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Bougainvillea Flower Extract Utilization as Eosin Substitute in Worm Eggs Microscopic Observation

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ABSTRACT

Introduction: Flavonoids, glycosides, alkaloids, phlobatannins, saponins, steroids, tannins, and terpenoids are all known components of bougainvillea flowers. The betalain compounds betasianin and betaxanthin, which give bougainvillea flowers their color, are present. Betalains are water-soluble pigments classified into betacyanins (red) and betaxanthins (yellow). The eosin dye, which can stain worm eggs, is comparable to the pigment in bougainvillea flowers. Because reagents are expensive, raw materials from the local area must be used instead. Local raw materials can replace eosin reagents. When examining intestinal nematode worm eggs, the extract of bougainvillea flowers can be used as an eosin reagent. We are interested in learning whether Bougainvillea flower extract can be used to observe worm eggs at a microscopic level instead of the expensive and difficult-to-come-by eosin.

Aim: This study aimed to ascertain whether Bougainvillea extract could be used instead of eosin to observe worm eggs at a microscopic level.

Method: Complete Randomised Design (CRD) is used in this quasi-experimental research. This study also aims to compare the efficacy of eosin reagent and bougainvillea flower extract in the protracted drying process. The Microbiology Laboratory, Department of Medical Laboratory Technology, Aceh Health Polytechnic, Ministry of Health, was the site of this study. Gampong Jawa, Banda Aceh, is where feces were sampled. Preparation of bougainvillea flower extract with a concentration of 100% as a substitute for 2% eosin on microscopic observations of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Hookworm* eggs to see the comparison of the drying time for reagents in worm egg preparations using red, pink and orange Bougainvillea flower extract with eosin control. Repeat three more times. T-test used for data analysis.

Result: When compared to the eosin control reagent, which dried in only 27.63 minutes in preparation for *Soil Transmitted Helminth* worm eggs, the average drying time for the reagents using natural reagents from the extract of red bougainvillea flowers was 23.96 minutes, that of pink bougainvillea flowers was 23.30 minutes, and that of orange bougainvillea flowers was longer at 28.40 minutes. A variety of bougainvillea species have different drying requirements for regeneration.

Conclusion: The time it took for red, pink, and orange Bougainvillea extract to dry, as well as the control eosin, indicates that there is a significant difference in the dry times between the treatments.

Keywords: Bougainvillaea extract, eosin reagents, worm eggs

INTRODUCTION

Due to its location in a tropical region with a consistent climate, Indonesia has a high biodiversity (Bahar & Veriyani, 2021). According to National Geographic Indonesia (2019), Indonesia's mainland biodiversity is second only to Brazil in

terms of importance [2]. The bougainvillea flower, also called Bougainvillea, is part of Indonesia's biodiversity.[3]

In order to adorn fences, ornamental plants called bougainvillea flowers are used [4]. South American native Bougainvillea is a very showy, flowering, and robust plant. This plant blooms vibrantly most of the year and is essentially disease- and pest-free. Flavonoids, glycosides, alkaloids, phlobotannins, saponins, steroids, tannins, and terpenoids have all been found in bougainvillea flowers [5]. Betalain compounds betasianin and betaxanthin are the color pigments in bougainvillea flowers [6]. Betalains are water-soluble pigments that can be divided into betacyanins (red color) and betaxanthins (yellow color) [7]. Bougainvillea flower dye is comparable to the eosin staining agent on worm eggs.

Eosin reagent, a native technique, is used in the most straightforward examination of intestinal nematode worm eggs. This reagent is acidic and orange-red. Using 2% eosin is intended so that worm eggs can be clearly distinguished from the surrounding dirt. 2% eosin also distinguishes between feces and feces, providing a red background for eggs with a yellowish hue[8].

The high cost of reagents opened the door for the use of locally available raw materials that can be used as eosin reagent substitutes, such as bougainvillea flower extract so that medical facilities that find it challenging to obtain or run out of eosin reagents can use alternative bougainvillea flower extract for examination of intestinal nematode worm eggs. We are interested in learning whether bougainvillea flower extract can be used as a substitute for eosin in microscopic observations of worm eggs. Eosin is one of the reagents used to examine intestinal nematode worm eggs, but it is expensive and difficult to obtain. This study sought to ascertain whether bougainvillea flower extract could be used to observe worm eggs under the microscope instead of eosin.

MATERIALS & METHODS

Complete Randomised Design (CRD) is the research methodology used in this quasi-experimental study. In this investigation, we compared the background dyes used in preparations for *Soil Transmitted Helminth* (STH) worm eggs between eosin reagent and the extract of red, pink, and orange bougainvillea flowers. In addition, this study aims to compare the efficacy of eosin reagent and bougainvillea flower extract in the protracted drying process. This study was carried out at the Microbiology Laboratory of the Department of Medical Laboratory Technology at the Health Polytechnic of the Aceh Ministry of Health. Gampong Jawa, Banda Aceh, is the site of the feces sampling.

The instruments used in this study were a microscope, *object glass*, *cover glass*, mortal, pestle, dropper pipette, measuring pipette, 25 ml measuring flask and beaker glass, scales, *stopwatch*, and marker. Red, pink, and orange bougainvillea flowers, eosin reagent, formalin, ether, 70% alcohol, handscoon, mask, laboratory coat, flannel cloth, *paper lens*, *tissue*, *aquadest*, and *Whatmann* filter paper were the examination tools used in this study.

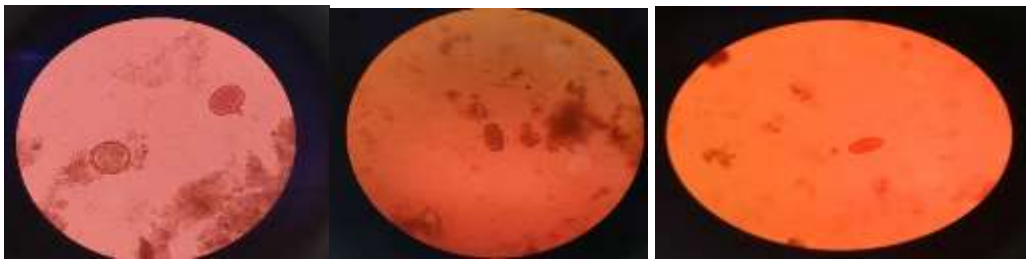
Making bougainvillea flower extract with a concentration of 100% as a substitute for 2% eosin on microscopic observations of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Hookworm* worm eggs to see the comparison of the drying time of the reagent in worm egg preparations using the extract of red, pink and orange bougainvillea flowers with eosin control. The first parameter observed in this research was a microscopic observation of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Hookworm* worm eggs using 2% eosin control, red, pink, and orange bougainvillea flower extract. The second parameter was the ratio of the drying times of the reagents to the worm egg preparations using the red, pink, and orange bougainvillea extract with the eosin control and repeated three times.

Photographic images were used to collect the background information on the coloring

of the worm egg preparations. Then, the T-test was used to perform statistical analysis on the data regarding how long it took the preparations to dry.

RESULT

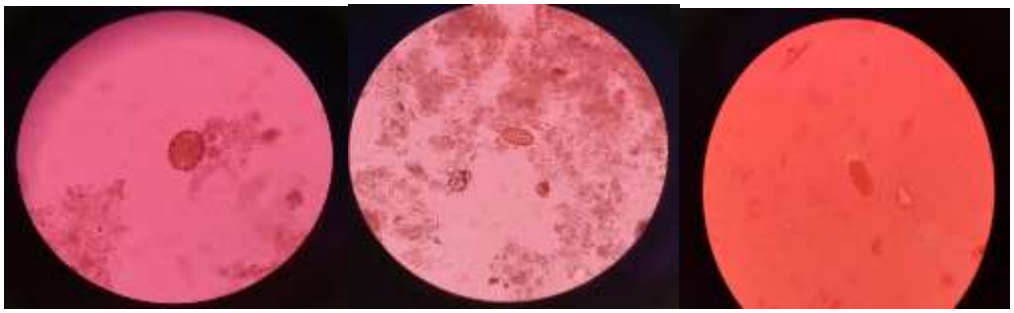
Photographic images were used to collect the background information on the coloring of the worm egg preparations. Then, the T-test was used to perform statistical analysis on the data regarding how long it took the preparations to dry. Results from Applying Red Bougainvillaea Flower Extract Against *Soil Transmitted Helminth* (*STH*) Worm Eggs. The figure below depicts the research findings on using red bougainvillea flower extract as a background for *STH* examination.



Ascaris lumbricoides *Trichuris trichura* *Hookworm*

Figure 1. Red bougainvillea flower extract contains worm eggs.

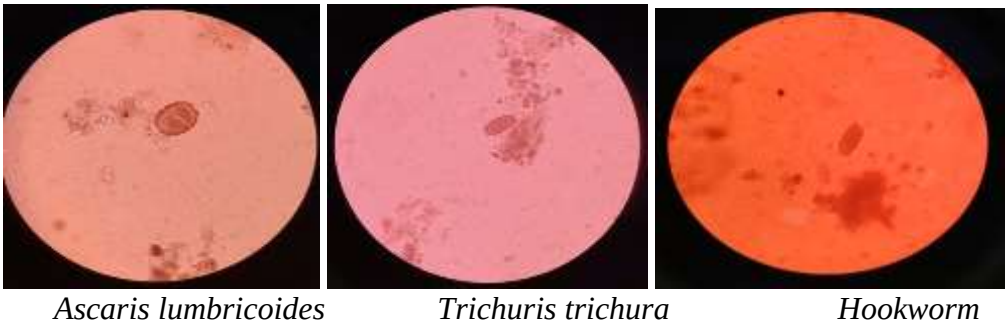
Results from Applying Pink Bougainvillaea Flower Extract Against *Soil Transmitted Helminth* (*STH*) Worm Eggs. The following figure shows the research findings using red bougainvillea flower extract as a background for *STH* examination.



Ascaris lumbricoides *Trichuris trichura* *Hookworm*

Figure 2. Pink bougainvillea flower extract contains worm eggs.

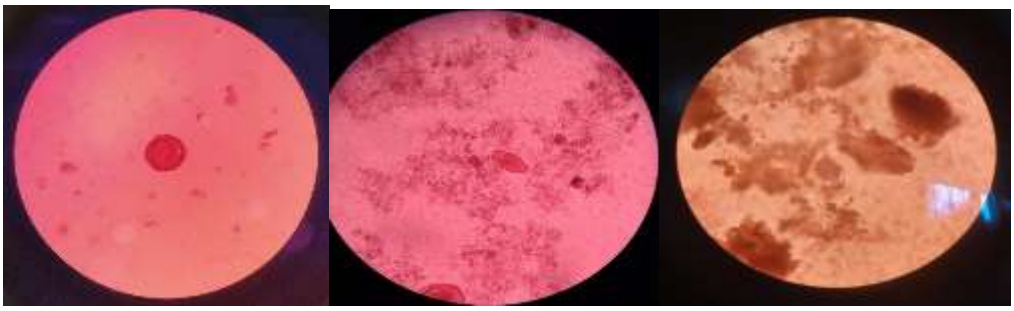
Results from Applying Orange Bougainvillaea Flower Extract Against *Soil Transmitted Helminth* (*STH*) Worm Eggs. The figure below depicts the research findings on using orange bougainvillea flower extract as a background for *STH* examination.



Ascaris lumbricoides *Trichuris trichura* *Hookworm*

Figure 3. Orange bougainvillea flower extract contains worm eggs.

Results from Applying Eosin as a Control for *Soil Transmitted Helminth* (STH) Worm Eggs. The following figure shows the investigation's findings into the background used for STH examinations using 2% eosin as a control.



Ascaris lumbricoides *Trichuris trichura* *Hookworm*

Figure 4. 2% eosin control contains worm eggs.

The results of measuring the average drying time of the reagents on preparations for *Soil Transmitted Helminth* worm eggs using eosin control and red, pink, and orange bougainvillea extract.

Table 1 shows the findings from measuring the average drying times of the reagents on eosin controls, red, pink, and orange bougainvillea flower extract, compared to *Soil Transmitted Helminth* worm egg preparations.

Table 1. Results of the average drying time of the reagent for preparations of *Soil Transmitted Helminth* worm eggs and red, pink, and orange bougainvillea flower extract versus eosin controls (in minutes).

Sample	Repetition I				Repetition II				Repetition III			
	P0	P1	P2	P3	P0	P1	P2	P3	P0	P1	P2	P3
1	30	19	36	46	10	36	35	31	29	22	22	22
2	21	30	30	31	29	29	30	35	20	37	28	31
3	10	37	37	37	17	44	15	12	26	42	11	16
4	16	11	25	17	23	27	20	27	22	22	25	15
5	23	30	31	33	30	29	28	33	25	28	32	30
6	25	54	60	44	46	47	56	56	35	37	31	26
7	25	28	33	28	27	29	30	30	22	30	29	37
8	27	18	31	23	25	14	9	11	27	20	27	23
9	10	23	23	23	34	38	39	38	11	33	25	11
10	36	32	17	27	16	13	20	13	22	20	17	23
Average (minutes)	22	28	32	31	26	31	28	29	24	29	25	23

Information:
P0 = Eosin Control
P1 = *Red* bougainvillea flower extract
P2 = *Pink* bougainvillea flower extract
P3 = O*range* bougainvillea flower extract

The outcomes of different reagents' average drying times

Table 2 shows the findings of the analysis of drying time variations using organic reagents from the extract of red, pink, and orange bougainvillea flowers and an eosin control in preparations for soil-transmitted helminth worm eggs.

Table 2. Results of the reagents' average drying time.

No	Reagents	N	Average (minutes)	Standard Deviation	P-value
1	Red bougainvillea flowers	30	23,96	8,26	0,192
2	Pink bougainvillea flowers	30	29,30	10,20	
3	Orange bougainvillea flowers	30	28,40	10,81	
4	Eosin Control	30	27,63	10,19	

According to Table 2, the average drying time for reagents using red bougainvillea flower extract is 23.96 minutes, pink bougainvillea flower extract is 23.30

minutes, and orange bougainvillea flower extract has a longer drying time, namely 28.40 minutes for Soil Transmitted Helminth worm egg preparations, compared to the eosin control reagent, which was only 27.63 minutes. The difference in drying time between the eosin control and the natural reagents made from the extract of red, pink, and orange bougainvillea flowers, according to the results of an ANOVA test, was 0.192 > alpha value of 0.05, proving that there was an actual difference between the treatments. The average drying time of the red, pink, and orange bougainvillea flower extract, as natural reagents, is longer than that of the eosin control reagent, as shown in Table.2.

DISCUSSION

The study's findings indicate that the red, pink, and orange bougainvillea flower extract can be used in place of eosin reagent to examine Ascaris lumbricoides, Trichuris trichiura, and hookworm (*Soil Transmitted helminth* worm) eggs. The shell's structure and the eggs' contents can be seen by reviewing *Soil Transmitted Helminth* worm egg preparations using red, pink, and orange bougainvillea flower extract.

betalain compounds betasianin and betaxanthin are the color pigments in bougainvillea flowers [6]. betalains are water-soluble pigments that can be divided into betacyanins (red color) and betaxanthins (yellow color) [7,9]. Using a primary camera at the time of shooting resulted in a difference in color contrast on the preparations during the documentation process. However, the worm egg structure was still clearly discernible. The eosin staining agent used to color worm eggs is comparable to the bougainvillea flower dye.[1011]

The native method uses eosin reagent and is the easiest way to examine intestinal nematode worm eggs. Where the reagent is acidic and has the color red-orange, it is intended that worm eggs stand out from the surrounding dirt when 2% eosin is used. In addition, 2% eosin gives yellowish eggs a red background and distinguishes feces from others [8]. [3]

The ANOVA test showed that there was a significant difference between treatments in the amount of time it took for the natural reagents - red, pink, and orange bougainvillea extract - to dry as compared to the control eosin, with a result of 0.192 > 0.05 alpha. The manufacturing process for red, pink, and orange bougainvillea flowers uses a 100% concentration of the flower extract, which does not add to distilled water. In contrast, the manufacturing process for bougainvillea reagent uses a 2% eosin concentration, which adds distilled water when 2 grams of eosin are added to 100 ml of aquadest. This results in a difference in drying time between the two products.[12]

CONCLUSION

The difference in the average drying time for the reagent using natural reagents: the extract of red bougainvillea flowers is 23.96 minutes, the extract of pink bougainvillea flowers is 23.30 minutes, and the extract of orange bougainvillea flowers has a longer time, namely 28.40 minutes when compared with the eosin control reagent which had a drying time of 27.63 minutes on *Soil Transmitted Helminth* egg preparations. The drying time of the reagent between the results of the ANOVA test on the drying time of red, pink, and orange bougainvillea flower extract and the control eosin showed a value of 0.192 > an alpha value of 0.05, indicating that there was a significant difference in the amount of drying time in each treatment. According to Table 4.2, eosin, the control reagent, takes longer on average to dry than the natural reagents made from red, pink, and orange bougainvillea flower extract.

Declaration by Authors

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