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Utilization Of Kuku Pancar (Lawsonia Inermis Lin) Leaves as a Substitute For Eosin Dye in The Examination of Soil Transmitted Helminth (STH) Eggs

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ABSTRACT

Nail surfacing leaves *(Lawsonia inermis lin*) are natural materials commonly used as dyes, because of the content of chemical compounds in them such as lawsone, flavonoids, tannins and anthocyanins, therefore nail surfacing leaves can be used as an alternative dye to replace 2% Eosin in STH worm egg examination, because it has the same properties as 2% Eosin and can produce an orange yellow color after the extraction process. The research objective is to determine the results of nail surfacing leaves *(Lawsonia inermis lin)* can be used as an alternative substance to replace Eosin in the examination of STH (*soil transmitted helminth*) worm eggs. The methods of this research is using is experimental, where this study looks at the clarity of soil transmitted helminth (STH) worm eggs on preparations, using coloring from the results of soaking the leaves of the nail surf as a substitute for Eosin. The results of the research is that the coloring results obtained in each variation of immersion time are good. The conclusion of this research is that the staining of worm eggs using the results of immersion of nail surfacing leaves for 12 hours, 24 hours, 36 hours, and 48 hours can be used as an alternative dye in the examination of STH worm eggs.

INTRODUCTION

Soil Transmitted Helminths (STH) are a class of intestinal nematode worms that often infect humans who ingest their eggs through the oral fecal route. This STH worm has several types, namely Ascaris lmbricoides, Trichuris trichiura, Necator americanus and Ancylostoma duodenale and Strongyloides stercoralis This helminth disease can also cause a person to experience iron deficiency anemia, micronutrient deficiencies, especially vitamin A, stunted growth, malnutrition, chronic diarrhea and a decrease in work productivity by as much as 40% (Alsakina et al., 2018).

Currently, more than 24% of the world's population is infected with helminthiasis and has spread to tropical and subtropical countries including Southeast Asia (Alsakina et al., 2018). Likewise, prevalence data regarding cases of soil-transmitted helminthiasis infection in Indonesia varies greatly based on several factors, namely geographical conditions, education, economy, environmental sanitation and community hygiene (Sandy et al., 2015). Infections that develop in the host body then grow into adult worms and reproduce by laying eggs, which are then transmitted through the soil. Roundworm and whipworm eggs are then excreted through the host's feces so that they can infect the soil environment, while hookworm eggs will be infective in the soil which develops into filiaform larvae (Sandy et al., 2015).

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The examination that will be carried out for intestinal worms or Protozoa is through examination of feces or feces. The simplest method of examining Intestinal Nematode worm eggs is to use 2% Eosin reagent. This reagent has an acidic composition and is orange red in color (Oktari & Mu'tamir, 2017). By using eosin we can more clearly see the difference between eggs and feces, eosin also gives a red background color to yellowish eggs (Kartini et al., 2021). But direct examination of preparations using 2% Eosin has a disadvantage, which requires a lot of reagents. So it is less economical and considering the price of eosin which is fairly expensive. Therefore, alternative dyes are needed that have the same function as eosin, using dyes from natural ingredients (Artanti et al., 2020).

A natural ingredient found in the leaves of henna nail (Lawsonia Inermis Linn) that produces a reddish-yellow molecule called lawsone. This compound is a phenol compound that belongs to a protein that can color well. As for some of the compound content in henna nail leaves are glycoside compounds, phytosterols, steroids, tannins and flavonoids. Which is where Flavonoids are compounds that belong to phenol compounds that can be found in nature. And flavonoids then affect plants in producing purple, red, blue, and yellow dyes (Supriningrum et al., 2018).

Researchers have conducted preliminary tests using the results of the soaking of nail surfacing leaves that have been soaked for 24 hours, then observed under a microscope and found Trichuris trichuira worm eggs with the morphology of the worm egg shape like a crock and has a bulge at both ends, brownish yellow in color and has 2 layers, namely albumin and hyaline, and contains the core of the egg. Therefore, the researcher wants to examine the nail surfacing leaves (Lawsonia inermis lin) as a substitute for Eosin 2%.

Researchers get the results of the color that is considered not too concentrated so that the conjecture arises “whether if doing soaking longer than the time previously done in the preliminary test will produce a more intense color, or will be the same” so that researchers are interested in doing time variations on soaking from the leaves of this nail pancar.

MATERIALS/METHODE

This study is an experimental study, which is this study will look at the clarity of Soil transmitted helminth (STH) eggs on preparations that use staining from the soaking of nail surfacing leaves as a substitute for Eosin. Alternative staining of nail surfacing leaves is obtained through the process of soaking nail surfacing leaves using 96% alcohol for 12 hours, 24 hours, 36 hours, and 48 hours.

The first stage that is done to get the coloring liquid from the nail surfacing leaves is to soak the nail surfacing leaves as much as 15 grams, using 96% alcohol as much as 5 ml, which is allowed to stand for 12 hours, then after 12 hours the nail surfacing leaves are removed and squeezed using gauze to filter the liquid that comes out, and ready to use, as well as soaking for 24 hours, 36 hours, and 48 hours. After all the soaking time is finished, the leaves of pancar kuku that have been soaked with a period of time are squeezed using gauze to get good coloring results and then can be directly used.

After the stain is ready to be used, fecal preparations are made using the direct or native preparation method, using two groups, namely the control group and the treatment group to see whether the worm eggs are able to absorb the color of the nail surfacing leaf bath or not, the control group in question is staining using 2% Eosin as a control, and nail surfacing leaves as a treatment group.

RESULTS AND DISCUSSION

The results of observations of direct coloring of preparations using the results of soaking the leaves of nail surfing in the examination of Soil transmitted helminth (STH) eggs are given an assessment with several categories including Good, Fair, Poor and Poor. There are three criteria that are seen, namely: worm eggs are clearly visible, the field of view is contrasted and the morphology of the worm eggs is clearly visible. From the assessment criteria, the observation results can be categorized into 4 categories, namely as follows:

a. Good: Has 3 criteria mentioned
b. Enough: Only has 2 criteria mentioned
c. Less: Only has 1 criterion mentioned
d. Poor: does not have any of the 3 criteria mentioned.

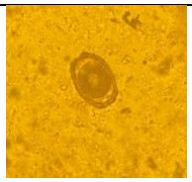
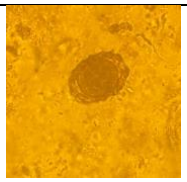
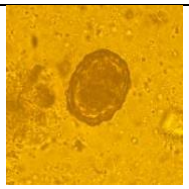
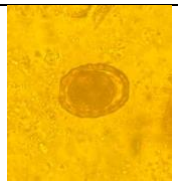
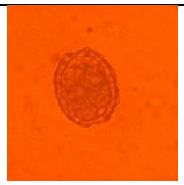
From several criteria and categories above, the results of the examination of soil transmitted helminth (STH) eggs using staining from the immersion results of nail surfacing leaves can be seen in the following table:

Table 1. Results of Staining Preparations Using the Immersion Results of Nail Surfacing Leaves (Lawsonia inermis lin).

Soaking Variation	immersion replication results					
	P1	P2	P3	P4	P5	P6
12 hours	3	3	3	3	3	3
24 hours	3	3	3	3	3	3
36 hours	3	3	3	3	3	3
48 hours	3	3	3	3	3	3
Eosin 2%	3	3	3	3	3	3

Description: 3 : Good 2: Fair 1 : Less 0: Bad

Table 2. Pictures of the results of coloring with the results of soaking the leaves of pancar kuku (Lawsonia inermis lin).

12 hours	24 hours	36 hours	48 hours	Eosin 2%
				

From the results of tables 1. and 2. it shows that all the results of coloring using the immersion results of the leaves of the pancar kuku give good results and can be used as a coloring agent in the examination of Soil transmitted helminth (STH) eggs, direct preparation method. Because it can provide a contrasting background and worm eggs can absorb color well so that the morphology can be seen clearly.

This study was conducted to determine whether the use of nail surfacing leaves (Lawsonia inermis lin) as a substitute for Eosin dye in the examination of Soil transmitted helminth (STH) eggs. Before conducting research with the use of nail surfacing leaves in the examination of STH worm eggs, an examination was first carried out using 2% Eosin as a gold standard or initial observation to see the positivity of feces and the actual picture of soil transmitted helminth (STH) worm eggs. After that, the examination of soil transmitted helminth (STH) eggs is carried out using the results of the nail surfacing leaf bath.

To get the results of the soaking of the nail surfacing leaves, the nail surfacing leaves that have been cleaned and weighed as much as 15gr and then put into a glass beaker and added 96% alcohol as much as 5 ml for the soaking process, in the soaking process there are

several variations of soaking time for nail surfacing leaves, namely there are those that will be soaked for 12 hours, 24 hours, 36 hours, and 48 hours. Then after each variation of time from soaking is complete, the leaves will be immediately removed and squeezed using gauze as a filter to get the coloring liquid from the soaking of pancar kuku leaves based on the length of time variation. However, if soaking is carried out beyond the limit of the time variation, the soaked pancar leaves will dry out and there will be no coloring liquid caused by evaporation from the alcohol added to the soaking process.

Based on tables 4.1 and 4.2, the results of research that has been carried out using nail surfacing leaf soaking to examine soil transmitted helminth (STH) eggs in the direct preparation method give good results, namely worm eggs can well absorb the color of the nail surfacing leaf soak so that their morphology can be seen clearly and a contrasting background so that it can easily distinguish between worm eggs and feces. Due to this leaf itself can produce orange yellow color. This color is produced by lawsone compounds, flavonoids and anthocyanins present in the leaves of this nail surf (Supriningrum et al., 2018).

The coloring results on the preparation also do not make the eyes easily tired or sore because the color produced is not too striking. In terms of cost, it is fairly cheap, and can be found around because it is a plant that grows wild and is also environmentally friendly because it is a natural ingredient. However, if it has been stored for too long and the juice from the bath begins to settle then turbidity occurs which causes this dye to be less good for use because it will make the preparation made dirty and difficult to read .

Based on the observation of the examination of worm eggs using the results of soaking the leaves of pancar kuku can provide good and clear microscopic results on pereparat. The optimal soaking result used is to use soaking for 12 hours, because the time is effective and the results are the same as other soaking time variations. Because worm eggs can be seen clearly and are able to absorb color well so that the morphology is clearly visible and the contrasting field of view makes it easy to identify worm eggs.

This is due to the anthocyanin content in the leaves of pancar kuku which can produce orange-yellow pigments that are easily dissolved if 96% alcohol is added and allowed to stand for the specified time. This method of removing color pigments from anthocyanins is based on previous research (Artanti et al., 2020) which utilizes the anthocyanin content in teak leaf buds. And also some studies that utilize alcohol to extract anthocyanins.

The anthocyanin content in the leaves of this nail transmitter is also an acidic anthocyanin, because after the pH test, the pH result is 3 which indicates that the anthocyanins in the leaves of the nail transmitter (Lawsonia inermis lin) have acidic properties. Based on its properties and characteristics, it can be said that eosin and anthocyanins have the same properties, namely acidic, so that they are able to give an orange red to orange yellow color, so that they can wash away the acidophilic components in worm eggs (Mutoharoh et al., 2020).

Because worm eggs are cationic which has alkaline properties in the cell when added to acidic dyes such as eosin and leaf soak pancar kuku (lawsonia inermis lin) which has acidic properties, the egg cells will be colored. This is in accordance with the coloring principle, namely acid staining for basic components and basic staining for acidic components. And in the results of this study obtained clear staining of worm eggs and a contrasting background that is in accordance with the coloring function (Oktari et al., 2022).

CONCLUSIONS

Soaking the leaves of pancar kuku for a variation of time 12 hours, 24 hours, 36 hours, and 48 hours can be used as a dye in the examination of soil transmitted helminth

(STH) eggs and the optimal coloring used is coloring with 12-hour soaking because it is more time efficient.

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