

Effect of the 10% nitric acid and 10% chromic acid ratio on the quality of maceration preparations

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ABSTRACT

Background: Maceration is a microtechnique used to examine xylem vessel anatomy by softening plant tissues in specific solutions so that tracheary elements can be observed clearly. However, the optimal ratio of acid solutions for producing durable and well-stained maceration slides is still rarely reported for *Piper betle*.

Objectives: This study compared two ratios (1:1 and 1:5) of 10% nitric acid and 10% chromic acid to evaluate their effects on the quality of maceration preparations from green betel (*Piper betle*) stems.

Methods: An experimental design was applied. Stem and petiole samples were processed through boiling, acid soaking, staining with safranin and methylene blue, graded alcohol dehydration, and fixation/clearing using xylol. Observations were conducted under 400× magnification, and findings were summarized descriptively.

Results: The 1:1 ratio produced more uniform staining and more resilient preparations than the 1:5 ratio. Slides prepared with the 1:5 ratio tended to be more fragile and exhibited less optimal staining, likely due to excessive chromic acid. Methylene blue staining was weak in both treatments, consistent with the dye's high water solubility and possible incomplete rinsing after acid treatment.

Conclusion: Maceration was effective for observing xylem anatomical structures in *P. betle*. A 1:1 ratio of 10% nitric acid to 10% chromic acid is recommended to obtain better slide quality and durability for microscopic observation.

Keywords: chromic acid, nitric acid, maceration, *Piper betle*, xylem anatomy

SDGs Relevance: This study supports SDG 4 (Quality Education) by improving laboratory-based learning and microscopy skills in plant anatomy, and contributes to SDG 15 (Life on Land) by strengthening basic anatomical observations useful for plant identification and structure–function studies.

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INTRODUCTION

Green betel (*Piper betle* L.) is widely utilized in Indonesia for traditional practices and medicinal purposes, and it belongs to the family Piperaceae, which includes several functional plants (Rahmawati & Kurniawati, 2016; Dewi et al., 2020). Understanding plant internal structures, particularly the vascular system, is essential for both botanical studies and biology education. Microtechnique-based slide preparation enables microscopic observation by producing thin, transparent specimens that can be penetrated by light (Gunawan, 2019; Faluti et al., 2022).

One commonly used approach is maceration, which softens whole or partial tissues by soaking them in water or specific chemical solutions. Maceration is especially useful for isolating and observing xylem tracheary elements and their secondary wall thickening patterns (Fahn, 1992). Because xylem is mechanically robust and provides structural support and water–mineral transport, its anatomical features are often distinct and informative (Aikmelisa & Waluyo, 2019; Ramdhini et al., 2021).

Acid-based maceration frequently employs nitric acid and chromic acid solutions to break down surrounding tissues and facilitate the separation of vascular elements. Nevertheless, excessive exposure to



strong acids can damage tissues and compromise staining quality, potentially leading to fragile preparations and poor visualization of cellular components (Dewi et al., 2020; Khristian & Inderiati, 2017).

This study addresses the practical gap regarding the optimal mixing ratio of 10% nitric acid and 10% chromic acid for producing maceration slides of *P. betle*. The novelty of this work lies in the direct comparison of two ratios (1:1 and 1:5) under the same processing workflow and staining scheme, with a focus on slide durability and staining uniformity. In addition, by optimizing a routine laboratory procedure, the study can enhance plant anatomy practicums (SDG 4) and support foundational plant structure investigations relevant to biodiversity learning (SDG 15). Accordingly, the objective of this research was to compare the effects of 1:1 and 1:5 ratios of 10% nitric acid and 10% chromic acid on the quality of maceration preparations from *P. betle*.

METHODS

Research design. This study used an experimental design to compare two maceration treatments based on the mixing ratio of 10% nitric acid (HNO_3) and 10% chromic acid.

Samples. The specimens consisted of stems and petioles of green betel (*Piper betle*).

Sample preparation and procedures. Samples were processed through (1) boiling, (2) soaking in the acid solutions with two ratios (HNO_3 10% : chromic acid 10% = 1:1 and 1:5), (3) staining using safranin and methylene blue, (4) graded dehydration using alcohol, (5) dealcoholization/clearing, and (6) fixation using xylol. Microscopic observations were conducted at 400 $\times$ magnification, and representative images were documented.

Laboratory involvement. All preparation and microscopic observations were conducted in a laboratory setting, following standard microtechnique workflows for plant maceration and staining.

Data analysis. The results were analyzed descriptively using a qualitative approach, emphasizing slide appearance, staining uniformity, and preparation durability.

RESULTS

Overall, maceration preparations showed observable xylem tracheary elements under 400 $\times$ magnification. However, slide quality differed between the two acid ratios.

Slides prepared using the 1:1 ratio exhibited more uniform safranin staining on xylem structures and generally maintained better integrity during handling and observation. In contrast, the 1:5 ratio often produced more fragile preparations with less optimal staining, which is consistent with the possibility of overexposure to chromic acid.

For methylene blue staining, both treatments resulted in weak color uptake, and plant tissues frequently retained a greenish appearance. This may be related to the dye's high solubility in water and/or insufficient rinsing after acid treatment, which can limit dye penetration and retention.

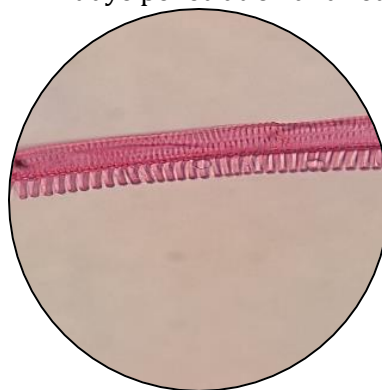


Figure 1. Safranin staining (HNO_3 10% : chromic acid 10% = 1:1), 400 $\times$ magnification.

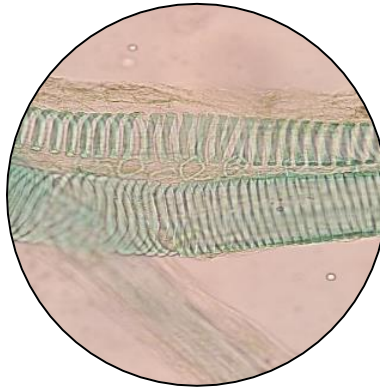


Figure 2. Methylene blue staining (HNO_3 10% : chromic acid 10% = 1:1), 400× magnification.



Figure 3. Safranin staining (HNO_3 10% : chromic acid 10% = 1:5), 400× magnification.

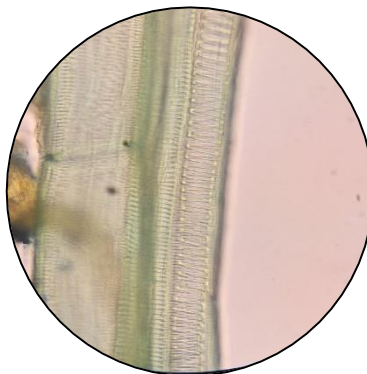


Figure 4. Methylene blue staining (HNO_3 10% : chromic acid 10% = 1:5), 400× magnification.

DISCUSSION

The clearer and more uniform safranin staining observed in the 1:1 treatment suggests that a balanced proportion of nitric acid and chromic acid can effectively soften surrounding tissues without excessively damaging xylem elements. Strong acids are effective softening agents, but overexposure may disrupt tissue integrity and reduce staining performance, especially for basophilic components such as nuclei (Dewi et al., 2020; Khristian & Inderiati, 2017). In this study, increasing the chromic acid proportion (1:5) was associated with more fragile slides, which likely reduced the stability of isolated elements during mounting and observation.

From a methodological perspective, potassium hydroxide (KOH) can support deproteinization via hydrolysis of peptide bonds, facilitating subsequent tissue separation (Hidayani et al., 2018). Nitric acid is highly corrosive and a strong oxidizer (Septiani et al., 2018), and it has been reported as a softening solution in plant organ preparation (Faluti et al., 2022).

Weak methylene blue staining across treatments aligns with reports that the dye is easily dissolved in water, potentially leading to poor retention on specimens (Avilia et al., 2024). Because staining aims to increase contrast for microscopic observation (Jannah et al., 2019), future work may test longer staining times, alternative dyes, or improved post-acid rinsing protocols.

SDGs implications. By optimizing a practical microtechnique workflow, the study can strengthen hands-on laboratory learning in biology education (SDG 4) and support basic plant anatomical investigations useful for biodiversity-related learning (SDG 15).

Limitations. This study employed descriptive qualitative evaluation without quantitative scoring of slide quality or statistical comparison. Future studies should incorporate standardized quality rubrics, replicate batches, and quantitative measures (e.g., staining intensity analysis).

CONCLUSION

Maceration effectively revealed xylem anatomical structures in *Piper betle* preparations. The 1:1 ratio of 10% nitric acid to 10% chromic acid produced more uniform staining and more durable slides than the 1:5 ratio. For routine plant anatomy practicums and microscopy observations, the 1:1 acid ratio is recommended to obtain better slide quality and handling stability. Improving rinsing and considering alternative blue stains may further enhance specimen contrast. Further studies should apply quantitative slide-quality assessment, evaluate different soaking durations, and test additional staining protocols to optimize visualization of xylem elements.

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Conflicts of Interest: The authors declare no conflicts of interest.

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