



# Effectiveness of Miana Leaves (*Plectranthus scutellaroides*) as a Natural Dye in the Identification of Soil-Transmitted Helminth (STH) Eggs

Erica Samosir<sup>1</sup>, Lestari Rahmah<sup>1</sup>

<sup>1</sup>Department of Medical Laboratory Technology, Politeknik Kesehatan Medan, Medan, Indonesia

## Correspondence

Erica Samosir  
Jl. Jamin Ginting KM. 13,5 Medan, North Sumatra  
20137  
Email: oktaviaerika769@gmail.com

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## Abstract

**Background:** The use of natural dyes in microscopic examinations, such as the identification of Soil-Transmitted Helminth (STH) eggs, provides a safer and more environmentally friendly alternative. Miana Leaves (*Plectranthus scutellaroides*), which contain high levels of anthocyanins, have the potential to serve as an effective natural dye for staining STH eggs in fecal samples. **Objectives:** This study aims to evaluate the staining efficacy of miana leaf extract (*Plectranthus scutellaroides*) in identifying STH eggs by comparing different extract concentrations.

**Materials and Method:** An experimental approach with descriptive data analysis was employed. Various concentrations of miana leaf extract (1:1, 1:2, and 1:3 ratios with distilled water) were tested on fecal samples positive for STH eggs. **Results:** The 1:2 concentration of miana leaf extract yielded optimal contrast and clear visualization of STH egg morphology, while the 1:1 concentration resulted in excessive staining and the 1:3 concentration produced pale staining. **Conclusions:** Miana leaf extract, particularly at a 1:2 concentration, is effective as a natural stain for STH egg identification. Further research should explore its application in broader parasitological diagnostic contexts.

## Keywords

Miana leaves, Natural dye, Staining concentration, STH eggs.



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## 1. Introduction

Miana also known as the sigresing plant in the Batak Toba community, has the scientific name *Plectranthus scutellaroides* and belongs to the Lamiaceae family. This plant is commonly found in gardens or fields as an ornamental plant (Surahmaida & Umarudin, 2019). In addition to its ornamental use, miana leaves are also utilized in traditional medicine due to their significant anti-inflammatory and antioxidant properties. The anthocyanins present in miana leaves act as natural pigments, imparting a purplish-red color to the extract, making it a potential safe and non-toxic natural dye (Salimi, 2021). Previous studies have demonstrated the use of miana leaf extract as an alternative dye for identifying Soil-Transmitted Helminth (STH) eggs, with varying results depending on the concentration used (Puspita et al., 2018; Permatasari et al., 2021).

STH infections are caused by the entry of nematode worms through soil contaminated with worm eggs. Common STH species include *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms such as *Ancylostoma duodenale*, and *Necator americanus* (Rahmawati, 2019). According to a survey by the Ministry of Health of the Republic of Indonesia in 2022, the prevalence of STH infections in Indonesia ranged from 2,5-62%, with the highest rates observed among preschool and primary school children (Yani et al, 2023). These infections have serious health consequences, such as malnutrition, anemia, and developmental disorders in children (Rahma et al., 2020). Thus, the screening and identification of STH eggs are crucial for preventing and controlling the spread of these infections.

Currently, a 2% eosin solution is frequently used in STH egg staining due to its acidic properties and ability to produce a contrasting red-orange color. However, the use of eosin poses several limitations, including the need for large quantities of reagents, economic inefficiency, the generation of hazardous waste, and potential carcinogenicity (Salnus et al., 2021; Sri Kartini & Angelia, 2021). This highlights the urgent need for safer and environmentally friendly alternatives. Miana leaf extract, as a biodegradable and safe natural dye, offers a practical solution, particularly for healthcare facilities in remote areas with limited access to synthetic chemicals. The use of this extract can help reduce hazardous waste and operational costs. This study aims to evaluate the effectiveness of miana leaf extract (*Plectranthus scutellaroides*) as a natural dye for the identification of Soil-Transmitted Helminth (STH) eggs and to determine the optimal concentration that provides the best visualization.

## 2. Materials and Methods

### 2.1 Types of Research

This true experimental study evaluated the effectiveness of miana leaf (*Plectranthus scutellaroides*) staining for identifying Soil-Transmitted Helminth (STH) eggs. Conducted at the Parasitology Laboratory, Polytechnic of the Ministry of Health Medan, it involved 30 fecal samples confirmed to be positive for STH eggs, randomly selected from the laboratory's specimen stock, and tested with three concentrations of miana leaf extract to determine the optimal staining. The independent variable was the concentration of miana leaf extract, while the dependent variable was the clarity of STH egg visualization under the microscope.

### 2.2 Miana Leaf Extract

100 g of miana leaves were weighed, washed, and air-dried. The leaves were then soaked in 100 mL of distilled water and centrifuged at 3000 rpm for 15 minutes to extract anthocyanins (Permatasari

et al., 2021).

### 2.3 Preparation of Miana Leaf Extract Test Solutions

Each concentration of 1:1; 1:2; and 1:3 involves mixing 10 drops of the extract with 10; 20; and 30 drops of distilled water (Permatasari et al., 2021).

### 2.4 Preparation of the Slide

The slide was cleaned, and 1-2 drops of the test solution (1:1; 1:2; and 1:3) are dripped on it. Approximately 2 mg of feces was mixed into the solution until homogeneous, then covered with a cover glass without air bubbles. The samples were observed using 10x and 40x magnification, and the results documented with a microscope camera (Permatasari et al., 2021).

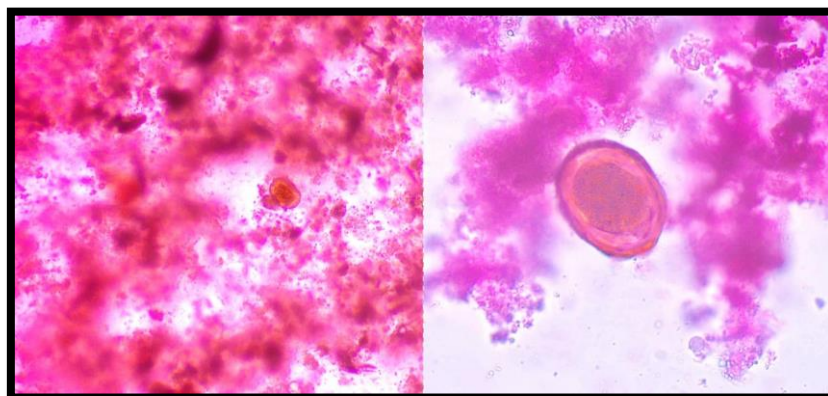
### 2.5 Data Analysis

The data were analyzed descriptively to compare staining effectiveness across the three concentrations, assessed based on the clarity of STH egg visualization (Permatasari et al., 2021).

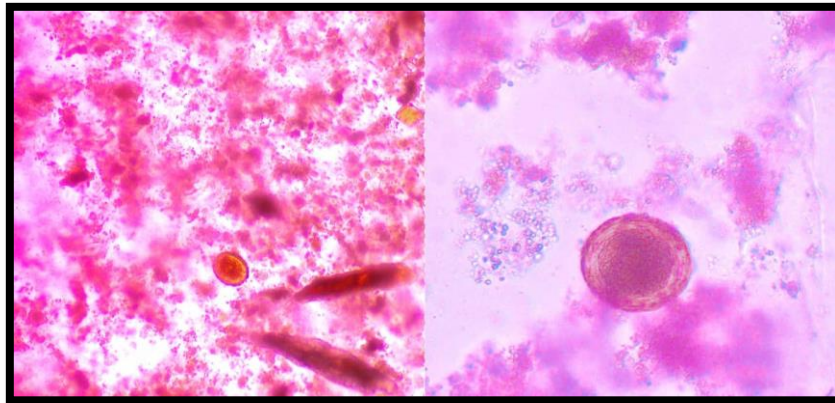
## 3. Results and Discussion

### 3.1. Results

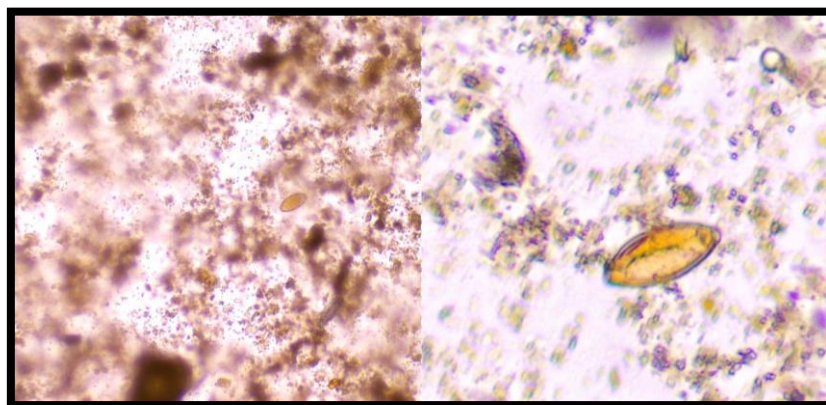
This study employed descriptive observation to assess the color contrast provided by miana leaf extract as a natural dye in identifying Soil-Transmitted Helminth (STH) eggs. This approach enable a detailed description of the contrast and visual clarity at different concentrations without requiring quantitative measurements. A 1:2 concentration of the extract was found to be optimal, providing sufficient color contrast for effective identification. The visualization of staining outcomes is shown in the following figure.



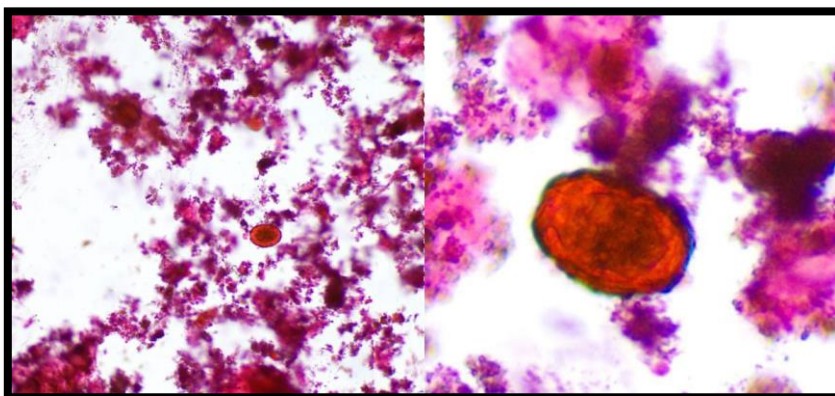
**Figure 1.** Staining results of *Ascaris lumbricoides* worm eggs at a concentration of 1:1, (Left) 10x magnification, (Right) 40x magnification



**Figure 2.** Staining results of *Ascaris lumbricoides* worm eggs at a concentration of 1:2, (Left) 10x magnification, (Right) 40x magnification



**Figure 3.** Staining results of *Trichuris trichiura* worm eggs at a concentration of 1:3, (Left) 10x magnification, (Right) 40x magnification



**Figure 4.** Staining results of *Ascaris lumbricoides* worm eggs in 2% eosin, (Left) 10x magnification, (Right) 40x magnification



In Figure 1, a 1:1 concentration produces overly intense staining, hindering identification and obscuring STH egg morphology. In contrast, Figure 2 demonstrates that a 1:2 concentration achieves balanced dye intensity, facilitating the clear visibility of STH egg morphology. Meanwhile, Figure 3 shows that a 1:3 concentration results in under-staining. Reducing both contrast and visibility of STH egg structures.

By comparison, although 2% eosin achieves effective staining, it poses environmental concerns as a class 3 carcinogen. Its higher cost further limits its suitability, especially for health facilities with constrained resources. In contrast, miana leaf extract not only provides adequate staining but is also more environmentally friendly and cost-effective than synthetic dyes.

### 3.2. Discussion

The findings from this study demonstrate that miana leaf extract, particularly at a 1:2 concentration, is effective as a natural dye for identifying STH eggs. This concentration provides optimal contrast, enhancing visualization of critical egg structures, such as the egg wall and internal contents. Due to its environmentally friendly properties, miana leaf extract is a viable alternative to synthetic dyes like eosin, particularly in resource-limited laboratory settings and for sustainable practices in public health laboratories (Ubaedilah & Supriyatna, 2023; Fati et al., 2020; Yusuf et al., 2018). This outcome aligns with previous studies, such as (Wakhidah & Silalahi, 2018), which indicate that natural dyes can yield satisfactory visualization. Although (Wakhidah & Silalahi, 2018) reported that natural dyes often produce lower intensity than synthetic dyes, the miana leaf extract in this study achieved comparable intensity at specific concentrations, likely due to variations in extraction methods or anthocyanin composition.

The application of miana leaf extract promotes sustainability by reducing the environmental impact associated with synthetic dye waste. While dye intensity remains stable at certain concentrations, color stability may decrease over time, especially with prolonged exposure to direct sunlight or improper storage (Puspita et al., 2018). Further research should investigate optimal storage conditions and potential additives to prolong extract shelf life, as well as standardized extraction methods to ensure consistency.

The color variation observed in this study, with a reddish-purple hue, differs from the yellowish-brown reported by (Permatasari et al., 2021). This discrepancy is likely due to differences in anthocyanin content influenced by factors such as plant growth location, maturity, and extraction processes. Standardizing extraction procedures is therefore critical to reduce variability and enhance result reproducibility.

Future studies are needed to improve the stability of miana leaf extracts and to evaluate their

applicability for other types of STH eggs under various diagnostic conditions. Additionally, incorporating quantitative methods, such as optical density measurements, would provide an objective assessment of staining effectiveness.

#### 4. Conclusions

Based on the findings, it can be concluded that miana leaf extract at a 1:2 concentration is effective as a natural dye for identifying STH eggs, yielding optimal results for visualizing egg structures. This natural dye shows significant potential for use in parasitology diagnostics, especially in resource-limited settings. Further research is recommended to evaluate its applicability to other helminth egg types and to develop methods for improving the stability and consistency of staining results.

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**Author Contributions:** ES: conceptualization and planning, data collection, data analysis, report writing and publication. LR: support data collection, data analysis and interpretation, discussion and development ideas

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