

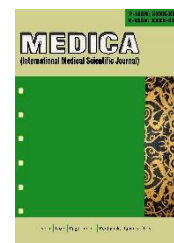
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Utilization of Red Dragon Fruit (*Hylocereus polyrhizus*) as an Alternative Dye for Threadworm Eggs (*Ascaris lumbricoides*)

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Abstract

The red dragon fruit (*Hylocereus polyrhizus*) contains a pigment called anthocyanin, which gives it its red color and has the potential to be used as a natural dye. In laboratory microscopic preparations, eosin is used as a standard dye. Natural dyes have the advantage of not causing negative effects like cancer or environmental damage. This study aimed to examine the potential of red dragon fruit (*Hylocereus polyrhizus*) as an alternative dye for the examination of *Ascaris lumbricoides* eggs. The study design used was static group comparison, with local red dragon fruit samples from Pontianak Tenggara District. Laboratory examination methods involved direct microscopic observation using a 2% eosin control. In this study, the juice from both the skin and flesh of the red dragon fruit was extracted and prepared in solutions of 80%, 90%, and 100% concentrations with four replications. The results showed that the skin of the red dragon fruit at concentrations of 80%, 90%, and 100% was not suitable for coloring the microscopic preparation for observing worm eggs. However, the juice from the flesh of the red dragon fruit at concentrations of 80%, 90%, and 100% could color the microscopic preparations, and the *Ascaris lumbricoides* eggs could be identified. At the 80% concentration of red dragon fruit flesh, the average score was 2, indicating low contrast background, weak color absorption by the eggs, and unclear egg details. At 90% and 100% concentrations, the average score was 3, indicating a clear contrast background, good color absorption by the eggs, and clear egg detail, similar to eosin 2% staining. This study concludes that the skin of red dragon fruit cannot be used for staining microscopic preparations, while the flesh of red dragon fruit can be used for staining *Ascaris lumbricoides* eggs, with optimal concentrations of 90% and 100%.

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1. INTRODUCTION

Intestinal parasitic infections have a relatively high prevalence in Indonesia, affecting approximately 60% of the 220 million population, with 21% of cases occurring in elementary school-aged children. Helminthiasis is a chronic endemic disease caused by one or more types of worms that enter the human body, with a high prevalence in children (Fatimah et al., 2012). Infections caused by worms can be diagnosed in several ways, one of which is through direct microscopic examination with eosin staining. A 2% eosin solution also provides a red background for the eggs, which are typically yellowish, and separates feces from contaminants. The direct microscopic method using eosin requires a significant amount of reagents and is specific for observing worm eggs in feces (Natadisastra, 2009). This staining principle is based on the Romanowsky method, which uses two dyes with acidic and basic properties (Darmadi & Dikna, 2022). The acidic pH of eosin allows it to stain the protein layer of the egg shell of *Ascaris lumbricoides*, turning it red. One natural pigment similar to eosin is anthocyanin, which is commonly found in plants.

Anthocyanin is a pigment responsible for providing red color and has the potential to be used as a natural food dye. It can serve as a safer alternative to eosin, as natural dyes are not carcinogenic and do not damage the environment, unlike synthetic dyes such as eosin (Sari, Suriani, & Chania, 2022; Balqis, et al., 2025). Natural dyes such as anthocyanin from dragon fruit (*Hylocereus polyrhizus*) can impart a red color to tissues without causing negative effects like cancer or environmental damage, which are associated with synthetic dyes like eosin.

Anthocyanin is a group of pigments that range from red to blue and are widely distributed in plants. It is classified as a flavonoid pigment. Flavonoid compounds are polar and can be extracted using polar solvents. Some polar solvents include ethanol, water, and ethyl acetate. The acidic condition affects the extraction results. The more acidic the environment, especially when approaching a pH of 1, the greater the amount of anthocyanin pigments that are present in the form of the flavilium cation or oxonium, which is colored, and absorbance measurements will show an increased amount of anthocyanin. Moreover, the more acidic the environment, the more the vacuolar cell walls break, allowing more anthocyanin pigment to be extracted (Simanjuntak et al., 2014).

The color and stability of anthocyanin pigments depend on the overall molecular structure. Substitution in the anthocyanin structures A and B influences the color. Under acidic conditions, the color of anthocyanins is determined by the number of substitutions on ring B. The greater the number of OH substitutions, the bluer the color, while methoxylation will cause the color to become redder. The stability of anthocyanins is affected by several factors, including pH, temperature, light, and oxygen (Samber et al., 2013). Anthocyanins are found in natural materials such as butterfly pea flowers, beetroot, red spinach, and dragon fruit. There are four types of dragon fruit: red flesh, white flesh, super red, and yellow flesh.

Red dragon fruit (*Hylocereus polyrhizus*) is a plant native to arid tropical regions. This fruit contains antioxidants such as vitamin C, flavonoids, and polyphenols. The red dragon fruit (*Hylocereus polyrhizus*) contains anthocyanin pigments that function as antioxidants. In addition to the fruit flesh, the skin of red dragon fruit also contains a significant amount of the natural colorant anthocyanin. Anthocyanin is a pigment responsible for providing red color, making it a potential natural food dye and an alternative to synthetic dyes that are safer for health (Yanty and Siska, 2018).

Dragon fruit also contains functional compounds in the form of polyphenols, which are a source of antioxidants. These compounds are fragile and must be extracted using a suitable method to preserve them. The best extraction quality of dragon fruit using

Microwave-Assisted Extraction (MAE) was found in the red dragon fruit skin, which produced the best color. The quality of the red dragon fruit flesh includes polyphenol content of 623.1 mg Gallic Acid Equivalent (GAE)/100 grams, antioxidant activity of 41.18%, anthocyanins at 84 mg/100 grams, betalains at 0.75 mg/100 grams, and a color value of 21.18. Gallic Acid Equivalent (GAE) is a general reference used to measure the number of phenolic compounds in a material (Harni et al., 2023). Miana leaves as an alternative dye to replace eosin in the examination of Soil-Transmitted Helminth eggs. The study found the optimal concentration ratio for miana leaf extract and distilled water to be 1:2 and 1:3 (Permatasari et al., 2021). The purpose of this study is to examine the potential of red dragon fruit (*Hylocereus polyrhizus*) as an alternative dye for the microscopic examination of *Ascaris lumbricoides* eggs.

2. METHOD

This study uses an experimental research design with a Static Group Comparison, where one group is subjected to a specific treatment and then observed for the effects of each dye concentration variation. The population of this study consists of red dragon fruit (*Hylocereus polyrhizus*) sold in the Pontianak Tenggara District. A survey found five fruit stores in the area. The samples used in this study were the skin and flesh of the red dragon fruit, which were made into solutions with concentrations of 80%, 90%, and 100% as alternative dyes for the microscopic examination of *Ascaris lumbricoides* eggs. A total of 24 samples were used, along with one control for the skin and one control for the flesh of the red dragon fruit. The sample criteria set for the study were mature, fresh, and undamaged or unspoiled red dragon fruit skin and flesh. The sampling technique used in this study was purposive sampling. The research was conducted from June 3, 2024, to June 6, 2024, in the Integrated Parasitology Laboratory of Poltekkes Kemenkes Pontianak.

The method used for the examination was the Native (Direct Slide) method. The Native (Direct Slide) method uses a microscope to detect feces containing worm eggs. The procedure involves placing a drop of 2% eosin (in this case, using a natural solution from red dragon fruit) onto a microscope slide, then picking up feces with a stick and homogenizing it. The slide is then covered with a cover slip and observed under a microscope with 100x or 400x magnification (Fuad, 2012). The data collection instrument used in this study was an Observation Sheet.

The analysis used was descriptive analysis with percentage distribution for each treatment, as this study aims to describe the ability of the skin and flesh solutions of red dragon fruit (*Hylocereus polyrhizus*) at concentrations of 80%, 90%, and 100% as alternative dyes for the microscopic examination of *Ascaris lumbricoides* eggs..

3. RESULTS AND DISCUSSION

This study on the Utilization of Red Dragon Fruit (*Hylocereus polyrhizus*) as an Alternative Dye for *Ascaris lumbricoides* Eggs was conducted at the Parasitology Laboratory of Poltekkes Kemenkes Pontianak in June 2024. The aim of this study was to determine whether the skin and flesh of red dragon fruit (*Hylocereus polyrhizus*), which contains anthocyanin as a color pigment, could be used as an alternative dye for *Ascaris lumbricoides* eggs. In this study, the initial stage involved the identification test of the red dragon fruit plant (*Hylocereus polyrhizus*), and the species classification result was *Hylocereus polyrhizus* (F. A. C. Weber) Britton & Rose.

Table 1. Observation results of the alternative staining of red dragon fruit flesh and skin (*Hylocereus polyrhizus*) at concentrations of 80%, 90%, and 100% on *Ascaris lumbricoides* eggs.

Sample code	Coloring Results (1-3)						Information
	Skin			Flesh			
	B80	B90	B100	C80	C90	C100	
R1	-	-	-	2	3	3	
R2	-	-	-	2	3	3	
R3	-	-	-	2	3	3	
R4	-	-	-	2	3	3	
Rata-rata	-						Value 3 is almost close to the eosin color 2%
		-	-	2	3	3	

Description:

1 : Background is not contrasting, worm eggs do not absorb color, egg parts are not visible.

2 : Background is less contrasting, worm eggs do not absorb color, egg parts are not clearly visible.

3 : Background is contrasting, worm eggs absorb color, egg parts are clearly visible.

Table 1 shows that the results of the coloring of the skin and flesh of red dragon fruit with concentrations of 80%, 90%, and 100% respectively with 4 repetitions and the 4 repetitions showed that the solution of red dragon fruit skin with concentrations of 80%, 90%, and 100% could not be used as an alternative dye for worm eggs, while for the solution of red dragon fruit flesh with concentrations of 80%, 90%, and 100% could color worm eggs, the most optimal concentrations were at concentrations of 90% and 100% because the coloring was close to coloring using 2% eosin.

DISCUSSION

The red dragon fruit has properties similar to eosin in terms of staining, specifically due to its anthocyanin content, which provides the red color. This anthocyanin is water-soluble, making it an effective natural dye. In the examination of parasitic worm eggs, the anthocyanin from red dragon fruit can provide a good contrast, allowing the eggs to be clearly visible with distinct shapes. Therefore, red dragon fruit can serve as an effective natural dye for the examination of STH (soil-transmitted helminths) eggs, much like eosin, which is widely used in egg staining. The solution made from the skin and flesh of red dragon fruit (*Hylocereus polyrhizus*) has an acidic pH of 2.5, whereas the pH of the skin solution was 6.7, and the pure flesh of the fruit had a pH of 5.6. At a pH of 1-3, anthocyanins are in an optimal condition to maintain stability and produce a consistent red color. At this pH range, anthocyanins do not degrade, as evidenced by the stable color of the solution even after 8 days.

Eosin itself is acidic, which means it can stain acidophilic components of tissues. In the context of parasitic worm egg staining, eosin is used to stain the cytoplasm of the eggs, which is an acidophilic component. Acidophilic components are structures or elements in tissues that can be stained by acidic dyes, such as eosin. Examples include mitochondria, secretory granules, collagen, and cytoplasm. The anthocyanin from red dragon fruit acts as an effective acidic dye for staining parasitic worm eggs. Its acidophilic properties allow it to bind to and color the acidophilic components in the egg, providing good contrast and clarifying the morphology of the eggs. The cytoplasm will appear pink due to the effect of eosin/anthocyanin, which is acidic. This staining helps in more clearly identifying parasitic worm eggs, as the contrast between the pink cytoplasm can be easily seen under a microscope. Thus, the acidic nature of the anthocyanin allows for effective staining of the

acidophilic components of the parasitic worm egg tissues.

On the other hand, the consistency of the solutions made from the skin and flesh of red dragon fruit (*Hylocereus polyrhizus*) differs. The solutions from the flesh of red dragon fruit at concentrations of 80%, 90%, and 100% have a liquid consistency and a darker color, while the solution from the skin of red dragon fruit has a gel-like consistency and a lighter color. Therefore, concentrations of 80%, 90%, and 100% of the skin solution are unsuitable for use as a dye. This is because the skin of red dragon fruit contains approximately 10.8% pectin. Pectin is a fiber component found in the middle lamella and primary cell walls of plants. Pectin is used as a gelling agent and stabilizer in fruit juices. In the food and pharmaceutical industries, pectin is used as a binder, gelling agent, stabilizer, and thickener. Pectin is a polygalacturonic acid that contains methyl esters (Yati et al., 2017).

Meanwhile, the flesh of red dragon fruit (*Hylocereus polyrhizus*) can be used as a dye because it has a lower pectin content (0.71 g of pectin per 100 grams of flesh) and a high water content (90%) (Lutfiyah et al., 2022). Red dragon fruit (*Hylocereus polyrhizus*) also contains anthocyanins, a group of pigments that range from red to blue and are widespread in plants. The total anthocyanin content in fresh flesh and skin of red dragon fruit is 159.7 ± 8.9 and 135.4 ± 9.3 mg/g dry weight, respectively (Khoo et al., 2022).

Red dragon fruit has properties similar to eosin in terms of staining, due to its anthocyanin content, which imparts a red color. This anthocyanin is water-soluble, making it an effective natural dye (Sari, Tazkiya, & Mafira, 2022; Sari, Suriani, & Adinda, 2022; Wahyuni, & Sabban, 2022). In parasitic worm egg examination, the anthocyanin from red dragon fruit provides a good contrast, allowing the eggs to be clearly visible with distinct shapes. Research indicates that a concentration of 80% anthocyanin gives the best results for egg staining. Therefore, red dragon fruit can serve as an effective natural dye for the examination of STH eggs, much like eosin, which is widely used in parasitic worm egg staining.

The results of the staining examination of *Ascaris lumbricoides* eggs in this study showed that at an 80% concentration of red dragon fruit flesh (*Hylocereus polyrhizus*), the *Ascaris lumbricoides* eggs were observed with a rating of 2, indicating that the background had low contrast, the eggs did not absorb the dye well, and the egg structure was not clearly visible. This was due to the fact that at an 80% concentration, the background contrast was insufficient and the egg membrane did not absorb the dye effectively, although the morphology of the egg was clearly visible, making the 80% concentration staining result good/optimal. This can be seen in the image of the staining results using 80% red dragon fruit flesh (*Hylocereus polyrhizus*) with *Ascaris lumbricoides* eggs stained using eosin, with a rating of 2.

For the examination of 90% and 100% red dragon fruit flesh (*Hylocereus polyrhizus*) solutions, the *Ascaris lumbricoides* eggs were rated 3 according to the evaluation criteria, which indicates a high contrast background, well-stained eggs, and clear visibility of the egg structure. However, at these concentrations, the alternative dye effectively stained the eggs but resulted in a less clear contrast with the background, and the dye was too intense, causing the morphology of the eggs to be less visible. The results from both of these concentrations were close to the criteria for eosin 2%, which provides a contrasting background and good dye absorption. However, when compared with the 80% red dragon fruit flesh solution staining, where the egg morphology is clearer, the staining at 90% and 100% concentrations is less optimal.

Meanwhile, the red dragon fruit skin (*Hylocereus polyrhizus*) solution could not be used as a dye due to its gel-like consistency. The solution was then diluted to 50% and 25%, which resulted in a consistency similar to that of the red dragon fruit flesh solution. However,

the staining results still showed a lack of contrast in the background, and the eggs did not absorb the dye.

The limitations of this study include the use of only one type of natural dye source, namely red dragon fruit, which may not represent the potential of all types of fruits or other plants as alternative dyes. In addition, this study was limited to certain dye concentrations (90% and 100%) without conducting wider variations in concentration, so it cannot be concluded whether lower concentrations can provide similar results. This study also did not test the long-term effects of using this dye on the quality of worm eggs or other organisms. In addition, the physical and chemical properties of dyeing using red dragon fruit still require further study to determine its stability and effectiveness in various environmental conditions.

4. CONCLUSION

It can be concluded that the flesh of red dragon fruit (*Hylocereus polyrhizus*) can be used as an alternative dye for *Ascaris lumbricoides* eggs. At an 80% concentration, the red dragon fruit flesh achieved an average rating of 2, while at 90% and 100% concentrations, it resulted in an average rating of 3. A rating of 3 indicates results close to staining with 2% eosin. Meanwhile, the skin of red dragon fruit (*Hylocereus polyrhizus*) at 80%, 90%, and 100% concentrations cannot be used as an alternative dye for *Ascaris lumbricoides* eggs due to its gel-like consistency and high pectin content, which can prevent proper dye absorption in the egg preparations. For future research, it is recommended to use the correct concentration when using red dragon fruit skin solution for egg staining and to explore the potential use of red dragon fruit as a dye for fungi.

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