Modification of Natural Dyes from Ethyl Acetate Extract of Red Angkak in Observations of Soil-Transmitted Helminth Eggs

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Modification of Natural Dyes from Ethyl Acetate Extract of Red Angkak in Observations of Soil-Transmitted Helminth Eggs

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ABSTRACT

An infection brought on by different kinds of parasitic worms in the intestines is known as a worm infestation. This species is classified as a soil-transmitted helminth (STH). Finding intestinal Nematode worm eggs can be done most easily using the native approach. Various examination components in preparations or specimens are observed with this procedure, which uses a 2% Eosin reagent. The non-biodegradable characteristics of eosin and the toxic waste it generates call for the replacement of this dye with a more environmentally friendly one. The anthocyanin content in red angkak can be used as an alternative dye for the observation of STH eggs. Finding out if red angkak ethyl acetate extract can be used as a dye to identify SHT infections during fecal exams is the goal of this study. This study uses a lab experiment using categorical descriptive features, where the dye's pH (basic and acidic) and the concentration of red angkak extract (pure, 1:1, 1:2, and 1:3) are varied. Based on the results, pure red angkak extract with an acidic pH works best as a dye substitute for the 2% Eosin reagent when staining STH worm eggs. In conclusion, red angkak ethyl acetate extract can be utilized as a natural color to distinguish STH worm eggs.

Keywords: Coloration, Eosin, Eggs of STH Worm. Red Angkak Ethyl Acetate Extract

INTRODUCTION

The tropical climate of Indonesia significantly influences the spread of endemic diseases, including soil-transmitted helminth (STH) infections. The prevalence of these worm infections in Indonesia remains high, with estimates ranging from 60% to 80% in areas with poor sanitation (Elmiyanti et al., 2022). According to the World Health Organization, 24% of people have been infected with soil-transmitted helminths (STH) (Munawaroh et al., 2022). Worm infections are still prevalent in Indonesia, with prevalence rates ranging from 2.5% to 62%. This high percentage is especially noticeable among the less fortunate and those who don't have access to sanitary conditions (Nadhira et al., 2023; Wahyuni et al., 2024).

Microscopic examination is one of the laboratory procedures used to identify helminthic diseases (Darwin, 2024). The gold standard for determining the presence of worm-caused infections is the examination of stool samples. The native method, which employs a 2% eosin reagent, is the most straightforward way to examine worm eggs (Phayana et al., 2024). This reagent can produce an orange-red tint and is acidic. The goal of the 2% eosin staining is to make it easy to distinguish the worm eggs from the nearby excrement (Ningsih et al., 2023). In addition to separating the excrement from the trash, the 2% eosin creates a crimson background against the yellowish eggs (Munawaroh & Shofi, 2023).

The simplest method for examining helminth eggs is the native method using 2% eosin reagent. Eosin itself is non-biodegradable, generates hazardous waste, and is flammable (Ningsih et al., 2023). Therefore, an alternative, more environmentally friendly staining method is needed. This can be achieved by utilizing natural dyes as an alternative, such as those derived from natural materials (Salnus et al., 2021; Suraini & Sophia, 2022).

One natural material that can be used as a natural staining reagent is red angkak. Red angkak extract or rice fermented with the mold of *Monascus purpureus* can be used as an alternative color (Rzkuloh et al., 2024). This extract is effective for staining Soil-Transmitted Helminth eggs because the structure of the eggshell and its contents can be clearly observed. The ethanol extract of red yeast rice contains anthocyanins, specifically rubropunctatin and monascorubrin, which are red pigments, making it suitable as a dye. The red color from these pigments serves as a natural alternative for staining Soil-Transmitted Helminth eggs, effectively replacing the 2% eosin stain (Puspita, 2020)

According to Rumondor et al. (2021), angkak is a product of rice fermented by the *Monascus purpureus* mold, which yields hues ranging to red and yellow. Monascorubin and rubropunktatin are two red pigments found in angkak (Lestario & Al-janati, 2023). Asia is the region where angkak is most commonly used as a colouring ingredient. Chinese cheese and the beverage known as anchu are colored with angkak. Japan also makes extensive use of the pigment angkak (Elkhateeb & Daba, 2023). They add red pigments to wine and yellow pigments to confections as colorants (Este & Mustoro, 2021). According to Apriani's (2016) research, red angkak solution can be utilized in wet preparations of dicot and monocot plant stems in place of dyes. Furthermore, it has been discovered that gram bacteria can also be colored by the pigments found on the body (Nurhidayat & Silviani, 2022).

As an alternative to synthetic red dyes commonly used in various foods, beverages, and bacterial staining, the red color from red yeast rice shows promising results. Furthermore, research on the use of STH egg dye derived from red yeast rice remains scarce, with no studies yet focusing on its potential as an alternative STH egg dye. This ethyl acetate extract of angkak is intended to take the place of the 2% eosin dye that was previously used for dyeing STH eggs. The purpose of this study is to evaluate the advantages of using ethyl acetate red angkak extract as a coloring agent to identify STH infections during fecal tests.

METHOD

This research is designed using experimental-based research, utilizing red fermented rice extract as a natural dye for staining cytological preparations. Four treatments in this research design compare the dye's pH and the concentration of red angkak ethyl acetate extract. The stock/pure solution or 100% pure extract concentration, a 1:1 concentration (2.5 ml of red angkak extract: 2.5 ml of ethyl acetate), a 1:2 concentration (1.7 ml of red angkak extract: 3.3 ml of ethyl acetate), and a 1:3 concentration (1.2 ml of red angkak extract: 3.75 ml of ethyl acetate) are the concentrations that were used**.** Pig excrement and red angkak extract make up the study's population. Pig excrement with STH worm eggs is what was used as a sample in the meantime. Because STH worm eggs are present in the feces of pigs, random sampling is the approach used for sampling (Munawaroh & Shofi, 2023).

The Production of Ethyl Acetate Extract

Extraction was carried out by soaking 25 g of red angkak powder in 100 ml of ethyl acetate. The mixture was left too steep for one week, with periodic stirring every twenty-four hours. The solution was removed from the residue after a week and kept in a glass bottle until needed (Nadhira et al., 2023).

Test Solution Generation

The ethyl acetate red angkak extract is prepared by dilution in an ethyl acetate ratio of 1:1 to create the test solution. To achieve this, mix 2.5 ml of red angkak ethyl acetate extract with 2.5 ml of ethyl acetate. For a 1:2 concentration, mix 1.7 ml of ethyl acetate red angkak extract with 3.3 ml of ethyl acetate; for a 1:3 concentration, mix 1.25 ml of red angkak ethyl acetate extract with 3.75 ml of ethyl acetate (Munawaroh & Shofi, 2023).

Analyzing Worm Eggs with 2% Eosin

To prevent oil, prepare and clean the glass objects. On a glass slide, put one or two drops of a 2% eosin solution. Once the rough edges have been removed, take around 2 milligrams of stick excrement and combine it with the 2% eosin solution. Cover the specimen with a cover slip until it is evenly coated to avoid air bubbles forming. Subsequently, an Optilab Advance from PT MICONOS was used to take pictures and the samples were examined under a microscope at magnifications ranging from 100 to 400 times (Munawaroh & Shofi, 2023).

Examining Worm Eggs with Ethyl Acetate Red Angkak Extract

One drop of red fermented rice extract and each test sample of the pure/stock solution at concentration ratios of 1:1, 1:2, and 1:3 should be placed on a clean glass slide. The process involves combining 1-2 drops of each concentration of ethyl acetate red angkak extract with excrement at the tip of a stick (± 2 mg) and homogenizing the mixture. After the rough section has been removed, cover the specimen with a cover glass until it is evenly covered to prevent air bubbles. Subsequently, an optilab was used to take pictures and the samples were examined

under a microscope at magnifications ranging from 100 to 400 times (Munawaroh & Shofi, 2023).

Data Collection

The data collection method in this research is to determine whether worm eggs or their background can have different colors, using the following assessment criteria:

1. Not contrasting, not absorbing color, the egg part is not visible.
2. Less contrast, less color absorption, and the egg part are less clear.
3. Less contrast, less color absorption, the egg part is clear.
4. Contrast, less color absorption, the egg part is clear.
5. Contrast, absorbing color, the egg part is clear (Munawaroh & Shofi, 2023)

Data Analysis

ANOVA tests were used to assess the research data. Then, DMRT was used to see if the treatments differed significantly from one another. The modified study criteria of Munawaroh & Shofi (2023) state that coloration can be evaluated based on 1, 2, 3, 4, and 5.

RESULT AND DISCUSSION

There are variations in the morphology of STH worm eggs, according to research done on the ethyl acetate extract of red angkak for the assessment of STH-positive worm eggs using 4 treatments and 2% eosin dye as a control. The study's findings are displayed in Table 1 and Figure 1 below.

Table 1 Observation Result of Coloring Morphology of STH Worm Eggs

pH	Temperature	Dilution	Repetition of Observation Result					
			1	2	3	4	5	Average
Acid	Room	Pure	3	4	4	4	4	$4,2^{c}$
		1 : 1	3	3	3	4	3	$3,2^{ab}$
		1 : 2	3	3	3	3	3	3^{ab}
		1 : 3	4	4	3	2	4	$3,4^{bc}$
	Cold	Pure	3	3	2	3	2	$2,6^{a}$
		1 : 1	3	4	4	4	4	$3,8^{bc}$
		1 : 2	2	4	3	3	3	3^{ab}
		1 : 3	3	3	4	4	4	$3,6^{bc}$
Basic	Room	Pure	3	4	4	4	4	$3,8^{bc}$
		1 : 1	4	3	3	3	3	$3,2^{ab}$
		1 : 2	4	2	3	4	4	$3,4^{bc}$
		1 : 3	4	3	4	4	3	$3,6^{bc}$
	Cold	Pure	4	3	4	4	4	$3,8^{bc}$
		1 : 1	3	4	4	3	4	$3,6^{bc}$
		1 : 2	4	2	4	4	4	$3,8^{bc}$

1 : 3	3	4	4	4	4	3,6^{bc}

Note: (1) Not contrasting, not absorbing color, the egg part is not visible. (2) Less contrast, less color absorption, and the egg part are less clear. (3) Less contrast, less color absorption, the egg part is clear. (4) Contrast, less color absorption, the egg part is clear. (5) Contrast, absorbing color, the egg part is clear. Numbers accompanied by the same letter indicate there is no significant difference based on Duncan's test 0.5%, n=5

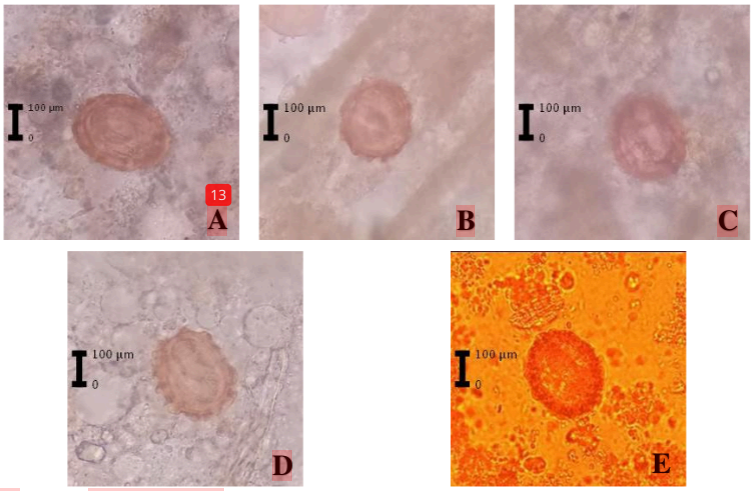

Figure 1 (A) Morphology of STH worm eggs with acid pH dye at room temperature, (B) Morphology of STH worm eggs with acid pH dye at cold temperature in a 1:1 ratio, (C) Morphology of STH worm eggs with alkaline pH dye at room temperature; (D) Morphology of STH worm eggs with alkaline pH dye at cold temperature in a 1:2 ratio, (E) Morphology of STH worm eggs with 2% Eosin dye. Magnification 40x.

Based on Figure 1, pictures A to E clearly show the morphology of the eggshell and contents of STH eggs which have three layers, namely vitelline, hyaline, and albuminoid. On the other hand, picture A shows that the background of the staining with 2% eosin is darker than the ethyl acetate of red angkak. However, the morphology of the egg is still clearly visible, with the shell and contents of the egg being brownish-yellow.

Table 1 presents the research findings indicating a substantial difference in the dyeing quality of the ethyl acetate red angkak extract, as compared to the control sample of 2% eosin. Following an analysis of variance (F test) utilizing statistical data processing software and a 5% confidence level DMRT test, the F test findings show that $F_{Calculated}$ (2,833) > F_{Table} at a

significance level of 0.002. We may conclude that dilution and pH impact the quality of STH worm egg staining. Based on further tests using 5% DMRT, the best treatment for the best dye is acidic pH with a pure ratio stored at room temperature. This suggests that as dye concentrations rise, the dye's quality improves and the composition of the eggs becomes more apparent (Munawaroh & Shofi, 2023).

The structure of the shell and the contents of the eggs can be visible after coloring, suggesting that the ethyl acetate extract of red angkak is an excellent dye for STH eggs. The reason for this is that red pigments that can be used as dyes, called anthocyanins, are present in red angkak extract. Specifically, rubropunktatin and monaskorubin are present (Puspita et al., 2020). It turns out that these red pigments can be utilized as a substitute for 2% Eosin and as a natural colorant for dyeing STH eggs.

The red angkak extract and 2% eosin have different colors due to the high quantities of fatty acids in the red angkak extract. The staining of STH worm eggs may also vary depending on the pH difference between the staining treatment and the eosin concentration. The amount of anthocyanin in the dye for STH worm eggs will depend on the pH of the dye (Doria et al., 2023). This is due to the fact that STH worm eggs with low anthocyanin concentration may exhibit less contrast than those with 2% eosin. The best observation results in acidic conditions with the red angkak extract dye demonstrate this, as the color of the red angkak is absorbed by the STH eggs but does not color the backdrop of the eggs. Anthocyanins are more stable in an acidic solvent environment (Ayun et al., 2023; Ghareaghajlou et al., 2021).

Temperature has a big impact on how STH egg staining turns out. Extreme temperature variations can impact the quality of coloring, leading to changes in color intensity, disruption of dye stability, and harm to the morphological structure of the eggs, among other effects (Alizadeh-Sani et al., 2020). Staining will be more successful at ideal temperatures, resulting in good contrast and more pronounced morphological characteristics, which will facilitate identification under a microscope. On the other hand, improper temperatures might cause less precise staining and make it more difficult to analyze the morphology of worm eggs (Wiryanti et al., 2022). This aligns with research indicating that storing dyes made from red yeast rice extract at room temperature provides an ideal storage condition. This is consistent with a study by Irawati et al. (2023) that found that the dye's temperature has a major impact on the caliber of the color preparation. When stored below room temperature, the color's intensity is more constant because the lower temperature minimizes microbial development, slows down cellular chemical reactions, and reduces water evaporation (Sugiastawa et al., 2021).

CONCLUSION

The findings of this research suggest that the ethyl acetate extract of red angkak can serve as an alternative dye for examining STH worm eggs, replacing the use of 2% eosin. The optimal dye for STH worm eggs is a pure extract with an acidic pH and stored at room temperature.

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