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Original Article

Extract of Teak Leaf Buds (Tectona grandis) as Natural Dyes Replacement of Eosin on HE Staining

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ABSTRACT

Hematoxylin and Eosin (HE) staining is a routine method in histopathology. In HE staining, eosin functions to stain the cytoplasm of the tissue. Continuous use of eosin may cause health problems due to its carcinogenic properties, thus requiring an alternative. One such alternative is teak leaf buds, which contain anthocyanin compounds. The aim of this study was to determine whether teak leaf bud extract can be used as a substitute for eosin in HE staining. The type of research used was a true experimental study with a post-test only control group design. The samples consisted of paraffin blocks, from which 10 slides were prepared: one control and nine treatment slides, with three repetitions for each concentration (20%, 30%, and 40%). Microscopic observations of HE staining using eosin showed a score of 3 (100%). Using 20% teak bud extract resulted in a score of 2 (100%), 30% concentration gave a score of 2 (33,3%) and score 3 (66,7%), while 40% concentration resulted in score 3 (100%). Kruskal-Wallis test showed eosin and teak leaf bud extract were not significantly different: for 20% concentration, p=0,083; for 30%, p=0,564; and for 40%, p=1,000 (p>0,05), indicating no significant difference.It can be concluded that 40% teak leaf bud extract is the best concentration and has the potential to replace eosin in HE staining. Future research is suggested to use 40% teak leaf bud extract with variations in staining time to further explore its potential as a substitute for eosin in histological staining.

Introduction

Histological staining is a method used to add color to cellular organelles, thereby facilitating their observation under a microscope. The purpose of staining is to enhance the visibility of tissue elements and allow differentiation of their components under the microscope. The HE staining process is one of the most frequently used techniques in histology. Hematoxylin functions as a basic dye, meaning it stains basophilic elements in the tissue blue (Setiawan, 2016).

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Eosin is a synthetic dye that belongs to the xanthene group. It is an acidic substance capable of binding to positively charged protein molecules in the cytoplasm and connective tissue. Eosin imparts an orange-red color to the cytoplasm and connective tissue and stains acidophilic structures such as mitochondria. The cytoplasm and collagen will appear pink when stained with eosin (Mutoharoh, Santoso, & Mandasari, 2020).

Prolonged use of eosin has the potential to cause negative effects on the body. Ingestion of eosin may lead to irritation of the gastrointestinal tract, inhalation may cause cyanosis, and direct contact with the skin can result in irritation. In addition, eosin is also considered to have carcinogenic properties (Wulandari, Widiyani, & Iswara, 2019).

Natural dyes derived from organic materials can be used to minimize the negative effects of eosin. One of the most popular types of natural dyes is anthocyanin. Anthocyanins are natural pigments found in various organisms in nature. They belong to the flavonoid group of compounds, are highly water-soluble, and are responsible for producing red, purple, blue, and yellow colors in plant parts such as fruits, vegetables, flowers, leaves, roots, tubers, legumes, and cereals (Fathinatullabibah, Khasanah, & Kawiji, 2014).

Research conducted by Mizan, Damayanti, & Nuroini (2021) proves that eosin contains fluorescein, which gives the cytoplasm a pink color, so that the anthocyanin content in some plants has the same properties or uses as the fluorescein content in eosin. Another plant containing anthocyanins is teak leaves. The anthocyanin compounds found in teak leaves contribute to red pigmentation. Teak tree leaves are brownish-green in color and when crushed, they release a red-colored sap. The use of young teak leaves produces a more intense red color compared to mature leaves (br Kembaren et al., 2014).

Based on the above background, the researchers conducted a study entitled "Teak Leaf Bud (Tectona grandis) Extract as a Natural Dye Alternative to Eosin in Hematoxylin-Eosin Staining." The purpose of this study was to determine the microscopic results of histological preparations using teak leaf bud (Tectona grandis) extract as a natural dye alternative to eosin in the HE staining process.

Methods

This study employed a true experimental design with a post-test only control group. The sample used was paraffin blocks of histological specimens obtained from the Anatomical Pathology Laboratory of Prof. W.Z. Johannes Kupang Regional General Hospital. Data were analyzed descriptively by assigning scores of 1, 2, and 3 to the HE-stained histological specimens, which were then presented in a frequency distribution table. The scoring was based on microscopic examination to assess the cell nucleus and cytoplasm. A score of 3 was given if the nucleus and cytoplasm were perfectly stained, a score of 2 if the cell nucleus and cytoplasm were partially stained, and a score of 1 if the cell nucleus and cytoplasm were not well stained. A Kruskal-Wallis test was then conducted to determine whether there were significant differences in the microscopic results of HE staining between the control and treatment groups.

Results

The preparation of teak leaf bud extract (Tectona grandis) used in this study was obtained from Oelnasi Village, Kupang Tengah Subdistrict, Kupang Regency, East Nusa Tenggara. In this study, teak leaf buds were macerated with the addition of 10% citric acid to clarify the red color of the pigment, as a preservative, and to equalize the pH with eosin, which is an acidic dye.

To maintain color intensity, an evaporator temperature below 60 °C was used to prevent anthocyanin degradation in the teak leaf bud extract. Furthermore, to prevent damage to anthocyanin pigments due to direct contact with sunlight and oxygen, glass bottles were used during the extraction process, which were tightly sealed, placed in a dark place, and wrapped in aluminum foil.

The microscopic images of HE-stained breast carcinoma preparations were assessed based on the clarity of nuclear and cytoplasmic staining and their distinguishability. Observations were made using a Cx-31 Trinocular connector camera microscope at 400x magnification. The following are the results of observations from control preparations with eosin and treatment preparations using teak leaf bud extract at concentrations of 20%, 30%, and 40%.

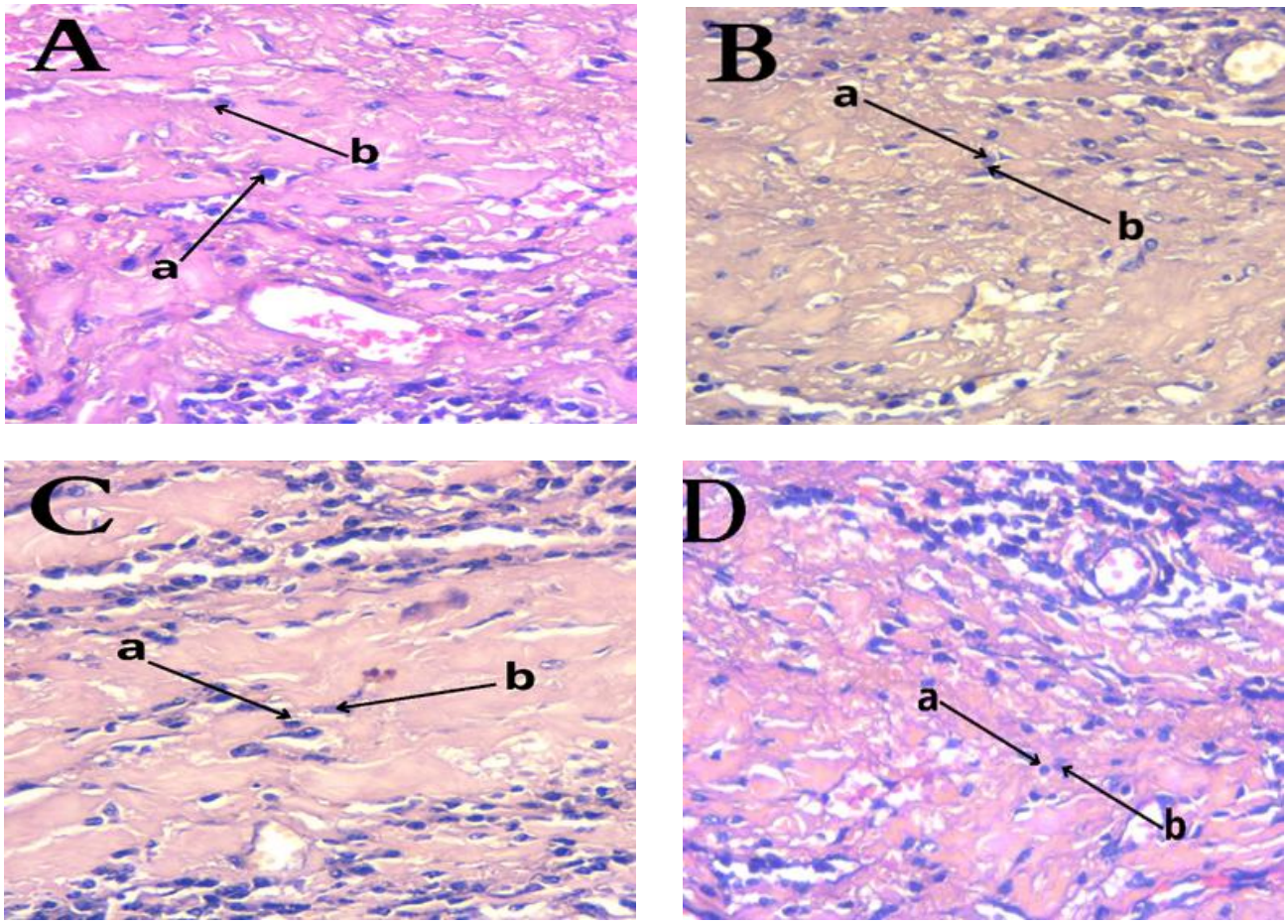

Figure 1. HE staining results of Ca mammae specimens (A.) Control; (B) 20% teak leaf bud extract; (C) 30% teak leaf bud extract; D) 40% teak leaf bud extract. (a: Cell nucleus; b: Cytoplasm)

A summary of the assessment results for HE staining using eosin and teak leaf bud extract as a substitute for eosin is presented in Table 1.

Table 1. Scoring assessment of he staining results

Treatment	Assessment Results						Total	
	Score 1 (Not Good)		Score 2 (Poor)		Score 3 (Good)			
	n	%	n	%	n			%
Eosin	0	0	0	0	1	100	1	100
Teak Leaf Bud Extract 20%	0	0	3	100	0	0	3	100
Teak Leaf Bud Extract 30%	0	0	1	33,3	2	66,7	3	100
Teak Leaf Bud Extract 40%	0	0	0	0	3	100	3	100

The results of the HE staining score for Ca mammae tissue specimens using eosin yielded a score of 3 (good) on slides with a percentage of 100%. The results of HE staining for Ca mammae tissue specimens using teak leaf bud extract as a substitute for eosin at a concentration of 20% yielded a score of 2 (poor) on all slides, namely 3 slides with a percentage of 100%, at a concentration of 30%, a score of 2 (poor) was obtained on 1 slide with a percentage of 33,3% and a score of 3 (good) on 2 slides with a percentage of 66,7%, and at a concentration of 40%, a score of 3 (good) was obtained on all slides, i.e., 3 slides with a percentage of 100%.

Discussion

HE staining can reveal the structure and morphology of tissues, as well as the presence and prevalence of certain tissue cells (Khristian & Inderiati, 2017). Hematoxylin is a basic dye that selectively stains acidic components within cells, resulting in a blue appearance. Eosin, on the other hand, is an acidic dye that stains the cytoplasm of cells, resulting in a pink color (Setiawan, 2016).

Based on microscopic observations using 400x magnification, in the control preparation stained with eosin, the cell nuclei were blue-violet and the cytoplasm was perfectly stained bright pink, with clear colors that could be distinguished and identified. In the group of specimens stained using 20% jati leaf bud extract, the color quality was poor, with cell nuclei appearing pale purple and cytoplasm stained a brownish pink, though they were still distinguishable and identifiable. In the group stained with 30% teak leaf bud extract, most specimens were stained nearly perfectly, with cell nuclei appearing blue-violet and cytoplasm pink, distinguishable and identifiable, though the specimen color lacked contrast. In the group of specimens stained using 40% teak leaf extract, the nuclei and cytoplasm were well stained, with the cell nuclei appearing blue-violet and the cytoplasm bright pink, the colors were clear, and the specimens could be distinguished and identified.

Good staining results were obtained at dilutions of 30% and 40%, with the cytoplasm appearing bright pink, while at a dilution of 20%, the results were less satisfactory, with the cytoplasm appearing brownish pink. Based on the research conducted, the intensity of the color produced is highly dependent on the concentration of the jati leaf bud extract. This is due to the difference in the amount of concentrated extract present in the solvent. Higher concentrations result in a greater amount of extract in the solvent, and thus higher anthocyanin and acid content. Anthocyanins are natural compounds that can stain tissues as an alternative to eosin. The more acidic the pH of the anthocyanins, the redder the color produced. Therefore, the higher the concentration of the extract, the more intense the red color produced.

After microscopic evaluation of the preparations, the data were statistically tested using the Kruskal-Wallis nonparametric test to assess how closely the color of each concentration of teak leaf bud extract preparation matched that of the control preparation. The color is considered to be close to the control or there is no significant difference (H0) if the significance value (p)>0,05, and not close to the control or there is a significant difference (H1) if the significance value (p)<0,05. Based on the results of the Kruskal-Wallis test, the significance values for the eosin and 20% teak leaf bud extract treatment were p=0,083, eosin and 30% teak leaf bud extract p=0.564, and eosin and 40% teak leaf bud extract p=1,000. The Kruskal-Wallis test results showed that all three p values were >0,05, indicating that the color of the preparations from the three concentrations was similar to the control or there was no significant difference between the control and the three extract concentrations.

The results of this study are not in line with the research conducted by Labai (2023), who used teak leaf bud extract as a substitute for eosin in Diff-Quik staining. There was a significant difference between the control slides and the treatment slides with a p-value <0,05 in the Kruskal-Wallis test, so the Mann Whitney test was continued and good results were obtained at a concentration of 30% with a p-value >0,05 (no difference), while poor results were obtained at concentrations of 40%, 50%, 60%, and 70% with a p-value <0,05 (significant difference). The differing results may be attributed to variations in the collection sites of teak leaf buds, anthocyanin content of the extract, solvent type, maceration time, addition of 10% citric acid, diluent solution, and sample type.

The Kruskal-Wallis test showed the highest significance value for the 40% extract (p=1,000), indicating that this concentration is optimal as a substitute for eosin in HE staining.

This study used three concentrations that produced different staining results. At 30% and 40% dilution, the cytoplasm was bright pink, while at 20% dilution, the results were less favorable, with the cytoplasm stained brownish pink. The intensity of the color produced is highly dependent on the concentration of the teak leaf bud extract. This is due to the difference in the amount of concentrated extract present in the solvent. The higher the concentration, the greater the amount of concentrated extract in the solvent, and the higher the content of anthocyanins and acids in the extract. The more acidic the pH of the anthocyanin, the redder the color produced. Therefore, the higher the extract concentration, the more intense the red color produced. For future research, extracts with higher concentrations can be used. Additionally, the duration of histological tissue staining using 40% teak leaf bud extract can be modified as a substitute for eosin in HE staining.

Conclusions

Microscopic results of HE staining of breast carcinoma tissue specimens using eosin yielded a score of 3 (100%), while 20% concentration of teak leaf bud extract yielded a score of 2 (100%), 30% concentration yielded a score of 2 (33,3%) and a score of 3 (66,7%), and at a concentration of 40%, a score of 3 (100%) was obtained. There was no significant difference between the microscopic results of breast carcinoma specimens stained with eosin and those stained with teak leaf bud extract as an alternative to eosin in HE staining, with a significance value of 20% for teak leaf bud extract (p=0,083), teak leaf bud extract 30% p=0,564, and teak leaf bud extract 40% p=1,000. The results of this study indicate that 40% teak leaf bud extract is the optimal concentration to replace eosin in HE staining. Based on these findings, further studies are

recommended to explore variations in staining time using 40% teak leaf bud extract as an alternative to eosin in HE staining.

Author contributions

AOW, AFTB, NKYS contributed to the study's concept and design. AFTB assisted with experimental studies and data acquisition. NKYS managed the literature search, data analysis, and statistical analysis. All authors participated in manuscript preparation, with AOW responsible for editing and review. All authors have read and agreed to the published version of the manuscript.

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Ethical approval statement

This study was approved by the Research Ethics Committee of Poltekkes Kemenkes Kupang with certificate number LB.02.03/1/0018/2024.

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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