OPTIMIZATION OF BUNI PIGMENT (*Antidesma bunius* L) AS A SUBSTITUTE FOR EOSIN IN THE EXAMINATION OF WORM EGGS

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ABSTRACT

Cases of helminthiasis still exist in the community with a low level of clean living behavior, the community's economy is still weak, and the level of education is relatively minimal. According to WHO (World Health Organization), more than half of the morbidity of the population in developing countries including Indonesia is caused by parasitic worm infections. A doctor can diagnose a positive patient for helminthiasis by conducting laboratory tests first. One of the examinations that need to be done is the examination of worm eggs. The examination requires materials such as eosin as a pigment to see the shape of the worm eggs so that they can distinguish one from the other. Eosin is a pigment produced by a reagent supply company for laboratory examination, which indirectly requires a fee to procure it. In addition, the material is flammable with category 3, and eyes are easily irritated. Therefore, it encourages researchers to develop the potential of natural materials to be used as a substitute for eosin. One of them is *Antidesma bunius* L which contains anthocyanins that can give color, there are several colors produced such as pale pink, pink, and dark pink. This study aimed to examine worm eggs using Berry pigment (Antidesma bunius L) as a substitute for eosin and use eosin as a control and analyze differences in the results of worm egg examination using Berry pigment (Antidesma bunius L) at several concentrations. The method used in this research is a laboratory experiment. The results of the Kruskal Wallis test showed a P value of 0.001 <0.05. This indicates that there are differences in the quality of the staining results to variations in the concentration of the berry ethanol extract can affect the quality of the staining results. Based on the results of the study, it can be concluded that the quality of the staining results is the same as that of Eosin 2%, which is found in berry extract (Antidesma bunius L) with distilled water concentration: extract (1:5).

Key Words : *Antidesma bunius* L, Eosin, Worm Eggs

INTRODUCTION

Helminthiasis/worm infection is a case that can happen to anyone, especially the age of children as long as the level of hygiene is not paid attention to. Cases of Helminthiasis still exist in the community with a low level of clean living behavior, the community's economy is still weak, and the level of education is relatively minimal. According to WHO (World Health Organization), more than half of the morbidity of the population in developing countries including Indonesia is caused by parasitic worm infections, this is following the conditions of Indonesia which is an area with a tropical climate[1]. The results of research conducted by Zahara (2021) stated that from 2012 to 2020 cases of helminthiasis still existed, even in a row in 2019 there were 55.5% positive helminthiasis in SDN 2 Malaka, North Lombok, and in 2020 there were 30, 8% positive for helminthiasis at SDN 47 Ampenan, East Lombok[2].

A doctor can diagnose a positive patient for helminthiasis by conducting laboratory tests first. One of the examinations that need to be done is the examination of worm eggs. The examination requires materials such as eosin as a pigment to see the shape of the worm eggs so that they can distinguish one from the other. Eosin is a pigment produced by a reagent supply company for laboratory examination, which indirectly requires a fee to produce it. In addition, the material is flammable with category 3, and eyes are easily irritated. In connection with this, it encourages researchers to develop the potential of natural materials to be used as a substitute for these materials [3].

Indonesia is a tropical area where many plants grow well and can be used for various purposes. One of them is Berry (Antidesma bunius L) which traditionally people use this plant to treat high blood pressure, heart palpitations, anemia, syphilis, anti-cancer, anti-radical, and as a source of natural pigment. Berry leaves contain several saponins, flavonoids, and tannins and the fruit contains anthocyanins, flavonoids, and phenolic acids[4]. Anthocyanin is the content of Berry (Antidesma bunius L) which can give the pigment to Berry (Antidesma bunius L), several colors can be produced such as pale pink, pink, and dark pink. Another research that has been conducted related to Berry is research from Ajmiati (2014), on several plants belonging to the genus Antidesma showing antibacterial activity including *A.madagascariensis*[5]. In addition, research conducted by Ritana et al (2013) stated that the extract of Berry(Antidesma bunius L) can be used as Lip Cream [6].

RESEARCH METHODOLOGY

This research is a quantitative study with a laboratory experimental design, namely making Berry juice (Antidesma bunius L) and examining worm eggs using Berry juice, and examining worm eggs using eosin as a control for Berry juice. The design of this study used Static Group Comparison, a group was subjected to a certain treatment, then observed the effect of the results of each color variation. The sample in this study was Berry with 2% eosin as a control. Sampling was conducted in Central Lombok, West Nusa Tenggara, where the most Berry (Antidesma bunius L) is planted. Then the stool samples used in this study were taken from stool samples that were positive for worms with 10% formalin preservative obtained from the Health Laboratory Testing and Calibration. This research was conducted at the Biology Laboratory and Traditional Medicine Laboratory of the Medica Farma Husada Polytechnic Mataram. The research instrument consisted of a microscope, object glass, deck glass, stick, dropper, filter paper, tissue, evaporator, water bath, micropipette, blue tip, yellow tip, pot, and materials such as; distilled water, 2% eosin solution, Berry pigment extract with several variations, stool sample (+) intestinal worm eggs in 10% formalin, tissue, handscoon, mask.

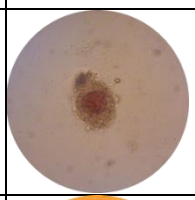
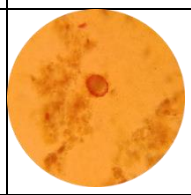
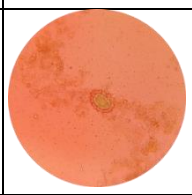
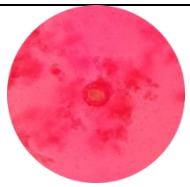
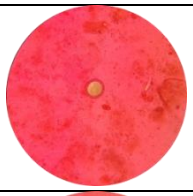
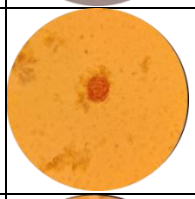
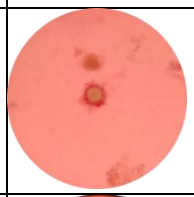
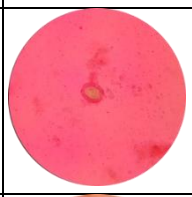
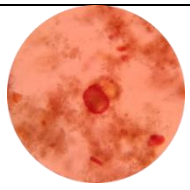
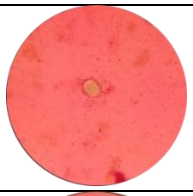
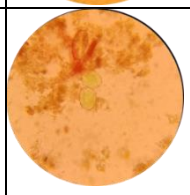
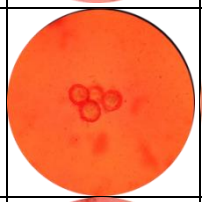
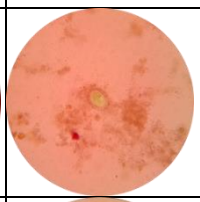
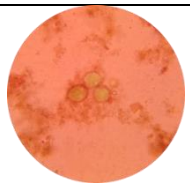
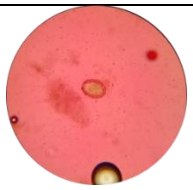
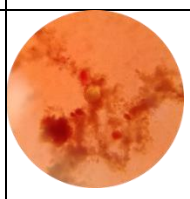
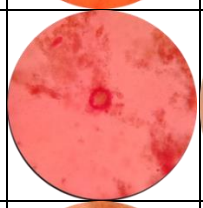
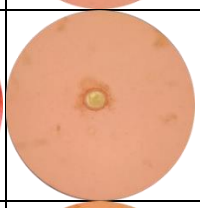
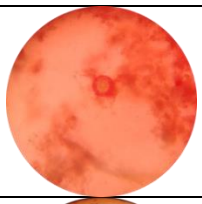
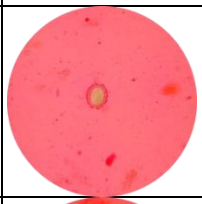
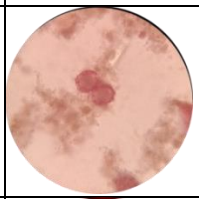
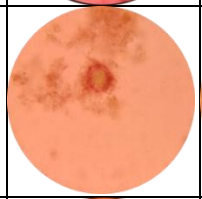
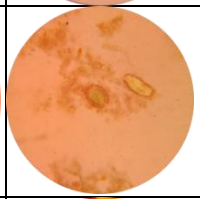
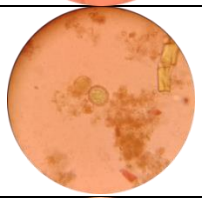
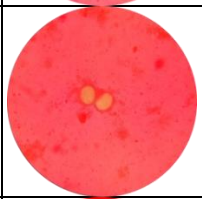
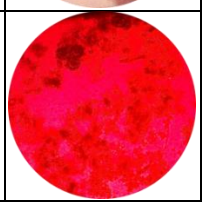
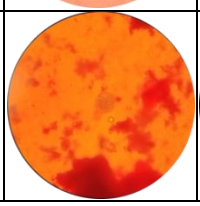
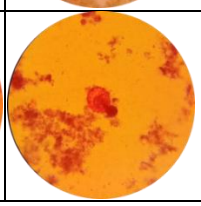
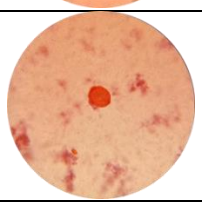
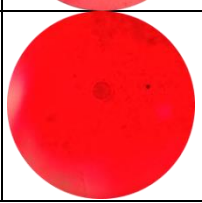
Making 2 grams of eosin was weighed and dissolved in 100 mL of distilled water. Extraction of Berry (Antidesma bunius L) was made using the extraction process using 70% ethanol as a solvent with the addition of 3% citric acid. The combination of 70% ethanol and 3% citric acid gave a higher total **anthocyanin** content. The choice of ethanol solvent is based on its polarity, which can dissolve semi-polar to polar secondary metabolites, including anthocyanin compounds in Berry so that the extracted compounds are expected to be maximized. The results of the maceration process are then evaporated to obtain a thick extract form using a vacuum rotary evaporator and a water bath. The extract obtained from the maceration process is 220 grams with a total yield of 38.26%. Extraction results are used for the examination of worm eggs[6].

The presence of worm eggs in feces can be detected by microscopic examination by painting using a variety of distilled water ratios with a concentration of 1:1, 1:2, 1:3, 1:4, and 1:5 berry extract. Each treatment was repeated 5 times so that the total sample was 25. Then observed using a microscope with a magnification of 100X to 400X. The data processing of this research uses Statistical Product and Service Solutions (SPSS) version 24 with data analysis using one-way ANOVA or Kruskal-Wallis hypothesis testing.

RESULTS AND DISCUSSION

Based on the results of the study, it was found that the ethanol extract of berry was 220 grams with a total yield of 38.26%. The extract was made in various concentrations with a ratio of distilled water: extract as much as 1:1; 1:2; 1:3; 1:4; and 1:5. For each concentration, 5 times were replicated and each concentration was controlled using 2% eosin pigment. The results of observations of worm eggs with pigment from the ethanol extract of berry can be seen in table 1.

Tabel 1. An overview of the results of staining worm eggs with berry ethanol extract pigment

Replication	**Concentration Variation (Distilled Water : Extract)**				
	1:1	1:2	1:3	1:4	1:5
1					
2					
3					
4					
5					
Eosin Control					

Based on the observations in table 1, it can be seen that the higher the concentration of berry, the more red and contrasting the colors produced. The red color produced by berry comes from anthocyanin substances. The red anthocyanin pigment can be extracted from berry[7]. The quality of the coloring results in this study can be assessed using a scale of 1 to 3. Data from the results of the study based on the scale of staining quality can be seen in Table 2.

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Tabel 2. Data of The Result Study

Replication	Concentration Variation (Distilled Water : Extract)				
	1:1	**1:2**	**1:3**	**1:4**	**1:5**
1	1	1	1	3	3
2	2	1	2	2	3
3	1	1	2	2	2
4	1	2	1	3	2
5	1	2	1	2	3
Eosin Control 2%	3	3	3	3	3

Description of Research Result Scale:

1: The field of view is not contrasting, the worm eggs do not absorb color, and the eggs are not visible.

2: The field of view is less contrasting, the worm eggs do not absorb color, and the eggs are not visible

3: The field of view is contrasting, worm eggs absorb color, and egg parts are visible

Based on Table 2, it can be seen that at a concentration of 1:1; 1;2; and 1:3 the average gives the results of staining with no contrasting field of view, worm eggs do not absorb color, the eggs are not visible. Meanwhile, at a concentration of 1:4 it gave an average coloration result with a less contrasting field of view, worm eggs did not absorb color, the eggs were not visible. While the average staining results at a concentration of 1:5 and a control of 2% eosin obtained results with a contrasting field of view, worm eggs absorb color, the eggs are visible. The rating of the coloring results can be seen from the mean rank value for each concentration which can be seen in table 3.

Tabel 3. Mean Rank for each concentration

Concentration (Distilled Water : Extract)	Data	Mean Rank
1:1	5	7.50
1:2	5	9.50
1:3	5	9.50
1:4	5	19.50
1:5	5	21.50
Eosin 2%	5	25.50
Total	**30**	

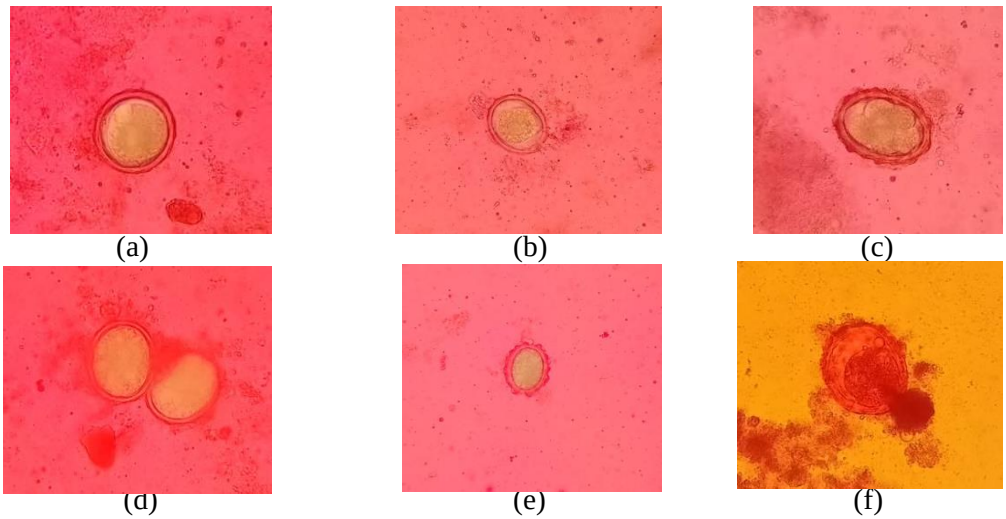
Based on Table 3, it is known that the mean rank that is close to the 2% eosin control is found in a concentration ratio of 1:5. The staining results seen at a concentration of 1:5 are close to the staining results on the 2% eosin control, namely the contrast field of view is visible, the worm eggs absorb the color, the eggs are visible. The greater the concentration of the added extract, the higher the mean rank value and closer to the mean rank value of the 2% eosin control. This is following the results of research conducted by (Putri et al, 2018) that the quality of worm egg preparations can be influenced by variations in the concentration of pigment[8].

The results of statistical tests using SPSS showed that the research data were not normally distributed and the data was not homogeneous with a P value of 0.000 <0.05. So it was continued with the non-parametric Kruskal Wallis test. The results of the Kruskal Wallis test showed a P value of 0.001 <0.05. This indicates that there are differences in the quality of the staining results to variations in the concentration of the berry ethanol extract so it can be concluded that variations in the concentration of the berry ethanol extract can affect the quality of the staining results. Find out the difference in each concentration treatment combination can be done by further testing using the Mann-U Whitney test. The results of the Mann-U Whitney further test can be seen in Table 4.

Tabel 4. Result of *Mann-U Whitney Post Test*

N0	Treatment Combination	Description
1	1:1 and 1:2	There is no difference in the quality of the coloring results
2	1:1 and 1:3	
3	1:2 and 1:3	
4	1:4 and 1:5	
5	**1:5 and Eosin 2%**	
6	1:1 and 1:4	
7	1:1 and 1:5	
8	1:2 and 1:4	
9	1:2 and 1:5	
10	1:3 and 1:4	There is a difference in the quality of the coloring results
11	1:3 and 1:5	
12	1:3 and Eosin 2%	
13	1:2 and 1:4	
14	1:2 and 1:5	
15	1:2 and Eosin 2%	
16	1:3 and 1:4	
17	1:3 and 1:5	
18	1:3 and Eosin 2%	
19	1:4 and Eosin 2%	
20	1:2 and Eosin 2%	

Based on the results of the Mann-U Whitney test in Table 4. it is known that the concentration treatment that has the same staining quality as the 2% eosin control is found in the concentration of distilled water: extract (1:5) with a p-value of 0.134> 0.05. Meanwhile, in the other treatment combinations, there were differences in the quality of the staining results with 2% eosin control. This shows that berry extract with a ratio of distilled water and extract concentrations (1:5) can be potential as a dye for worm eggs to replace 2% eosin. The results of staining of 1:5 berry extract and eosin control can be seen in Figure 3.1.



(a) (b) (c)

(d) (e) (f)

Figure 3.1. The results of observations of worm eggs (a) distilled water concentration: berry extract 1:5 1st replication (b) 2nd replication (c) 3rd replication (d) 4th replication (e) 5th replication (f) Eosin control 2%

The results of staining worm eggs at distilled water concentration compared to 1:5 Berry extract had a staining quality with the color of worm eggs being yellow and the background was red. While the eosin pigment shows a yellowish background. Eosin is a pigment commonly used for staining worm eggs. Eosin pigment is a reagent that has an acidic pH so that it can color acidophilic components of worm eggs such as collagen, secretory granules, and mitochondria. However, eosin pigment is carcinogenic with a class 3 carcinogen, flammable with a category 3, and easily irritating to the eyes[9]. So based on the results of the study that Berry can be used as an alternative pigment that is safe for eosin at an inexpensive cost.

Berry is a plant with green to dark red fruit. Young Berry is bright green then turns dark red when mature. Berry used in this study is a fruit with a red color that has a sour taste. The red color of the Berry is considered the same as the red color of eosin. In addition, the sour taste in Berry can color the components of worm eggs which are acidophilic so that the staining results on the Berry extract have the same staining quality as 2% eosin.

The red color of the Berry is an anthocyanin substance produced by the Berry. Anthocyanins are natural pigments in the subclass of flavonoids. Berry contains flavonoid polyphenols and organic acids. The total anthocyanin in Berry was 141.94 ± 4.32 mg per 100 grams of fresh ripe fruit. According to the results of the study, the total anthocyanin content in Berry was 274,012 (mg/100g)[10]. Anthocyanins have three groups, namely anthocyanidins, aglycones, and glucosides. Anthocyanin is the core aglycone of anthocyanins which causes the formation of red, blue, and yellow colors in vegetables and fruits[11].

CONCLUSION

Based on the results of the study, it can be concluded that the quality of the staining results is the same as that of Eosin 2%, which is found in berry extract (Antidesma bunius L) with distilled water concentration: extract (1:5) and the quality of worm egg preparations can be influenced by variations in the concentration of the pigment.

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