JURKES

ISSN : 2089-3906

JURNAL KESEHATAN

JURNAL ILMU-ILMU KEPERAWATAN, KEBIDANAN, FARMASI & ANALIS KESEHATAN

DOI : https://doi.org/10.52221/jurkes

Red Dragon Fruit Peel Extract (*Hylocereus lemairei*) as an Alternative Dye in Worm Egg Staining

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Article Information

Revised: October, 2023

Available online: October, 2023

Keywords

Worm infection, eosin dye, dragon fruit dye

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ABSTRACT

Helminthiasis is still a problem in Indonesia, and worm infections often occur without symptoms, so the disease receives less attention. Identification can be done by several methods, one of which is by direct examination using 2% eosin dye. However, 2% eosin dye is included in synthetic dyes that can harm ul to health. The alternative used is to utilize natural ingredients. Red dragon fruit Hylocereus lemairei is a natural material that contains anthocyanin substances where in an acidic atmosphere anthocyanin substances provide a red colour so that it can be used as an alternative dye to replace eosin. The aim of this study was to determine the microscopic picture of worm egg preparations using alternative dyes of red dragon fruit peel extract. The research method used is an experimental method. The results showed that the red dragon fruit peel extract dye with a concentration variation of 2%, 5%, and 10% did not colour the worm eggs. So the red dragon fruit peel extract dye is still not optimal for use as an alternative dye for coloring worm eggs.

INTRODUCTION

Cases of worms are still a major problem in Indonesia, transmission of worms can be through direct contact such as feet, hands or nails that are contaminated with soil containing worm eggs. This infection is usually asymptomatic, so this disease receives less attention. There are many ways to diagnose an infection, one of which is a direct examination using a dye (Artanti et al., 2020).

Dyes are organic compounds that are used to sharpen and clarify the picture of cells so that it makes it easier to observe under a microscope. Dyes that are widely

used in clinical laboratories are synthetic dyes. However, several studies have shown that the use of chemical/synthetic materials can have side effects on human health, and dyes are generally needed in limited quantities and have an expiration date. So we need alternative dyes that have the same function, namely dyes from natural ingredients. Natural ingredients can be found in plants, herbs and fruits that contain anthocyanins, namely substances that can give a red to blue color.(Gresby, 2013).

Anthocyanin belongs to the class of flavonoid compounds, anthocyanin substances are stable at pH 3.5 in an acidic environment, which acts to give a red color and pH 8 in an alkaline environment gives a blue color which can be used as an alternative dye from natural ingredients so that it is safer for health and pollution. environment. This extra natural coloring of dragon fruit skin with the addition of citric acid solvent can last for 7 days, but even though it can last 7 days this dye can be used as an alternative dye to replace synthetic dyes if the stock of dyes is limited. One of the synthetic dyes that is often used to examine worm eggs is eosin(Khuzaimah, 2018).

Eosin dyes are included in synthetic dyes which contain the compound eosin y (bromo fluorescein acid) which gives a slightly yellowish red color, and the compound eosin b (dibromo dinitro derived from fluorescein) which gives a bluish red color. The eosin content that can color worm eggs is the eosin y component (bromo fluorescein acid). The quality standard for eosin dye is that it can color the visual field, color part of the worm eggs and the dye is easily absorbed. The alternative dye used in this study was red dragon fruit peel extract which contains anthocyanin compounds which give a red color and are acidic in nature similar to eosin dyes. The use of alternative dyes has the same standards as eosin dyes even though the resulting colors are different but according to the standards can color the eggs of worms, these alternative dyes can be used. The principle of eosin staining is to give a red background to worm eggs and their parts. The aim is to clarify the various elements, and microscopic images of worm eggs and separate worm eggs from the dirt around them. The component of worm eggs that is stained by eosin which is acidic is called acidophilic and microscopic picture of worm eggs and separating worm eggs from the dirt around them. The component of worm eggs that is stained by eosin which is acidic is called acidophilic and microscopic picture of worm eggs and separating worm eggs from the dirt around them. The component of worm eggs that is stained by eosin which is acidic is called acidophilic (Siregar et al., 2019).

Previous research by Anita Oksari, et al (2017) showed that red fruit juice (Pandanus sp) with a concentration ratio of 1:2 could be used as an alternative dye in examining worm eggs. Based on this background, the researchers conducted the same study, but there were differences in the dyes used, namely red dragon fruit peel extract (Hylocereus lemairei) as an alternative dye for coloring worm eggs.

METHOD

Research participants

This research is experimental in nature because it conducted an experiment of natural dyes of red dragon fruit skin extract as an alternative dye to replace eosin in worm egg examination. The sample used in this study was red dragon fruit skin (Hylocereus lemairei). Sampling used a purposive sampling technique, which means that the researcher took the sample and determined the special characteristics and certain criteria, namely the inclusion and exclusion criteria. The inclusion criteria included red dragon fruit (Hylocereus lemairei), dragon fruit that was not rotten, dragon fruit skin bright red and fresh. Whereas the exclusion criteria

included super red dragon fruit (Hylocereus costaricensis), red dragon fruit (Hylocereus polyrhizus) and dragon fruit skin that was brown and had holes due to storage time.

Research procedure

1. Pre-analytical stage

1.1 Preparation of worm egg suspension

In the previous suspension, separation of eggs from feces had been carried out using a sedimentation technique in which the principle was to separate the suspension from the supernatant with the help of centrifugal force so that the worm eggs could settle. The suspension is not expired and is still good so it can be used for identification (Maulida, 2016).

1.2 Preparation of eosin staining reagent

Pay attention to the expiration date printed on the packaging, the packaging must be intact, the contents must not harden and there is no change in color (Maria et al., 2018).

1.3 Preparation of red dragon fruit peel alternative coloring

The tools and materials to be used are prepared, the red dragon fruit skin is separated first from the fruit and then washed.

1.4 Checking the pH

Measure the pH value using a pH meter that has previously been calibrated with a buffer solution of pH 4.01-6.86 The pH meter is turned on until it is stable. The electrodes were rinsed with distilled water and then dried with filter paper, then the electrodes were dipped into the dye solution, left for a while until a stable reading was obtained. Then record the pH value.

2. Analytical stage

2.1 Preparation of red dragon fruit peel extract

2.1.1 Red dragon fruit peel extract concentration of 2%

Dragon fruit skin was mashed using a blender, then the results of the dragon fruit skin that had been blended were weighed as much as 5 grams put into an Erlenmeyer and added 2% citric acid solvent until the volume was 250 ml. Soaked for 24 hours at room temperature, then filtered to obtain maserate.

2.1.2 Red dragon fruit peel extract concentration of 5%

The red dragon fruit skin was mashed using a blender, then the results of the dragon fruit skin that had been blended were weighed as much as 12.5 grams, put into an Erlenmeyer and added 2% citric acid solvent until the volume was 250 ml. Soaked for 24 hours at room temperature, then filtered to obtain maserate.

2.1.3 Red dragon fruit peel extract concentration of 10%

Red dragon fruit skin is mashed using a blender, then the blended results are weighed as much as 25 grams put into an Erlenmeyer and added 2% citric acid solvent until the volume is 250 ml. Soaked for 24 hours at room temperature, then filtered to obtain maserate.

2.1.4 Red dragon fruit peel extract concentration of 20%

The red dragon fruit skin was mashed using a blender, then the results of the dragon fruit peel that had been blended were weighed as much as 50 grams, put into the elenmeyer and added 2% citric acid solvent until the volume was 250 ml. soaked for 24 hours at room temperature then filtered to obtain macerate(Khuzaimah, 2018).

2.2 Concentration of red dragon fruit peel extract

Red dragon fruit peel extract with concentrations of 2%, 5%, and 10% after maceration to obtain macerate was then concentrated using a water bath at 50°C for 1 hour.

2.3 Preparation of inaga fruit skin extract pH 6.3

Dragon fruit peel extract pH 3.4 was added with NaOH until the pH was 6.3.

2.4 Examination of worm eggs using eosin dye as a control

Worm egg suspension is stirred until homogeneous, take a glass object and drop 1-2 drops of worm egg suspension then add 1-2 drops of eosin dye, cover using a cover glass. After that, an examination was carried out under a microscope with an objective lens magnification of 10x or 40x(Maulida, 2016).

2.5 Examination of worm eggs using alternative dyes

Worm egg suspension stirred until homogeneous, took a glass object and dripped 1-2 drops of worm egg suspension then added an alternative dye 1-2 drops of red dragon fruit peel extract, covered using a cover glass. After that, an examination was carried out under a microscope with an objective lens magnification of 10x or 40x (Sari et al., 2020).

Instruments

The tools used in this study include, analytical balance, blender, beaker, measuring cup, stir bar, Erlenmeyer, filter paper, pH meter, microscope, filter, glass object, glass deck, and dropper pipette.

The materials used in this study were red dragon fruit skin, worm egg suspension, citric acid, and aquadest.

The measurement results in this study were stained if the visual field was colored and the part of the worm egg, namely the cell wall, could be stained.

Unstained if the visual field is colorless and the cell wall of the worm egg is not stained.

Data analysis

Data obtained from examination of red dragon fruit peel extract (Hylocereus lemairei) as an alternative dye to eosin is presented in tabular form and explained in a narrative manner with an overview conclusion.

Microscopic examination of worm eggs using alternative dyes, colored or unstained red dragon fruit peel extract.

RESULTS AND DISCUSSION

1. Plant Determination Test Results

   Previously, a red dragon fruit plant determination test was carried out in the laboratory of the Faculty of Biology, Galuh University. The identification results showed that the test sample used as the test material was a red dragon fruit with the species (Hylocereus lemairei). With the following test results:

   Classification :

   Regnum : *Plantae*
   Devisio : *Spermatophyta*
   Subdivisio : *Angiosperms*
   Class : *Dicotyledoneae*
   Order : *Carryophyllales*
   Familia : *Cactaceae*
   Genus : *Hylocereus*
   Species : *Hylocereus lemairei*

   Description

   Shrubs, long roots reach 20-30 cm, with a depth of 50-60 cm. brownish, and grow with stringy. The stem is triangular in shape, greenish in color and has blackish thorns and the stem is succulent (contains a lot of water), branching about 5-8 pieces at a growing point. Flowers have scales on the crown. Elongated funnel shape, size 30 cm or more, the crown is greenish, the petals are red, and the inside is whitish. The fruit has scales on the surface and the fruit has a red color and flesh that varies depending on the species. The seeds are black and shiny.

2. Extract Manufacturing

   Red dragon fruit peel extract (Hylocereus lemairei) was previously macerated using 2% citric acid solvent treated with several concentrations, namely concentrations of 2%, 5%, 10% and 20%. with the following results:

Table 1
Extract organoleptic test results

Concentration Extract	Organoleptic Results	
2%	Form	Liquid Extract
	Color	Light pink
	Aroma	Special scent
5 %	Form	Liquid Extract
	Color	Red pink
	Aroma	Special scent
10%	Form	Liquid Extract
	Color	Red
	Aroma	Special scent
20%	Form	Liquid Extract
	Color	Red
	Aroma	Special scent

Based on the organoleptic results of the dragon fruit peel extract, it was found that the higher the concentration, the more intense the color was given.

Table 2
The results of observations of worm egg preparations using natural dyes of red dragon fruit skin extract without concentration and eosin dye

Sample repetition	Dragon fruit skin extract dye Concentration				NaCl dye	Eosin Color
	2%	5%	10%	20%		
1	-	-	-	-	-	+
2	-	-	-	-	-	+

Information:
(+): Colored
(-): Unstained

Based on the results of the research presented in table 4.2, it was shown that the coloring of the red dragon fruit peel extract with two repetitions of the examination gave results where the worm eggs were not stained by alternative dyes or the worm eggs did not absorb the dye.

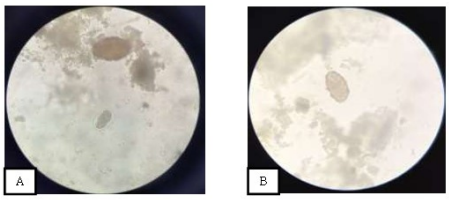

Figure 1 Eggs A.) Ancylostoma duadenale and B). Ascaris lumbricoides unfertilized using a red dragon fruit peel extract dye with a concentration of 2% pH 3.4 and pH 6.3 at 40 X objective lens magnification

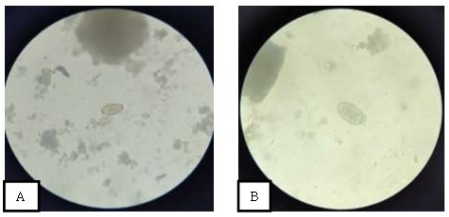

Figure 2 Eggs A). *Trichiuris trichiura* and B). *Ascaris lumbricoides* was not fertilized using red dragon fruit peel extract dye concentration of 5% pH 3.4 and pH 6.3 at 40 X objective lens magnification

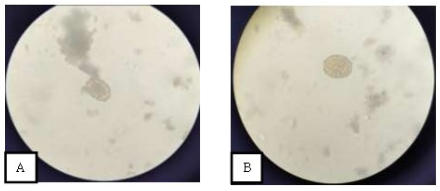

Figure 3 *Ascaris lumbricoides* Eggs A). fertilized and B). embryos using red dragon fruit skin extract dye concentration of 10% pH 3.4 and pH 6.3 at 40 X objective lens magnification.

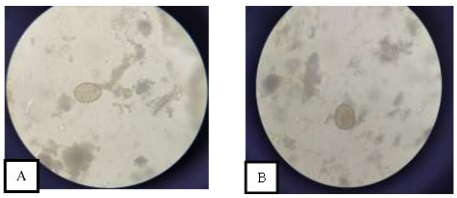

Figure 4 *Ascaris lumbricoides* eggs with red dragon fruit peel extract A). Concentration of 20% pH 3.4 and B). 20% concentration pH 6.3 at 40X lens magnification.

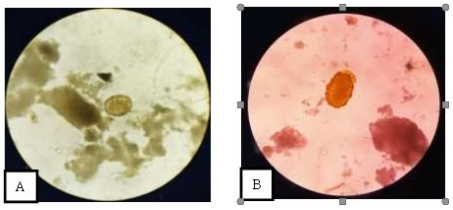

Figure 5 Fertilized and unfertilized *Ascaris lumbricoides* eggs at 40x objective lens magnification using A). NaCl and B). 2% eosin dye.

The results of observations using red dragon fruit skin extract coloring at a concentration of 2% pH 3.4 found *Ancylostoma duadenale* eggs with the characteristics of an oval shape with a cell wall consisting of 1 layer, namely a clear or transparent layer and colorless. And the results of observations at a concentration of 2% pH 6.3 showed that *Ascaris lumbricoides* eggs were not fertilized with oval-shaped characteristics, there were 2 layers of the cell wall, namely the albumin layer which was coarse and irregularly tortuous, the hyaline layer was smooth, the cell wall was colorless. The description of the worm eggs and the field of view given from the two variations of pH are the same, that is, they are less clear and colorless.

The results of observations using a red dragon fruit skin extract coloring concentration of 5% pH 3.4 showed *Trichiuris thichiura* eggs with oval-shaped characteristics, having 2 protrusions called mucoid plugs and having 2 layers of cell walls consisting of an outer layer and an inner layer which transparent and colorless. And the results of observations at a concentration of 5% pH 6.3 found *Ascaris lumbricoides* eggs unfertilized with oval shape characteristics, there were 2 cell walls consisting of albumin layers which were coarse and irregular winding, smooth hyaline layers, colorless cell walls . The description of the worm eggs and the field of view given from the two variations of pH are the same, that is, they are less clear and colorless.

The results of observations using red dragon fruit peel extract dyes at a concentration of 10% pH 3.4 and pH 6.3 showed that *Ascaris lumbricoides* eggs were fertilized and embryonic with oval-shaped characteristics, there were 3 layers of cell walls consisting of a winding outer layer rough, the second layer is hyaline and the third layer is vitelline. The appearance of worm eggs and the field of view of the two pH variations are the same, that is, they are less clear and colorless.

The results of observations using dragon fruit skin extract coloring at a concentration of 20% pH 3.4 and pH 6.3 showed embryon *Ascaris lumbricoides* eggs with oval-shaped characteristics, there were 3 layers of cell walls consisting of a rough winding outer layer, the second layer hyaline, vitelline third layer. The appearance of worm eggs and the field of view of the two pH variations are the same, namely clear/bright and colorless.

The results of observations using NaCl showed that *Ascaris lumbricoides* worm eggs were fertilized with oval-shaped characteristics, there were 3 layers of cell walls consisting of a coarse winding outer layer, a second hyaline layer, and a third vitelline layer, the visual field provided was clear and colorless .

The results of observations with 2% eosin dye showed that *Ascaris lumbricoides* worm eggs were not fertilized with oval-shaped characteristics, there were 2 layers of cell walls consisting of a coarse winding outer layer, and a second layer of hyaline, the field of view given was red, the walls Worm eggs can be stained.

After the determination test was carried out on red dragon fruit, the test results obtained were red dragon fruit with the species (*Hylocereus lemairei*). Next, extract the red dragon fruit skin dye with varying concentrations of 2%, 5%, 10% and 20% and concentrated the extract by heating it at

50°C. The result of the thick extract obtained was a color change in the anthocyanin substance, this was due to the heating process. Based on previous research, it was explained that the higher the heating temperature, the lower the color stability so that the red color decreases where the anthocyanin structure decomposes from the aglycone form to chalcone (colorless). (Dragon & Nizori, 2020).

Examination of red dragon fruit peel extract (*Hylocereus lemairei*) with two repetitions showed the same results, namely the lack of dye absorption by the worm eggs so that the worm eggs could not be stained, this could be due to several factors that could affect the chemical structure of the anthocyanin substance in the dye. naturally so that the anthocyanins are colorless or cannot color worm eggs, namely: temperature, pH, and light

In this study, the pH of the dye was checked where the red dragon fruit skin extract dye had an acidic pH of 3.4. The acidic atmosphere is caused by the influence of the addition of citric acid which is used as a solvent. After testing the color stability of the pH for 3 days there was a change in pH to become increasingly acidic, this was because the longer the anthocyanins were left, the pH value decreased and became more acidic.

In contrast to the pH of the dragon fruit peel extract dye which has been adjusted to the pH of eosin, namely pH 6.3 where after the addition of NaOH, the pH value is getting closer to neutral or alkaline but does not cause a color change in the dragon fruit peel extract. Based on previous research, it was explained that the dye condition of dragon fruit peel extract after color stability and absorbance tests were carried out at pH 2-6, macerate dissolved in citrate buffer at each pH did not find any color change (Hidayah, 2013).

The temperature used for storage of dragon fruit peel extract dye was room temperature (25°C). The coloring of dragon fruit peel extract with the addition of citric acid after being stored at room temperature (25°C) lasted for 3 days and did not change color. Based on previous research, anthocyanin dyes in red dragon fruit are more stable stored at cold temperatures (14°C) than at room temperature (25°C) because temperature has an important role in anthocyanin stability, low storage temperatures can inactivate enzymes, so as to maintain stability and slow down the degradation of anthocyanins (Nasrullah et al., 2021).

In the red dragon fruit skin extraction process, where the anthocyanin dye is stored in a dark cupboard and left for 24 hours. Storage in a dark place is used to avoid light which can accelerate anthocyanin degradation.

Based on the results of observations of worm eggs using red dragon fruit peel extract dyes with several concentration variations, namely 2%, 5%, 10% and 20%, it shows the best picture at a concentration of 20%, where the visual field is colorless, looks clearer/brighter, the eggs are (morrula) and the egg cell wall consisting of albumin, hyaline and vitelline is more pronounced. This is because the higher the concentration used, the more concentrated the color of the extract, thus giving a better color. Whereas at concentrations of 2%, 5% and 10% they provide a colorless field of vision and parts of the worm eggs, namely the cell wall, are less clearly visible, this is because the concentration is too low where the dye given is less concentrated. (Oktari & Mu'tamir, 2017).

While the results of observing worm eggs using NaCl where the results obtained were not much different from using red dragon fruit peel extract dyes, when observed microscopically it provides a clear field of view and the shape of worm eggs is clear

and colorless. A different thing is obtained from staining using 2% eosin dye, the dye given can be absorbed and the visual field is more red, and parts of the egg cell wall can be stained. So that the red dragon fruit peel extract dye is still not optimal to be used as an alternative dye for worm egg staining.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of research on red dragon fruit peel extract (Hylocereus lemairei) as a natural dye for coloring worm eggs, it can be concluded that red dragon fruit peel extract dyes with concentration variations of 2%, 5%, 10%, and 20% do not color worm eggs, so the dye red dragon fruit peel extract is still not optimal for use as an alternative dye for worm eggs staining.

Suggestions

Future researchers are expected to be able to conduct research by utilizing different natural materials with suitable solvents to be used as alternative dyes. In the future researchers are expected to be able to conduct research by utilizing different natural materials with suitable solvents to be used as alternative dyes.

BIBLIOGRAPHY

Artanti, D., Sari, YES, & Ariana, D. (2020). Differences in the quality of preparations for roundworm eggs (Ascaris lumbricoides, Linn) using teak stems and teak leaf buds. National Health Seminar, 21–30. http://jurnalrsam.stikesrsanwarmedika.ac.id/index.php/prosenakes/article/view/36/64

Gresby, A. (2013). Utilization of young teak leaf filtrate (Tectona grandis) as an alternative coloring agent for maceration preparations of grass jelly (Cyclea barbata) stems. Thesis, 54–60.

Hidayah, T. (2013). Stability Test of Pigments and Antioxidants Extracted from Natural Dyes from Dragon Fruit Peels (Hylocereus undatus). Oncogene, 29(18), 2616–2627. http://www.ncbi.nlm.nih.gov/pubmed/20154724%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3378055%0Ahttp://lib.unnes.ac.id/ en/eprint/19663

Khuzaimah, S. (2018). Pigment Stability Test Of The Extraction Of Natural Colors From Dragon Fruit (Hylocereus undatus) peels. Journal of Agrotekbis, 2(258–4272), 1–10.

Maulida, A. (2016). Differences in Quality of Roundworm Eggs (Ascaris lumbricoides, Linneaeus 1758) Using Eosin Stain and Giemsa Stain. [Thesis]., 59.

Naga, B., & Nizori, A. (2020). Characteristics of Red Dragon Fruit Peel Extract (Hylocereus Polyrhizus) With The Addition Of Various Concentrations Of Citric Acid As A Natural Food Coloring. Journal of Agricultural Industrial Technology, 30(2), 228–233. https://doi.org/10.24961/j.tek.ind.pert.2020.30.2.228

Nasrullah, N., Husain, H., & Syahrir, M. (2021). The Influence of Temperature and Heating Time on the Stability of Anthocyanin Pigments Citric Acid Extract of Red Dragon Fruit Peels (Hylocereus polyrizus) and Applications in Foodstuffs. Chemica: Scientific Journal of Chemistry and Chemistry Education, 22(1), 43. https://doi.org/10.35580/chemica.v22i1.21728

Oktari, A., & Mu'tamir, A. (2017). Optimization of Red Fruit Juice (Pandanus sp.) In Examination of Worm Eggs. Journal of Laboratory Technology, 6(1), 8. https://doi.org/10.29238/teknolabjournal.v6i1.85

Siregar, S., Krisdianilo, V., & Rizky, VA (2019). RHODAMIN B. 2(1), 31–39.

Sari, YES, Artanti, D., & Rozi, F. (2020). Optimization of Stem Soaking of Teak Trees (Tectona grandis) in Examination of Soil Transmitted Helmint. Journal of TechnolMedical Laboratory1(1)1–6. https://doi.org/10.36932/teklabmed.v1i1.30.

Maria, T., Wieke, S., Doni, S., & Anik, N. (2018). Medical Laboratory Technology Teaching Materials (Quality Control). Jakarta: Ministry of Health of the Republic of Indonesia. http://bppsdmk.kemkes.go.id