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Ethanol Extract of Fresh Peel of Balangkasua Fruit (*Lepisanthes alata* (Blume) Leenh) as an Alternative Reagent in the Identification of *Trichuris trichiura* Eggs

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Abstract
Introduction: *Soil-Transmitted Helminths (STH) remain a major public health concern, particularly in areas with limited sanitation. Conventional staining using 2% eosin, although effective, poses drawbacks due to its non-biodegradability, toxicity, and flammability.* ***Objective:*** *This study aimed to evaluate the potential of ethanol extract from the fresh peel of Balangkasua fruit (Lepisanthes alata (Blume) Leenh) as a natural alternative reagent for the identification of Trichuris trichiura eggs.* ***Method:*** *A laboratory-based experimental design was conducted using fecal samples containing STH eggs. The fruit peel extract was prepared through maceration with ethanol and tested at concentrations of 20%, 40%, 60%, 80%, and 100%, while 2% eosin served as the positive control. Staining quality was assessed based on egg color contrast, shell clarity, internal morphology, shape and size clarity, and background staining.* ***Result and Discussion:*** *Results showed that the 100% extract yielded the highest score (13), categorized as good staining quality, while lower concentrations (20%–80%) were classified as moderate. The positive control consistently achieved superior results (score 19, very good). Statistical analysis using the Kruskal–Wallis test revealed significant differences among treatment groups ($p < 0.05$).* ***Conclusion:*** *These findings demonstrate that L. alata peel extract possesses potential as an eco-friendly alternative stain, although further optimization of extraction methods, pigment stabilization, and validation across various STH species are necessary before replacing synthetic eosin in routine diagnostics.*

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Introduction

Soil-Transmitted Helminths (STH) are intestinal nematodes whose life cycle requires specific soil conditions to reach the infective stage (Kadarsan, 2005 as cited in Oktari, 2017). The simplest examination method for detecting intestinal nematode eggs is the native method using 2% eosin reagent. This reagent is acidic and orange-red in color. The use of 2% eosin aims to clearly differentiate helminth eggs from surrounding fecal debris. Eosin also provides a red background against the yellowish eggs, making them more distinguishable from fecal matter (Oktari & Mu'tamir, 2017). However, eosin has limitations, as it is non-biodegradable, produces toxic waste, and is flammable. These drawbacks open opportunities for utilizing local natural resources as alternatives to replace eosin (Salnus & Arwie, 2020).

One potential plant is *Balangkasua* (*Lepisanthes alata*), as its fruit peel contains anthocyanins that produce a reddish-purple color and show potential as a natural dye. The peel of *Balangkasua* fruit is red, and as it ripens, it turns purple, which is strongly associated with anthocyanin content, a type of flavonoid. Plants containing anthocyanins belong to a pigment group that ranges in color from red to blue and are widely distributed in plants. Anthocyanins share similar characteristics with eosin, being acidic and red in color. This study explores the utilization of this plant as an alternative dye with properties comparable to eosin. Since anthocyanins are classified as flavonoids with polar characteristics, their extraction is carried out using polar solvents (Simanjuntak *et al*., 2014).

In recent years, research on anthocyanin-based natural dyes has gained significant attention, particularly as environmentally friendly alternatives to synthetic stains such as eosin for microscopic identification of *Soil Transmitted Helminths* (STH) eggs. Natural dyes are considered safer, more sustainable, and more accessible compared to synthetic chemicals, which may pose toxicological and environmental concerns. Several recent studies have reported promising results from various plant sources containing anthocyanins as potential substitutes for synthetic stains.

Nuswantoro *et al*. (2025) found that ethanolic extracts of red beans (*Phaseolus vulgaris*) and hibiscus (*Hibiscus rosa-sinensis*), extracted for 48–72 hours, produced staining quality comparable to eosin. Likewise, Charisma, Rahayu, and Arianing (2024) reported that a 1:1 infusion of hibiscus and roselle (*Hibiscus sabdariffa*) effectively stained STH eggs, although eosin remained superior in contrast sharpness. Tanjung, Maulidayanti, and Situmorang (2025) demonstrated that 75% mangosteen peel (*Garcinia mangostana*) extract yielded the most optimal staining quality, emphasizing that moderate concentration often provides the best balance between contrast and clarity. Similarly, Artanti *et al*. (2024) utilized the pressed extract of red amaranth stems (*Amaranthus tricolor L*), while Faatiha *et al*. (2025) examined purple sweet potato (*Ipomoea batatas L.*) juice—both showing satisfactory staining ability attributed to their anthocyanin content.

Furthermore, Anggraini and Afrianti (2025) showed that teak leaf (*Tectona grandis*) extract achieved the best results at intermediate concentrations, suggesting that excessive pigment concentration may reduce image clarity due to residue accumulation. Collectively, these studies indicate a growing research trend focusing on the optimization of extraction methods, pigment stabilization, and diagnostic validation of anthocyanin-based natural dyes. In this context, the exploration of *Lepisanthes alata* (Balangkasua) peel extract serves as a valuable contribution, offering a locally available, eco-friendly, and cost-effective alternative for diagnostic staining in the identification of STH eggs.

In this study, a preliminary test was conducted to detect the presence of anthocyanins in the fresh peel of *Balangkasua* fruit (*Lepisanthes alata* (Blume) Leenh). The results showed a positive reaction, indicated by a red color change, confirming the presence of anthocyanins in the fruit. This finding further strengthens the potential of *Balangkasua* fruit as a natural dye alternative with characteristics similar to eosin, making it applicable for staining Soil-Transmitted Helminth eggs.

Method

This study is a laboratory-based experimental research with a quasi-experimental design, conducted in 2025 at the Pathology Laboratory of Borneo Lestari University. The research samples consisted of feces containing Soil-Transmitted Helminth (STH) eggs. The main materials used were the peel of *Balangkasua* fruit (*Lepisanthes alata*), 96% ethanol as a solvent, 2% eosin solution as the control, distilled water, HCl, and NaOH. The equipment included a rotary evaporator, light microscope, glass slides, cover slips, dropper pipettes, and basic tools for preparing microscopic specimens.

The peel of *Balangkasua* fruit was separated from the pulp, washed, and cut into small pieces. The extraction process was carried out using the maceration method to produce extracts with several concentration variations. The obtained extract was then subjected to phytochemical testing and qualitative identification of anthocyanin compounds.

The staining process was performed by applying both *Balangkasua* extract and 2% eosin solution (as a comparator) onto fecal preparations containing helminth eggs, which were then observed under a microscope at 10x and 40x magnifications. The observed parameters included egg color contrast, shell clarity, internal morphological details, clarity of shape and size, and background staining (negative contrast). Data analysis was conducted using a t-test to determine significant differences between the effectiveness of *Balangkasua* extract and 2% eosin.

Result and Discussion

1. Result

The plant material used in this study was taxonomically identified at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan. The specimen was determined to be Lepisanthes alata (Blume) Leenh., locally known as Balangkasua.

Table 1

Frequency Distribution of the Quality Assessment of *Trichuris trichiura* Egg Preparations

Treatment	Scoring					
	Egg Color Contrast	Shell Clarity	Internal Morphological Details	Clarity of Shape and Size	Background Color (Negative Contrast)	Total
20%	1	2	3	3	1	10
40%	1	2	2	3	1	9
60%	1	2	2	3	1	9
80%	1	2	2	3	1	9
100%	2	3	3	3	2	13
K (+)	4	4	4	4	4	19

Interpretation:

- 17–20: Very good staining, parasite eggs are easily identified
- 13–16: Good staining, sufficient for routine examination
- 9–12: Moderate staining, identification requires caution
- 5–8: Poor staining, not suitable as a substitute for 2% eosin

The quality assessment of *Trichuris trichiura* egg preparations indicates that the concentration of *Lepisanthes alata* fruit peel extract affects several staining aspects (egg color contrast, shell clarity, internal morphology details, shape/size clarity, and background color). Among the five tested concentrations, 100% extract yielded the highest total score (13), which falls within the "good" staining category (range 13–16). In contrast, concentrations of 20%–80% scored between 9 and 10, classified as "moderate" staining. This suggests that while the results are acceptable, microscopic identification requires greater caution due to suboptimal contrast and morphological detail.

The positive control (assumed 2% eosin) consistently showed superior performance with a total score of 19 ("very good" staining), indicating that while *L. alata* extract is promising, the current formulation cannot fully replace synthetic eosin in diagnostic quality. Component-wise analysis shows that egg color contrast and negative background contrast improved most noticeably at 100% concentration (score 2 for both), whereas lower–medium concentrations tended to produce weaker background contrast (score 1). Shell clarity and internal morphology maintained moderate scores (2–3) at medium to high concentrations, but did not reach the well-defined clarity observed in the positive control (score 4).

Figure 1. *Trichuris trichiura* egg Stained with 2% Eosin

Based on Figure 1, egg color contrast, shell clarity, internal morphological details, egg shape/size clarity, and negative background contrast appear very clear.

Figure 2. *Trichuris trichiura* egg Stained with 20% Extract

In Figure 2, egg color contrast is unclear (score 1), shell clarity appears blurred (score 2), internal morphology is fairly visible (score 3), egg shape/size is fairly clear (score 3), while negative background contrast interferes with observation (score 1).

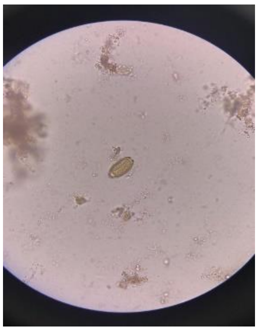

Figure 3. *Trichuris trichiura* egg Stained with 40% Extract

In Figure 3, egg color contrast is unclear (score 1), shell clarity is fairly clear (score 3), internal morphology is less visible (score 2), egg shape/size is difficult to distinguish (score 2), and negative background contrast interferes with observation (score 1).

Figure 4. *Trichuris trichiura* egg Stained with 60% Extract

In Figure 4, egg color contrast is unclear (score 1), shell clarity is fairly clear (score 3), internal morphology is less visible (score 2), egg shape/size is difficult to distinguish (score 2), and negative background contrast interferes with observation (score 1).

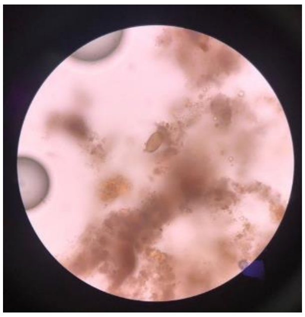

Figure 5. *Trichuris trichiura* egg Stained with 80% Extract

In Figure 5, egg color contrast is unclear (score 1), shell clarity is fairly clear (score 3), internal morphology is less visible (score 2), egg shape/size is difficult to distinguish (score 2), and negative background contrast interferes with observation (score 1).

Figure 6. *Trichuris trichiura* egg Stained with 100% Extract

In Figure 6, egg color contrast appears dull (score 2), shell clarity is fairly clear (score 3), internal morphology is fairly visible (score 3), egg shape/size is fairly clear (score 3), and negative background contrast is suboptimal (score 2).

Table 2
Results of Kruskal–Wallis Test

	KWT	KCT	DMI	KBU	LB
Kruskal-Wallis H	27.000	14.503	13.337	16.691	24.652
df	6	6	6	6	6
Asymp. Sig.	.000	.024	.038	.010	.000

a. Kruskal Wallis Test
b. Grouping Variable: Perlakuan

The statistical test results showed that the significance values (sig) for all treatment groups of *L. alata* fruit peel extract concentrations were less than 0.05. This indicates that each treatment concentration produced statistically significant differences in the staining quality of STH eggs. In other words, the greater the variation in extract concentration, the more pronounced the differences in staining outcomes.

2. Discussion

The results of the study showed that the peel extract of *Lepisanthes alata* was able to provide staining quality for the preparations of Soil Transmitted Helminths (STH) eggs, particularly *Trichuris trichiura*. The extract concentration had a significant effect on color clarity and morphological details, as demonstrated by the Kruskal–Wallis test with $p < 0.05$ in all variables. The 100% concentration produced the highest total score (13), categorized as good staining quality, whereas concentrations of 20–80% only reached the moderate category. Although it has not yet matched the positive control of 2% eosin (score 19, very good category), these findings confirm that *L. alata* extract has potential as a natural alternative stain in microscopic examinations.

These findings are in line with recent research exploring anthocyanin-rich plant extracts as substitute stains for STH egg identification. Nuswantoro *et al*. (2025) reported that ethanolic extracts of red beans (*Phaseolus vulgaris*) and hibiscus (*Hibiscus rosa-sinensis*), extracted for 48–72 hours, achieved staining quality comparable to eosin. This underscores the importance of extraction techniques—including solvent selection and duration—in determining the effectiveness of natural colorants. Similarly, Charisma, Rahayu, and Arianing (2024) found that a 1:1 infusion mixture of hibiscus and roselle flowers could effectively stain STH eggs, although eosin still provided superior definition. These observations suggest that anthocyanin-based natural dyes hold great promise but require further refinement to achieve diagnostic quality comparable to synthetic stains.

Consistent with this, Tanjung, Maulidayanti, and Situmorang (2025) observed that a 75% mangosteen peel (*Garcinia mangostana*) extract produced optimal staining results compared to both lower and higher concentrations. Excessively high concentrations, however, tended to leave pigment residues that obscured morphological details. This finding emphasizes that increasing concentration does not always enhance staining quality; purification and removal of non-coloring compounds are necessary to achieve clearer visualization.

Regarding pigment stability, anthocyanins are particularly sensitive to pH changes and oxidative degradation. The enzyme polyphenol oxidase (PPO) can accelerate anthocyanin oxidation, resulting in reduced color intensity and contrast (Rahmayani & Pasala, 2025). This observation corresponds with the present study, where low to moderate concentrations yielded less distinct contrasts—likely due to pigment degradation or residual impurities in the extract. Therefore, maintaining optimal pH and limiting oxidation are crucial for ensuring anthocyanin stability.

A component-wise assessment revealed that increasing extract concentration enhanced the visibility of certain structural features, particularly color and background contrast. However, shell transparency and internal morphological definition remained moderate and did not reach the clarity achieved by eosin. This indicates that although higher concentrations improve the general visualization of STH egg structures, the pigment intensity and spectral range of *L. alata* extract remain insufficient to fully replicate eosin's diagnostic performance.

Based on these observations, a 100% extract concentration can be considered the most effective among those tested for routine application, offering consistent and adequate staining quality. Nevertheless, further optimization is required before *L. alata* extract can completely replace eosin 2%. Such improvements include optimizing extraction parameters (solvent composition, duration, and temperature) to enhance pigment yield, adjusting pH and inhibiting PPO activity to maintain color stability, combining with complementary stains such as hematoxylin to enhance contrast, performing long-term stability assessments, and conducting broad diagnostic validation involving multiple STH species and evaluators under field conditions.

Although statistical analysis confirmed significant differences among the tested concentrations ($p < 0.05$), the most pronounced practical improvement occurred only at the 100% concentration. Thus, while the statistical effect of concentration was evident, only the highest concentration approached the operational standards required for diagnostic use, whereas eosin still demonstrated superior staining capability.

In conclusion, these results align with the growing body of evidence suggesting that anthocyanin pigments from natural plant sources have great potential as sustainable and environmentally friendly alternatives for microscopic staining. However, to achieve equivalence with synthetic dyes such as eosin, further refinement is needed in extraction optimization, pigment stabilization, purification, and practical validation. This study contributes valuable evidence supporting the development of locally derived, eco-friendly staining reagents that could eventually substitute synthetic eosin in the microscopic examination of STH eggs.

Conclusion

The peel extract of Lepisanthes alata has been proven to possess potential as a natural dye for Soil Transmitted Helminths (STH) egg preparations, particularly Trichuris trichiura. The extract concentration significantly influenced staining quality, with the 100% concentration producing the best result (good category), although it has not yet surpassed the positive control of 2% eosin (very good category). Differences in staining quality across concentrations were closely related to anthocyanin content, where higher concentrations provided better contrast and morphological detail compared to lower concentrations.

Thus, L. alata extract shows promising potential as an eco-friendly alternative stain in microscopic examinations. However, optimization of extraction methods, pigment stabilization, and further testing on various types of STH are still required. A concentration of 100% is recommended as the most effective starting point, although the positive control still demonstrates superior staining quality for routine diagnostic purposes.

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