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# Effectiveness of Coleus blumei (Mayana) Leaf Crude Extract as an Alternative Staining Reagent for Urine Sediment Staining

Jeziel Khyl P. Ariamado, Mary Claire D. Busio, Angelito Miguel A. De Vera, Ross Alexandrei L. Fider, Janna Grace C. Gaa, Kristel Kaye A. Jiao, and Mary Jane E. Alba

**Abstract**

This study evaluated the effectiveness of Coleus blumei (Mayana) leaf crude extract as a potential alternative staining reagent for urine sediment staining. The study was conducted as an experimental laboratory investigation comparing Mayana leaf extracts prepared using 70% ethanol and 95% ethanol. The prepared stains were applied to urine sediment specimens and assessed based on selected staining criteria, including comparability to standard staining performance, stain stability, color enhancement, clarity of staining, cost-effectiveness, practicality, time efficiency, component differentiation, and recommendation potential. The findings showed that the 70% ethanol Mayana extract obtained a higher overall mean rating of 4.25 compared with the 95% ethanol extract, which obtained a mean rating of 2.25. The reported Mann-Whitney test result showed a p-value of 0.05, indicating a statistically significant difference between the two extract preparations at the 0.05 level of significance. The 70% ethanol extract performed better particularly in comparability to standard stains, stain stability, and color enhancement. The findings suggest that 70% ethanol may be a more suitable solvent concentration for preparing Mayana-based staining reagent for urine sediment observation. However, the study should be interpreted as a preliminary experimental evaluation, since full validation against Sternheimer-Malbin stain was not directly performed.

**Keywords:** *Coleus blumei; Mayana leaf extract; natural stain; urine sediment; ethanol extraction; laboratory staining*

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## 1. Introduction

Urine sediment examination remains an important component of routine urinalysis because it allows the microscopic observation of formed elements such as red blood cells, white blood cells, epithelial cells, casts, and crystals. These elements may provide useful clinical information in the assessment of urinary tract conditions, renal involvement, inflammation, infection, and other pathological processes. In standard clinical microscopy, staining reagents are often used to improve the visibility, contrast, and morphological differentiation of urine sediment components. Standardized urinary sediment examination requires careful preparation and interpretation because diagnostic usefulness depends on the accurate recognition of sediment elements (Japanese Association of Medical Technologists, 2017; Palaoro & Angerosa, 2014).

In response to concerns related to cost, access, chemical handling, and environmental disposal, natural plant-based dyes have gained attention as possible alternatives or adjuncts to synthetic staining reagents. Plant-derived

pigments are of interest because they may be locally available, biodegradable, cost-effective, and potentially safer for laboratory personnel and the environment. Anthocyanin-containing plant extracts have shown promise as biological staining materials when properly extracted and applied (Alshamar & Dapson, 2021).

*Coleus blumei*, commonly known in the Philippines as Mayana, is a widely available ornamental plant recognized for its highly pigmented leaves. Its coloration is associated with anthocyanins and other phytochemical constituents that may contribute to its staining potential. Because Mayana is locally abundant and visually rich in pigment, it presents a practical candidate for exploratory laboratory studies on natural staining reagents. Previous work on *Coleus blumei* dye extraction indicates that its pigments can be extracted using ethanol-based procedures (Maung Maung et al., 2018).

In urine sediment staining, the effectiveness of a reagent depends on its capacity to improve microscopic visibility without distorting the morphology of cells, casts, crystals, or other sediment elements. A natural stain that produces sufficient color enhancement and maintains structural clarity may be useful in teaching laboratories, preliminary microscopy work, or resource-limited settings. However, such alternatives must be evaluated carefully because staining performance may vary depending on extraction solvent, concentration, preparation stability, specimen type, and observation criteria.

The present study therefore examined the effectiveness of *Coleus blumei* (Mayana) leaf crude extract as an alternative staining reagent for urine sediment staining. Specifically, the study compared Mayana leaf extracts prepared using 70% ethanol and 95% ethanol and evaluated their staining performance based on selected criteria such as comparability to standard staining performance, stain stability, color enhancement, clarity of staining, cost-effectiveness, practicality, time efficiency, component differentiation, and recommendation potential. The study is best understood as a preliminary experimental evaluation of Mayana-based staining preparation, with emphasis on identifying which ethanol concentration produced better staining outcomes for urine sediment observation.

The study aimed to determine the effectiveness of *Coleus blumei* (Mayana) leaf crude extract as an alternative staining reagent for urine sediment staining. Specifically, it sought to: (1) prepare Mayana leaf crude extract using 70% ethanol and 95% ethanol as extraction solvents; (2) compare the staining performance of the 70% and 95% ethanol Mayana extracts based on selected microscopic staining criteria; (3) determine which extract preparation produced better staining quality for urine sediment observation; and (4) assess the potential usefulness of Mayana leaf crude extract as a preliminary natural staining reagent for laboratory microscopy applications.

## 2. Review of Related Literature

### *2.1 Plant-Based Natural Dyes as Biological Staining Reagents*

The search for natural alternatives to synthetic laboratory stains has gained attention because many conventional dyes are associated with chemical handling concerns, disposal requirements, and potential environmental burden. Plant-based pigments have been explored as possible staining materials because they are generally accessible, biodegradable, and capable of producing visible coloration in biological specimens. In histological and cytological contexts, natural dyes are particularly relevant when they can provide adequate contrast, preserve morphology, and produce sufficient differentiation of microscopic structures.

Anthocyanin-containing plant extracts have been examined as potential substitutes or complements to conventional staining reagents. Alshamar and Dapson (2021) demonstrated that anthocyanins from a single botanical source could be used as a replacement for hemalum and eosin in selected staining applications, suggesting that plant pigments may have legitimate staining value when properly extracted and applied. The relevance of plant-based dyes is not limited to color production alone. Their usefulness depends on whether the pigment can attach to or interact with biological structures in a manner that improves microscopic visualization.

In laboratory practice, a stain must enhance recognition of cells and other formed elements without obscuring morphology. Thus, while plant pigments offer practical and environmental advantages, their laboratory use requires

empirical evaluation rather than mere assumption of equivalence with standard reagents. This issue is central to the present study, which evaluates Mayana leaf crude extract as a preliminary alternative staining reagent for urine sediment microscopy.

### *2.2 Anthocyanins and the Pigment Potential of Coleus blumei*

*Coleus blumei*, also referred to taxonomically as *Plectranthus scutellarioides*, is known for its vivid leaf coloration. The red, purple, and bluish tones observed in many plant tissues are commonly associated with anthocyanins, which are water-soluble flavonoid pigments. Anthocyanins are found in plant flowers, fruits, stems, and leaves, and their coloration properties have made them significant in food, pharmaceutical, cosmetic, biomedical, and material applications (Kong et al., 2003; Nistor et al., 2022). Their relative non-toxicity, biodegradability, and strong coloring capacity make them relevant to exploratory studies on natural staining reagents.

The potential of *Coleus blumei* as a staining source is linked to the anthocyanin-rich pigmentation of its leaves. Maung Maung et al. (2018) examined the extraction and characterization of purple *Coleus blumei* natural dyes, showing that its pigment can be extracted and characterized using ethanol-based procedures. This provides a methodological basis for investigating Mayana leaf extract in staining-related applications. The plant's local availability in the Philippines further strengthens its practical relevance, especially in settings where low-cost, locally sourced, and environmentally safer laboratory materials are being explored.

However, anthocyanin behavior is influenced by several conditions, including solvent type, concentration, pH, light exposure, and storage conditions. Nistor et al. (2022) emphasized that anthocyanins are chemically active phytochemicals, and their stability may vary depending on environmental and preparation factors. For staining applications, this means that the pigment source alone is insufficient; the extraction method and solvent concentration must also be considered. This supports the present study's comparison of 70% and 95% ethanol Mayana extracts.

### *2.3 Extraction Conditions and Solvent Concentration in Natural Dye Preparation*

Extraction is a critical stage in the preparation of plant-based staining reagents because it affects pigment yield, stability, and staining behavior. The original manuscript adopted an ethanol-based extraction procedure patterned after the work of Maung Maung et al. (2018), in which *Coleus blumei* leaves were washed, crushed, mixed with ethanol, stored at low temperature, stirred, filtered, and centrifuged before use. Ethanol is commonly used in natural dye extraction because it can dissolve a range of plant pigments and phytochemical constituents while remaining relatively accessible for laboratory preparation.

The concentration of ethanol may influence how much pigment is extracted and how the resulting dye interacts with biological material. A more aqueous ethanol mixture, such as 70% ethanol, may allow extraction of water-soluble pigments such as anthocyanins while also preserving sufficient solvent activity. A higher ethanol concentration, such as 95%, may behave differently because reduced water content can affect pigment solubility, extraction balance, and staining performance. Since anthocyanins are water-soluble pigments, the solvent environment is particularly important in determining the quality of the resulting extract (Kong et al., 2003; Nistor et al., 2022).

In the context of the present study, solvent concentration is not merely a technical preparation detail but one of the main experimental comparisons. The study evaluates whether 70% ethanol or 95% ethanol produces a more effective Mayana-based stain for urine sediment observation. This is methodologically important because the usefulness of a natural stain depends not only on the plant source but also on the extract preparation that produces the clearest and most stable staining effect. Adjacent natural-product work similarly indicates that crude plant extracts require dose- or concentration-specific optimization and phytochemical interpretation before their functional performance can be generalized (Laxamana et al., 2025).

### *2.4 Urine Sediment Staining and the Role of Standard Stains*

Urine sediment examination is a routine component of clinical microscopy. It involves the observation of formed elements such as erythrocytes, leukocytes, epithelial cells, casts, crystals, and microorganisms. Staining may improve the contrast and visibility of these components, particularly when unstained preparations provide insufficient differentiation. Standard urine sediment stains are used because they help distinguish cellular structures and casts during microscopic analysis.

The clinical and laboratory value of urine sediment staining is linked to the accurate recognition of sediment elements. Palaoro and Angerosa (2014) examined urine sediment cytology using fresh samples and stained smears, reinforcing the relevance of staining methods in enhancing urinary sediment evaluation. The Japanese Association of Medical Technologists (2017) also emphasized the importance of standardized procedures in urinary sediment examination, including proper preparation and microscopic interpretation. These references support the need for consistency, clarity, and morphological preservation when assessing any staining reagent.

Sternheimer-Malbin stain remains a relevant point of comparison because it is specifically associated with urine sediment microscopy. However, the present manuscript indicates that the actual Sternheimer-Malbin reagent was not directly available for experimental comparison. This limitation is important. The study can compare the performance of 70% and 95% ethanol Mayana extracts, but it should not overclaim that Mayana extract is already equivalent to Sternheimer-Malbin. At this stage, Mayana extract is better described as a preliminary natural staining candidate requiring further validation against standard stains under controlled laboratory conditions. Comparable reagent-validation work in histopathology shows that reagent chemistry and endpoint control can shape morphology preservation and interpretive quality, reinforcing the need for controlled protocol validation when proposing alternative laboratory reagents (Morcilla et al., 2025).

### *2.5 Synthesis and Literature Gaps*

The reviewed literature supports the general possibility of using plant-derived pigments as biological staining materials. Anthocyanins are recognized for their strong coloration, water solubility, and biological relevance, and *Coleus blumei* has been shown to contain extractable pigments that may be useful in dye-related applications (Kong et al., 2003; Maung Maung et al., 2018; Nistor et al., 2022). Literature on urine sediment examination also confirms that staining can improve microscopic visualization and interpretation of urinary formed elements (Japanese Association of Medical Technologists, 2017; Palaoro & Angerosa, 2014).

Despite this foundation, several gaps remain. First, the staining usefulness of *Coleus blumei* extract in urine sediment microscopy is still insufficiently established. Second, the role of solvent concentration in producing an effective Mayana-based staining reagent requires empirical testing. Third, available evidence does not yet support a strong claim that Mayana extract can replace Sternheimer-Malbin stain in routine clinical use. The present study responds to these gaps by comparing 70% and 95% ethanol Mayana leaf crude extracts using selected staining performance criteria. However, the findings should be interpreted as preliminary and should be followed by validation against standard stains, larger specimen sets, and more rigorous microscopy evaluation protocols. This cautious framing is consistent with adjacent crude-extract screening, where preliminary bioactivity findings require fractionation, reproducibility testing, and safety validation before translation into routine laboratory application (Diaz et al., 2025).

## 3. Methodology

### *3.1 Research Design*

This study used a laboratory-based experimental comparative design to evaluate the staining performance of *Coleus blumei* (Mayana) leaf crude extract as a potential alternative reagent for urine sediment staining. The experiment compared two Mayana extract preparations using different ethanol concentrations, namely 70% ethanol

and 95% ethanol. The purpose was to determine which preparation produced better staining outcomes based on selected microscopic and practical evaluation criteria.

### *3.2 Materials and Specimen Source*

The primary plant material used in the study was *Coleus blumei* or Mayana leaves. The leaves were prepared as crude extract using ethanol-based extraction. Urine sediment specimens were used as the biological material for microscopic staining. The paper indicates that urine specimens were obtained through voluntary donation, with attention to confidentiality and safe handling. However, the number of urine specimens, number of slides, number of trials, and number of evaluators were not specified in the manuscript.

### *3.3 Preparation of Mayana Leaf Crude Extract*

The extraction procedure was adapted from the ethanol-based extraction method described for purple Coleus blumei natural dye preparation. The Mayana leaves were first washed with distilled water and allowed to stand at room temperature to remove excess surface moisture. Five grams of leaves were then crushed using a mortar and pestle. The crushed plant material was placed in an Erlenmeyer flask and mixed with ethanol. Two extract preparations were considered in the study: one using 70% ethanol and another using 95% ethanol.

The extract mixture was stored overnight in a refrigerator at approximately 4°C. After refrigeration, the mixture was stirred using a magnetic stirrer for two hours while exposed to controlled heating conditions. The extract was then filtered using filter paper to remove solid residues. The remaining extract was centrifuged to further separate plant residues from the liquid staining solution. The prepared extract was transferred into a screw-capped amber bottle, properly labeled, and stored in a refrigerator before use.

### *3.4 Staining Procedure*

Urine sediment was prepared for direct microscopic examination. A small portion of urine sediment was placed on a clean microscopic slide. The prepared Mayana staining solution was added to the specimen, and a coverslip was carefully placed over the preparation to avoid air bubbles. The stained slide was allowed to stand for approximately three minutes before microscopic examination. The urine sediment preparations were observed under high power objective, specifically HPO 40x.

The study originally intended to compare the Mayana extract with Sternheimer-Malbin stain as a standard urine sediment stain. However, the manuscript indicates that Sternheimer-Malbin stain was not available during the conduct of the experiment. Therefore, the actual experimental comparison was limited to the 70% ethanol and 95% ethanol Mayana extract preparations. Any comparison with Sternheimer-Malbin should therefore be treated as literature-based rather than directly experimental.

### *3.5 Evaluation Criteria*

The prepared slides were evaluated using selected staining criteria. These included comparability to standard stain performance, stain stability, color enhancement, clarity of staining, cost-effectiveness, practicality and time efficiency, component differentiation, and recommendation potential. The stained urine sediment was also assessed in terms of its ability to support the observation of sediment components, particularly cellular and crystalline structures such as calcium oxalate crystals.

### *3.6 Data Analysis*

The staining performance of the 70% ethanol and 95% ethanol Mayana extract preparations was summarized using mean ratings. The manuscript reported an overall mean rating of 4.25 for the 70% ethanol Mayana extract and 2.25 for the 95% ethanol Mayana extract. A Mann-Whitney test was used to compare the two extract preparations. The reported p-value was 0.05, which was interpreted as statistically significant at the 0.05 level of significance.

### *3.7 Ethical Considerations*

The study observed basic ethical considerations in the handling of urine specimens. Participation in specimen donation was voluntary. The confidentiality and privacy of specimen donors were to be protected. The researchers also observed laboratory safety measures, including the use of personal protective equipment and proper handling protocols. The study did not involve animal testing, and the manuscript states that no humans or animals were harmed during the experimentation.

## 4. Results and Discussion

### *4.1 Overall Staining Performance of 70% and 95% Ethanol Mayana Extracts*

The study compared the staining performance of *Coleus blumei* (Mayana) leaf crude extract prepared using 70% ethanol and 95% ethanol. Based on the reported findings, the 70% ethanol Mayana extract obtained a higher overall mean rating of 4.25, while the 95% ethanol Mayana extract obtained a lower overall mean rating of 2.25. The Mann-Whitney test yielded a reported p-value of 0.05, which was interpreted as statistically significant at the 0.05 level of significance.

**Table 1**. *Comparative Staining Performance of 70% and 95% Ethanol Mayana Leaf Extracts*

| Evaluation criterion | 70% ethanol Mayana stain | 95% ethanol Mayana stain | Interpretation |
|---|---|---|---|
| Overall mean rating | 4.25 | 2.25 | 70% ethanol extract showed higher overall staining performance. |
| Comparability to standard stains | 4.67 | 2.33 | 70% ethanol extract performed better. |
| Stain stability | 4.33 | 2.33 | 70% ethanol extract performed better. |
| Color enhancement | 4.33 | 2.00 | 70% ethanol extract performed better. |
| Clarity of staining | No separate numerical mean reported | No separate numerical mean reported | No difference was reported. |
| Cost-effectiveness | No separate numerical mean reported | No separate numerical mean reported | No difference was reported. |
| Practicality and time efficiency | No separate numerical mean reported | No separate numerical mean reported | No difference was reported. |
| Component differentiation | No separate numerical mean reported | No separate numerical mean reported | No difference was reported. |
| Recommendation potential | No separate numerical mean reported | No separate numerical mean reported | No difference was reported. |
| Mann-Whitney test result | $p = 0.05$ | $p = 0.05$ | Difference was interpreted as significant at alpha = 0.05. |

The results indicate that the 70% ethanol Mayana extract was rated more favorably than the 95% ethanol extract in the overall assessment. The most evident advantages of the 70% preparation were observed in comparability to standard stains, stain stability, and color enhancement. These results suggest that the 70% ethanol preparation produced a more favorable staining effect for urine sediment observation than the 95% ethanol preparation.

However, several criteria were reported as having the same mean across the two preparations, particularly clarity of staining, cost-effectiveness, practicality and time efficiency, component differentiation, and recommendation potential. This means that the superiority of the 70% ethanol extract should not be interpreted as

uniform across all performance indicators. Rather, its advantage appears strongest in the visual and stability-related aspects of staining.

### *4.2 Visual Clarity and Observation of Urine Sediment Components*

The manuscript reports that the 70% ethanol Mayana stain provided better clarity and reduced confusion in distinguishing calcium oxalate crystals compared with the 95% ethanol preparation. It was also described as producing better visualization of morphological structures in the urine sediment sample. These findings suggest that 70% ethanol may be more suitable for extracting or preserving pigment properties that improve the visual contrast of selected urine sediment components.

In urine sediment microscopy, visual clarity is essential because formed elements must be identified based on their morphology, color contrast, and relationship to the background field. A stain that produces excessive coloration, weak coloration, background interference, or structural distortion may reduce interpretive usefulness. Based on the reported observations, the 70% ethanol Mayana extract appeared to provide a more useful balance between color enhancement and morphological visibility.

The 95% ethanol Mayana stain was described as providing some visual overview but not meeting the expected criteria for effective staining in the investigation. The original manuscript suggests that the 95% ethanol preparation may have affected the appearance of stained components and may have been less reliable for distinguishing structures. This should be interpreted cautiously, however, because the study did not provide microscopic images, replicate counts, evaluator agreement, or detailed scoring distributions.

### *4.3 Comparative Interpretation of the 70% and 95% Ethanol Extracts*

The difference between the 70% and 95% ethanol preparations may be explained by the nature of plant pigments, particularly anthocyanins, which are water-soluble compounds. A 70% ethanol solution contains a higher water proportion than 95% ethanol, which may support better extraction or functional behavior of water-soluble pigments. In contrast, a more concentrated ethanol solution may not necessarily improve staining performance if the target pigment has better solubility or stability in a more aqueous solvent environment.

This interpretation is consistent with the literature discussed earlier, where anthocyanins were identified as relevant plant pigments because of their strong coloration and possible staining potential. Studies on anthocyanins and natural dyes suggest that extraction conditions affect pigment yield, stability, and practical application (Kong et al., 2003; Maung Maung et al., 2018; Nistor et al., 2022). In this study, the stronger performance of the 70% ethanol preparation suggests that solvent concentration is an important technical factor in preparing Mayana-based staining reagent.

Still, the reported results should be framed as preliminary. The study did not directly validate the Mayana extract against Sternheimer-Malbin stain under controlled side-by-side conditions. The manuscript indicates that the standard stain was unavailable and that comparison with Sternheimer-Malbin was based partly on literature rather than actual experimental testing. Therefore, the findings support the statement that 70% ethanol Mayana extract performed better than 95% ethanol Mayana extract within this experiment, but they do not yet establish Mayana extract as a validated replacement for Sternheimer-Malbin stain.

### *4.4 Discussion*

The findings provide preliminary evidence that *Coleus blumei* leaf crude extract prepared with 70% ethanol may have useful staining potential for urine sediment microscopy. The higher overall mean rating of the 70% extract suggests better perceived staining performance compared with the 95% extract. Its stronger ratings in comparability to standard stains, stain stability, and color enhancement are particularly relevant because these criteria relate directly to the visual usefulness of a staining reagent.

The result is also methodologically meaningful because it shows that the effectiveness of a natural stain depends not only on the plant source but also on the preparation method. Mayana is valued as a possible natural staining source because of its pigmentation, but pigment presence alone does not guarantee laboratory usefulness. The solvent concentration, extraction process, storage condition, and staining procedure all affect the final performance of the reagent. In this study, 70% ethanol appeared more suitable than 95% ethanol for producing a Mayana-based urine sediment stain.

The findings are generally aligned with literature suggesting that plant-based dyes and anthocyanin-rich extracts can have biological staining potential when properly processed (Alshamar & Dapson, 2021; Maung Maung et al., 2018). The observed advantage of the 70% ethanol preparation may also be consistent with the water-soluble nature of anthocyanins, which can be affected by extraction environment and solvent composition (Kong et al., 2003; Nistor et al., 2022). In practical terms, this supports continued investigation of Mayana extract as a low-cost and locally available staining candidate.

However, the study has important limitations. First, the manuscript does not specify the number of urine specimens, number of stained slides, number of raters, scoring scale, or inter-rater agreement. Second, the p-value of 0.05 is exactly at the conventional threshold for significance, which should be interpreted cautiously. Third, the absence of direct Sternheimer-Malbin comparison limits the strength of any claim that Mayana extract can replace standard urine sediment stains. Fourth, important reagent properties such as pH, shelf life, stain stability over time, reproducibility, and batch-to-batch consistency were not fully reported.

Overall, the results support a limited but useful conclusion: within the reported experiment, 70% ethanol Mayana leaf crude extract showed better staining performance than 95% ethanol Mayana leaf crude extract for urine sediment observation. The study provides a reasonable preliminary basis for further validation, but additional controlled testing is needed before the extract can be recommended for routine clinical microscopy.

## 5. Conclusions, Recommendations, and Implications

### *5.1 Conclusions*

Based on the findings of the study, *Coleus blumei* (Mayana) leaf crude extract demonstrated preliminary potential as a natural staining reagent for urine sediment staining. However, its effectiveness depended on the ethanol concentration used in the extraction process. The 70% ethanol Mayana extract obtained a higher overall mean rating of 4.25, while the 95% ethanol Mayana extract obtained a lower mean rating of 2.25. The reported Mann-Whitney test result of $p = 0.05$ indicated a significant difference between the two extract preparations at the 0.05 level of significance.

The 70% ethanol Mayana extract performed better particularly in terms of comparability to standard staining performance, stain stability, and color enhancement. These findings suggest that 70% ethanol was more suitable than 95% ethanol for preparing the Mayana-based staining reagent in this experiment. The stronger performance of the 70% preparation may be associated with the water-soluble nature of plant pigments such as anthocyanins, which may respond better to a more aqueous ethanol concentration.

The study further concludes that 70% ethanol Mayana extract may improve the visibility of selected urine sediment components, particularly crystalline structures such as calcium oxalate crystals, without excessively obscuring the microscopic field. Nevertheless, the findings should be interpreted as preliminary. Since Sternheimer-Malbin stain was not directly used as an actual experimental control, the study cannot yet establish Mayana extract as a validated replacement for standard urine sediment stains. Rather, the evidence supports its possible use as a candidate natural stain that requires further controlled validation.

### *5.2 Recommendations*

Future studies should conduct a direct comparative evaluation between 70% ethanol Mayana extract and Sternheimer-Malbin stain using the same urine sediment specimens, standardized slide preparation procedures, and blinded evaluator ratings. This would allow a stronger assessment of whether Mayana extract can approximate or supplement standard urine sediment staining procedures.

The researchers should also increase the number of urine specimens, slides, and replicate trials to improve the reliability of the findings. A larger specimen set should include urine sediments with different formed elements such as red blood cells, white blood cells, epithelial cells, casts, and crystals. This would determine whether Mayana extract is useful only for selected sediment components or whether it can support broader urine sediment evaluation.

It is also recommended that future experiments standardize and report the scoring system used for stain evaluation. The number and qualifications of raters should be stated, and inter-rater agreement should be measured. This is important because staining quality involves visual assessment, which can be affected by evaluator subjectivity.

Further testing should examine the pH, shelf life, storage stability, light sensitivity, and batch-to-batch consistency of the Mayana extract. Since natural pigments may degrade over time, storage conditions such as temperature, light exposure, and container type should be evaluated systematically.

Finally, future research may explore the staining performance of Mayana extract in other biological specimens, such as sputum, vaginal smears, cerebrospinal fluid sediment, or simple cytological preparations. However, such applications should be tested separately and should not be assumed from the present urine sediment findings.

### *5.3 Implications of the Study*

The study has practical implications for laboratory education and exploratory clinical microscopy. If validated further, Mayana leaf extract may provide a locally available and low-cost natural staining candidate for teaching laboratories, preliminary staining exercises, or resource-limited settings. Its use may also encourage students and laboratory personnel to explore sustainable alternatives to synthetic reagents while maintaining awareness of scientific validation requirements. In health professions education, such laboratory innovations are most defensible when linked to competency development, quality assurance, and curriculum-practice alignment in licensure-based programs (Bermido et al., 2025).

Methodologically, the study highlights the importance of extraction conditions in natural dye research. The difference between the 70% and 95% ethanol preparations shows that the plant source alone does not determine staining effectiveness. Solvent concentration, pigment behavior, specimen type, and evaluation criteria must be carefully controlled when developing natural laboratory reagents.

The study also contributes to the broader discussion on environmentally safer laboratory materials. Natural stains may reduce reliance on synthetic dyes, but they must still meet standards of clarity, reproducibility, stability, and diagnostic usefulness. Therefore, the main implication of this study is not that Mayana extract should immediately replace standard stains, but that it deserves further controlled investigation as a possible natural staining reagent for urine sediment microscopy. Related plant-based monitoring research also illustrates how locally available biological materials can support applied laboratory inquiry when the procedures remain standards-based and the interpretation is cautious (Ylagan et al., 2025).

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