

Effect Of Red Amber (*Amaranthus Tricolor L.*) Extract on Soil-Transmitted Helminth Worm Egg Morphology as a Substitute for 2% Eosin

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Submission date: 20-6-2025; Date of receipt: 10-7-2025

Abstract

The prevalence of worm disease in Indonesia is around 45-65%. The cause of worm infections that are still common in Indonesia is intestinal nematodes. The intestinal nematode group inhabits the digestive tract and is classified within the Soil-Transmitted Helminths group. This group requires contact with warm and moist soil to reach an infective form. Clinically important soil-transmitted helminth species that infect humans are *Ascaris Lumbricoides* (roundworm), *Trichuris Trichiura* (whipworm), *Anylostoma Duodenale*, and *Necator Americanus*. This study aimed to determine the effect of red spinach extract (*Amaranthus tricolor L.*) on the morphology of soil-transmitted helminth eggs as a substitute for 2% Eosin. The research method used was experimental research, and the design used was Statistical Group Comparison. The results of this study are that red spinach extract (*Amaranthus tricolor L.*) can be used as an alternative coloring in the microscopic examination of soil-transmitted helminth worm eggs. A concentration of 1:1 shows the optimal concentration that can color Soil Transmitted Helminth worm eggs.

Keywords: Red Spinach Extract, Soil-Transmitted Helminths

Introduction

Worm infestation is one of the most common infections worldwide. According to the World Health Organization (WHO), in 2023, approximately 1.5 billion people, or 24% of the world's population, were infected with worms. In Southeast Asia, soil-

transmitted helminth infections reached 500 million people, and 11 countries were categorized as endemic, including Indonesia. Indonesia ranks second in Southeast Asia for the need for worm treatment in children, with a percentage of 15%, after India, which is the priority, with a percentage of 61%. Worm infestation remains a significant public health issue, especially in Indonesia. The prevalence of worm infestation in Indonesia is still high, at around 45-65%. Poor sanitation in tropical and subtropical regions can reach 80% due to poor sanitation. Indonesia, with its tropical climate and high humidity, allows worm eggs and larvae to thrive (Rahmayanti, 2024). Pekanbaru City is one of the cities where cases of worm infestation are still prevalent. In 2012, data from the Pekanbaru City Health Office recorded 2,285 cases of worm infestation from 20 community health centers (Kartini, 2016).

The most common cause of worm infections in Indonesia is intestinal nematodes. This group of intestinal nematodes lives in the digestive tract and belongs to the soil-transmitted helminth group. This group requires contact with warm, moist soil to reach its infective form. Clinically important soil-transmitted helminth species that infect humans include *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale*, and *Necator americanus* (Arisandi, 2024).

Examination is essential to identify worm infections, both on live worms and on smears. The worms are examined depending on the type of parasite. Intestinal worms or protozoa are examined through feces (Rusmanto, 2012). The native method using 2% eosin reagent is the simplest method for examining intestinal nematode eggs. This reagent has an acidic composition and is orange-red (Hurbelubun, 2011).

The native method using 2% eosin requires a large amount of reagent and is quite expensive; therefore, alternative dyes are needed to visualize the morphology of

intestinal nematode eggs. Several plants can be used as alternative natural dyes, including red spinach (*Amaranthus tricolor* L.). The pigment found in red spinach is anthocyanin, which gives plants and fruits their purplish-red color (Juliastuti et al., 2021).

Based on research by Ningsih, Salnus, and Novriani (2023), hibiscus flower extract (*Hibiscus rosa-sinesis* L.) can be used as an alternative dye in the examination of soil-transmitted helminth eggs, but it is not as good as eosin dye because it can only differentiate worm eggs from feces and does not absorb into the worm egg cells.

Based on this background, the authors are very interested in knowing whether red spinach extract (*Amaranthus tricolor* L.) can be an alternative dye for the examination of soil-transmitted helminth eggs, replacing 2% eosin.

Method

The type of research used is Experimental research, where this research will see the clarity of the shape and color of worm eggs in preparations using red spinach extract (*Amaranthus tricolor* L.) as an alternative dye in the examination of Transmitted Helminth worm eggs with concentration variations of 1:1, 1:2, 1:3, 1:4, 1:5, and 2% eosin as a control. This research uses a Static Group Comparison design, namely, a group is given a certain treatment, then the effect of the results of each variation in staining time is observed. The population in this study was all fecal samples positive for soil-transmitted helminth eggs. The samples used in this study were fecal samples positive for soil-transmitted helminths in specimens at the Thamrin Pekanbaru Clinical Laboratory. The results of staining Soil Transmitted Helminths eggs using red spinach extract (*Amaranthus tricolor* L.) were compared with the results of staining Soil Transmitted Helminths eggs using 2% Eosin as a control.

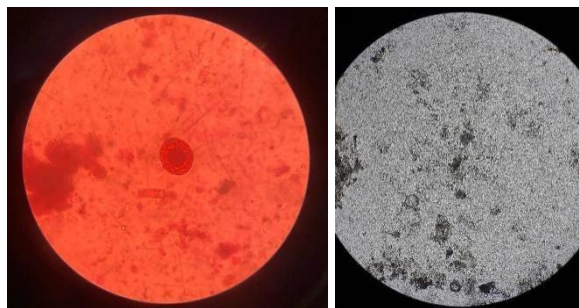
The data processing for this study refers to the effectiveness research criteria. The results of this study were scored 1, 2, and 3, with the following criteria referring to research (Oktari and Mutamir, 2017):

1. A score of 1 is given if the field of view lacks contrast, the worm eggs do not absorb color, and the parts of the worm eggs are not clearly visible.
2. A score of 2 is given if the field of view lacks contrast, the worm eggs do not absorb color, and the parts of the worm eggs are not clearly visible.
3. A score of 3 is given if the field of view has contrast, the worm eggs absorb color, and the parts of the worm eggs are clearly visible.

Results

In a study on optimizing red spinach (*Amaranthus tricolor* L.) extract, fecal samples positive for Soil-Transmitted Helminths (STH) eggs were used. The red spinach extract was then diluted with distilled water at 1:1, 1:2, 1:3, 1:4, and 1:5 dilutions. The dilutions were then observed using a microscope at 10x to 40x magnification. The following comparisons were made by observing the color of the worm eggs in the results of staining with 2% Eosin, without staining, and red spinach extract concentrations of 1:1, 1:2, 1:3, 1:4, and 1:5. The results of the study for each treatment are shown in the following figure:

Figure 1: Staining using eosin and without eosin



A

B

A = Staining with Eosin.

B = Without Staining.

Figure 2: Coloring with Red Spinach Extract Concentration 1:1



Figure 3: Coloring with Red Spinach Extract at a Concentration of 1:2



Figure 4: Coloring with Red Spinach Extract Concentration 1:3



Figure 5: Coloring with Red Spinach Extract Concentration 1:4



Figure 6: Coloring with Red Spinach Extract Concentration 1:5



Based on Figure 1, when compared with Figures 2 to 6, which are stained with spinach extract, it is found that Figure 2, which is stained with 1:1 red spinach extract, gives almost the same results as Figure 1A, which is stained with 2% Eosin. The quality of staining using 1:1 red spinach extract and 2% Eosin provides a bright background color, a clear egg shape, and can be distinguished from feces. This is different from worm eggs without being stained, which can be seen in Figure 1B and cannot be distinguished from feces.

Discussion

Optimization of Red Spinach Extract

This study used fecal samples positive for Soil-Transmitted Helminths (STH) as test samples. This study examined whether there were significant differences between alternative staining methods using red spinach extract at concentrations of 1:1, 1:2, 1:3, 1:4, and 1:5 compared to 2% Eosin staining as a control for the test samples.

After the study, the results of the comparisons of red spinach extract at concentrations of 1:1, 1:2, 1:3, 1:4, and 1:5 were included in the effectiveness research criteria. The test results were given scores of 1, 2, and 3, based on the research conducted by Oktari and Mutamir (2017).

The results of the 1:1 comparison of red spinach extract were given a score of 2 because the visual field lacked contrast, the helminth eggs did not absorb the color, and the egg parts were not clearly visible. Meanwhile, the results of a 1:2 to 1:5 ratio were given a score of 1 because the visual field lacked contrast, the worm eggs did not absorb the dye, and the worm egg parts were not clearly visible.

Staining using red spinach extract at a 1:1 concentration showed quite good results when observed microscopically; the background of the visual field lacked contrast, the worm eggs did not absorb the dye, and the egg parts were not clearly visible. Furthermore, microscopically, the color of the eggs and feces was clear and distinguishable.

Anthocyanins belong to the flavonoid group. The pigment colors produced by anthocyanins are red, orange, blue, and violet. Red spinach is one of the plants that produces red anthocyanins (Saati et al., 2019). This is supported by research conducted by Charolin Pebrianti, RB. Ainurrasyid and Sri Lestari Purnamaningsih (2015) stated

that red spinach produces anthocyanin levels of 6350 ppm in the leaves and 2480 ppm in the stems. Therefore, red spinach can be used as an alternative to eosin due to its high anthocyanin content.

Plant dyes or pigments are extracted using solvents that match the polarity of the substance to be extracted. Extraction of flavonoid compounds is recommended under acidic conditions because the acid denatures plant cell membranes, then dissolves the anthocyanin pigments so they can be released from the cells, and prevents flavonoid oxidation. Anthocyanin pigment content in plants is influenced by several factors, especially sunlight (intensity), air temperature, and pH. Anthocyanins are stable at pH 3-5 and temperatures of 50°C, and during storage at 4°C (Eppang et al., 2020).

One study using plant extracts as dyes was conducted by Fasya Fatarani Nadhira, Mamat Rahmat, Yuliansyah Sundara Mulia, and Zuri Rismiarti (2023). They used teak wood extract as an alternative to eosin in the examination of soil-transmitted helminth eggs. They found that teak wood extract was effective as an alternative dye to 2% eosin in the examination of soil-transmitted helminth eggs. This is due to teak wood being a plant containing anthocyanins.

This study concluded that red amaranth (*Amaranthus tricolor* L.) can be used as an alternative dye for soil-transmitted helminth eggs. The results of this study are also supported by research conducted in 2022 by Lina Yunda Artanti, Hendra Budi Sungkawa, Herlinda Djohan, Ari Nuswantoro and Riska Alfianita, which stated that the juice of red spinach stems can be used as an alternative dye for Soil Transmitted Helminth eggs, which at a concentration of 1: 1 produces a contrasting color close to Eosin.

Conclusion

Based on the results of research conducted on the effectiveness of red spinach extract (*Amaranthus tricolor* L.) as an alternative stain for microscopic examination of soil-transmitted helminth eggs, it can be concluded that:

1. Red spinach extract (*Amaranthus tricolor* L.) can be used as an alternative stain for microscopic examination of soil-transmitted helminth eggs, but not as an eosin stain because it only differentiates helminth eggs from feces and does not penetrate the helminth egg cells.

2. A 1:1 concentration indicates the optimal concentration for staining soil-transmitted helminth eggs.
3. Staining with red spinach extract allows for visualization of the morphology of soil-transmitted helminth eggs.

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