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RESEARCH ARTICLE

Analysis of the staining quality of soil transmitted helminths (STH) eggs in *Tectona grandis* Linn. f.-glycerol wet mount preparations against preparation storage periods

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Abstract

Soil-transmitted helminth (STH) infections remain a significant public health problem in Indonesia. Microscopic identification of STH eggs relies on wet mount preparations, which require clear, stable, and well-contrasted staining. Although leaf extract of *Tectona grandis* Linn. f. contains anthocyanins and tannins with potential as a natural dye, data on the storage stability and long-term microscopic quality of wet mounts stained with this extract are limited. This study evaluated the staining efficacy (clarity, contrast, and preservation of egg morphology) and temporal stability of wet mount preparations stained with 30% (v/v) ethanolic *T. grandis* leaf extract in glycerol, compared with **2%** glycerol eosin as a positive control. Faecal samples positive for STH were collected from 30 participants and prepared as wet mount slides, which were examined macroscopically and microscopically during 1–3 weeks of storage. Two expert observers independently assessed slide quality using a 3-point scoring scale, supplemented by evaluation of microscopic colour intensity. The microscopic quality of *T. grandis*–stained preparations improved over time, with proportion rated "very good" increasing from 20% in week 1 to 60% in week 3. The Friedman test showed a significant effect of storage time on microscopic quality (p-value = 0.029). Compared with eosin *T. grandis* preparations showed no significant differences in egg morphology preservation and colour contrast (p-value > 0.05), indicating comparable staining efficacy. In conclusion, 30% (v/v) ethanolic *T. grandis* leaf extract in glycerol is a promising natural alternative stain for STH egg wet mounts, with efficacy comparable to eosin and with quality maintained and improving, over 3 weeks of storage.

1. INTRODUCTION

Soil-transmitted helminth (STH) infections remain a common public health problem, particularly in developing countries (1). According to WHO data from 2023, approximately 1.5 billion people, or 24% of the world's population are infected with STH. Vulnerable groups include preschool-aged children (over 260 million), school-aged children (654 million), adolescent girls (108 million), and pregnant and lactating women (138.8 million). The high prevalence is largely attributable to poor sanitation and limited access to clean water (2). STH diagnostics faces several challenges, including a shortage of trained laboratory personnel, large sample volumes that demand considerable time and resources, and limited microscopy equipment in many healthcare facilities. In addition, the sensitivity of diagnostic methods, which depends on staining quality and on specimen conditions that often vary, can reduce accuracy, particularly in low-intensity infections (3). Microscopic examination of STH eggs in faecal samples with staining reagents remains the gold standard (2,3). Good staining facilitates egg identification, which is essential for accurate, diagnosis, and treatment. Direct wet

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preparations are made by mixing faeces with a staining reagent under a cover glass (4). The wet preparation must be observed immediately because they dry out quickly (5). Once a preparation has dried, eggs can no longer be identified and a new slide must be made (6). Preserved wet mount preparations (temporary preserved preparations) are therefore needed.

Direct faecal usually uses 2% eosin, a synthetic dye. Synthetic dyes add to laboratory costs, and they may contain compounds that degrade poorly in the environment, contributing to soil pollution and harming flora and fauna (7). mproper handling of eosin may result in occupational exposure through ocular, dermal, and inhalation routes. Furthermore, concerns regarding its potential carcinogenic effects have been reported (8). Replacing synthetic dyes with natural dyes is attractive for three reasons. First, natural dyes are generally cheaper and can be sourced locally, reducing dependence on imported materials that are expensive and difficult to obtain (9). Second, they can reduce hazardous chemical waste from laboratories. Third, because they derived from biodegradable materials and leave no toxic residues, they are more environmentally friendly and support more sustainable laboratory practices. Young teak leaves (*Tectona grandis* Linn. f.) are a promising natural dye source. In addition, the use of young *T. grandis* Linn. f. extract can also minimize the potential for pollution, as this natural material typically decomposes naturally and is safer if released into the environment (10). These compounds contain anthocyanins, which contribute to reddish coloration, and relatively high concentrations of tannins, which provide stable brownish to yellowish hues and may enhance the preservation of preparations by reducing decay and deterioration (11).

Compared to other plants that also containing these compounds, teak leaves have several advantages (12). First, their phytochemical content is relatively stable, yielding extract of consistent compositen across growing conditions. Second, they are abundant and widely cultivated in tropical regions, including Indonesia. Third, previous studies have also reported good colour fastness and significant antimicrobial and antioxidant activity (13,14). Other natural materials, such as turmeric (*Curcuma longa*) and papaya leaves (*Carica papaya*), have been investigated as alternative dyes, but mainly in histology and textile industry rather than in diagnostic parasitology (15). Compared with these materials, *T. grandis* has shown the ability to stain STH eggs, yet it remains little explored as a natural staining agent in parasitological microscopy. It is therefore a promising candidate for an environmentally friendly, effective, and sustainable dye in parasitic diagnostics. Together, these factors provide a strong scientific rationale for using teak leaves as a raw material for natural dyes and other bioactive applications.

Delays in reading STH preparations are commonly caused by large sample volumes, insufficient human resources, and limited equipment. A previous study by Novita and Yuliana (15) showed that wet mount prepared with a 30% eosin-glycerol under a cover glass kept their quality for up to 6 weeks at room temperature, but no storage period has been established for preparations using natural dyes. Our study therefore evaluated storage durations of 1, 2 and 3 weeks. Short initial periods (days to weeks) are commonly used in stability studies of natural bioactive compounds to detect the earliest and most pronounced changes in components such as flavonoids and anthocyanins and to establish a baseline before long-term evaluations (16). Glycerol is hygroscopic and helps prevent wet mounts from drying out (17). The edges of the cover glass were seal to reduce evaporation. Our study used 30% glycerol because glycerol-water mixtures at about 30% have been reported to extract polyphenols, flavonoids, and leaf pigments effectively. Glycerol also acts as a humectant and molecular protectant that helps maintain the stability of bioactive extracts and may extend the shelf life of natural dye reagents.

In resource-limited settings, particularly in primary healthcare centres and endemic rural areas, microscopic examination is often delayed by high sample volume, limited personnel, and restricted microscope access. The ability to store wet mounts for several weeks without loss of staining quality is therefore highly valuable, and a storage period of 1–3 weeks reflects realistic workflows in settings where re-preparing slides is not always feasible. Evidence-based storage limits could improve diagnostic efficiency, reduce reagent use, and repeated sample handling, and support more sustainable parasitological diagnostic.

We hypothesised that bioactive compounds in *T. grandis* leaf extract, such as anthocyanins and tannins, bind to components of the STH egg wall to produce contrasting, stable staining, and that 30% glycerol maintains moisture and limits pigment degradation, preserving egg clarity and morphology for up to three weeks. This study aimed to evaluate the staining quality of STH eggs in *T. grandis*–glycerol wet mount preparations and the effect of storage duration (1–3 weeks) on observation quality.

2. MATERIALS AND METHODS

2.1. Study Design and Ethics

This experimental study evaluated the staining quality and storage stability of wet mount preparations of soil-transmitted helminth (STH) eggs stained with a young teak (*T grandis* Linn. f.) leaf extract–glycerol solution. The study was conducted at the Department of Medical Biology, Universitas Riau, Indonesia, from 2024-2025. Preparations were stired for 1, 2, and 3 weeks and compared with a positive control (eosin-glycerol) and a negative control (distilled water-glycerol). The study was conducted in accordance with the ethical standards of the institutional research ethics committee and was

approved under ethical approval by the Health Research Ethics Committee of the Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Indonesia (Ethical Clearance Certificate No. 059/KE/06/2024).

Thirty pig farmers from Tiwir Village, Moyudan, Yogyakarta were recruited by purposive sampling. The inclusion criteria were active involvement in pig farming, residence in the study area, willingness to provide stool samples, and no anthelmintic treatment within the previous three months. All participants gave written informed consent after receiving an explanation of the study objectives, procedures, benefits and risks. They were assured that participation was voluntary and that their data would remain confidential.

Stool samples were collected from all consenting participants and screened microscopically by direct smear for STH eggs. Stool samples positive for STH were obtained from 30 samples of participants. The species identified were *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms. Positive samples were used to prepare the wet mounts. For each sample, approximately 0.2 g of stool was then emulsified in 1 mL of sterile physiological saline solution (0.9% NaCl) to obtain a homogeneous suspension.

2.2. Preparation of Materials

Young *T. grandis* L.f. buds, including lower parts of the bud (rolled or partially opened, yellow to reddish in colour), were collected from the campus area of Universitas Muhammadiyah Semarang, Semarang, Central Java, Indonesia. Botanical identification was performed by Mudyawati, Ph.D., Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta. A voucher specimen was deposited at the UGM Herbarium (accession no. UGM-Herbarium-2025-TG015).

The leaves were washed under running water, cut into small pieces, and ground with a mortar and pestle. Fifty grams of ground material was weighed on an analytical balance and macerated in 100 mL of 96% ethanol (50% w/v) in a closed glass container for 24 hours in a dark cabinet to minimize photodegradation. The mixture was filtered through Whatman filter paper using a funnel and the filtered was stored in a dark brown bottle until use.

2.3. Preparation of Staining Solutions

All solutions were prepared by mixing 3 mL of the aqueous or ethanolic component with 7 mL of glycerol (total volume 10 mL), giving 30% extract and 70% glycerol by volume (v/v). Therefore, the correct and precise designation of each solution is 1) Treatment: 30% (v/v) T. grandis extract in glycerol, prepared from the 50% (w/v) ethanolic extract; 2) Positive control: 30% (v/v) eosin–glycerol, prepared from commercial 2% eosin Y in water (C.I. 45380; Merck, Cat. No. 115935); and 3) Negative control: 30% (v/v) distilled water in glycerol.

The glycerol was analytical grade ((≥99.5%, Cat. No. 49770; Sigma-Aldrich, Merck, PT. Multi Anugerah Putra Mandiri, Jakarta, Indonesia), chosen to avoid contaminants that could interfered with the staining or degrade the natural pigments. All solutions were stored in amber bottles at room temperature, protected from light.

2.4. Preparation of Wet Mount Slides

One drop (about 50 µL) of staining solution was placed on a clean glass slide. A small amount of STH-positive stool suspension, collected with the tip of a wooden stick, was added and gently mixed. The mixture was covered with a coverslip, and additional coverslips were placed along the edges of the main coverslip to stabilise the preparation. Preparations were first examined under a light microscope (Model DM500, Leica, PT Biogen Scientific, Jakarta, Indonesia) with 10x and 40x objectives to confirm the presence of STH eggs. They were then stored in closed boxes at room temperature and evaluated at 1,2, and 3 weeks. The three groups were processed identically except for the staining solutions, a total of 90 wet mount preparations were prepared, with 30 independent replicates for each storage period (n = 30).

2.5. Process and Evaluation Criteria

Preparations were assessed macroscopically and microscopically (10× and 40× objectives). Two parasitology experts evaluated all preparations independently, blinded to the treatment groups (extract vs. control) and storage durations.

Staining quality was assessed using predefined macroscopic and microscopic criteria. Macroscopic evaluation assessed the physical condition of the mounting media, particularly its degree of dryness. A score of 1 was assigned when the preparation showed signs of drying or damage, and a score of 2 when it remained in good condition with no visible drying.

Microscopic evaluation assessed the clarity and contrast of staining of the helminth eggs, the background, and other visible components, using the following scale: A score of 1 indicates poor staining quality, which egg structure is unclear or invisible; background and other components more dominantly stained. A score of 2 indicates moderate staining quality, which eggs coloured, background faintly stained, and other components stained but not dominantly. A score of 3 indicates good staining quality, which egg structure clearly visible and distinct, background colourless or nearly so and components faintly stained.

Representative photomicrographs of each score level and staining group served as visual references. Inter-rater reliability was assessed with Cohen's Kappa (κ). The result was κ = 0.81, indicating almost perfect agreement according to Landis and Koch (Table 1).

2.6. Statistical Analysis

Scores were analysed as ordinal data. Changes in staining quality across storage times were analysed using the Friedman test. If the Friedman test showed a statistically significant difference, pairwise comparisons (week 1 vs. week 2, week 1 vs. week 3, and week 2 vs. week 3) were performed using the Wilcoxon signed-rank test with Bonferroni correction (adjusted α = 0.0167) to control the family-wise error rate. Differences in staining quality among the three staining groups at each storage time point were analysed using the Kruskal–Wallis test, followed by Dunn's post hoc test for pairwise comparisons. All statistical analyses were performed using (SPSS versi 26, R versi 4.4.2). The significance level was set at p-value < 0.05.

3. RESULTS AND DISCUSSION

The observation results showed that 30% *Tectona grandis* Linn. f. (teak) leaf extract–glycerol preparation stained soil-transmitted helminth (STH) eggs microscopically and macroscopically, did not dry out and remain stable up to 3 weeks.

3.1. Macroscopic Observations

Macroscopic observations were conducted to evaluate the physical condition and stability of *T. grandis* Linn. f.-glycerol wet mount preparations containing STH eggs across different storage periods. The assessment focused on the condition of the preparation medium, including the presence or absence of visible signs of drying or deterioration. A two-point scoring system was applied to assess the macroscopic condition of each preparation, with scores indicating whether the preparation showed signs of drying or remained in good physical condition. Each preparation was evaluated at predetermined storage intervals to identify changes in macroscopic stability over time. The results of the macroscopic observations are presented in Table 2. All preparations (100%) remained in good condition at every time point, with no drying, cracking, or colour change. Statistical analysis showed no significant difference across storage durations (p-value = 1.000).

3.2. Microscopic Observations

Microscopic observations were conducted to evaluate the staining quality and morphological preservation of STH eggs in *T. grandis* Linn. f.-glycerol wet mount preparations across different storage periods. The assessment focused on the clarity and visibility of the egg structures, the staining contrast between the eggs and the background, and the staining intensity of other visible components. The evaluation aimed to determine the consistency and effectiveness of staining over time using a three-point scoring system ranging from poor to very good staining quality. The results of the microscopic evaluation are presented in Figures 1–3. In the 30% *T. grandis* Linn. f.-glycerol group, 80% preparations were rated good and 20% very good in week 1. The proportion rated very good rose to 30% in week 2 and 60% in week 3 (Table 3).

Tabel 1. Reference scale by Landis and Koch

κ value	Results
< 0.00	Poor
0.00–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

Table 2. Results of macroscopic observations of wet mount leaf preparation of teak-glycerol 30%

Treatment	Week	Score 1 (%)	Score 2 (%)
Eosin - glycerol 30%	1	0	100
Eosin - glycerol 30%	2	0	100
Eosin - glycerol 30%	3	0	100
Aquades - glycerol 30%	1	0	100
Aquades - glycerol 30%	2	0	100
Aquades - glycerol 30%	3	0	100
Teak Leaf - glycerol 30%	1	0	100
Teak Leaf - glycerol 30%	2	0	100
Teak Leaf - glycerol 30%	3	0	100

Table 3. Results of macroscopic observations of wet mount leaf preparation of teak-glycerol 30%

	Week I			Week II			Week III		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Score 1	0%	100%	0%	0%	100%	0%	0%	100%	0%
Score 2	70%	0%	80%	60%	0%	70%	40%	0%	40%
Score 3	30%	0%	20%	40%	0%	30%	60%	0%	60%
Total	100%	100%	100%	100%	100%	100%	100%	100%	100%

Note: T1; Eosin-glycerol 30%. T2; Aquadest-glycerol 30%. T3; Teak Leaf-glycerol 30%.

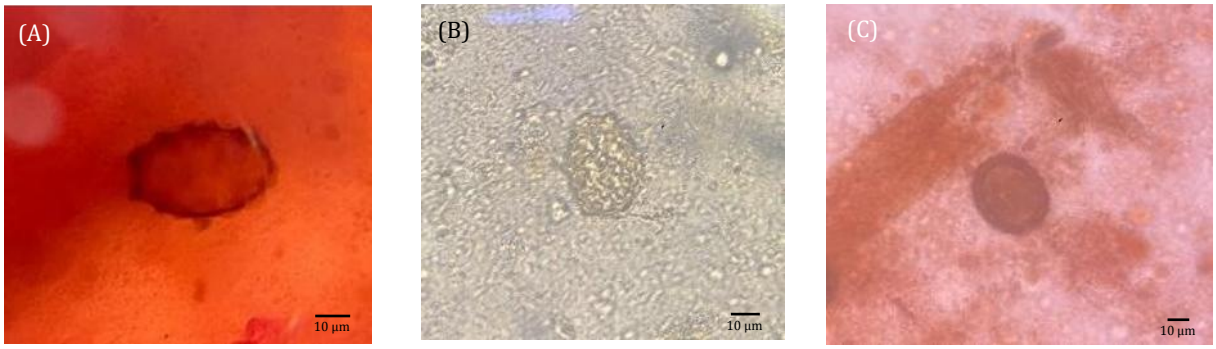


Figure 1. Eggs of *Ascaris* sp. with 40x objective lens enlargement stored for 1 week. (A) Eosin-glycerol 30%. (B) Aquades-glycerol 30%. (C) Leaf-glycerol 30%.

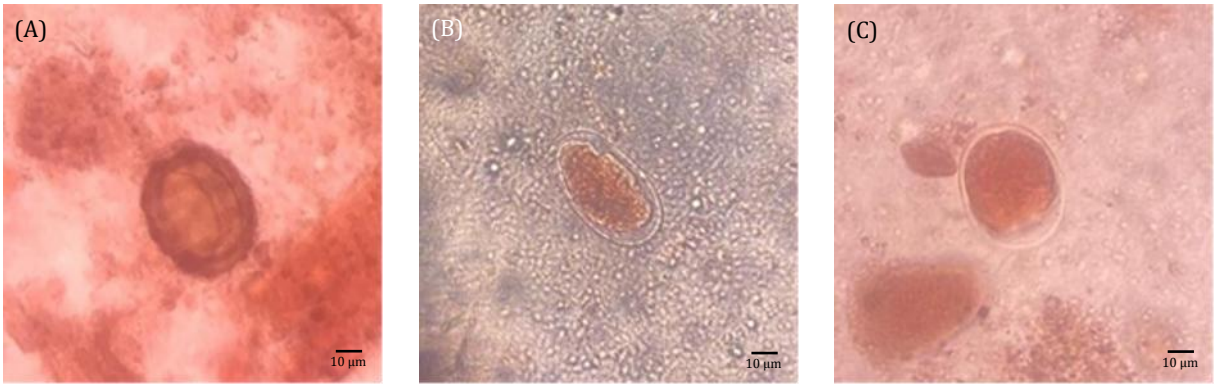


Figure 2. Eggs of *Ascaris* sp. with 40x objective lens enlargement stored for 2 weeks: (a) eosin-glycerol 30%, (b) aquades-glycerol 30%; (c) leaf-glycerol 30%.

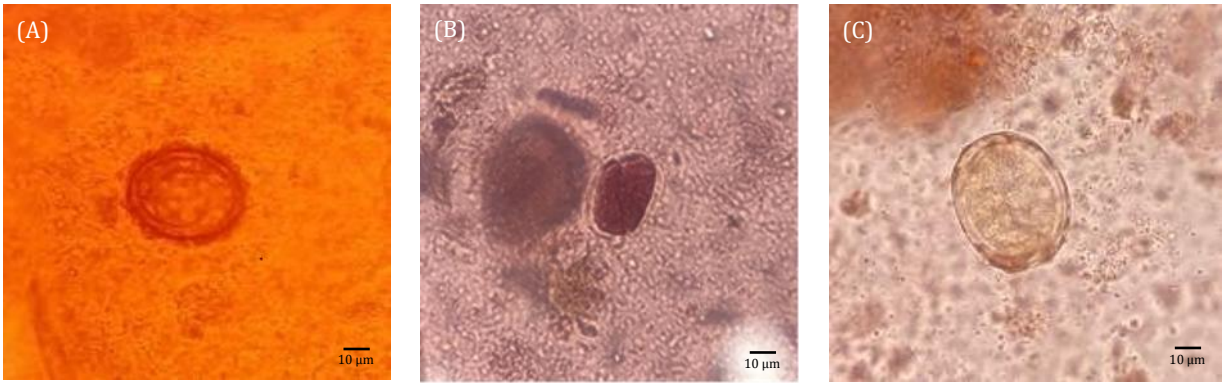


Figure 3. Eggs of *Ascaris* sp. with 40x objective lens enlargement stored for 3 weeks. (A) eosin-glycerol 30%. (B) Aquades-glycerol 30%. (C) Leaf-glycerol 30%

In week 1, the eggs were visible but weakly stained and their morphology was less distinct. The background and stool debris were more prominent, so contrast was low and some eggs were hard to distinguish from debris. By week 2, the eggs were more distinctly coloured and the background less prominent, giving better contrast and clearer morphological features. By week 3, the eggs were most intensely and distinctly stained against a relatively faint background. The characteristic structures were clearly visible: the morula of hookworm eggs, the albuminoid and hyaline layers of *Ascaris lumbricoides* eggs, and the outer layer of *Trichuris trichiura* eggs.

In the positive control (30% eosin–glycerol), quality improved over time but remained lower. In week 1, 100% of preparations were rated poor. In week 2, 70% remained poor and 30% were good. In week 3, 80% were good. The improvement was associated with reduced background staining and clearer egg structures. The negative control (glycerol without dye) was rated poor (score 1) throughout, because without a staining agent the eggs remained transparent and were difficult to distinguish from stool material

The Friedman test showed a significant difference in microscopic staining quality across the three storage durations ($\chi^2(2) = 10.24$, p-value = 0.006), with mean ranks increasing from week 1 to week 3. Post-hoc Wilcoxon signed-rank test with Bonferroni correction showed a significant difference only between week 1 and week 3 (adjusted p-value = 0.015); week 1 vs. week 2 and week 2 vs. week 3 were not significant. Thus, staining quality significantly was macroscopically stable but microscopically improved significantly after three weeks of storage.

This study investigated whether *T. grandis* Linn. f. leaf extract can serve as an alternative natural dye for STH wet mount preparations and how storage time affects preparation quality. Previous work (18) showed that this extract can replace 2% eosin in direct examination, but its use in wet mounts and its optimal storage period had not been examined. Unlike studies that evaluated staining only immediately after slide preparation, the present study examined how staining changes with storage. This matters in practice because slides are often stored for re-examination or documentation, and color stability affects diagnostic quality.

All preparations remained in good condition for three weeks. This is consistent with glycerol's hygroscopic properties: it retains moisture, limits evaporation, and its viscosity holds the specimen in position (19). Drying can produce artefacts that interfere with examination, so the combination of extract and glycerol maintained the physical integrity of the specimens. Because macroscopic quality did not vary, glycerol appears to act mainly as a stabiliser and does not itself determine staining quality, as the unstained negative control confirms.

Microscopic quality improved with storage rather than deteriorating. The proportion of "very good" preparations rose from 20% to 30% to 60% over weeks 1–3. This pattern may reflect the time needed for natural dye compounds to diffuse into, and be retained by, the egg structures. Early on, staining may not yet have reached optimal intensity. With longer contact between dye, glycerol, and eggs, coloration strengthened, and the background became relatively less prominent, improving contrast.

Stronger staining does not automatically mean better quality, since excessive staining can reduce contrast between eggs and background. In this study, however, the increase remained within a range that supported egg identification up to week 3. The significant week 1–week 3 difference, without a significant difference between adjacent weeks, suggests gradual accumulation of staining rather than an abrupt change. Staining varied with egg type, which may reflect differences in the structure and permeability of the egg walls. The dye highlighted the morula in hookworm eggs, the albuminoid and hyaline layers in *A. lumbricoides*, and the outer layer in *T. trichiura*. This matter because identification depends on characteristic morphology as well as presence (20).

The extract was prepared by macerating young teak leaf buds in 96% methanol (50 g in 100 mL). Young leaves were chosen for their high content of anthocyanins and tannins, which can bind proteins and cell wall components (21,22). The staining probably results from several constituents acting together rather than from one pigment. Teak leaves also contain flavonoids, anthraquinones, naphthoquinones, and phenolic acids (e.g., gallic, vanillic, ferulic, chlorogenic, sinapic, p-coumaric, and cinnamic acids) (23,24). Quinones isolated from *T. grandis* (tectoleafquinone, anthratectone, tectoquinone, tectograndone) have been associated with red, orange, yellow, and reddish-brown colouration. Their conjugated structures absorb visible light. During storage, oxidation of phenolics and flavonoids may produce additional coloured quinone derivatives, which could explain the increasing red colouration (23). Tannins may further enhance retention of colored compounds on egg structures through hydrogen bonding and hydrophobic interactions with proteins, and glycerol may help protect these compounds from degradation. However, because chromatographic and spectrophotometric characterization of the extract was not performed, the contribution of individual compounds cannot be determined, and this mechanism remains hypothetical (25).

The positive control (30% eosin–glycerol) exhibited a more reddish background, which may have interfered with microscopic observation. This finding highlights the importance of staining characteristics in maintaining background clarity during microscopic examination. In contrast, the T. grandis preparations exhibited clearer backgrounds and higher staining quality scores over the storage period. These findings suggest that the teak leaf extract–glycerol solution may offer advantages in maintaining microscopic clarity during storage. Differences between our findings and previous reports may be attributable to variations in preparation techniques, smear thickness, dominant STH species, and storage conditions, including temperature and humidity.

3.3. Limitation and Implications

The sample size was small (n = 10), limiting generalisability. Only one extract concentration (30%) was tested, so no dose–response relationship could be assessed. Storage was at room temperature only, and storage durations were limited to 1–3 weeks. Variation in the source and condition of the teak leaves may affect extract consistency. The extract was not

chemically characterised. Despite these limitations, the study suggests that *T. grandis*–glycerol is an environmentally friendly, low-cost alternative to eosin, particularly useful for resource-limited laboratories where slide reading is often delayed.

4. CONCLUSIONS

Wet mount preparations stained with 30% *T. grandis* Linn. f. extract-glycerol showed no significant macroscopic differences across 1,2 and 3 weeks of storage, but showed significant microscopic differences: staining quality of the STH eggs improved with longer storage (significant between weeks 1 and 3). The extract-glycerol combination is therefore an effective, physically stable natural staining medium for STH wet mounts. Further research should examine different glycerol and extract comparison with other natural or synthetic dyes, chemical stability of the extract during storage, toxicity and safety, alternative storage media, longer storage period and varied environmental conditions.

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REFERENCES

1. Holland C, Sepidarkish M, Deslyper G, Abdollahi A, Valizadeh S, Mollalo A, Mahjour S, Ghodsian S, Ardekani A, Behniafar H, Gasser RB, Rostami A. Global prevalence of Ascaris infection in humans (2010–2021): A systematic review and meta-analysis. Infect Dis Poverty. 2022;11(1): 113. https://doi.org/10.1186/s40249-022-01038-z
2. Andargie Y, Alelign A, Tekeste Z. Prevalence and associated risk factors of soil-transmitted helminth infections among schoolchildren in Mekan Eyesus town, northwestern Ethiopia. Parasite Epidemiol Control. 2024;27:e00379. https://doi.org/10.1016/j.parepi.2024.e00379
3. Dana D, Vlaminck J, Mekonnen Z, Ayana M, Vogt F, Verdonck K, Tweya H, Timire C, Geldhof P, Levecke B. Diagnostic sensitivity of direct wet mount microscopy for soil-transmitted helminth infections in Jimma Town, Ethiopia. J Infect Dev Ctries. 2020;14(61):66S–71S. https://doi.org/10.3855/jidc.11733
4. Aulia K, Sitepu R. Comparison of feat examination results using direct slide and sedimentation methods for detecting soil transmidet helmint (STH) worm infection in primary school students. Medistra Med J. 2023;1(1):1–6. https://doi.org/10.35451/mmj.v1i1.1937
5. Lebu S, Kibone W, Muoghalu CC, Ochaya S, Salzberg A, Bongomin F, Manga M. Soil-transmitted helminths: A critical review of the impact of co-infections and implications for control and elimination. PLoS Negl Trop Dis. 2023;17(8):e0011496. http://dx.doi.org/10.1371/journal.pntd.0011496
6. Ngwese MM, Manouana GP, Moure PAN, Ramharter M, Esen M, Adégnika AA. Diagnostic techniques of s oil-transmitted helminths: Impact on control measures. Trop Med Infect Dis. 2020;5(2):93. https://doi.org/10.3390/tropicalmed5020093
7. Adhikary K, Ganguly K, Maity M. General microbiology and parasitology, virology and mycology with lab manual. First Edition. India: Taurean Publications; 2025. https://www.researchgate.net/publication/394023558_General_Microbiology_and_Parasitology_Virology_and_Mycology_With_Lab_Manual
8. Zaman RF, Khanum H, Nargis S, Das PK. Comparison of saline, iodine and koh wet mount preparations for occurrence of parasites in stool samples from patients attending ICDDR,B. Bangladesh J Zool. 2018;45(2):159–70. https://doi.org/10.3329/bjz.v45i2.35711
9. Suryanti V, Kusumaningsih T, Marliyana SD, Setyono HA, Trisnawati EW. Identification of active compounds and antioxidant activity of teak (*Tectona grandis*) leaves. Biodiversitas. 2020;21(3):946–52. https://doi.org/10.13057/biodiv/d210313
10. Kolli PK, Obbalareddy S, Yejella RP, Athili LD, Ponnada S. A review on tectona grandis. Int J Res Pharm Chem. 2022;12(3):39–39. https://doi.org/10.33289/IJRPC.12.3.2022.12(39)
11. Laohaphatanalert K, Gavinlertvatana P, Apisittiwong T, Boonsiriwit A. Potentiality of using teak (*Tectona grandis*) leaf to produce antioxidant herbal tea. Pakistan J Life Soc Sci. 2024;22(2):3165–73. https://doi.org/10.57239/PJLSS-2024-22.2.00231
12. Novita I, Yuliana L. Utilization of natural dyes solutions and glycerol for the quality and durability of direct wet mount preparations storage in educational laboratories. 2022;4(2):50–7. https://doi.org/10.35916/thmr.v4i1.65
13. Nurtiana W. Anthocyanin as natural colorant : A review. 2019;1(1):1-7. https://dx.doi.org/10.33512/fsj.v1i1.6180

14. Warnasih S, Hasanah U. Phytochemical characterization and tannin stability test from kluwek (*Pangium edule* Reinw). 2018;1(2):44–9. https://doi.org/10.33751/jsi.v1i02.1000

15. Novita I, Yuliana L. Differences in techniques and mounting solutions for wet mount preparations in the production of preserved specimens in educational laboratories. J Labora Med. 2023;7(1):1-5. https://doi.org/10.26714/jlabmed.7.1.2023.1-5

16. Cahuantzi V, Herrera RP, Verónica N, Alonso Z, Leza DS, Mendoza MSB, Pérez E. Freeze-drying as a stabilization strategy for natural dyes derived from *Lawsonia inermis* L. and *Indigofera suffruticosa*. Analytica. 2025;6(3):1–29. https://doi.org/10.3390/analytica6030022

17. Kaewsangsai S, Panyadee P, Panya A, Pandith H, Wangpakapattanawong P, Balslev H, Inta A. Ethnobotany, cytotoxicity and color stability of karen natural colorants. Plats. 2025;14(9):1348. https://doi.org/10.3390/plants14091348

18. Rumicha TD, Hirko F, Fikreyesus S, Yetenayet F, Tola B. Drying temperature and storage duration for the retention of bioactive compounds of selected wild edible plants from ethiopia. 2025;13(8):e70782. https://doi.org/10.1002/fsn3.70782

19. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2095–128. https://doi.org/10.1016/S0140-6736(12)61728-0

20. Soenjono S, Setiawan B, Wulandari RS, Suyana S, Martiningsih MA. Synthetic food coloring as an alternative to worm egg staining *Ascaris lumbricoides*. 2021;9(B):1541–3. https://doi.org/10.3889/oamjms.2021.7495

21. Rungruangkitkrai N, Mongkholrattanasit R, Ounu P, Chatvivatpornchai N, Boonyarit J, Laohaphatanaleart K, Chollakup R. Current research in green and sustainable chemistry enhancing sustainable silk textiles : Optimization of teak leaf extract dyeing and antibacterial efficacy. Curr Res Green Sustain Chem. 2025;10(April):100457. https://doi.org/10.1016/j.crgsc.2025.100457

22. Wang J, Zhao Y, Sun B, Yang Y, Wang S, Feng Z, Li J. The structure of anthocyanins and the copigmentation by common micromolecular copigments: A review. Food Res Int. 2024:176:113837. https://doi.org/10.1016/j.foodres.2023.113837

23. Asarina S, Haeruni N. Evaluation of glycerol use in the preparation of semi-permanent helminth egg mounts. J Pengelolaan Lab Pendidik. 2019;1(2):37–40. https://doi.org/10.14710/jplp.1.2.37-40

24. Leischner U, Reifarth M, Brill MS, Schmitt F, Hoeppener S, Jess DU. PRIAMOS : A technique for mixing embedding media for freely adjusting pH value and refractive index (RI) for optical clearing (OC) of whole tissue samples. J Microsc. 2025;300(3):302-314. https://doi.org/10.1111/jmi.70022

25. Smith-togobo C, Fatau AA, Kpor F, Simpong DL, Yiadom GO, Asiamah EA. *Tectona grandis* (teak tree) young leaf extract as a histological stain. 2023;16(2):17–41. https://doi.org/10.4236/jbise.2023.162002