

# Evaluation of Purple Cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) Aqueous Soaking Solution as a Potential Alternative Stain for Soil-Transmitted Helminth Eggs

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## Abstract

Worm infestation is a public health issue in Indonesia, caused by soil-transmitted helminth infections. Main worms causing infections are *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworm. The 2% eosin dye stains worm eggs for diagnosing helminth infections via microscopic examination. Purple cabbage has anthocyanin pigments that can produce various colors. This study explains staining differences from varying soaking times for purple cabbage as an alternative dye to eosin in examining soil-transmitted helminth eggs. The research method was quasi-experimental, and purposive sampling was used. The native method (direct examination) was employed. The sample size was 27. The treatment was replicated nine times at each time variation: 24, 36, and 48 hours. Results showed that worm egg preparations with purple cabbage soaking water for 24 and 36 hours produced a blurred visual field, the eggs did not absorb the dye, and parts were not visible. Preparations with 48-hour soaking produced a bright visual field, eggs absorbed the dye, and parts were visible. The Cochran Q test results for purple cabbage soaked for 24, 36, and 48 hours showed an Asymp.Sig value <0.000, with a significance value < $\alpha$  0.05, indicating differences in staining results. This study indicates that a 48-hour purple cabbage soaking solution shows potential as a natural dye. However, it is not yet a suitable alternative to eosin for examining soil-transmitted helminth (STH) eggs, as it only stains eggs without adequate background contrast, especially the red coloration provided by eosin. While promising, further optimization of preparation, concentration, and staining protocol is needed to enhance effectiveness.

**Keywords:** soil-transmitted helminth; cabbage; worm

## Introduction

Soil-transmitted helminth (STH) infections remain a major public health problem in Indonesia, particularly in tropical and subtropical regions, with the highest prevalence among preschool and school-aged children. These infections are closely associated with poor hygiene, inadequate sanitation, and frequent

exposure to contaminated soil, leading to adverse health outcomes such as malnutrition, impaired growth, and reduced cognitive development.<sup>1,2</sup> The main causative species include *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworms such as *Necator americanus* and *Ancylostoma duodenale*.<sup>3</sup>

Microscopic examination is the standard method for diagnosing STH infections, commonly using 2% eosin staining to enhance the visibility and contrast of helminth eggs.<sup>3</sup> However, eosin is a synthetic dye with several limitations, including relatively high cost, limited biodegradability, and potential safety risks due to its chemical properties.<sup>4</sup> These drawbacks highlight the need for safer, more sustainable, and cost-effective alternative staining agents.

Natural dyes have gained attention as potential alternatives due to their biodegradability and lower toxicity. One promising source is purple cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), which contains anthocyanins—pigments responsible for its red-purple color. Anthocyanins exhibit pH-dependent color changes and have been widely studied for their natural dye potential.<sup>5,6</sup> Despite their advantages, their application is influenced by stability factors such as pH, temperature, and light exposure.<sup>7,8</sup>

Previous studies have demonstrated the potential of plant-based dyes for helminth egg staining, including red spinach extract and teak leaf extract, which showed adequate staining performance.<sup>9,10</sup> Nevertheless, the use of purple cabbage—particularly in the form of an aqueous soaking solution—remains limited. More importantly, no study has specifically optimized the immersion time of purple cabbage soaking solution as a direct staining agent for STH egg identification.

Therefore, this study aims to evaluate the effectiveness of purple cabbage (*Brassica oleracea*



var. capitata f. rubra) soaking solution and to determine the optimal immersion time for its use as a natural alternative stain to eosin in the microscopic examination of STH eggs.

## Materials and methods

### Study Design

A quasi-experimental study was conducted to evaluate the effectiveness of purple cabbage (*Brassica oleracea* var. *capitata f. rubra*) extract as a natural staining agent for helminth egg visualization. This design was selected due to the limited feasibility of controlling all experimental variables, while still enabling comparison across different treatment conditions. The primary variable investigated was immersion duration.

### Sample and Experimental Design

A total of 27 experimental units were included in this study, consisting of purple cabbage samples subjected to three immersion durations: 24 h, 36 h, and 48 h. Based Federer calculation, a minimum of nine replications per group was required, resulting in 27 samples in total.

Samples were selected using a purposive sampling approach based on predefined quality criteria, including bright purple coloration, firm stem structure, crisp leaf texture, and absence of wilting. These criteria were applied to ensure homogeneity of the raw materials and to reduce experimental bias.

A single pooled fecal sample positive for *Ascaris lumbricoides* and hookworm was used for all replications to ensure biological consistency. A total of 27 purple cabbage aqueous extracts were prepared (3 immersion times  $\times$  9 replications)."

### Study Setting

The study was conducted from August 2024 to July 2025 at the Integrated Microbiology Laboratory, Health Polytechnic of the Ministry of Health, Pontianak, Indonesia. All experimental procedures were performed under controlled laboratory conditions.

### Data Collection

Primary data were obtained through direct observation of helminth egg staining following treatment with purple cabbage extract. Observations focused on two main parameters: (1) clarity of the microscopic field and (2) staining absorption by the egg structures. Secondary data were collected from relevant scientific literature to support the methodological framework and interpretation of results.

### Instruments and Measurement

Microscopic examination was performed using a light microscope, and immersion duration was controlled using a calibrated timer. Observations were recorded using a standardized scoring sheet. Staining quality was assessed using a dichotomous scale: a score of 0 indicated poor staining (unclear field, no dye absorption, and indistinct egg morphology), whereas a score of 1 indicated effective staining (clear field, dye absorption, and well-defined egg morphology).

### Data Processing and Management

Data processing included editing, coding, and tabulation. Data were first checked for completeness and consistency. Each treatment group was coded as follows: 24 h (A), 36 h (B), and 48 h (C). The coded

data were subsequently organized into tabular form for statistical analysis.

### Statistical Analysis

All statistical analyses were performed using SPSS software. Descriptive (univariate) analysis was conducted to summarize the distribution of staining outcomes. Inferential analysis was performed using Cochran's Q test to evaluate differences in staining effectiveness across the three related treatment groups. This test was selected due to the binary nature of the outcome variable and the repeated-measures structure of the data. A significance level of  $p < 0.05$  was considered statistically significant.

### Result

A total of 2700 g of fresh purple cabbage (*Brassica oleracea* var. *capitata f. rubra*) was processed as the primary material for extract preparation, using a ratio of 100 g cabbage to 100 mL distilled water. The study was conducted from March 21 to March 23, 2025, at the Integrated Laboratory of the Health Polytechnic, Ministry of Health, Pontianak, Indonesia. Purple cabbage samples were obtained from Flamboyan Market, while fecal samples confirmed positive for Soil-Transmitted Helminths (STH) infection were obtained from the Pontianak Laboratory Unit.

The maceration process was initiated on March 21, 2025, with immersion durations of 24 h, 36 h, and 48 h. Microscopic examinations were subsequently performed using a light microscope at 100 $\times$  and 400 $\times$  magnifications.

### Organoleptic Characteristics

The organoleptic evaluation of the purple cabbage extract showed consistent color characteristics across all immersion durations. The extract exhibited a reddish-purple coloration at 24 h, 36 h, and 48 h, indicating no visible variation in macroscopic appearance.

**Table 1. Organoleptic characteristics of purple cabbage soaking solution at different immersion times**

| Immersion Time | Color          |
|----------------|----------------|
| 24 h           | Reddish-purple |
| 36 h           | Reddish-purple |
| 48 h           | Reddish-purple |

### Phytochemical Analysis

Phytochemical screening confirmed the presence of anthocyanin compounds in the extract. A positive reaction was indicated by a color transition from reddish-purple to greenish-blue, followed by gradual fading, confirming the presence of anthocyanins as the pigment responsible for staining.

**Table 2. Phytochemical test results for anthocyanins in purple cabbage soaking solution**

| Test Parameter | Result       |
|----------------|--------------|
| Anthocyanin    | Positive (+) |

### pH Measurement

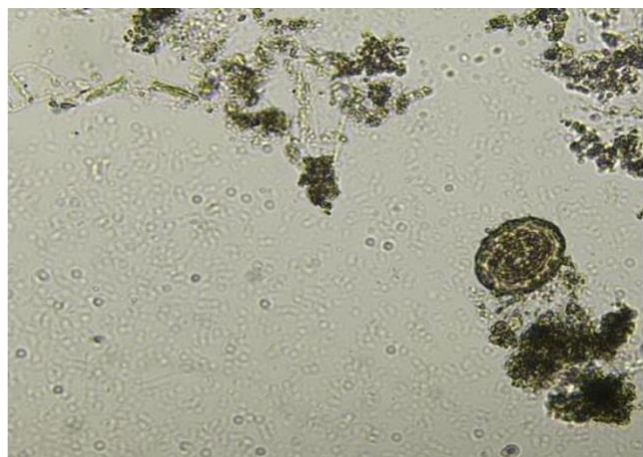
The pH of the extract decreased with increasing immersion duration, indicating a progressive increase in acidity.

**Table 3. pH values of purple cabbage soaking solution at different immersion times**

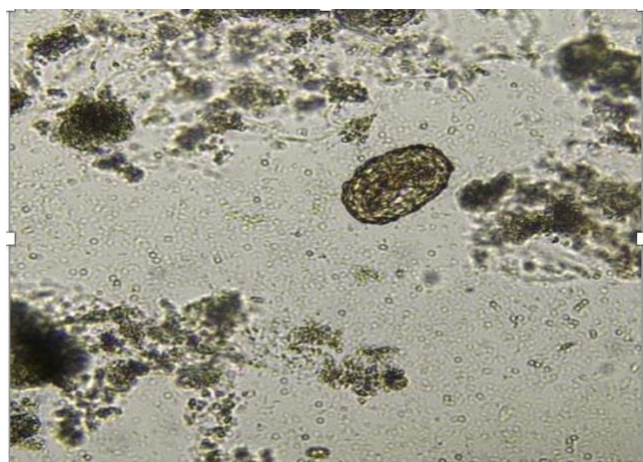
| Immersion Time | pH   |
|----------------|------|
| 24 h           | 3.62 |
| 36 h           | 3.47 |
| 48 h           | 3.18 |

#### Univariate Analysis of Staining Outcomes

Staining effectiveness was evaluated using a dichotomous scoring system across nine replications per treatment group. The results demonstrated that immersion durations of 24 h and 36 h consistently produced poor staining outcomes (score = 0), whereas 48 h immersion resulted in optimal staining (score = 1) across all replicates.



**Figure 1. 24 Hours staining results of helminth eggs using purple cabbage soaking solution**



**Figure 2. 36 Hours staining results of helminth eggs using purple cabbage soaking solution**

**Table 4. Staining results of helminth eggs using purple cabbage soaking solution**

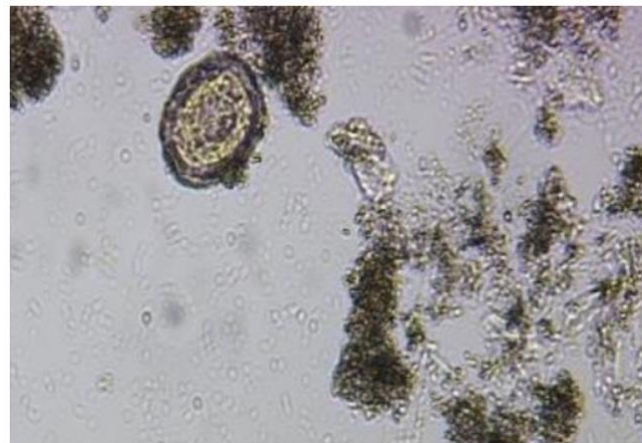
| Replication | Eosin 2% | 24 h | 36 h | 48 h |
|-------------|----------|------|------|------|
| 1           | 1        | 0    | 0    | 1    |
| 2           | 1        | 0    | 0    | 1    |
| 3           | 1        | 0    | 0    | 1    |
| 4           | 1        | 0    | 0    | 1    |
| 5           | 1        | 0    | 0    | 1    |
| 6           | 1        | 0    | 0    | 1    |
| 7           | 1        | 0    | 0    | 1    |
| 8           | 1        | 0    | 0    | 1    |
| 9           | 1        | 0    | 0    | 1    |

#### Scoring criteria:

Score 0 = unclear field, no staining absorption, indistinct egg morphology

Score 1 = clear field, staining absorbed, well-defined egg morphology

These findings indicate that only the 48-hour immersion achieved staining performance comparable to the standard eosin 2% staining.



**Figure 3. 48 Hours staining results of helminth eggs using purple cabbage soaking solution**

#### Multivariate Analysis

Statistical analysis using Cochran's Q test revealed a significant difference in staining outcomes among the three immersion durations. The analysis yielded a Cochran's Q value of 27.000 with a significance level of  $p < 0.001$ .

**Table 5. Cochran's Q test results for staining effectiveness**

| Cochran's Q | df | p-value |
|-------------|----|---------|
| 27.000      | 3  | <0.001  |

Since the p-value was lower than the significance threshold ( $\alpha = 0.05$ ), the null hypothesis was rejected, indicating a statistically significant difference between treatment groups. These results confirm that immersion duration significantly influences staining effectiveness, with the 48-hour immersion identified as the optimal condition for visualizing STH eggs, including *Ascaris lumbricoides* and hookworm species.

#### Discussion

This study utilized faecal samples confirmed positive for Soil-Transmitted Helminths (STH) as test specimens to evaluate the effectiveness of purple cabbage extract as an alternative staining agent. The primary objective was to determine whether significant differences existed in staining outcomes of helminth egg preparations using purple cabbage immersion at 24 h, 36 h, and 48 h compared to conventional eosin staining.

In routine parasitological examinations, 2% eosin is commonly used as a counterstain due to its ability to stain cytoplasmic components and connective tissue in shades of red to orange.<sup>11</sup> This staining

mechanism is attributed to the interaction between eosin, which is negatively charged (acidic), and protein components of helminth egg structures that are positively charged (basic), allowing effective binding.<sup>12</sup> The primary purpose of staining in helminth examination is to enhance visualization, distinguish eggs from debris, and provide a contrasting background.<sup>13</sup>

In contrast to conventional methods, this study explored a natural staining alternative derived from purple cabbage, which contains anthocyanin pigments. The extraction of anthocyanin pigments was achieved through maceration of cabbage in distilled water for varying durations. This method is consistent with previous studies utilizing natural pigments, such as anthocyanins from teak leaves,<sup>10</sup> as well as studies employing other solvents like alcohol for pigment extraction. The use of distilled water as a solvent is advantageous due to its polar nature, facilitating the dissolution of anthocyanins,<sup>14</sup> in addition to being readily available and cost-effective.<sup>15</sup>

The organoleptic assessment revealed that all immersion durations produced a consistent reddish-purple color, indicating that macroscopic appearance alone was insufficient to differentiate extract quality. However, phytochemical analysis confirmed the presence of anthocyanins, as evidenced by the characteristic color change from reddish-purple to greenish-blue followed by gradual fading. This observation is consistent with the known properties of anthocyanins as water-soluble pigments responsible for red, purple, and blue coloration in plants.<sup>8</sup>

The pH measurements demonstrated a progressive decrease in pH with increasing immersion time, indicating increasing acidity. The extracts exhibited pH values of 3.62, 3.47, and 3.18 at 24 h, 36 h, and 48 h, respectively. This trend is important because anthocyanin stability and color expression are strongly influenced by pH. Under acidic conditions (pH < 7), anthocyanins typically exhibit red to purple hues.<sup>16</sup> Furthermore, anthocyanins are known as pH-sensitive pigments, displaying red coloration under strongly acidic conditions and shifting toward blue or colorless forms at higher pH levels.<sup>17</sup>

Microscopic observations demonstrated that immersion duration significantly affected staining performance. At 24 h and 36 h, all replicates showed poor staining outcomes, characterized by unclear microscopic fields, lack of dye absorption, and indistinct egg morphology. These findings suggest that anthocyanin concentration at shorter immersion durations was insufficient to produce effective staining. This limitation is likely due to incomplete extraction of anthocyanin compounds into the solvent, as the maceration process had not yet reached optimal efficiency.

In contrast, the 48 h immersion produced optimal staining results across all replicates, with clear microscopic fields, effective dye absorption, and well-defined egg morphology. This indicates that prolonged immersion enhances anthocyanin extraction, thereby increasing staining effectiveness. These findings are consistent with previous research demonstrating that longer maceration times lead to higher anthocyanin

yield and improved extract quality. For instance,<sup>18</sup> reported that extended maceration time significantly increased anthocyanin yield from purple sweet potato, with optimal results observed at 30 h.

Statistical analysis using Cochran's Q test further supported these observations, demonstrating a significant difference in staining outcomes across the three immersion durations ( $p < 0.001$ ). This confirms that immersion time is a critical factor influencing the staining capability of purple cabbage extract.

Anthocyanins, the bioactive compounds in purple cabbage, are flavonoid-derived pigments with amphoteric properties, enabling them to react with both acidic and basic environments.<sup>19</sup> Their water solubility and color variability under different pH conditions make them suitable candidates for natural staining applications.

The staining procedure employed in this study followed a direct (native) examination method, in which a small amount of fecal sample was mixed with staining solution on a glass slide, homogenized, and observed under a microscope at 100× and 400× magnifications.<sup>20</sup> This method allows rapid visualization of helminth eggs without complex preparation steps.

Despite its potential, the use of purple cabbage extract as a staining agent presents several limitations. The stability of anthocyanin pigments is highly dependent on environmental factors, including pH, temperature, and light exposure. Prolonged storage or exposure to high temperatures may lead to pigment degradation and color, reducing staining effectiveness. In this study, the extract was stored at room temperature (20–25 °C) in dark conditions to minimize degradation. Previous studies have shown that high temperatures can accelerate anthocyanin degradation, while stability is better maintained under acidic conditions.<sup>6</sup>

The findings of this study demonstrate that purple cabbage extract is capable of staining STH eggs, including *Ascaris lumbricoides* and hookworm. However, unlike eosin, which provides a red-colored background, the cabbage extract produces a relatively clear background, which may affect contrast. Nevertheless, this characteristic is consistent with previous findings using other natural dyes, such as teak leaf extract, which also produced a clear background while effectively staining helminth eggs.<sup>12</sup>

The results of this study indicate that purple cabbage soaked in water for 48 hours demonstrates potential as a natural dye. However, it cannot yet be considered a suitable alternative to eosin in the examination of soil-transmitted helminth (STH) eggs, as it only stains the worm eggs without producing adequate background contrast, particularly the characteristic red coloration provided by eosin. Therefore, while the extract shows promise, further optimization in terms of extraction method, concentration, and staining protocol is required to enhance its effectiveness.

## Conclusion

Immersion duration significantly affects the staining effectiveness of purple cabbage (Brassica

oleracea var. capitata f. rubra) soaking solution for Soil-Transmitted Helminth (STH) eggs. Soaking solutions obtained after 24 h and 36 h immersion produced poor staining, while 48 h immersion yielded clearer visualization with more effective dye absorption.

However, the purple cabbage soaking solution cannot fully replace eosin, as it does not provide adequate background contrast. Cochran's Q test confirmed a significant difference among treatments ( $p < 0.001$ ), highlighting immersion time as a key factor influencing staining performance.

### Conflict of interest

Authors state no conflict of interest.

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