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Analysis of the Effectiveness of Red Andong Leaf Extract (*Cordyline fruticosa*) as an Alternative to 2% Eosin in the Examination of *Soil-Transmitted Helminth* Eggs

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Abstract. *Microscopic examination of fecal specimens is an important method for detecting Soil-Transmitted Helminth (STH) eggs. Eosin 2% staining is standardly used to provide high diagnostic contrast; however, natural dye alternatives are increasingly sought to advance eco-friendly and safer laboratory diagnostics. This preliminary laboratory study aimed to evaluate the effectiveness of red andong leaf (Cordyline fruticosa) extract as a natural alternative to 2% eosin in direct slide examinations of STH eggs. The extract was prepared by 70% ethanol maceration, acidified with 0.1 N HCl, and concentrated at 45–50°C. Three concentrations (40%, 60%, and 80%) were evaluated without independent experimental replication, alongside distilled water (negative control) and 2% eosin (positive control). Preparations were microscopically assessed at 10× and 40× objective magnifications using a five-parameter operational scoring rubric on a 1–4 scale. Results demonstrated that 40%, 60%, and 80% extract concentrations failed to produce adequate staining contrast, with preparations remaining largely transparent. Although 0.1 N HCl maintained the visible red color of the extract, it did not produce sufficient microscopic contrast for clear visualization of the STH eggs. The mean staining score was the same across all extract concentrations (1.8), compared with 2.0 for distilled water and 4.0 for 2% eosin. As a preliminary descriptive observation, red andong leaf extract did not demonstrate adequate staining performance as a direct substitute for 2% eosin under the tested conditions. Further studies should optimize the extraction solvent, pH, staining time, pigment concentration, and the interaction between plant pigments and biological structures, with appropriate independent experimental replication. This study may contribute to the exploration of plant-derived staining materials in support of SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production); however, the potential health and environmental benefits of the extract require further evaluation.*

Keywords: *Red Andong Leaf Extract; Anthocyanin; Coloring; STH Worm Eggs; Fecal Preparations*

1. Introduction

Soil-transmitted helminths (STH), or *soil-transmitted* worm infections, are a common health problem in tropical and subtropical regions. This group includes intestinal nematodes such as *Ascaris lumbricoides, Trichuris trichiura,* and *hookworms (Necator americanus and Ancylostoma duodenale)*. STH infections are associated with poor sanitation and can impact anemia, malnutrition, and community health and productivity (Djuardi et al., 2021; Chen et al., 2024).

Microscopic examination of feces is an important method for detecting helminth eggs. Staining helps clarify the structure of the eggs and other components in the specimen. 2% eosin was used as a positive control because it provides good contrast in the examination of

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STH eggs, while distilled water was used as a negative control. However, the use of synthetic dyes has prompted the search for safer, more economical, and environmentally friendly natural materials (Enwuru et al., 2021; Nizar et al., 2023).

Red andong leaves (*Cordyline fruticosa*) have the potential to be used as a natural dye due to their anthocyanin, flavonoid, and tannin content (Al-Snafi, 2020; Andani et al., 2023; Tematio Fouedjou et al., 2023; Utami, 2021). Anthocyanin pigments impart a red to purplish color and are relatively stable under acidic conditions (Al-Alwani et al., 2019; Ayun et al., 2022; Supriyanto & Triana, 2021). Previous research has reported that soaking andong leaves in 2 N HCl can stain STH eggs, although the staining intensity was lower than that of synthetic dyes (Wahyuni et al., 2024).

This study used a different approach, namely extraction by maceration using 70% ethanol, the addition of 0.1 N HCl, and evaporation at a low temperature of 45–50°C (Lestari et al., 2025). This approach was used to obtain a more concentrated extract and maintain the pigment. The aim of the study was to analyze the effectiveness of red andong leaf extract at concentrations of 40%, 60%, and 80% compared to 2% eosin in direct slide examination of STH worm eggs.

Furthermore, this investigation supports global green-laboratory initiatives aligned with SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production) by evaluating botanical alternatives to synthetic chemical stains (United Nations, 2022).

2. Methods

2.1 Study Design and Location

This study used a descriptive laboratory experimental design with a qualitative comparative approach. The study was conducted from February 23 to March 12, 2026, at the Microbiology Laboratory of the DIII Medical Laboratory Technology Study Program, STIKes Panrita Husada Bulukumba. The independent variable was red andong leaf extract *(Cordyline fruticosa)*, while the dependent variable was the quality of STH egg observation.

2.2 Extract Preparation and Concentration Variations

The young leaves of red andong were washed, cut, dried at room temperature, and then weighed to obtain 100 g. The sample was macerated with 1000 mL of 70% ethanol for two consecutive 24-hour periods at room temperature. The filtrate was filtered, 0.1 N HCl was gradually added to lower the pH, and the solution was then evaporated using a water bath at 45–50°C until a concentrated extract was obtained. The concentration variations were prepared from the extract stock by dilution with distilled water: 80% (8 mL extract + 2 mL distilled water), 60% (6 mL + 4 mL), and 40% (4 mL + 6 mL).

2.3 Staining and Microscopic Observation

Fecal samples positive for STH eggs were used to prepare direct-slide preparations. The preparations were treated with red andong leaf extract according to the treatment concentrations. Eosin 2% was used as the positive control and distilled water as the negative control. The preparations were examined using a light microscope at 10× objective magnification for screening and 40× objective magnification for identification. The

observation parameters included egg color contrast, shell clarity, morphological details, clarity of egg shape and size, and background color.

To standardize microscopic evaluation, an operational scoring rubric (scale 1–4) was established across five parameters :

- Score 1 (Poor): Absence of color contrast, transparent background, or unidentifiable egg morphology.
- Score 2 (Fair): Minimal contrast, faint outline of egg shell visible, poor internal detail.
- Score 3 (Good): Clear egg outline and shell structure visible with moderate background contrast.
- Score 4 (Optimal): High contrast, sharp shell definition, distinct internal morphology, and optimal background differentiation (equivalent to 2% eosin).

2.4 Data Analysis

The observation data were tabulated and analyzed descriptively and comparatively using SPSS. Because independent experimental replication was not performed for each treatment, inferential statistical testing was not conducted. Therefore, the results were compared descriptively based on the staining scores and mean scores obtained for each treatment.

Because independent experimental replication was not performed, the numerical scores should be interpreted as descriptive observations under the tested laboratory conditions rather than as evidence of statistically significant differences among treatments.

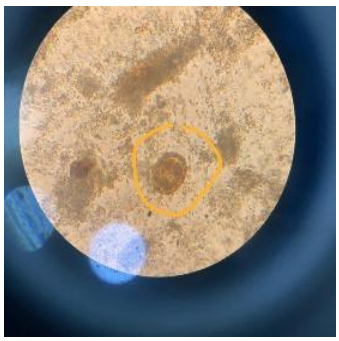
3. Results and Discussion

3.1 Characteristics of the extract

The red andong preparations were liquid at all tested concentrations. The concentrated extract appeared dark red, while dilution to 80% and 60% produced red preparations and 40% produced a paler red preparation. The measured pH was approximately 3–4 for the higher concentrations and 4–5 for the lower concentration. Addition of 0.1 N HCl lowered the extract pH and maintained the red coloration. This observation is consistent with the known pH sensitivity of anthocyanins, which tend to retain red coloration under acidic conditions.

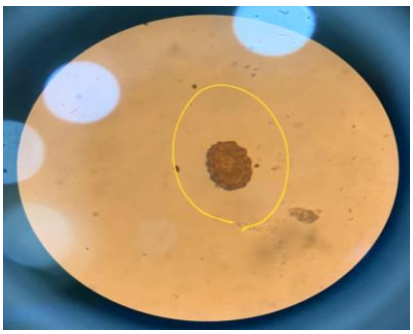
3.2 Microscopic staining performance

Extract 40%

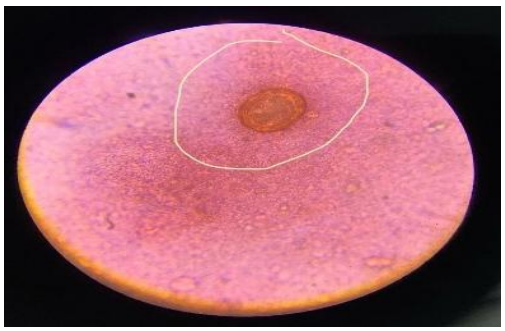


Extract 60%

Extract 80%



Negative Control (Distilled Water)



Positive Control (2% Eosin)

Figure 1. Observation Results of Preparations with Different Extract Concentrations, Distilled Water, and 2% Eosin

The results showed that 2% eosin as a positive control produced clear staining, making STH eggs easier to observe, while distilled water as a negative control did not produce staining and the preparations appeared transparent. Red andong leaf extract at concentrations of 40%, 60%, and 80% also did not produce optimal staining at 10× and 40× magnification. The structures of *Trichuris trichiura* and *Ascaris lumbricoides* eggs were difficult to observe because no clear staining was visible. Increasing the extract concentration and adding 0.1 N HCl did not improve the staining results. Therefore, red andong leaf extract did not demonstrate adequate staining performance for STH eggs under the tested conditions.

The visual comparison in Figure 1 shows that the 40%, 60%, and 80% red andong preparations produced substantially less contrast than the 2% eosin preparation. The three red andong concentrations also showed no visible improvement in staining quality with increasing concentration.

Table 1. Staining-quality scores for each treatment

Observation parameter	40%	60%	80%	Distilled water	2% eosin
Egg color contrast	1	1	1	1	4
Shell clarity	2	2	2	3	4
Morphological detail	2	2	2	2	4
Egg shape and size	3	3	3	3	4
Background color	1	1	1	1	4

The scoring results show that 2% eosin obtained the highest score for all five parameters. The three red andong concentrations had identical scores for each parameter, indicating that increasing the concentration from 40% to 80% did not improve the observed staining quality under the conditions used. Although some structural features, including the egg shell, morphology, and egg shape and size, could still be visualized, the staining provided insufficient contrast for clear microscopic identification.

3.3 Descriptive comparison of mean scores

Descriptive analysis using SPSS showed that the mean staining score for red andong leaf extract at concentrations of 40%, 60%, and 80% was 1.8 for each concentration, while distilled water and 2% eosin had mean staining scores of 2.0 and 4.0, respectively. The highest mean score was obtained for 2% eosin, indicating the best staining quality among the treatments. The three red andong extract concentrations showed identical mean scores and remained lower than 2% eosin. Although the extract produced lower staining scores than 2% eosin, its potential as a natural staining material warrants further investigation under optimized extraction and staining conditions. Because independent experimental replication was not performed for each treatment, the data were analyzed descriptively without inferential statistical testing.

Therefore, the mean scores presented in Table 2 should be interpreted as descriptive summaries of the five observational parameters rather than as treatment means derived from independent experimental replications. The results should not be interpreted as evidence of statistically significant differences among treatments.

Table 2. Total and mean staining scores

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
total	5	9	20	11.40	4.827
mean	5	1.8	4.0	2.280	0.965
Valid N (listwise)	5				

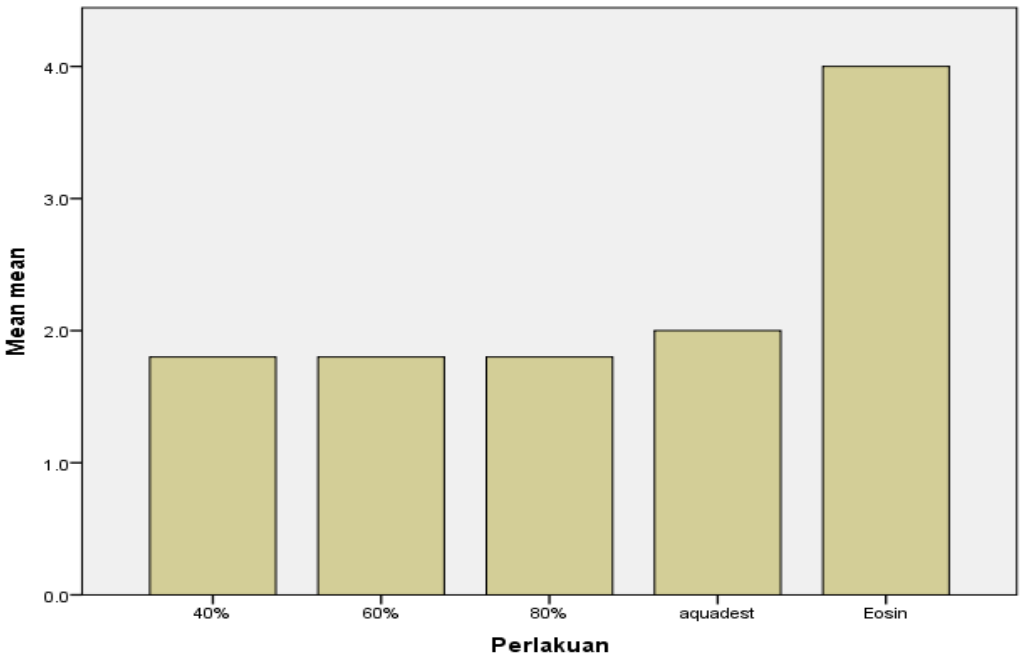


Figure 2. Bar chart of mean values based on treatment

The findings indicate that red andong leaf extract did not perform as well as 2% eosin as a staining reagent for STH egg preparations. Although the extract retained a visible red color after acidification, the pigment did not generate sufficient contrast in the fecal preparation. This distinction is important because the visible color of an extract does not necessarily translate into effective microscopic staining.

Anthocyanin is a plausible source of the red coloration observed in the extract. Its color stability is strongly influenced by pH, and acidification can favor colored forms of the pigment. In the present study, 0.1 N HCl helped maintain the red appearance of the extract, but this did not translate into improved staining of the parasite preparation. Therefore, pigment stability in solution and staining performance on biological structures should be considered as separate processes.

The extract may therefore remain visibly red while failing to produce sufficient microscopic contrast. Anthocyanin color stability and staining performance are different properties: the former concerns the maintenance of pigment color in solution, whereas the latter concerns the interaction, adsorption, or retention of pigment on the biological structures being examined. STH eggs, including *Ascaris lumbricoides* and *Trichuris trichiura*, have distinct shell structures that may influence the interaction between plant pigments and the egg surface. Therefore, maintaining a red color in the extract does not necessarily indicate adequate microscopic staining performance for the observation of STH eggs.

The absence of improvement from 40% to 80% suggests that simply increasing extract concentration was insufficient under the tested conditions. Possible explanations include limited extraction of the relevant pigment fraction, the choice of 70% ethanol as solvent, insufficient interaction between the pigment and the target structures, and the staining procedure itself. Previous research has demonstrated the potential of plant-derived materials as natural stains, but differences in extraction and application procedures can produce different staining outcomes (Nuswantoro et al., 2025; Supriyanto & Triana, 2021; Wahyuni et al., 2024).

The methodology used for this study also indicates that the extraction conditions may be relevant to the outcome. The sample was macerated with 70% ethanol, acidified with 0.1 N HCl, and concentrated at 45–50°C. Although these conditions were selected to obtain and maintain the red pigment, Although these conditions were selected to obtain and maintain the red pigment, they may not have produced an extract with sufficient staining performance for STH egg structures. Future studies should therefore systematically compare solvent concentration, extraction time, pH, staining time, and pigment concentration, with appropriate independent experimental replication.

The result also emphasizes the value of using a positive control. The consistently higher scores obtained with 2% eosin show that the microscopic preparation and observation procedure were capable of producing clear contrast. The relatively poor performance of the red andong extract therefore appears to be associated mainly with the staining material and/or staining conditions rather than with the observation system itself.

The findings should also be compared with previous work using (*Cordyline fruticosa)*. (Supriyanto & Triana, 2021 ; Wahyuni et al., 2024) investigated andong leaf preparations as alternative staining materials. Differences between their findings and the present study may reflect differences in extraction or preparation methods, concentration, pH, staining procedures, observation criteria, and experimental design. Thus, the present findings indicate

that the specific preparation tested here was inadequate under the conditions used, rather than establishing that (*Cordyline fruticosa)* cannot be used as a natural staining source.

A major limitation of this study is the absence of independent experimental replication. Consequently, the findings should be interpreted as descriptive observations under the tested laboratory conditions rather than as evidence of statistically significant differences between treatments. Future studies should include independent replicates and systematically optimize solvent type, extraction conditions, pH, staining time, and pigment concentration.

Conclusions

Under the conditions tested, red andong leaf extract (*Cordyline fruticosa*) at concentrations of 40%, 60%, and 80% did not demonstrate adequate staining performance as an alternative to 2% eosin for direct-slide examination of STH eggs. The three extract concentrations produced the same mean staining score of 1.8, compared with 2.0 for distilled water and 4.0 for 2% eosin. Because independent experimental replication was not performed, these findings should be interpreted as preliminary descriptive observations rather than evidence of statistically significant treatment effects. Although the extract was not effective as a direct substitute for 2% eosin under the tested conditions, (*Cordyline fruticosa)* remains a potential plant-derived staining material for further investigation. Future studies should optimize the extraction solvent, pH, staining time, pigment concentration, and interactions between plant pigments and biological structures using independent experimental replication. This study may contribute to the exploration of plant-derived laboratory materials in support of SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production); however, the potential health and environmental benefits of the extract require further evaluation.

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Conflicts of Interest

The authors declare no conflict of interest.

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