MEDFARM: Jurnal Farmasi dan Kesehatan, Vol. 15, No. 1, 2026, Page 117-124
e-ISSN : 2715-9957
p-ISSN: 2354-8487

Stability Test Of Miana Leaf Extract (*Coleus Atropurpureus*) With The Addition Of Glycerol In The Diagnosis Of Parasitic Infections

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ABSTRACT

Worm infections (Helminthiasis) are among the most common infections affecting children. Worm infections caused by soil-transmitted helminths result from contact with the eggs or larvae of these parasites, which develop in moist soil. One method for detecting parasitic worm infections is the Direct slide method using 2% eosin. Miana leaves (Coleus atropurpureus) are plants that contain anthocyanin pigments, producing purple, red, or blue colors, so extracts from miana leaves can be used in the identification of Soil Transmitted Helminth eggs. However, the stability and effectiveness of miana leaf extracts during storage are currently unknown. This study aims to determine the storage stability of miana leaf extract at 2–6℃ with the addition of 0.5% and 1% glycerol in the diagnosis of helminth infections. This is a true experimental study with a post-test control design. Miana leaf extract was obtained through a maceration process using distilled water and 2% acetic acid (1:7) as solvents. Glycerol 0.5% and 1% were then added to the miana leaf extract, which was stored at 2–6℃ and tested for staining stability on days 0, 5, 10, and 15. The results of the study on day 0 showed a score of 3 for all treatments. The extract without glycerol showed a decrease in stability on day 5 and onwards, as did the extract with the addition of 0.5% glycerol, which showed a decrease in stability on day 10 and onwards, while the addition of 1% glycerol maintained color stability until day 15. The results of the study can be concluded that the addition of 1% glycerol effectively maintains the stability of miana leaf extract for 15 days at a temperature of 2–6℃ in the diagnosis of helminth infection .

Keywords*: Glycerol; Miana leaves; Soil-Transmitted Helminth Eggs*

Introduction

Parasitic worm infections are infections caused by parasitic worms that live in the intestines and belong to the intestinal nematode group. These types of nematodes spread through soil and are known as *Soil Transmitted Helminths* (STH). Types of STH worms include *Ascaris lumbricoides*, *Trichuris trichiura*, *Hookworm*, *Strongyloides stercoralis* (Permatasari *et al*., 2021). Parasitic worm infections caused by *Soil Transmitted Helminths* are one of the most common types of infections worldwide. The number of people infected with STH is estimated to be around 1.5 billion people or 24% of the total population in the world (Setiawan *et al*., 2022). The spread of STH worm infections occurs, among other ways, through soil contamination

by feces containing STH worm eggs or larvae (Islawati *et al*., 2024). Fecal examinations can be used to detect worm infections, with several methods including the native method, flotation NaCl, or lugol (Septiani, 2022). Eosin is an acide and orange red in color.

The use of 2% eosin aims to make worm eggs clearly visible compared to surrounding feces 2% eosin provides a red background that makes the yellowish eggs stand out, thereby separating worm eggs from feces (Oktari & Mu'tamir, 2017). The continuous long-term use of eosin has carcinogenic properties because it contains chemicals such as picric acid and chlorate. Its negative effects include irritation of the eyes, skin, and mucous membranes, as well as the potential to cause cancer. In addition, eosin waste can pollute the environment. Miana leaves (*Coleus atropurpureus*) come in various colors, ranging from brownish purple to black, depending on the type. Some leaves also have green or yellow edges. The difference in the color of miana leaves is influenced by the amount of pigments they contain, such as anthocyanins. Anthocyanins are a group of flavonoids that can react in acidic and alkaline environments to produce purple, red, or blue pigments. In acidic conditions, anthocyanins produce a dark red color, while in alkaline conditions, the color changes to purple or blue (Krisdianio & Khairiyah, 2023).

Figure 2.1 Miana (*Coleus artopurpureus*)
(Source*: Personal Collection*)

The production process for this pigment is relatively easy and inexpensive because the raw materials are always available and abundant in nature. Research by Suraini & Sophia (2022) shows that the stability of anthocyanins can be affected by pH, temperature, and exposure to light. The results of research by Hidayah *et al*. (2014) show that the natural color of dragon fruit skin, which contains anthocyanin, is stable at a pH of 3-5. Anthocyanin is non toxic and can be extracted using method, tape technique, and sedimentation method (Regina *et al*., 2018). The gold standard used for stool examination is the direct slide method. This method can use a 2% eosin solution using materials that are not harmful to human health or the environment and can also be extracted with distilled water (Puspita *et al.,* 2018).

Water-based dye solutions are easy to use during extraction and application but are prone to degradation during storage. Organic compounds in the solution tend to degrade if stored for too long, especially under suboptimal storage conditions. The

water content in the solution also has the potential to support microorganism growth, which can accelerate solution degradation (Rahayuningsih *et al*., 2016). Ethanol and glycerol are substances that can be used to preserve extracts. Ethanol can be harmful to the environment, polluting water and soil, and potentially damaging ecosystems. Glycerol, on the other hand, is non toxic and safe for use in many applications, including cosmetics and food (Kowalska *et al*., 2021).

Methodology

This study is an experimental study with a posttest-only control group design. Research hypothesis: 0.5% and 1% glycerol can maintain the stability of miana leaf extract (*Coleus atropurpureus*) in the diagnosis of parasitic worm infections.

1. Extraction of Miana Leaves by Maceration
    a. Remove any dirt or foreign objects from the miana leaves and wash them thoroughly under running water. Then, chop the leaves into small pieces and blend until smooth.
    b. Weigh out 84g of miana leaves, then place them in a dark glass container with a lid.
    c. Add 21 mL of distilled water as a solvent and 147 mL of 2% acetic acid (ratio 1:7) to a dark glass container with a lid, then extract by maceration for 3 × 24 hours, stirring occasionally
    d. Filter the miana leaf infusion using filter paper to obtain the filtrate.
    e. Centrifuge the filtrate at 7000 rpm for 10 minutes; the resulting supernatant is the liquid extract of miana leaves (Almajid et al., 2021).

2. Addition of Miana Leaf Extract with 0.5% Glycerol
    a. Place 50 mL of miana leaf extract into a dark glass container, then add 0.25 mL of glycerol and mix thoroughly
    b. Store the extract at 2–6°C for 5 days, 10 days, and 15 days

3. Preparation of Miana Leaf Extract Using 1% Glycerol
    a. Place 50 mL of miana leaf extract into a dark glass container, then add 0.5 mL and mix thoroughly
    b. Store the extract at 2–6°C for 5 days, 10 days, and 15 days.

4. Examination of *Soil-Transmitted Helminth* Eggs
    a. Prepare a clean, grease-free microscope slide
    b. Place one drop of miana leaf extract solution (without glycerol) onto the microscope slide
    c. Take a small amount of feces (about the size of a toothpick tip) and homogenize it.
    d. Cover the specimen with a cover slip
    e. Observe the specimen under a microscope using 10x and 40x objective lenses
    f. Repeat the procedure for miana leaf extracts with 0.5% and 1% glycerol added, before storage and after storage for 5 days, 10 days, and 15 days.

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The research data were analyzed descriptively in tabular form. Based on a 3-point Likert scale (3, 2, and 1):
Score 3 = High-contrast field of view; worm eggs absorb color; worm egg parts are clearly visible.
Score 2 = Low-contrast field of view; worm eggs do not absorb color well; worm egg parts are not clearly visible.
Score 1 = No contrast in the field of view, worm eggs do not absorb color, parts

Result and Discussion

Table 1. Results of Miana Leaf Extract Staining with Glycerol Addition in *Soil Transmitted Helminth* (STH) Egg Examination (*Personal documentation, 2025*)

Test Time	Without Glycerol	0,5% Glycerol	1% Glycerol
0	3	3	3
5	2	3	3
10	2	2	3
15	2	2	3

Description:
Score 3 = High contrast field of view, worm eggs absorb color, worm egg segments are clearly visible.
Score 2 = Field of view lacks contrast, worm eggs absorb color poorly, parts of the egg are unclear.
Score 1 = Field of view lacks contrast, worm eggs do not absorb color, parts are unclear (Riwanti, 2020).

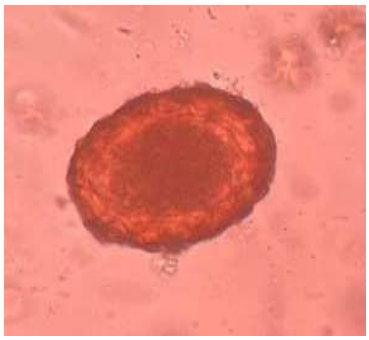
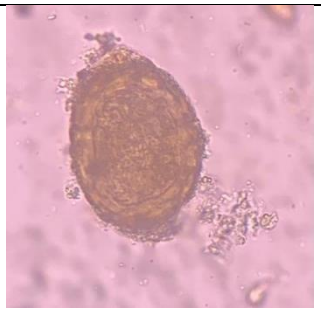
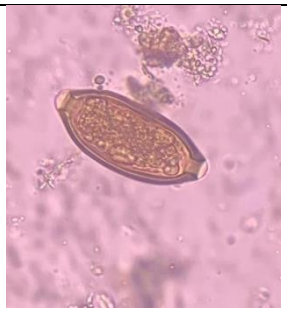
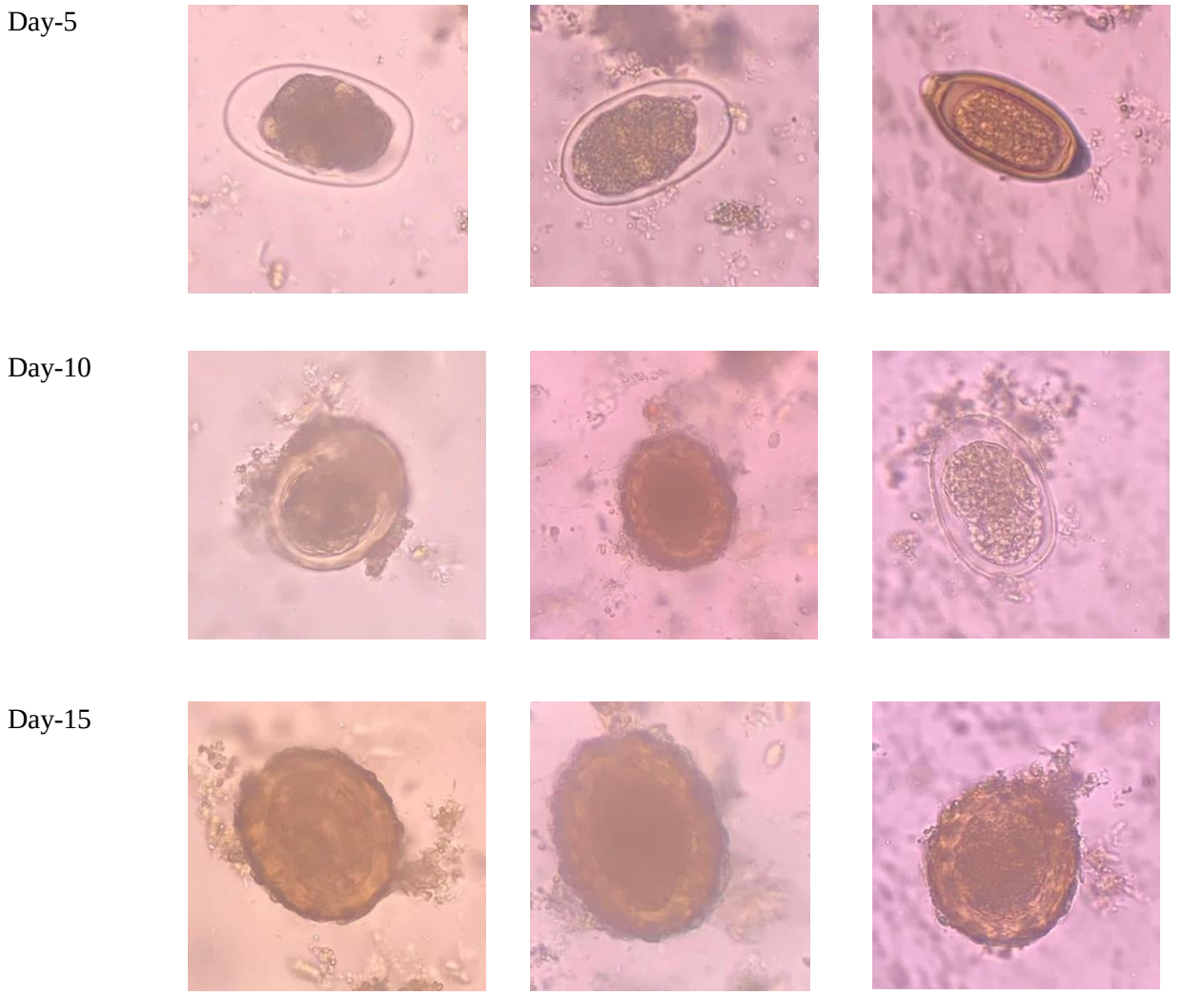


Figure 1. *Ascaris lumbricoides* eggs stained with 2% eosin
(*Personal documentation, 2025*)

Table 2. Microscopic Observation Results of *Soil Transmitted Helminth* Eggs at 400x Magnification (Personal documentation, 2025)

Time	Miana Leaf Extract		
	Without Glycerol	0.5% Glycerol	1% Glycerol
Day-0			



Based on Table 1 and Table 2, it can be seen that immediate examination of both the glycerol-free extract and the extracts containing 0.5% and 1% glycerol yielded excellent staining results on the Likert scale, with a score of 3. This score indicates that the field of view has good contrast, the eggs absorb the stain well, and the morphological structure of the worm eggs is clearly visible. However, over time, the stability of the staining decreased, particularly in the glycerol-free extract. On day 5, extracts of without glycerol starting from showed a decrease in stability to a score of 2, characterized by reduced contrast in the field of view, poor color absorption by the worm eggs, and less distinct egg sections. In contrast, extracts with the addition of 0.5% and 1% glycerol maintained good dyeing stability (**score 3**). The decrease in stability became more pronounced on days 10 and 15, with miana leaf extracts without and with the addition of 0.5% glycerol showing reduced dyeing results indicated by a score of 2. Only 1% glycerol maintained good dyeing stability (**score 3**).

Research on the stability test of miana leaf extract with the addition of glycerol in the diagnosis of worm infections was conducted at the Parasitology Laboratory of

the John Paul II Health Academy in Pekanbaru. The samples used were fresh miana leaves that were blended and then added with distilled water and 2% acetic acid (1:7). The stability of anthocyanins in polar solvents combined with organic acids such as acetic acid can optimally increase the stability of anthocyanins (Priska *et al*., 2018). Miana leaf extract was obtained through a maceration process for 3x24 hours. Maceration is a simple method and the most widely used in the extraction process to avoid the destruction of thermolabile compounds (Isma, 2022). Then, the maceration results were filtered using filter paper to obtain the filtrate. The filtrate was centrifuged at a speed of 7000 rpm for 10 minutes to obtain the supernatant, which was used as the test material. This study aimed to examine the effect of adding glycerol as a preservative on the shelf life of miana leaf extract in diagnosing worm infections.

The glycerol concentrations added to the miana leaf extract in this study were 0.5%, 1%, and as a control, 2% eosin was used because 2% eosin has the ability to produce different colors between the field of view and worm eggs. 2% eosin is an acidic solution that is red in color. Eosin 2% is also used in stool examinations to provide a red background and distinguish STH worm eggs from feces. Based on the results of **Table 1 and 2**, it was found that during observation, the addition of 0.5% and 1% isomerol to the miana leaf extract significantly increased the number 2 show that during observation Based on the results of Tables 1 and 2, it was found that durin Immediately after treatment, both samples without and with 0.5% and 1% glycerol showed excellent staining results based on the Likert scale with a score of 3.

This score indicates that the field of view is contrasting, the eggs absorb color well, and the morphological structure of the worm eggs is clearly visible. However, over time, the stability of the staining decreased, especially in extracts without added glycerol. On day 5, extracts without glycerol began to show a decrease in stability to a score of 2, indicating less contrast in the field of view, poor color absorption by the worm eggs, and less clear visibility of the egg sections, while extracts with the addition of 0.5% and 1% glycerol still maintained good staining stability (**score 3**).

The decline in stability was more apparent on days 10 and 15, where miana leaf extracts without and with 0.5% glycerol showed a decline in staining, indicated by a score of 2, and only extracts with 1% glycerol addition maintained good stability (score 3). Anthocyanins found in miana leaves are natural pigments derived from water-soluble flavonoids. These pigments are amphiphilic, meaning they can react in both acidic and basic conditions. Anthocyanins in acidic conditions produce red colors, while in basic conditions, they produce purple and blue colors (Salnus *et al*., 2021). This flavonoid compound contains two benzene rings connected by three carbon atoms and can be extracted with polar solvents, including methanol, ethanol, and acetic acid. In this study, the solvent used was a combination of acetic acid and distilled water. Acidic conditions will affect the extraction results; the more acidic the conditions, the more vacuole cell walls will break, resulting in more anthocyanin pigments being extracted (Ayun & Khomsiyah, 2022).

Glycerol added to miana leaf extract aims to maintain the stability of the anthocyanins contained in the extract, thereby extending the shelf life of the extract, which can be used as a dye in the diagnosis of worm infections. Glycerol is a simple trihydric alcohol in the form of a thick, clear, odorless liquid with a sweet taste

(Khairati, 2022). Research by Khanna *et al*. (2014) shows that glycerol can act as a preservative in semi-permanent preparations due to its hygroscopic properties, which prevent premature drying of the preparation. Research by Fhadila *et al*. (2024) found that glycerin is commonly used as a humectant due to its ability to absorb moisture from the environment and reduce water evaporation, thereby maintaining the stability of preparations and increasing their spreadability.

Conclusion

1% glycerol solution was able to maintain the stability of miana leaf extract until day 15 at 2–6°C for the diagnosis of soil-transmitted helminth infections, whereas miana leaf extract without glycerol showed a decline in quality starting on the fifth day of storage.

Declaration of Competing Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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