**

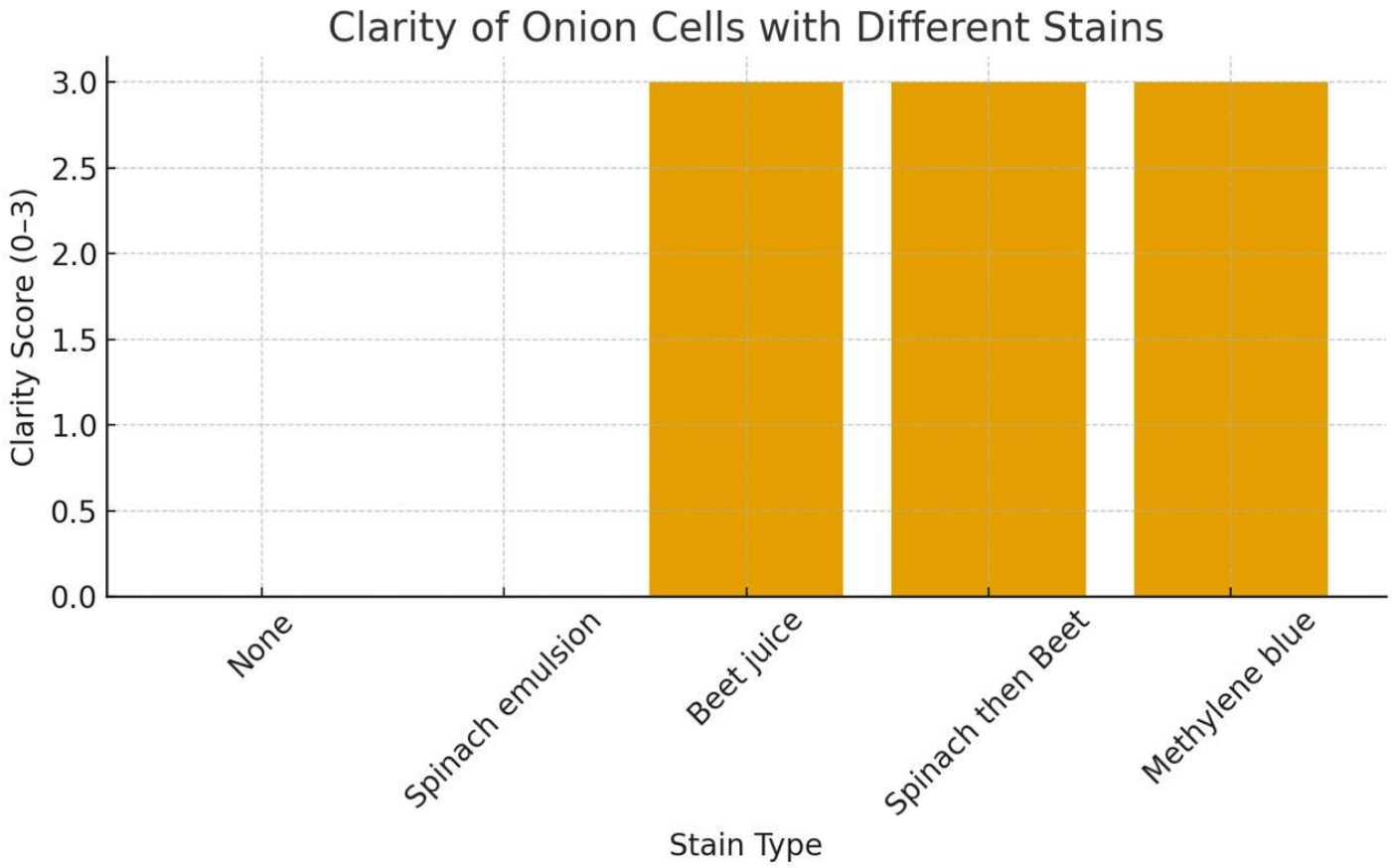
Score Key: 0 = Not visible, 1= Faint, 2= Clear, 3= Very Clear

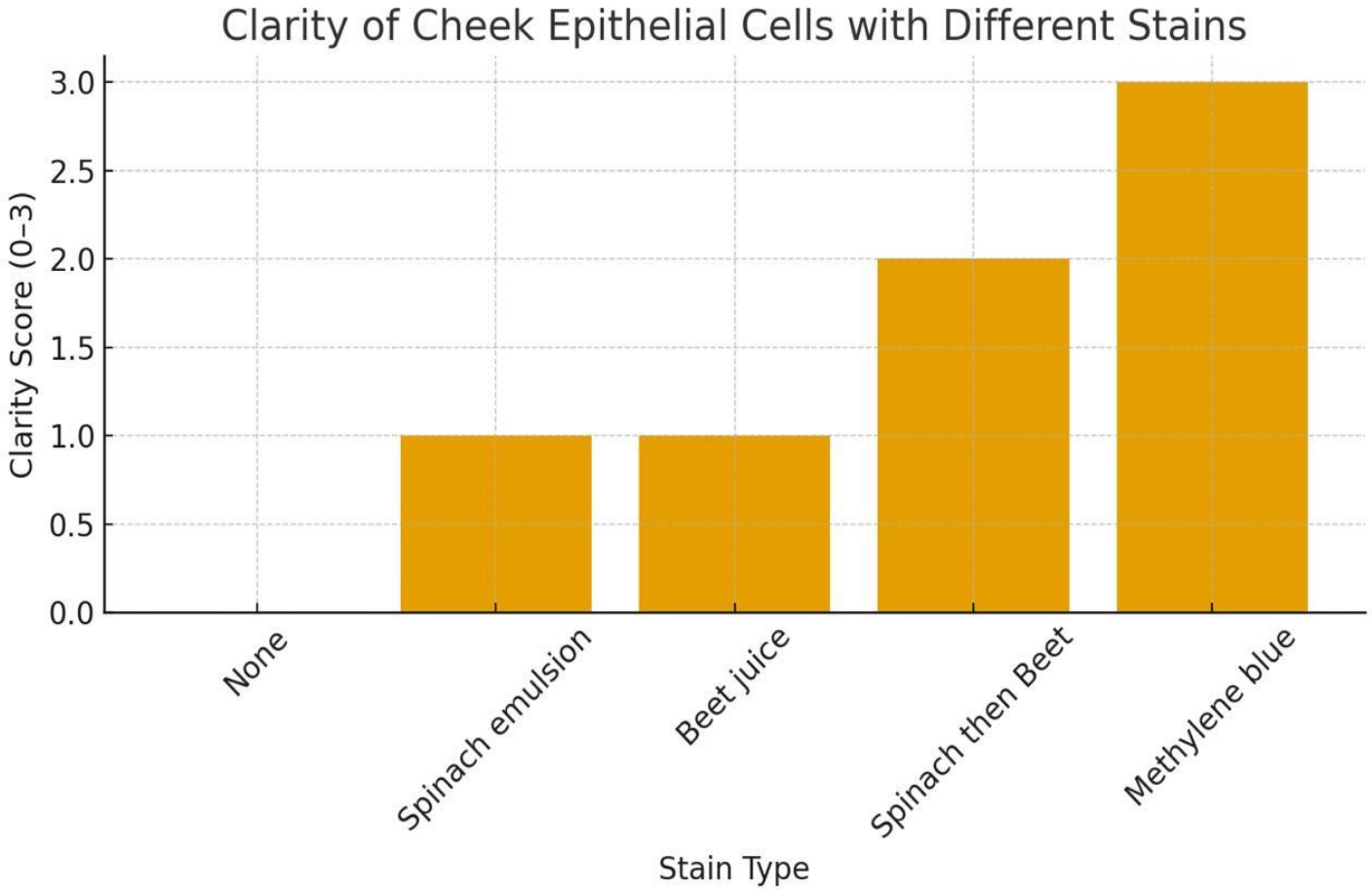
Data Table for Cheek Epithelial cell vs different stains

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 emulsion	**30s**	**No**	**Yes**	**1**
B 3	Cheek Epithelial cell	Beet juice	**30s**	**No**	**Yes**	**1**
B 4	Cheek Epithelial cell	Spinach then Beet	**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

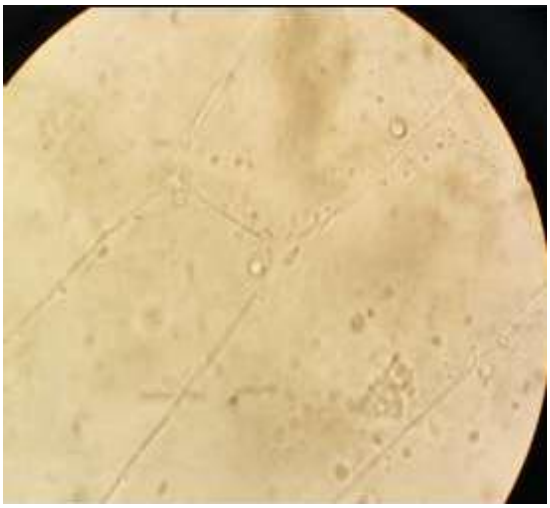


Figure 2.onion(spinach)

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

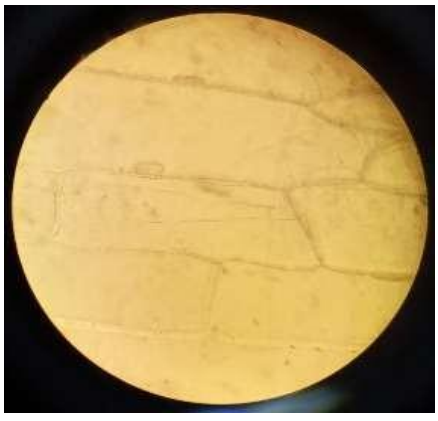


Figure 3 onion –Beet extract

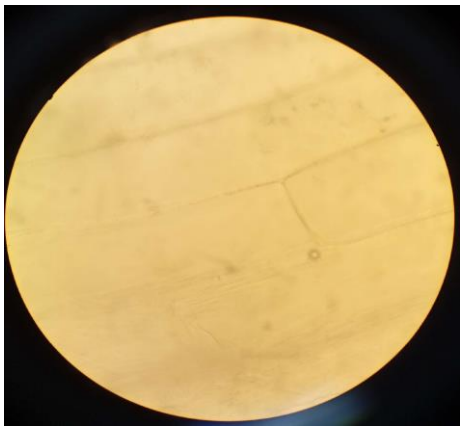


Figure 4. onion (spin + beet) dual

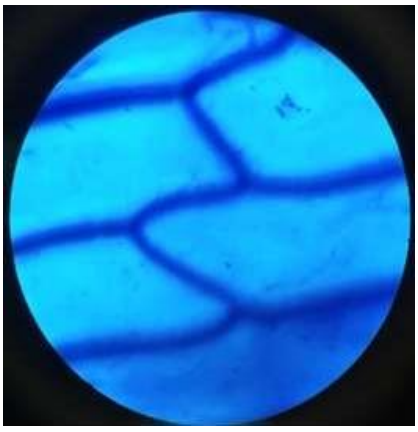


Figure 5 onion-methylene blue

- 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).

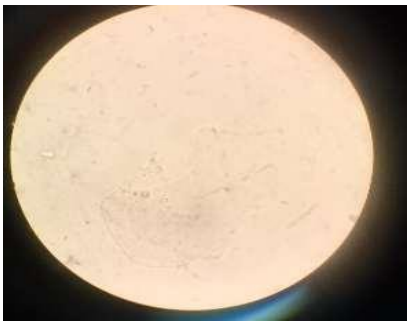


Figure 6 Cheek cells- No stain

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

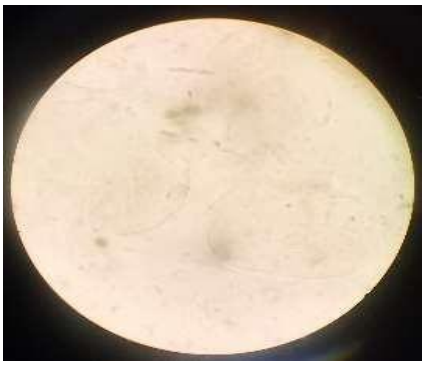


Figure 7 Cheek cells – Spinach

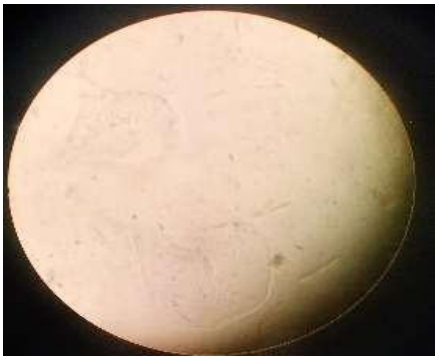


Figure 8 Cheek cells-Beet

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

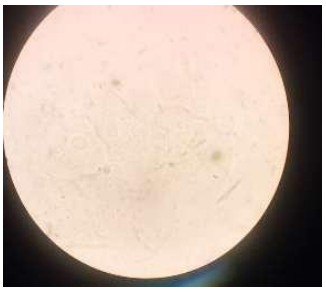


Figure 9 Cheek cells-dual

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

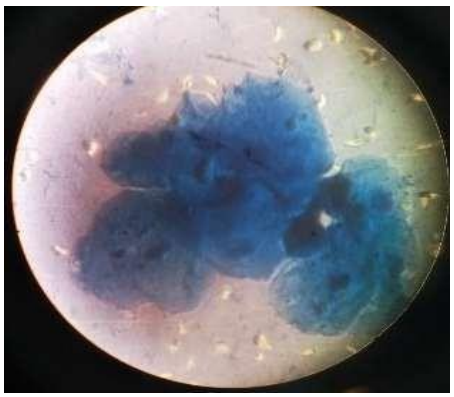


Figure 10 cheek cells-methylene blue

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 juice 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.

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.

REFERENCE:

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.