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Original Article

Utilization of Teak Leaf Extract as a Substitute for Eosin in Hematoxylin Eosin Staining

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ABSTRACT

Hematoxylin–Eosin (HE) staining is widely used in histopathology to visualize tissue morphology. Eosin, a synthetic dye commonly used to stain cytoplasmic components, may cause environmental and health concerns, encouraging the search for safer natural alternatives. Young teak leaves (*Tectona grandis*) contain anthocyanin pigments with potential as natural staining agents. This study evaluated the effectiveness of teak leaf extract as a substitute for eosin in HE staining of uterine tissue. A *quasi-experimental post-test only control group design* was applied. Young teak leaves were extracted through maceration using 95% ethanol for seven days, followed by filtration and evaporation to obtain a concentrated extract. Uterine tissue preparations were stained using hematoxylin combined with 75% teak leaf extract, while eosin staining served as the control. Microscopic evaluation at 400x magnification was performed by an anatomical pathology specialist using a staining quality scoring system. The control group stained with eosin demonstrated optimal staining quality, with clear bluish-purple nuclei and bright pink cytoplasm, achieving a good score in all slides. In comparison, teak leaf extract produced pale purple nuclei and brownish-pink cytoplasm that remained distinguishable morphologically, though with lower staining quality. Overall, teak leaf extract demonstrated potential as a natural alternative to eosin, but further optimization is needed to improve staining performance.

Introduction

Histotechnology is the process of processing tissue into histological slides through several stages, from creating paraffin blocks to staining, allowing the slides to be observed under a microscope. The quality of the slides is highly dependent on how the tissue specimen is handled, particularly during the staining process (Utami et al., 2025; Fathinatullabibah, Khasanah, & Kawiji, 2014).

HE is a commonly used stain in histology to assess cellular structure within tissues (Golberg et al., 2024; Dibal, Garba, & Jacks, 2022). This stain utilizes the chemical properties of acidic and basic dyes to differentiate cellular components. Hematoxylin, as a basic dye, binds to nucleic acids, and mucin stains the cell nucleus dark blue. Meanwhile, eosin, as an acidic dye, binds to basic cellular components such as cytoplasm, keratin, and collagen, producing a pink color (Fitrianingsih, 2022; Haliza et al., 2025). The use of HE in combination allows for clear and contrasting visualization of cellular structures, making it a standard staining method in histological analysis (Nadhira et al., 2023; Marques & Soares, 2022).

Citation: .....

In laboratories, the limitations of automated equipment force technicians to come into direct contact with hazardous materials during tissue processing and staining. Eosin, a hazardous component used in anatomical pathology laboratories to stain tissue, has the potential for significant negative impacts. The synthetic dye eosin is known to be carcinogenic and can cause organ damage. Furthermore, contact with (Jumardi et al., 2023; Hidayat & Dewi, 2024).

Dyes come in two types: synthetic and natural. Natural dyes are derived from plant sources such as vegetables, flowers, leaves, stems, and roots, and are used to color tissues for microscopic examination. Each type of tissue or cell has different characteristics, resulting in varying levels of dye absorption. Natural dyes are considered safer because they do not contain toxic or carcinogenic substances, and their waste does not pollute the environment. One natural dye frequently used in research is young teak leaves (Nadhira et al., 2023; Kembaren et al., 2014).

Several studies have investigated the use of teak leaf extract as a substitute for eosin; however, most previous research has relied on soaking techniques or has focused primarily on cytology preparations and parasitological examinations (Liyanda & Kristanto, 2017). In contrast, the present study employed a maceration–evaporation extraction method to obtain a concentrated anthocyanin-rich extract and specifically evaluated its staining performance on uterine histological tissue preparations (Imran et al., 2024). This approach was expected to enhance pigment extraction efficiency and provide clearer insight into the staining affinity of teak leaf extract toward cytoplasmic structures in HE staining (Lowe, Anderson, & Anderson, 2023).

Teak leaves (*Tectona grandis*) have great potential as a natural dye. One important component of the leaves is anthocyanin, a flavonoid pigment that can produce colors such as red, blue, purple, violet, magenta, and orange in various plant parts, including leaves, flowers, fruit, roots, tubers, legumes, cereals, and vegetables. In teak leaves, anthocyanin produces the red color (Nadhira et al., 2023; Mutoharoh, Santoso & Mandasari, 2020). Based on research conducted by Jumardi et al. (2023), the combination of hematoxylin and teak leaves in a ratio of 1:1 and 1:2 gave good results. Meanwhile, a mixture with a ratio of 2:1 showed less satisfactory results. Meanwhile, research by Sari & Hariyanto (2020) found that soaking teak leaf buds as a substitute for eosin in HE staining gave the best results after 48 hours of soaking. However, these results were not able to stain the cytoplasm optimally. This indicates that the soaking method is less effective in extracting pigments from teak leaves. Therefore, to obtain more optimal staining results, further testing is needed using more efficient methods such as extraction through maceration to the evaporation stage, in order to obtain a thick extract rich in color pigments. Several previous studies have shown that teak leaves, especially the youngest leaves, contain natural pigments consisting of anthocyanin, peophiptin, β-carotene, pelargonidin 3-glucoside, pelargonidin 3,7-diglucoside and chlorophyll.

Methods

Quasi-experimental research type with *a post-only control group design*, namely observing the results of HE staining on histological preparations using eosin as a control and teak leaf extract as an alternative to eosin. This study employed a true experimental design using a post-test only control group approach. It was conducted from February to April 2025 at the Pharmacy Laboratory and the Anatomical Pathology Laboratory of Prof. Dr. W.Z. Johannes Regional Hospital, Kupang. The samples used were paraffin blocks of histological preparations obtained from the Anatomical Pathology Laboratory of Prof. WZ Johannes Kupang Regional Hospital.

Preparation of Teak Leaf Extract

Fresh young teak leaves (*Tectona grandis*) were thoroughly washed with distilled water and air-dried at room temperature for seven days. The dried leaves were then ground into a fine powder using a laboratory blender. A total of 500 g of teak leaf powder was macerated in 2500 mL of 95% ethanol using a 1:5 (w/v) ratio. The maceration process was carried out for seven days at room temperature with manual stirring three times daily.

After maceration, the mixture was filtered using Whatman No. 1 filter paper to obtain the filtrate, which was then concentrated using a rotary evaporator at 50 °C until a thick extract was produced. This process yielded 86 g of concentrated extract with a yield of 17,2%. The extract was subsequently diluted with distilled water to obtain a final concentration of 75% prior to staining.

The pH of the extract was measured using a digital pH meter and found to be 5,2. The extract was stored in a dark brown glass bottle at 4 °C to minimize anthocyanin degradation due to light and temperature exposure.

Histological Preparation and Staining Procedure

A paraffin-embedded uterine tissue block obtained from the Anatomical Pathology Laboratory was sectioned using a rotary microtome at a thickness of 3,5 µm. A total of six slides were prepared, consisting of one control slide stained with eosin and five treatment slides stained using teak leaf extract.

The staining procedure was performed as follows: deparaffinization in xylene I and II for 5 minutes each; rehydration through graded ethanol series (100%, 96%, 80%, and 70%) for 2 minutes each; washing with distilled water; hematoxylin staining for 8 minutes; rinsing under running water for 5 minutes; differentiation with 1% acid alcohol for 3 seconds; bluing in lithium carbonate solution for 30 seconds; washing with distilled water;

staining with 75% teak leaf extract for 3 minutes; dehydration through graded ethanol; clearing in xylene; and finally mounting using entellan and coverslip application.

Microscopic Evaluation

Microscopic observations were conducted using an Olympus CX-31 trinocular microscope equipped with a camera at 400x magnification. Staining quality was evaluated by an anatomical pathology specialist based on nuclear clarity, cytoplasmic staining intensity, and contrast between cellular components. The staining quality was scored as follows:

Score 1 indicated poor staining quality with indistinct nucleus and cytoplasm;
Score 2 indicated moderate staining quality with identifiable but suboptimal contrast; and
Score 3 indicated good staining quality with clear visualization of nucleus and cytoplasm.
Descriptive analysis was performed by calculating the average staining scores for each group.

Study Limitation

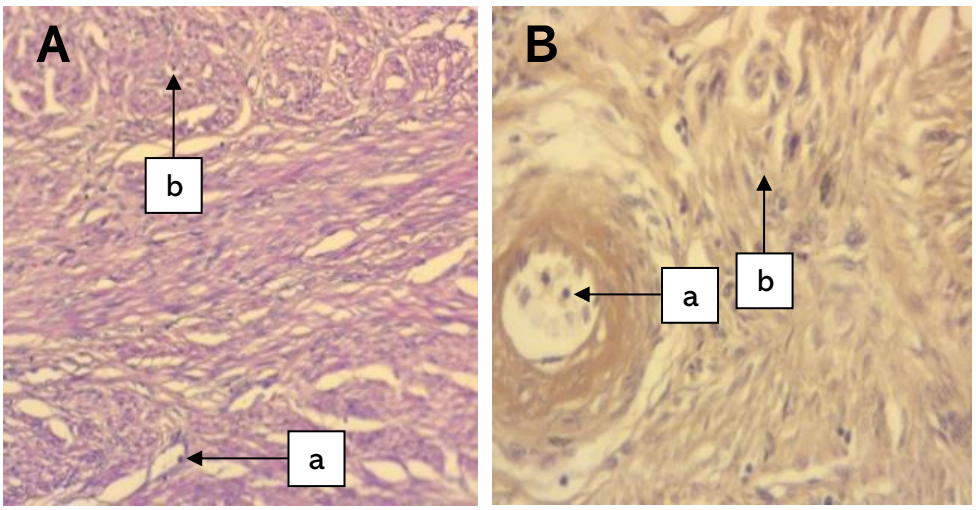
This study utilized a limited number of tissue samples and slides; therefore, comprehensive statistical analysis could not be performed. Future studies with larger sample sizes are recommended to enhance the validity and reproducibility of the findings.

Results

Teak leaf extract was prepared using the maceration method with 95% ethanol solvent for seven days and stirred 3 times a day, with a ratio of powder and solvent of 1:5 after 7 days the sample is filtered to produce a filtrate, then continued to the rotary evaporator until the solvent evaporates and becomes a thick extract and produces a brownish red color. Then dilution is carried out using distilled water.

In this study, the sample used was uterine tissue that had been processed into paraffin blocks through histological stages, starting from tissue selection to the process of making blocks . The sample used was 1 paraffin block obtained from the Anatomical Pathology Laboratory of Prof. Dr. WZ Johannes Kupang Regional General Hospital. The paraffin block was sectioned using a rotary microtome at a thickness of 3,5 µm. From the results of the cuts, 6 preparations were obtained, consisting of 1 preparation for control using eosin and 5 preparations for treatment using teak leaf extract with a concentration of 75%.

The microscopic appearance of the uterine tissue was evaluated based on nuclear and cytoplasmic staining characteristics. Observations were made using a Cx-31 Trinocular microscope equipped with a camera connector, at a magnification of 400x. The results of the microscopic observations are shown in Figure 1.



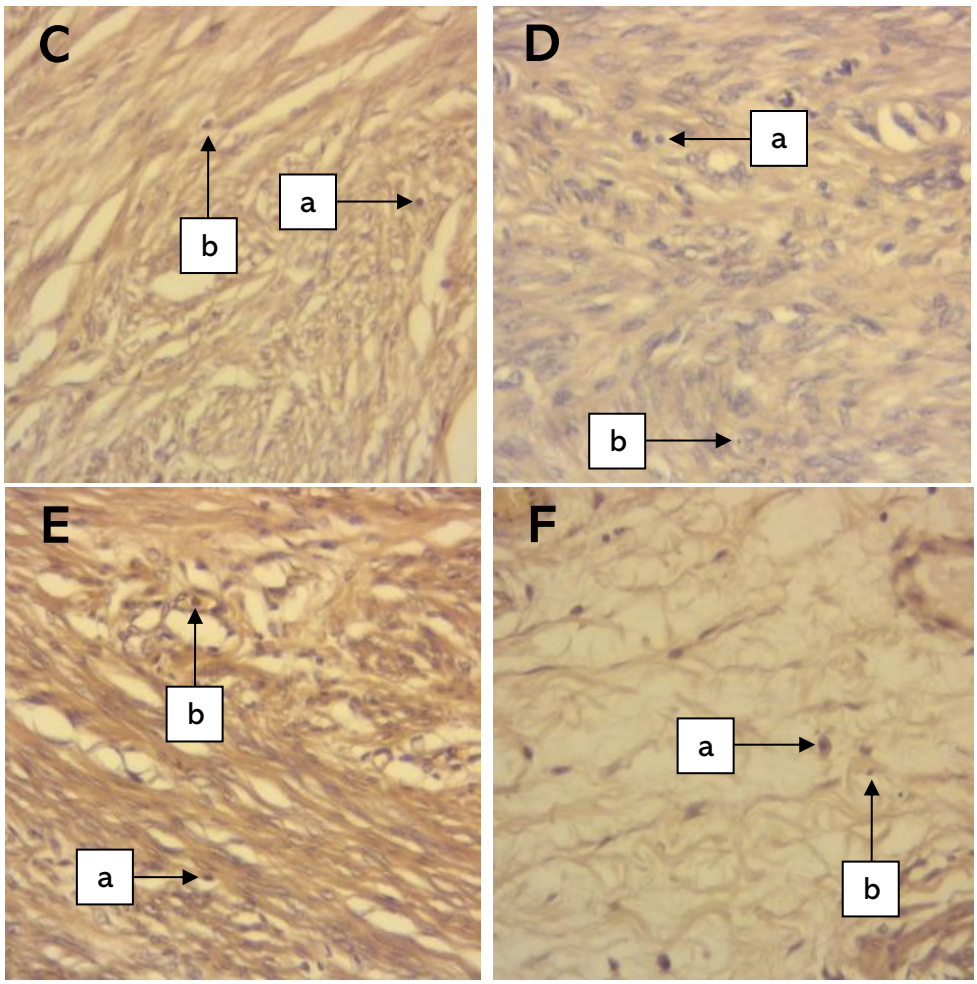


Figure 1. HE Staining Results of Uterus Preparations (A) Control; (B) Teak Leaf Extract Slide I; (C) Teak Leaf Extract Slide II; (D) Teak Leaf Extract Slide III; (E) Teak Leaf Extract Slide IV; (F) Teak Leaf Extract Slide V
Information:
a. Showing the core
b. Showing cytoplasm

The teak leaf extract produced a brownish-red concentrated solution after maceration and evaporation processes and demonstrated the ability to stain both nuclear and cytoplasmic components of uterine tissue, although its staining intensity was lower than that of eosin. Microscopic observations showed that eosin provided optimal cytoplasmic coloration with strong contrast between the nucleus and cytoplasm, whereas teak leaf extract resulted in a brownish-pink cytoplasmic appearance with pale purple nuclei; nevertheless, cellular morphology and tissue structures remained clearly identifiable despite the reduced contrast. Quantitatively, the average cytoplasmic staining score in the eosin control group was 5,5 compared to 2,76 in the teak leaf extract group, with all eosin-stained slides scoring 3 (good) and all teak leaf extract slides scoring 2 (moderate/less good). Due to the limited sample size, statistical analysis was not performed; however, these descriptive findings suggest that teak leaf extract has potential as a natural staining agent but requires further optimization.

The staining assessment was conducted in collaboration with an anatomical pathology specialist. The results of this study obtained the average number of cytoplasm stained using eosin and teak leaf extract can be seen in table 1. The cytoplasm stained using eosin in the control treatment was 55 with an average cytoplasm of 5,5. In the treatment using teak leaf extract, slide I found the number of cytoplasm 23 with an average cytoplasm of 2,3, on slide II found the number of cytoplasm 33 with an average cytoplasm of 3,3, on slide III found the number of cytoplasm 30 with an average cytoplasm of 3, on slide IV found the number of cytoplasm 22 with an average cytoplasm of 2,2, on slide V found the number of cytoplasm 30 with an average cytoplasm of 3.

Table 1. Average Amount of Cytoplasm Stained Using Eosin and Teak Leaf Extract

Treatment	Amount of Cytoplasm	Cytoplasm Amount (N)
Control	55	5,5
Teak leaf slide I	23	2,3
Teak leaf slide II	33	3,3
Teak leaf slide III	30	3
Teak leaf slide IV	22	2,2
Teak leaf slide V	30	3

HE staining of uterine tissue using eosin yielded a score of 3 (good) for all slides, with a 100% score. Meanwhile, HE staining using teak leaf extract as an eosin substitute yielded a score of 2 (moderate) for all five slides observed, also with a 100% score.

The recapitulation of the assessment data on HE staining using eosin and teak leaf extract as a substitute for eosin is presented in Table 2.

Table 2. Score Assessment of HE Staining Results on Uterine Tissue Using Eosin and Teak Leaf Extract

Treatment	Assessment Results						Total	
	Score 1 (Not good)		Score 2 (Not good)		Score 3 (Good)			
	n	%	n	%	n	%	n	%
Eosin	0	0	0	0	1	100	1	100
Teak Leaf Extract	0	0	5	100	0	0	5	100

Discussion

Staining is the process of giving color to the cut tissue so that the tissue structure appears more contrasting and can be observed under a microscope. One of the routine staining methods that is often used is HE staining which is able to stain the nucleus, cytoplasm, and connective tissue. In this study, the results on the control preparation using eosin showed purplish blue cell nucleus staining and bright pink cytoplasm with good color sharpness, so that tissue elements are easily distinguished and identified. Meanwhile, in the preparation stained with teak leaf extract, the staining results were less than optimal with the cell nucleus appearing pale purple and the cytoplasm brownish red although the tissue structure can still be distinguished and identified.

The findings of the present study differ from those reported by Labai (2023) who used teak leaf extract as a substitute for eosin in *diff quik staining*. In this study, teak leaf extract with concentrations of 70%, 60%, 50% and 40% obtained different results from eosin because the concentration used was quite concentrated and good results were obtained at a concentration of 30%. Meanwhile, in the study, researchers using teak leaf extract with a concentration of 75% obtained less good results. Different results can be caused by the solvent and maceration time used, the anthocyanin content of the extract, the type of sample used and differences in dilution solutions (Nadifah et al., 2024; Rahmawanti, Setyowati & Mukhlis, 2021).

The present study demonstrated that young teak leaf extract possesses staining capability toward uterine tissue structures in HE staining, where the anthocyanin compounds in teak leaves contributed to the observed coloration of cytoplasmic components. Anthocyanins are flavonoid pigments capable of producing red, purple, or blue hues depending on environmental pH conditions (Marques & Mariutti, 2025). In conventional HE staining, eosin acts as an acidic dye that strongly binds to basic cytoplasmic proteins, collagen, and muscle fibers, resulting in a bright pink appearance, and acidic conditions enhance ionic interactions between eosin and positively charged cytoplasmic proteins. However, in this study, teak leaf extract produced less optimal staining, likely because the extract pH and anthocyanin stability did not fully support strong binding affinity to cytoplasmic structures.

Anthocyanin pigments are highly sensitive to pH, temperature, oxygen, and light exposure; under acidic conditions they tend to produce red coloration and remain more stable, whereas neutral or alkaline conditions can lead to pigment degradation and color fading (Gómez, Lafiosca, & Giovannini, 2024). The extract used in this study had a pH of 5,2, which may not have been sufficiently acidic to achieve staining intensity comparable to eosin. In addition, staining quality may have been affected by extract storage conditions, as anthocyanin degradation can occur rapidly when exposed to direct light and room temperature, making proper storage in dark containers and low temperatures essential to maintain pigment stability.

One factor influencing anthocyanin stability in this study was light exposure, which plays a crucial role in the formation and degradation of anthocyanin. Therefore, teak leaf extract should be stored in a dark place or in a brown glass bottle at low temperatures. However, in this study, the teak leaf extract was stored in a clear bottle and placed in a bright place. Furthermore, the type of solvent used and the pH value during the dyeing process also influence the absorption of anthocyanin dyes, thus impacting the quality of the tested preparations.

A limitation of this study lies in the lack of pH treatment, either acidic or basic, on the teak leaf extract. This treatment should have been performed to determine the pH content of the extract and compare it with the pH of the eosin dye. Overall, these findings are consistent with previous studies indicating that teak leaf extract can function as a natural dye, although further optimization is required to achieve staining quality comparable to eosin. Variations in concentration, extraction methods, solvent types, tissue types, staining duration, and pH treatment may contribute to differences in staining performance across studies. Future research should focus on pH adjustment, mordant addition, concentration variation, and extended staining duration to enhance cytoplasmic staining intensity, while larger sample sizes and quantitative image analysis are recommended to strengthen evidence regarding the effectiveness of teak leaf extract as an alternative histological.

Conclusions

Young teak leaf (*Tectona grandis*) extract demonstrated the ability to stain nuclear and cytoplasmic components in uterine histological preparations, indicating its potential as a natural substitute for eosin in HE staining; however, the staining quality remained inferior to eosin, as the contrast between the nucleus and cytoplasm was less distinct. The average cytoplasmic staining score in the teak leaf extract group was lower than in the eosin control group, suggesting that further optimization is required before practical laboratory application can be recommended. Future studies should focus on improving staining quality through pH adjustment, concentration optimization, mordant addition, improved extraction techniques, and controlled storage conditions to enhance anthocyanin stability and cytoplasmic staining affinity.

Author contributions

MAV, AWD, and JC Contributed to the research design, data collection, data analysis, data processing, and manuscript review. All authors have read and approved the final version of the manuscript.

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Ethical approval statement

The study received ethical approval from the Ethics Committee of Poltekkes Kemenkes Kupang with certificate number No.LB.02.03/1/00 0 8/2025.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

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