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Original Research

*Potentiated extracts of Karamunting (*Rhodomyrtus tomentosa*), Senduduk (*Melastoma malabathricum*), and Jernang (*Daemonorops draco*) fruits as natural eosin substitutes in Hematoxylin–Eosin histopathological staining*

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Abstract: Histopathological staining is essential for the assessment of tissue morphology, with hematoxylin–eosin (H&E) staining being the most widely used technique. However, eosin poses several limitations, including carcinogenic potential, tissue damage, flammability, and environmental hazards. Natural pigments derived from *Rhodomyrtus tomentosa* (karamunting), *Melastoma malabathricum* (senduduk), and *Daemonorops draco* (jernang) fruits represent promising safer alternatives due to their richness in chromogenic compounds. This study aimed to evaluate the potential of these fruit extracts as substitutes for eosin in H&E histopathological staining. A true experimental design was employed, comprising qualitative phytochemical screening, gas chromatography–mass spectrometry (GC–MS) profiling, extract pH analysis, and histopathological staining of pancreatic tissue obtained from diabetic white rats (*Rattus norvegicus*). Staining performance was assessed using extract concentrations of 70%, 80%, and 90%, with conventional H&E staining as a positive control and unstained sections as a negative control. Phytochemical analysis revealed that senduduk extracts contained flavonoids, anthocyanins, and tannins; karamunting extracts contained flavonoids, anthocyanins, alkaloids, saponins, and tannins; and jernang extracts contained flavonoids, anthocyanins, and tannins. GC–MS analysis identified 19 metabolites in senduduk, 27 in karamunting, and 4 in jernang extracts. All extracts exhibited acidic pH values. Among the tested conditions, the 90% karamunting extract produced the most satisfactory staining, yielding a distinct reddish coloration of the cytoplasm and connective tissue with adequate contrast to delineate pathological alterations, whereas other extracts and concentrations resulted in suboptimal staining quality. To our knowledge, this is the first study to comparatively evaluate the staining performance of these three natural fruit extracts on pancreatic tissue from diabetic rats using an integrated phytochemical, metabolomic, and pH profiling approach. The findings indicate that a 90% karamunting fruit extract has potential as a natural substitute for eosin in H&E histopathological staining.

Keywords: Histopathological staining; Hematoxylin–eosin substitute; Natural dyes; Rhodomyrtus tomentosa; Diabetic rat pancreas

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INTRODUCTION

Histopathology stains help visualize tissue specimens under a microscope.[1] Hematoxylin–Eosin staining remains the gold standard in histopathology because it provides clear contrast between nuclear and cytoplasmic components.[2] However, the eosin component presents several practical limitations related to its chemical properties, including irritant potential, waste-management concerns, and handling risks associated with hazardous laboratory reagents, so it requires an alternative stain to minimize the potential dangers caused by the dye. Various plant-derived extracts, such as *Hibiscus sabdariffa* (Roselle)[3], *Curcuma Longa rhizome* (Turmeric)[4], *Beta vulgaris* (Beetroot), and several species within the *Melastomataceae* family, have been investigated as potential substitutes for eosin.[5] Although many of these extracts yield pink-to-red cytoplasmic staining, their performance has been inconsistent, and their diagnostic reliability remains uncertain.[6] A summary of previous natural dyes and their reported limitations is presented in **Table 1.**

Table 1. Summary of previously investigated natural cytoplasmic stains

Plant Extract	Target Tissue	Performance Reported	Key Limitations
Lawsonia inermis (henna)[7]	Liver biopsies	Strong cytoplasmic staining at 1% concentration; contrast comparable to eosin	Variation with extraction method; weaker staining intensity under certain conditions (temperature and accentuator type)
Curcuma longa (turmeric)[8]	Oral mucosa; general tissues	Yellow–orange cytoplasmic staining; acidic dye with affinity for collagen, muscle, and epithelium; provides Hematoxylin counterstaining with contrast similar to eosin	Variable intensity; sometimes weaker contrast than eosin. Shorter shelf life than *Zingiber officinale*
Zingiber officinale (ginger)[9]	Oral histopathology	Outperformed *Curcuma longa* in staining quality, efficacy, and shelf life; cost-effective, biodegradable, and lower hazard profile	Staining intensity varied with extraction conditions; suboptimal temperature or accentuators reduced contrast; requires optimization for consistent results
Beta vulgaris (beetroot)[6]	Oral histopathology	Cytoplasmic staining observed	Low detail resolution; weak nuclear contrast
Hibiscus rosa-sinensis[6]	Oral histopathology	Pink/red staining, weak staining	Very low staining intensity; poor morphological clarity

Eosin is acidic and stains basic structures in tissue such as cytoplasm, collagen, and other tissue structures.[10] Eosin has a pink or red color. Plants that have potential as dyes are karamunting (*Rhodomyrtus tomentosa),* senduduk (*Melastoma malabathricum*), and jernang (*Daemonorops draco*). Karamunting and Senduduk have high anthocyanin content, are acidic, and red, making them potential as natural dyes[11], and jernang has a deep red color. Senduduk is starting to be developed as a dye for connective tissue preparations[12] and Jernang is used

by the Anak Dalam Tribe in Muara Kilis as a clothing dye due to its flavonoid and anthocyanin content.[13,14]

The high flavonoid and anthocyanin potential in karamunting, senduduk and jernang has the potential to be developed as a histopathological dye that can visualize disease in tissue and reduce the effects of eosin contained in H&E dye. Research related to tissue dye substitutes is starting to be developed using natural ingredients such as extracts of jambolan fruit[15], mangosteen peel, hibiscus, young teak leaves[16], purple cabbage[17], beetroot and turmeric.[18] However, the dyes produced from these extracts are less than optimal in visualizing tissue under a microscope. Studies that have been able to stain tissue structures include safflower seeds[19], black plums, henna[20], ginger[21], and senduduk fruit[12]. However, these studies did not visualize the tissue damage caused by disease, so the disease-related tissue damage was not visualized and could not be analyzed. Currently, the number of Occupational Accidents (KK) and Occupational Diseases (PAK) in laboratories has reached 1,310 cases of KK and 234,370 cases of PAK. Therefore, research into safe alternative dyes is needed to reduce the number of KK and PAK.[22]

The use of natural extracts as a substitute for eosin in H&E staining as a histopathology stain is a safe innovation for health and the environment for examining abnormalities and determining the level of tissue damage caused by disease. The production of dyes from the fruit extracts of karamunting (*Rhodomyrtus tomentosa*), senduduk (*Melastoma malabathricum*), and jernang (*Daemonorops draco*) was carried out through maceration extraction, concentration, and phytochemical testing. The maceration method prevents the destruction of thermolabile secondary metabolites[23]. Concentration at 50°C prevents the destruction of bioactive compounds.[24] In a study by Romaidha et al.[12], the maceration extraction and concentration method at 50°C optimized the staining results. The potential of natural tissue dyes as a substitute for eosin in H&E staining is safer, more environmentally friendly, and more cost-effective.[4]

Histopathology staining involves five stages: 1. Tissue collection 2. Fixation with 4% formaldehyde 3. Dehydration, clearing, embedding, and sectioning 4. H&E staining 5. Histopathology reading.[25] For pancreatic tissue, the staining must clearly reveal structural details and pathological changes while ensuring safety for laboratory personnel and the environment. Although karamunting, senduduk, and jernang fruits contain strong anthocyanin and flavonoid pigments, there has been no study evaluating their ability to stain pathological tissue, including diseased pancreatic tissue, and no comparative assessment among the three plant extracts has been conducted. This gap highlights the need for further research, and the present study addresses this need by providing the first evaluation of karamunting, senduduk, and jernang fruit extracts as natural substitutes for eosin in staining pancreatic tissue from diabetic rats, integrating phytochemical analysis, pH characterization, and histopathological assessment to determine their staining potential and ability to visualize disease-related tissue changes. The development of natural histopathological dyes has the potential to reduce laboratory exposure to hazardous chemicals and decrease the incidence of PAK.[26]

MATERIAL AND METHOD

This study used a control and treatment group, using a qualitative research design. The research design was true experimental. The data scale used was nominal. The data analysis was qualitative descriptive[27] by describing the structure, changes, and significance of pathological development of the pancreas of diabetic *Rattus norvegicus* using microscopy results from staining extracts of Karamunting, Senduduk, and Jernang fruit. This research has obtained ethical clearance from

the Health Research Ethics Committee (KEPK) of Dr. Soebandi University with No. 1297/KEPK/UDS/VI/2025 so that it is suitable for experimental testing.

This study aimed to analyze the potential of Karamunting *(Rhodomyrtus tomentosa*), Senduduk (*Melastoma malabathricum*), and Jernang (*Daemonorops draco*) fruit as natural dyes. The histopathology of tissue consisted of three stages:

1. Preparation of Karamunting, Senduduk, and Jernang Fruit

Karamunting, Senduduk, and Jernang fruit were separated and selected for ripe and unrotten fruit. Each fruit was washed to remove sand, dust, or other foreign substances that could potentially affect the extraction results. Each fruit was weighed to the tune of 5000 grams. The cleaned fruit was air-dried and sorted to remove foreign substances from the sample. Next, it was chopped.[27]

2. Preparation of Karamunting, Senduduk, and Jernang Fruit Extracts and Phytochemical Testing

Extraction was carried out using the maceration method. Maceration has the advantage of extracting compounds without the need for heating and without the risk of degradation of natural metabolite compounds.[28] Maceration was carried out with 96% ethanol with a ratio of 1:10 (5000 grams of simplicia:50 liters of 96% ethanol) for 3 x 24 hours at room temperature. The 96% ethanol served as the solvent due to its polar nature, which binds polar anthocyanins and flavonoids.[23] Concentration was carried out using a rotary evaporator at 50°C to optimize the potential of the natural dyes. The concentrated extract was diluted to determine the concentration of the histopathological dye treatment using the formula (M1.V1 = M2.V2).[12] The concentrated extract was then subjected to quantitative phytochemical testing using Gas Chromatography-Mass Spectrometry (GC-MS) in the integrated laboratory of the Islamic University of Indonesia. GC-MS used an Rtx-5MS column (5% diphenyl / 95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol) with helium as the carrier gas and temperature for ionsource is 250°C and interface is 300 °C. The pH test was performed on the extract using the weight to volume (w/v) formula, for concentrations of 70% (70 grams of concentrated extract in 100 ml of distilled water), concentrations of 80% (80 grams of concentrated extract in 100 ml of distilled water), and concentrations of 90% (90 grams of concentrated extract in 100 ml of distilled water). After the extract was diluted to the specified concentration, the pH test was performed using a pH meter calibrated with calibration reagents (acidic at pH 4 and basic at pH 10).

3. Preparation, Staining, and Reading of Histopathology Preparations

This study utilized pancreatic tissue diabetic *Rattus norvegicus* (mice) preparation at pharmaceutical laboratory, undergraduate pharmacy study, STIKES Borneo Cendekia Medika with all procedures approved by the Research Ethics Committee of Dr. Soebandi University, number: 1297/KEPK/UDS/VII/2025. This study used the pancreas of diabetic *Rattus norvegicus*. Male rats weighing ≥200 g were acclimatized for 10 days before baseline fasting blood glucose was measured on day 11 using a photometric method. Diabetes was induced on day 11 with Streptozotocin (ZTC) administered intramuscularly at a dose of 55 mg/kg, prepared at a concentration of 4 mg/mL in citrate buffer, with the pH of the solution not measured. Animals were monitored for 24 hours following induction, after which fasting blood glucose was reassessed; rats with glucose levels ≥500 mg/dL were considered diabetic, and all rats successfully achieved diabetic status.[12] Diabetic animals were euthanized 3–7 days after ZTC administration, and the pancreas was excised and fixed in 10% Neutral Buffered Formalin to ensure adequate tissue preservation for sectioning. The pancreas tissue underwent deparaffinization, rehydration, and hematoxylin staining, followed by substitute staining using Karamunting, Senduduk, and Jernang fruit extracts, and subsequent dehydration, clearing, and mounting for histopathological examination.[25] The study adhered to the 3R principle: Replacement by using tissue sections instead of live animals for repeated tests; Reduction by minimizing animal numbers; and Refinement through careful monitoring and humane euthanasia. Three extracts at three concentrations

were tested, with one positive control (H&E) and one negative control (distilled water), each repeated three times.[30]

The staining process begins with deparaffinization using xylol 1 and 2 for 10 minutes each, immersion in absolute alcohol for 1 minute, 96%, 70%, and 50% alcohol for 1 minute. Then, it is soaked in water for 2 minutes. It is immersed in Harris haematoxylin staining solution for 1-3 minutes and then rinsed with water for 2 minutes and immersed in bluing reagent for 1 minute and rinsed with water for 2 minutes. In the positive control, eosin is used for 1-2 minutes, the negative control is used distilled water, and the extract treatment is for 15 minutes. Next, immersion in 96% alcohol, absolute alcohol, Xylol 1, 2, and 3 for 1 minute each and continued with the process of mounting the preparation and reading the tissue.

Staining results are assessed by one board-certified Anatomical Pathologist, using the following scale:

Table 2. Assessment of Tissue Staining Results

Description	Qualification	Ordinal Scale
Red staining in the connective tissue and cytoplasm is unclear, the colour on the slide is not uniform, the pathologic development cannot be described	Poor	1
Red staining in the connective tissue and cytoplasm is quite good, the colour on the slide is quite good, the pathologic development can be described	Fair	2
Red staining in the connective tissue and cytoplasm is good, the colour on the slide is uniform, the pathologic development can be described	Good	3

RESULTS AND DISCUSSION

1. Phytochemical Test Results

a. Qualitative Phytochemical Testing

Based on Table 3, the results of qualitative phytochemical analysis revealed that the three fruit extracts included distinct arrays of secondary metabolites. The flavonoid and anthocyanin assays yielded a red solution, while the tannin assay exhibited a bluish-black hue, indicating that *Melastoma malabathricum* (Senduduk) is positive for flavonoids, anthocyanins, and tannins. No alkaloids or saponins were detected, as evidenced by the absence of brick-red or yellowish-white precipitates in the alkaloid test and the lack of stable foam in the saponin test.

Among the examined samples, *Rhodomyrtus tomentosa* (Karamunting) exhibited the most intricate composition of phytochemicals. Flavonoids, anthocyanins, alkaloids, tannins, and saponins exhibited beneficial effects. The flavonoid test exhibited a brick red coloration upon the addition of hydrochloric acid, followed by a greenish hue at the introduction of sodium hydroxide. Alkaloids were identified by the formation of brick-red or yellowish-white precipitates, while tannins were indicated by a bluish-black solution. The presence of saponin was validated by the formation of a stable foam around 1 cm in height, indicating significant surfactant action.

Extracts of *Daemonorops draco* (Jernang) demonstrated the presence of flavonoids, anthocyanins, and tannins, as evidenced by a characteristic red hue in the flavonoid and anthocyanin assays and a bluish-black reaction in the tannin assay. Alkaloids and saponins were absent, similar to *M. malabathricum*, as evidenced by the lack of precipitate or foam formation.

The results indicate that *R. tomentosa* fruit extract possesses a broader spectrum of chromogenic and bioactive compounds compared to the other extracts. The detection of flavonoids, anthocyanins, and tannins in all samples suggests their potential application as natural dyes for histopathology staining. The

presence of alkaloids and saponins in R. tomentosa may augment its staining efficiency, as demonstrated by subsequent histological assessments.

b. Quantitative Phytochemical Testing Using GC–MS

Based on Table 4, the gas chromatography–mass spectrometry (GC–MS) analysis of *Rhodomyrtus tomentosa* (Karamunting) fruit extract identified 27 chemical constituents with varying abundances. The chromatographic analysis revealed that the extract predominantly consisted of fatty acids, glycerides, terpenoids, and phenolic compounds. These possess chromogenic and bioactive properties beneficial for histology staining.

Linoleic acid ($C_{18}H_{32}O_2$) was the predominant component identified, including a total peak area of 27.96%, indicating that it constituted the majority of the extract. Palmitic acid (2.30%), palmitoleic acid (3.94%), oleic acid (0.30%), and icosanoic acid (0.43%) were among the other fatty acids identified. These lipid constituents may facilitate the dye's solubility, adherence to tissues, and uniform staining. We additionally identified glyceride derivatives, including glyceryl 1,3-dipalmitate (2.46%), glyceryl monooleate (0.20%), glyceryl 1,3-distearate (0.54%), diolein (8.95%), and 1,3-distearoyl-2-palmitoylglycerol (0.66%). This indicates the presence of intricate lipid structures that may facilitate the adherence of dyes to tissue matrices.

Phloroglucinol constituted 4.27% of the phenolic compounds. This is a recognized chromogenic phenol that may play a significant role in cytoplasmic coloration due to its acidic functional groups. We identified terpenoid chemicals such as dammarenediol-I (total peak area 16.36%), cedrane (0.31%), and sitosterol (0.34%). Dammarenediol-I, a triterpenoid compound, is associated with significant molecular stability and may account for the intensity and longevity of staining. Menthyl acetoacetate (13.11%) and cyclohexylidenecyclohexane (13.35%) were identified in substantial quantities, indicating a diverse chemical profile and potential interactions that may have influenced staining behavior.

The GC–MS data indicate that the fruit extract of *R. tomentosa* comprises a complex mixture of fatty acids, phenolic compounds, glycerides, and terpenoids. The extract's inclination towards fundamental cellular constituents, particularly those within the cytoplasm and connective tissue, is corroborated by its high content of acidic fatty acids and phenolic compounds. The chemical composition of this material likely accounts for the superior staining properties of the 90% Karamunting extract, hence endorsing its application as a natural eosin substitute in hematoxylin-eosin histopathology staining.

Based on Table 5, the gas chromatography–mass spectrometry (GC–MS) analysis of *Melastoma malabathricum* (senduduk) fruit extract identified 19 chemical constituents with diverse structural classifications and varying relative abundances. The chromatographic profile predominantly consisted of lipid-derived compounds, including fatty acids, glycerides, aldehydes, and triterpenoid constituents. These factors may influence the staining properties of the extract in histopathological applications.

Diolein (19.70%) and dammarenediol-I, which appeared in three peaks with a cumulative relative area of 29.45%, were the predominant chemicals identified. This indicates that triterpenoid constituents comprise a significant portion of the extract. Additional fatty acids were linoleic acid (5.84%), palmitic acid (1.10%), and octacosanoic acid (0.58%). This indicates that the entire chemical profile was acidic, potentially facilitating adhesion to basic tissue components. Linoleoyl chloride (5.03%) and unsaturated aldehydes, including 9,12-octadecadienal (0.64%) and 9-octadecenal (2.68%), were also identified. These chemicals may contribute to chromogenic interactions in tissue sections.

Glyceride derivatives, including glyceryl 1,3-dipalmitate (2.82%), glyceryl 1,3-distearate (1.88%), and monolinolein (total peak area of 11.02%), indicate the presence of complex lipid structures that could enhance dye solubility and tissue

penetration. Cyclohexylidenecyclohexane (12.39%) and various other hydrocarbon derivatives contributed to the chemical diversity of the extract.

The GC–MS analysis indicates that *M. malabathricum* fruit extract contains several lipid-based and triterpenoid compounds, with diolein and dammarenediol-I being the most significant. The extract possesses an acidic composition and many chromogenic compounds; nonetheless, the predominance of nonpolar lipids may result in inconsistent and low-contrast staining. This aligns with the observation that senduduk extract does not stain as effectively as Karamunting extract.

Based on Table 6, the gas chromatography–mass spectrometry (GC–MS) analysis of *Daemonorops draco* (Jernang) fruit extract identified four primary chemical constituents, indicating a relatively simple phytochemical profile compared to the karamunting and senduduk extracts. The chromatogram predominantly comprised phenolic and esterified fatty acid molecules. These chemicals possess chromogenic and binding properties that are advantageous for histology staining.

Pterostilbene (41.36%) and p-tert-butylphenyl salicylate (40.21%) were the predominant compounds identified. Collectively, they constituted almost 80% of the total peak area. Pterostilbene is a phenolic stilbenoid compound characterized by notable aromatic conjugation. This may enhance color intensity and chromogenic stability. Similarly, p-tert-butylphenyl salicylate, a phenolic ester, contains aromatic and hydroxyl functional groups that may improve interactions with essential tissue components. The presence of these phenolic compounds supports the reported responses related to anthocyanins and tannins in the qualitative phytochemical analysis.

Methyl palmitoleate (11.56%) and methyl oleate (6.88%) were identified as minor components. These fatty acid methyl esters may influence the solubility and distribution of the pigment in tissue sections, but they are unlikely to directly impact the specificity of the staining.

The GC–MS data indicate that *D. draco* fruit extract contains many phenolic chromophores, but exhibits limited chemical diversity. The elevated quantity of phenolic compounds suggests potential for vibrant coloration; nevertheless, the absence of a varied array of acidic dyes and other lipid components may restrict staining uniformity and contrast. This compositional characteristic may clarify the poorer histological staining effectiveness of Jernang extract compared to karamunting extract under the experimental conditions employed.

2. Testing the acidity level of Potential of Hydrogen (pH) of Sendunia, Karamunting and Jernang Extracts

Based on Table 7, the pH values of Senduduk (*Melastoma malabathricum*), Karamunting (*Rhodomyrtus tomentosa*), and Jernang (*Daemonorops draco*) fruit extracts at concentrations of 70%, 80%, and 90% indicated varying acidity profiles among the three natural dye contenders. All extracts exhibited pH values ranging from acidic to nearly neutral, with distinct variations in size and concentration-dependent trends.

The Senduduk extract exhibited elevated acidity at all tested concentrations, with pH values of 2.10, 2.02, and 2.03 at concentrations of 70%, 80%, and 90%, respectively. This minor alteration indicates that the buffering is highly stable; nevertheless, it also reveals that the environment is significantly acidic, which may lead to excessive cytoplasmic staining or tissue injury during histological processing.

The acidity of Karamunting extract was moderate, with pH values increasing from 3.16 at 70% to 3.36 at 90%. This pH range closely resembles that of standard eosin solutions, which typically perform optimally in mildly acidic environments. The gradual increase in pH with higher extract content signifies improved staining equilibrium and a reduced likelihood of overstaining, supporting the superior histopathological efficacy shown for the 90% Karamunting extract.

The pH values of Jernang extract are significantly higher, ranging from 5.60 at 70% to 6.75 at 90%, approaching neutrality. The pH levels impede the adherence of basic cytoplasmic and extracellular matrix components, perhaps resulting in diminished staining intensity and contrast. The relatively low acidity of jernang extract may account for its inferior staining capability compared to Karamunting and Senduduk extracts.

pH testing indicates that Karamunting extract possesses the optimal acidity profile for application as a natural eosin substitute. This is due to its pH range facilitating optimal interaction with basic tissue components while minimizing the potential for tissue injury. These findings corroborate the histological staining results and affirm that 90% Karamunting extract is the optimal selection.

3. Tissue Analysis Results

An anatomical pathologist analyzed pancreatic tissue from *diabetic Rattus norvegicus* subjected to extracts from Karamunting (*Rhodomyrtus tomentosa*), Senduduk (*Melastoma malabathricum)* and Jernang (*Daemonorops draco)* fruits to assess their effectiveness as eosin substitutes (Figure 1). The standard hematoxylin-eosin (H&E) staining, employed as the positive control, provided the optimal histological contrast. The evident crimson staining of the connective tissue and cytoplasm demonstrated this. This dye facilitated the visualization and comprehension of pancreatic tissue structure. The exocrine glands and islets of Langerhans were clearly visible, as indicated by the blue and orange arrows. The negative control (staining solely with hematoxylin) had no reddish tint in the connective tissue or cytoplasm. This obscured the tissue structure and any indications of pathology.

Histological staining utilizing Karamunting fruit extract demonstrated concentration-dependent differences in staining quality. Among the examined quantities, the 90% Karamunting extract had the most favorable staining outcome, rendering the connective tissue and cytoplasm a pronounced crimson hue, providing sufficient contrast to differentiate between normal and pathological characteristics of pancreatic tissue. The staining intensity was slightly inferior to that of the positive control; nonetheless, the tissue morphology was effectively preserved and comprehensible. At an 80% concentration, Karamunting extract induced faint reddish staining, obscuring cell visibility and complicating the differentiation of tissue components. This complicated the interpretation of histology. The 70% concentration facilitated the observation of certain cellular characteristics; nevertheless, the inadequate penetration of the red pigment compromised the contrast and overall staining quality.

In contrast, Senduduk fruit extract exhibited poor staining performance across all tested concentrations. At both 90% and 80% concentrations, reddish coloration was observed to penetrate the connective tissue and cytoplasm; however, this was accompanied by noticeable cellular swelling and distortion, which obscured cellular details and compromised tissue integrity. At the 70% concentration, cellular components were partially visible, but insufficient penetration of the reddish pigment resulted in inadequate contrast, preventing reliable histopathological assessment.

Histological staining utilizing Jernang fruit extract, conversely, proved ineffective at all tested concentrations. At 90%, a faint crimson hue emerged, accompanied by significant cellular swelling, which compromised tissue stability and obscured cellular characteristics. The pigment failed to infiltrate the tissue adequately at the 80% concentration, resulting in continuing cellular expansion, which further obscured the histological examination. At 70%, certain cellular components were discernible, however, the insufficient reddish staining precluded a clear distinction between the cytoplasmic and connective tissue regions.

Overall, our findings indicate that the type and concentration of plant-derived extracts significantly influence their staining efficacy. The 90%

karamunting fruit extract had the highest reliability and efficiency in staining, suggesting its potential as a natural alternative to eosin in hematoxylin-eosin histological staining of pancreatic tissue. In contrast, jernang extract did not achieve satisfactory staining quality under the examined experimental conditions, likely due to limitations in pigment penetration and unfavorable physicochemical properties.

Figure 1 shows that at a concentration of 90%, the red pigment begins to penetrate the cells, but this causes them to swell. At a concentration of 80%, the red pigment cannot penetrate, causing the cells to swell. At a concentration of 70%, the red pigment cannot penetrate the cytoplasm and connective tissue. The marked cellular swelling could plausibly originate from the high ethanol fraction in these extracts, which acts not only as a membrane fluidizer but also as an osmolyte. Ethanol is known to disrupt membrane fluidity and increase permeability, permitting uncontrolled water influx; similar effects have been demonstrated in mammalian cells where ethanol interferes with normal volume-regulatory mechanisms. More recent evidence shows that ethanol alters the membrane–water interface by promoting water penetration into the lipid bilayer, thereby changing the conformational dynamics of transmembrane proteins, including ion channels. In addition to its direct effects on membrane lipids, ethanol can be metabolically incorporated into membrane phospholipids which further modifies membrane topology and activates intracellular signaling pathways.[31] The low pH of the extract may exacerbate this problem by altering ion gradients promoting water influx via a Donnan effect and disturbing intracellular ionic equilibrium. Together, these stresses may overwhelm the cells' regulatory volume decrease mechanisms, leading to swelling.[32]

In the testing process, samples of karamunting, senduduk and jernang fruit were extracted using the maceration method and concentrated using a rotary evaporator. In the concentration process, 5000 ml of macerate produced approximately 50 grams of concentrated extract. The concentrated extract was stored in a tightly closed, airtight glass container, at a low temperature of 4 degrees Celsius or room temperature of 25-30 degrees Celsius and not transparent to light. Storage of the extract results ensures the quality of secondary metabolite compounds, especially anthocyanins[33] as a dye that has the potential to be a substitute for eosin. The extract results were tested for potential dyes by qualitative and quantitative phytochemical testing, testing the acidity of the Potential of Hydrogen (pH) and testing on pancreatic histopathological tissue. Qualitative phytochemical testing revealed compounds including flavonoids, anthocyanins, alkaloids, tannins, and saponnins. Karamunting fruit extract contains flavonoids, anthocyanins, alkaloids, tannins, and saponnins, while senduduk and jernang fruit extracts contain flavonoids, anthocyanins, and tannins.

The same secondary metabolites found in all three fruit extracts are flavonoids and anthocyanins. Anthocyanins are pigments found in plants with potential as natural dyes. Anthocyanins act as natural antioxidants. Anthocyanins belong to the flavonoid group. Anthocyanins are glucoside compounds, causing the red, violet, and blue colors.[34] Anthocyanins contain cationic compounds in their chemical structure, resulting in interactions between anthocyanin molecules and polynucleotides within the nucleus. The color will appear consistent across cell nuclei because the anthocyanin binding mechanism occurs simultaneously in each nucleus, especially under uniform environmental conditions such as pH, dye concentration, and fixation. Thus, the detected color is the interaction between anthocyanin molecules and polynucleotides in the nucleus.[12]

Anthocyanins are a class of flavonoid compounds. Flavonoids are secondary metabolites found in plants. Flavonoids are polar compounds because th2ey have a hydroxyl group (-OH) that will form hydrogen bonds.[35] Flavonoids can be divided into several subgroups depending on the carbon in the C ring where the B ring is attached and the level of unsaturation and oxidation of the C ring.

Flavonoids whose B ring is attached to position 3 of the C ring are called isoflavones. Flavonoids whose B ring is attached to position 4 are called neoflavonoids, while flavonoids whose B ring is attached to position 2 can be further divided into several subgroups based on the structural features of the C ring. These subgroups include flavones, flavonols, flavanones, flavanonols, flavanols, or catechins, anthocyanins and chalcones.[36] These compounds have an important role in plant stress tolerance and are very relevant to human health, especially because these compounds have anti-inflammatory and antimicrobial properties. In addition, flavonoids have several applications in the food industry as preservatives, colorants, and antioxidants, as well as in other industries such as cosmetics and pharmaceuticals. Thus, flavonoids have numerous functions in various aspects, particularly in health and as colorants.[37] Anthocyanins have the potential to increase affinity for tissue pigment aggregation. Cation aggregates on surfaces with a negative charge density, for example, in the granular content of inflammatory cells, thus potentially staining inflamed cells.[38]

Several studies have confirmed that anthocyanins possess various biological activities beyond their function as color pigments, including immunomodulatory, antioxidant, hepatoprotective, antitumor, and anti-inflammatory properties. The extraction and purification of anthocyanins from natural plants is the basis for their application. The extraction process involves anthocyanins and large amounts of sugars, organic acids, and proteins. Increasing these compounds significantly impacts the stability, physiological activity, and delivery of the final anthocyanin product. Separation and purification of the crude extract are key to obtaining anthocyanins with high stability, strong physiological activity, and high quality.[39] Therefore, in this study, each stage of the extraction process was monitored, starting from the preparation of the simplex, solvent, temperature, time, and homogenization process to produce a high-quality extract. In addition to flavonoid anthocyanins, tannins are secondary metabolites found in the three extracts. Tannins are a class of polyphenolic compounds that can form complexes with proteins. The structure of tannins consists of a benzene ring (C6) bonded to a hydroxyl group (-OH). Tannins act as antioxidants.[40] Extract storage greatly affects the stability of anthocyanins, especially light, temperature, pH, enzymes, metal ions, antioxidants, and oxygen. The stability of anthocyanidins is influenced by the B-ring chain in the anthocyanidin structure and the presence of methoxy or hydroxyl groups.[29]

Anthocyanins in their flavylium cation form (predominant under acidic conditions) bind tissue components via a combination of electrostatic attraction, hydrogen bonding, and hydrophobic (π–π) interactions rather than purely ionic interactions. The structural behavior and color expression of anthocyanins are controlled by a well-defined series of pH-dependent equilibria, each associated with distinct chemical species. At pH < 3, anthocyanins predominantly exist as the flavylium cation, a highly conjugated and strongly pigmented form that produces red to orange hues and represents the most stable and reactive state. This cationic form carries a positive charge, enabling potential electrostatic interactions with negatively charged cellular components. As pH rises to 6–7, hydration and tautomerization convert the flavylium cation into the quinoidal anhydrobase, which exhibits a violet coloration and reduced charge density, altering both its chromatic intensity and binding affinity. At pH 7–8, further deprotonation yields the anionic quinone base, which appears blue but is only moderately stable. At pH > 11, anthocyanins shift into dianionic quinone forms, characterized by blue–green tones but very low stability due to rapid structural degradation. These pH-dependent transitions are critical in histological staining because only the low-pH flavylium-rich environment preserves strong coloration and maintains the cationic state necessary for interaction with tissue components. These multifaceted interactions allow anthocyanins to associate with cytoplasmic proteins and collagen, particularly at negatively charged or hydrophobic amino acid domains.[41] In

contrast, eosin Y is an anionic, acidic dye that selectively targets positively charged (basic) amino acid residues, such as lysine, arginine, and histidine, in proteins via strong electrostatic salt bridges. Consequently, even though anthocyanin-based staining may visually resemble eosin's pink–red cytoplasmic coloration, the chemical basis of binding is distinct, involving different kinds of molecular forces and target sites.[42] This mechanistic difference suggests that while anthocyanins can serve as a natural counterstain, they do not replicate eosin's binding behavior on a molecular level.

Anthocyanin is stable at low pH. The stability of anthocyanin pigments is influenced by pH, storage temperature of 10 degrees Celsius for ten days.[43] In reading the staining results, it was found that 90% Karamunting extract produced good-quality staining in pancreatic histopathology. To further evaluate this performance, we conducted blinded scoring by one board-certified pathologist, who examined randomized and de-identified pancreatic sections stained with Karamunting 90% and standard eosin Y. Staining quality was assessed using a 1–3 ordinal scale (1 = poor, 2 = fair, 3 = good). These blinded comparative evaluations support the interpretation that Karamunting 90% provides staining of sufficient clarity and contrast to be considered a potential alternative to eosin for pancreatic tissue preparations.

CONCLUSION

This study demonstrates that *Rhodomyrtus tomentosa* (Karamunting) is the effective natural fruit extract among the three evaluated for usage as a natural substitute for eosin in hematoxylin-eosin histological staining. The exceptional staining efficacy of the 90% Karamunting extract is attributed to its elevated concentration of chromogenic compounds, as evidenced by phytochemical screening and GC–MS analysis. The gently acidic pH closely approximates the optimal conditions for eosin-based staining. Extracts of *Melastoma malabathricum* (Senduduk) and *Daemonorops draco* (Jernang) exhibited poor staining properties. The colors failed to penetrate well, the cells were deformed, and the contrast remained insufficient, regardless of the quantity used. Future research should focus on enhancing extraction methods, pH adjustment, and formulation stability to further augment the staining efficacy of Karamunting extract. For it to become a more commonly utilized eco-friendly alternative dye in normal histopathological practice, it requires quantitative image analysis, toxicity assessment, and validation across many tissue types.

AUTHORS' CONTRIBUTIONS

Iqlila Romaidha; Coordinating research ensuring the successful implementation of research starting from managing permits, making extracts, preparing and implementing tissue preparation staining; **Rima Agnes Widya Astuti;** extraction of Karamunting, Sendunia and Jernang fruit for coloring and drafting of articles; **Ariva Syiva'a;** Reading and validation the results of tissue preparations. **Ledisda Apriana:** Supervision and reviewing; **Nurlaili Khoirunnisa;** suitability of the article template to be submitted.

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DATA AVAILABILITY STATEMENT
The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT
The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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Annex:

Table 3. Qualitative Test Results of *Melastoma malabathricum*, *Rhodomyrtus tomentosa*, and *Daemonorops draco* Extracts

Sample Name	Flavonoid	Antosianin	Alkaloid	Tannin	Saponin
***Melastoma malabathricum* (Senduduk)**	+	+	-	+	-
Information	The solution turns red when HCl is added and changes to Red	Red Solution	No brick-red or yellowish-white precipitate forms	Bluish-Black Solution	No Foam forms
***Rhodomyrtus tomentosa* (Karamunting)**	+	+	+	+	+
Information	The solution turns brick-red when HCl is added and changes to greenish when NaOH is added	Red Solution	A brick-red or yellowish-white precipitate forms	Bluish-Black Solution	Foam forms 1 cm
***Daemonorops Draco* (Jernang)**	+	+	-	+	-
Information	The solution turns brick-red when HCl is added and changes to greenish when NaOH is added	Red Solution	No brick-red or yellowish-white precipitate forms	Bluish-Black Solution	No Foam forms

Table 4. Results of quantitative phytochemical testing of karamunting (*Rhodomyrtus tomentosa*) fruit using the GC–MS method

Peak	SI	Compound Formula	Compound Name	Retention Time	Percentage Area
1	89	$C_6H_6O_3$	*Phloroglucinol*	4.910	4.27
2	95	$C_{13}H_{56}O^{+2}$	*Hydron;methane;hydrate*	12.365	0.22
3	96	$C_{16}H_{32}O_2$	*Palmitat acid*	13.586	2.30
4	91	$C_{18}H_{32}O_2$	*Linoleic acid*	15.433	26.15
5	74	$C_{18}H_{32}O_2$	*Linoleic acid*	15.575	1.81
6	85	$C_{35}H_{68}O_5$	*Glyceryl 1,3-dipalmitate*	16.742	2.46
7	88	$C_{16}H_{28}$	*Cyclohexadecadien*	17.837	0.25
8	83	$C_{21}H_{40}O_4$	*Glyceryl Monooleate*	17.875	0.20

9	87	$C_{18}H_{32}$	*1,1':3',1''-Tercyclohexane*	17.942	0.24
10	81	$C_{18}H_{34}O_2$	*Oleic acid*	17.986	0.30
11	89	$C_{12}H_{20}$	*Cyclohexylidenecyclohexane*	18.273	13.35
12	86	$C_{14}H_{24}O_3$	*Menthyl acetoacetate*	18.306	13.11
13	86	$C_{39}H_{76}O_5$	*Glyceryl 1,3-distearate*	18.478	0.54
14	70	$C_{19}H_{40}S$	*1-Nonadecanethiol*	20.544	0.26
15	86	$C_{18}H_{31}ClO$	*Linoleoyl chloride*	21.914	2.06
16	88	$C_{15}H_{26}$	*Cedrane*	21.983	0.31
17	87	$C_{31}H_{62}O$	*16-Hentriacontanone*	24.086	0.36
18	74	$C_{29}H_{50}O$	*Sitosterol*	24.610	0.34
19	84	$C_{16}H_{32}O$	*Hexadecanal*	25.224	0.29
20	86	$C_{16}H_{30}O_2$	*Palmitoleic acid*	25.372	3.94
21	74	$C_{31}H_{62}O$	*16-Hentriacontanone*	25.508	0.53
22	88	$C_{12}H_{22}O$	*Cyclododecanone*	26.858	0.31
23	89	$C_{30}H_{52}O_2$	*Dammarenediol-I*	27.004	13.12
24	87	$C_{30}H_{52}O_2$	*Dammarenediol-I*	27.138	3.24
25	85	$C_{20}H_{40}O_2$	*Icosanoic acid*	27.850	0.43
26	78	$C_{55}H_{106}O_6$	*1,3-distearoyl-2-palmitoylglycerol*	30.851	0.66
27	83	$C_{39}H_{72}O_5$	*Diolein*	34.364	8.95

Table 5. Results of quantitative phytochemical testing using the GC–MS method for Senduduk fruit (*Melastoma malabathricum)*

Peak	SI	Compound Formula	Compound Name	Retention Time	Percentage Area
1	96	$C_{16}H_{32}O_2$	*Palmitat Acid*	13.557	1.10
2	92	$C_{18}H_{32}O_2$	*Linoleic acid*	15.346	5.84
3	84	$C_{35}H_{68}O_5$	*Glyceryl 1,3-dipalmitate*	16.745	2.82
4	89	$C_{12}H_{20}$	*Cyclohexylidenecyclohexane*	18.270	12.39
5	81	$C_{39}H_{72}O_5$	*Diolein*	18.304	19.70
6	84	$C_{39}H_{76}O_5$	*Glyceryl 1,3-distearate*	18.487	1.88
7	70	$C_7H_{12}Cl_2$	*1,2-Dichlorocycloheptane*	19.715	0.45
8	67	$C_{28}H_{56}O_2$	*Octacosanoic acid*	20.561	0.58
9	85	$C_{18}H_{31}ClO$	*Linoleoyl chloride*	21.930	5.03

10	84	$C_{18}H_{32}O$	*9,12-Octadecadienal*	21.994	0.64
11	86	$C_{31}H_{62}O$	*16-Hentriacontanone*	24.098	0.40
12	86	$C_{26}H_{50}$	*13-Hexacosyne*	25.388	5.23
13	78	$C_{30}H_{52}O_2$	*Dammarenediol-I*	25.492	3.44
14	82	$C_{21}H_{38}O_4$	*Monolinolein*	25.686	5.44
15	84	$C_{21}H_{38}O_4$	*Monolinolein*	25.825	5.58
16	90	$C_{30}H_{52}O_2$	*Dammarenediol-I*	27.002	20.32
17	88	$C_{30}H5_2O_2$	*Dammarenediol-I*	27.154	5.69
18	32	$C_{13}H_{23}^-$	*3-(2,3-Dimethylbutyl)-4-methylcyclohexene*	34.233	0.80
19	72	$C_{18}H_{34}O$	*9-Octadecenal*	34.477	2.68

Table 6. Results of quantitative phytochemical testing using the GCMS method for Jernang (*Daemonorops draco*)

Peak	SI	Compound Formula	Compound Name	Retention Time	Percentage Area
1	91	$C_{17}H_{32}O_2$	*Methyl Palmitoleate*	13.130	11.56
2	90	$C_{19}H_{36}O_2$	*Methyl Oleate*	14.902	6.88
3	81	$C_{16}H_{16}O_3$	*Pterostilbene*	13.586	41.36
4	84	$C_{17}H_{18}O_3$	*p-tert-Butylphenyl salicylate*	15.433	40.21

Table 7. Test results for measuring the acidity level of Potential of Hydrogen (pH) of senduduk, karamunting and jernang extracts

Name Extract	Concentration	pH
Senduduk	70%	2.10
	80%	2.02
	90%	2.03
Karamunting	70%	3.16
	80%	3.26
	90%	3.36
Jernang	70%	5.60
	80%	6.21
	90%	6.75

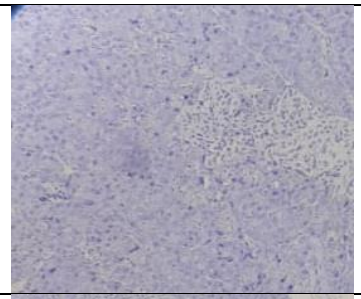
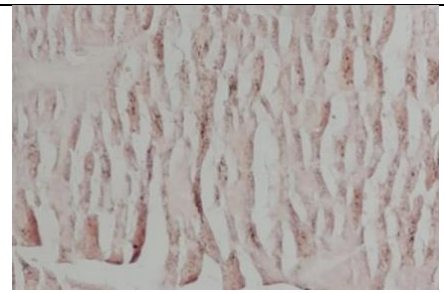
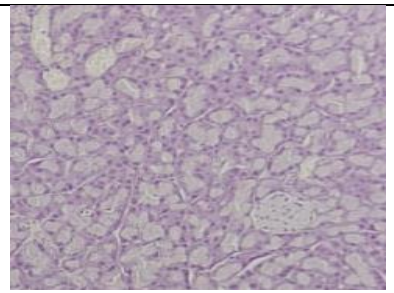
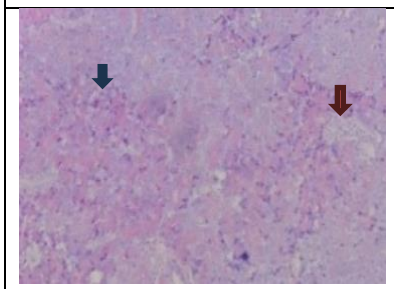
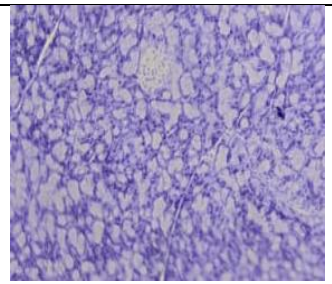
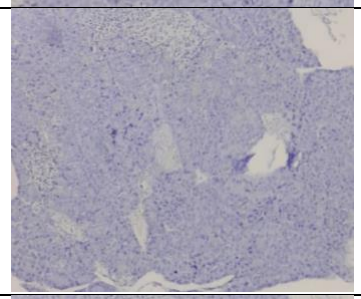
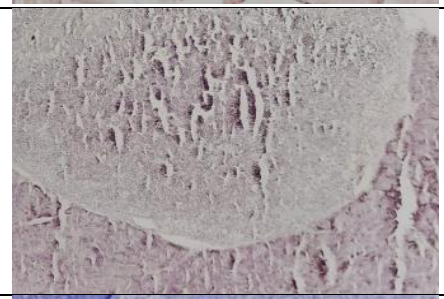
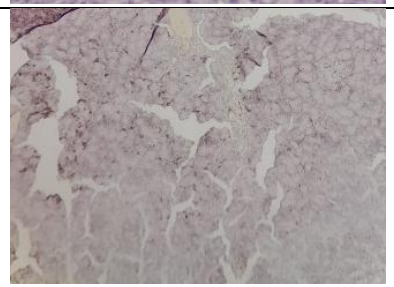
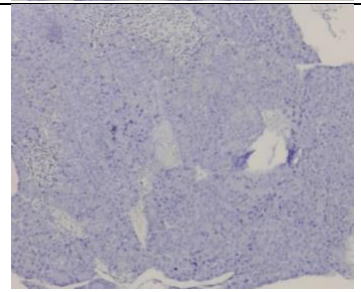
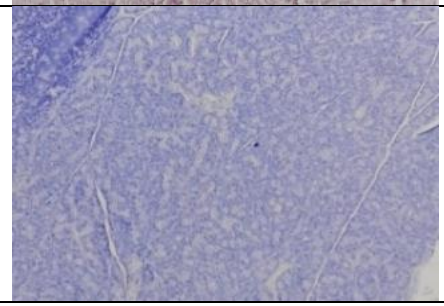
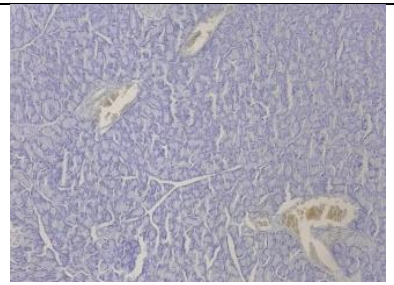
Control		Experimental Group (Concentration)			Natural Plant
Positive (hematoxylin-eosin)	**Negative (Hematoxylin)**	**70 %**	**80 %**	**90%**	
					Karamunting
					Senduduk
Blue arrows represent exocrine glands, **orange arrows** represent islets of Langerhans.					Jernang

Figure 1. Results of Histological Staining of Pancreatic Tissue for 3 Experimental Group