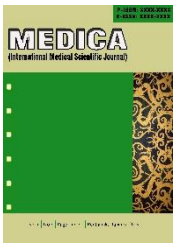
MEDICA

(International Medical Scientific Journal)
Vol.7, No.2, May 2025, pp. 67 - 74
ISSN 2622-660X (Online), ISSN 2622-6596 (Print)
https://journal.ahmareduc.or.id/index.php/medica



The Effect of Different Soaking Times of Gambir (*Uncaria gambir (Hunter) Roxb.*) on the Examination of *Ascaris lumbricoides* Eggs as an Alternative Stain to Eosin

Tantri Rahmatika[1]✉, Ari Nuswantoro[1], Laila Kamilla[1]

[1] Department of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Pontianak, Pontianak, West Kalimantan, Indonesia

Info Article

Article History:
Received:
15 May 2025
Accepted:
29 May 2025
Published:
31 May 2025

Keywords:
Ascaris lumbricoides eggs
Eosin
Gambir

Abstract

Ascaris lumbricoides is a type of helminth that can infect humans. One laboratory method for its examination is the use of 2% eosin staining with the direct slide method. However, eosin poses potential hazards to both the environment and human health, thus prompting the need for an alternative, eco-friendly staining agent. Gambir (*Uncaria gambir (Hunter) Roxb.*) contains red catechu, which imparts a red coloration to solutions. This study aims to evaluate the staining differences based on varying soaking times of Gambir extract for staining *Ascaris lumbricoides* eggs as a natural alternative to eosin. This research is a quasi-experimental study using purposive sampling. The Gambir samples were soaked in 96% ethanol for 1 hour, 1 hour 30 minutes, and 2 hours. Each treatment was replicated 9 times, resulting in a total of 27 samples. The staining was conducted using the direct slide method to examine *Ascaris lumbricoides* eggs. The results showed that Gambir soaked for 1 hour yielded a staining quality of 41.67%, 1 hour 30 minutes resulted in 68.51%, and 2 hours resulted in 87.96%, while the 2% eosin control achieved a staining quality of 100%. Based on the results of this study on the variation in soaking time of gambier (Uncaria gambir (Hunter) Roxb.) as an alternative dye to eosin in the examination of Ascaris lumbricoides eggs, it can be concluded that the staining success rate increases with longer soaking durations.

Corresponding Author:
✉ Tantri Rahmatika
Department of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Pontianak, Pontianak, West Kalimantan, Indonesia
Email: tantri.rahmatika03@gmail.com

1. INTRODUCTION

Soil-Transmitted Helminths (STH) are nematode worms whose transmission occurs through soil. The most common STHs that infect humans are *Ascaris lumbricoides* (roundworm), Trichuris trichiura (whipworm), and hookworms such as Necator americanus and Ancylostoma duodenale (Ramayanti, 2018). *Ascaris lumbricoides* is a type of worm that belongs to the STH group. This parasite can infect humans and cause a disease known as ascariasis.

Ascaris lumbricoides lives in the human small intestine and can affect digestion, nutrient absorption, and metabolism, ultimately leading to malnutrition in affected individuals (Masangcay et al.,, 2021; Fauziah, et al., 2022; Gabain, Ramsteijn, & Webster, 2023). Humans are the only host for Ascaris lumbricoides, and the disease it causes is called ascariasis. This parasite can infect both adults and children, but it is more commonly found in children due to their lack of understanding of transmission pathways, poor personal hygiene habits such as not washing hands before eating, consuming unclean food and drinks, and defecating in open areas. This infection is usually not acute and often goes unnoticed, allowing the parasite to safely live in the human body. However, if left untreated, the parasite can continue to grow and may cause serious health issues, potentially leading to death (Sihombing, & Gultom, 2018).

Infection with *Ascaris lumbricoides* can be diagnosed using direct smear examination, which aims to detect the presence of worm eggs in feces using 2% eosin solution and a cover glass. The use of 2% eosin helps to distinguish worm eggs from fecal matter by providing a red background that contrasts with the yellowish appearance of the eggs (Artanti, Sari, & Ariana, 2020). However, eosin is non-biodegradable and generates hazardous waste, necessitating the need for an environmentally friendly alternative staining agent (Suraini & Sophia, 2022).

Eosin is highly toxic and dangerous, especially when it contaminates water, making the water unfit for use. Eosin contains aromatic rings that make it resistant to degradation, thus being highly carcinogenic and harmful to human health, potentially damaging the digestive tract, liver, kidneys, and lungs, and causing redness, swelling, and pain, and it is particularly dangerous if it comes into contact with the cornea (Bukhari et al., 2022). One plant that can be used as an alternative stain for *Ascaris lumbricoides* egg staining is gambir, which contains red catechu, a sap that gives the red color to gambir derived from tannins and catechins (Firdausni, Yeni, & Failisnur, 2019).

Gambir is a semi-processed product made from extracts of the leaves and twigs of the Uncaria gambir Roxb. plant. The extraction process involves heating through boiling, pressing, precipitation, draining, molding, and drying (Sofyan & Failisnur, 2016). There are two types of gambir products: raw gambir (crude gambir) and processed gambir (with high catechin content) (Suharman, 2018). The color produced comes from the tannins and catechins in gambir; the tannin component in gambir is in the form of catechu-tannic acid, a flavonoid compound that acts as a dye (Firdausni, Yeni, & Failisnur, 2019).

The condensed tannin pigments are responsible for producing the red color (Wulansari et al., 2012). Catechins are flavonoid compounds, classified as secondary metabolites and polyphenolic antioxidants, which are evenly distributed in plant tissues (Zainal et al., 2022). In both water and ethanol solvents, gambir appears brown due to the catechin compounds (Latirah, Indrawati, & Purba, 2015). Traditionally, gambir is used as a complement to betel chewing and as a medicinal product (Dhalimi, 2006).

In West Kalimantan, particularly in Seluan Village, North Putussibau District, Kapuas Hulu Regency, gambir has been traditionally used by the lower-middle-income community as a remedy for chickenpox (Varicella) and this practice continues to this day

(Supraningsih, et al., 2024). Gambir is also used in the textile and batik industries as a dye resistant to sunlight, as well as in the leather tanning industry to prevent decomposition. In the cosmetics industry, gambir is utilized as a raw material to produce astringents and lotions that soften the skin, improve skin elasticity, and enhance skin tone (Dhalimi, 2006). This study aims to evaluate the staining differences based on varying soaking times of Gambir extract for staining *Ascaris lumbricoides* eggs as a natural alternative to eosin.

2. METHOD

This study employed a quasi-experimental research design. This design is a development of the true experimental design, which is often difficult to implement in practice. While the quasi-experimental design includes a control group, it does not fully control for external variables that may influence the implementation of the experiment. This design was chosen due to the practical difficulty of obtaining a true control group for research purposes (Sugiyono, 2013).

The population of this study consisted of gambir obtained from Beringin Market in Singkawang, totaling 1 kilogram of gambir product. The research samples were gambir extracts soaked for 1 hour, 1 hour and 30 minutes, and 2 hours. The type of data used in this study was primary data. Primary data were obtained from the examination of *Ascaris lumbricoides* eggs that had been stained with gambir solution for 1 hour, 1 hour and 30 minutes, and 2 hours. Data collection was conducted through observation, which involves gathering factual information about the real world through direct observation. The instruments used in this study included scoring sheets and a timer.

Data processing was carried out after data collection. At this stage, raw data collected from observations were processed and analyzed to produce meaningful information (Masturoh & Anggita, 2018). The data consisted of information on whether the egg preparations were stained or unstained by the gambir soaking treatments. The results of the specimens were then observed and the data analyzed using percentage-based statistical analysis.

3. RESULTS AND DISCUSSION

The study on the effect of varying soaking durations of gambir using 96% alcohol as a solvent—specifically 1 hour, 1 hour and 30 minutes, and 2 hours—for staining *Ascaris lumbricoides* eggs as an alternative dye to eosin was conducted at the Integrated Parasitology Laboratory, Medical Laboratory Technology Department, Poltekkes Kemenkes Pontianak. A total of 27 samples were treated in this study. The data obtained from each treatment group are presented in the table and calculations below.

Table 1. Results of the Gambir Soaking Stain Study

Sample Code	Microscopic Examination				2% Eosin Control
	Contrast (1–3)	Background (1–3)	Homogeneity (1–3)	Egg Shell Layer (1–3)	
A1	1	1	1	2	3
A2	1	1	1	1	3
A3	1	1	1	3	3
A4	1	1	1	1	3
A5	1	1	1	3	3
A6	1	1	1	2	3
A7	1	1	1	2	3

A8	1	1	1	2	3
A9	1	1	2	1	3
Total A	9	9	10	17	27
B1	2	2	2	2	3
B2	2	2	2	3	3
B3	2	2	2	2	3
B4	1	1	1	2	3
B5	2	2	2	1	3
B6	3	3	2	3	3
B7	2	2	2	2	3
B8	2	2	2	3	3
B9	2	2	2	3	3
Total B	18	18	17	21	27
C1	3	3	3	2	3
C2	3	3	3	2	3
C3	3	3	2	2	3
C4	3	3	2	2	3
C5	3	3	3	2	3
C6	3	3	3	1	3
C7	3	3	3	3	3
C8	3	3	3	2	3
C9	3	2	2	2	3
Total C	27	26	24	18	27

Explanation:
Gambir soaking with 96% alcohol for 1 hour: A
Gambir soaking with 96% alcohol for 1 hour and 30 minutes: B
Gambir soaking with 96% alcohol for 2 hours: C
$\frac{f}{N}$ *x 100%*
f = Frequency
N = number of sample

Percent A:

Contrast A $\frac{f}{N}$ x 100% = $\frac{9}{27}$ x 100% = 33,33%

Background A $\frac{f}{N}$ x 100% = $\frac{9}{27}$ x 100% = 33,33%

Homogeneity A $\frac{f}{N}$ x 100% = $\frac{10}{27}$ x 100% = 37,03%

Egg shell layer A $\frac{f}{N}$ x 100% = $\frac{17}{27}$ x 100% = 62,96%

$\sum$ A = $\frac{33{,}33 + 33{,}33 + 37{,}03 + 62{,}96}{4}$ = 41,67%

Percent B:

Contrast B $\frac{f}{N}$ x 100% = $\frac{18}{27}$ x 100% = 66,66%

Background B $\frac{f}{N}$ x 100% = $\frac{18}{27}$ x 100% = 66,66%

Homogeneity B $\frac{f}{N}$ x 100% = $\frac{17}{27}$ x 100% = 62,96%

Egg shell layer B $\frac{f}{N}$ x 100% = $\frac{21}{27}$ x 100% = 77,77%

$\sum$ B = $\frac{66,66 + 66,66 + 62,96 + 77,77}{4}$ = 68,51%

Percent C:

Contrast C $\frac{f}{N}$ x 100% = $\frac{27}{27}$ x 100% = 100%

Background C $\frac{f}{N}$ x 100% = $\frac{26}{27}$ x 100% = 96,29%

Homogeneity C $\frac{f}{N}$ x 100% = $\frac{24}{27}$ x 100% = 88,88%

Egg shell layer C $\frac{f}{N}$ x 100% = $\frac{18}{27}$ x 100% = 66,66%

$\sum$ C = $\frac{100 + 96,29 + 88,88 + 66,66}{4}$ = 87,95%

Based on table 1 and the calculations, the results of microscopic observations showed that the percentage of stained *Ascaris lumbricoides* egg preparations varied according to the soaking duration. The percentage of staining observed at 1 hour was 41.67%, at 1 hour and 30 minutes was 68.51%, and at 2 hours was 87.95%, compared to the 2% eosin control group which showed a staining percentage of 100%.

DISCUSSION

Based on the table and percentage calculations regarding the different soaking durations of *Uncaria gambir (Hunter) Roxb*.) using 96% alcohol as a solvent to replace 2% eosin—at intervals of 1 hour, 1 hour and 30 minutes, and 2 hours—for staining *Ascaris lumbricoides* eggs, the resulting percentages of successful staining were 41.67% for 1 hour, 68.51% for 1 hour and 30 minutes, and 87.95% for 2 hours. In comparison, the 2% eosin control group achieved a 100% staining rate. These findings indicate that the 2-hour gambir soaking duration yielded a higher staining effectiveness compared to the shorter durations. This suggests that the longer the soaking time, the more intense the resulting coloration. A higher staining percentage reflects better staining quality in terms of contrast, background, homogeneity, and visibility of the egg shell layer under microscopic observation.

Ascaris lumbricoides eggs are classified into three types: fertilized, unfertilized, and decorticated eggs. Fertilized eggs possess an outer albuminoid layer characterized by a brown, coarse, and mammillated surface, followed by a thick, transparent hyaline layer, and an inner vitelline membrane that is thin yet strong. Unfertilized eggs are typically longer and more slender than fertilized eggs. Decorticated eggs are fertilized eggs that have lost their cortical layer, leaving a smooth and transparent outer surface. The absorption of stain differs across these layers due to structural differences. The albuminoid layer, for instance, tends to retain a darker color compared to the hyaline layer (Khasanah et al., 2023).

In this study, staining with gambir solution for 1 hour generally produced a yellowish color on the contrast and background, with poor homogeneity, resulting in uneven staining across the specimen. Staining with a 1 hour and 30 minute soaking duration produced similar results but with a slightly more orange-red hue and slightly improved distribution. The best staining quality was observed in the 2-hour soaking group, where most samples exhibited a red coloration comparable to that achieved with 2% eosin, with good contrast, background clarity, and generally even homogeneity throughout the slide. Poor contrast, background staining, and homogeneity in shorter soaking durations may be attributed to insufficient pigment extraction. Additionally, low homogeneity could result from inadequate mixing of the fecal suspension and staining solution.

In this study, 2% eosin served as the control dye in the direct smear method, which involves adding eosin to the fecal sample to better distinguish parasitic eggs from surrounding debris by providing a red background (Nurfadillah, Ridwan, & Arwie, 2021). Eosin is commonly used in microscopic examination of protozoa and helminth eggs and also serves as a fecal diluent. The use of eosin enhances the visibility of parasite eggs in fecal matter (Maulida, 2016). Eosin is a synthetic xanthene dye that is reddish in color, acidic, and negatively charged, allowing it to easily bind to basic proteins (Khasanah et al., 2023). The results of this study confirmed that 2% eosin yielded the highest staining quality in all assessed aspects—contrast, background, homogeneity, and egg shell layer visualization—compared to the tested gambir staining solutions. Fungal elements and debris observed during staining were distinguishable from helminth eggs and could have originated either from the fecal sample or from contamination on the cover glass.

Gambir is a plant-derived substance that can be used as an alternative dye for staining *Ascaris lumbricoides* eggs. It contains red catechu, a reddish pigment derived from tannins and catechins (Firdausni, Yeni, & Failisnur, 2019). Gambir is a semi-processed product made from the extract of leaves and twigs of the Uncaria gambir Roxb. plant. The extraction process involves boiling, pressing, precipitation, draining, molding, and drying (Sofyan & Failisnur, 2016). The red coloration produced by gambir originates from tannins and catechins, specifically in the form of catechu-tannic acid, a flavonoid compound responsible for its dyeing properties (Firdausni, Yeni, & Failisnur, 2019).

In this study, 96% alcohol was used as the solvent for gambir powder. This choice was based on preliminary tests showing that gambir is soluble in alcohol. Although alcohol is an effective solvent for extracting coloring agents, it is highly volatile (Walidah, Supriyanta, & Sujono, 2014). Therefore, the soaking solution must be stored in a closed container and used promptly to prevent evaporation, which could otherwise lead to concentration and thickening of the solution.

4. CONCLUSION

Based on the results of this study on the variation in soaking time of gambier (Uncaria gambir (Hunter) Roxb.) as an alternative dye to eosin in the examination of Ascaris lumbricoides eggs, it can be concluded that the staining success rate increases with longer soaking durations. Specifically, gambir soaking for 1 hour resulted in a staining percentage of 41.67%, 1 hour and 30 minutes yielded 68.51%, and 2 hours achieved 87.96%. In comparison, the control using 2% eosin achieved a 100% staining success rate. For future research, it is recommended to explore other plant-based materials (simplisia) with more defined dye content as the primary component, in order to further advance scientific knowledge in developing alternative dyes for helminth egg staining to replace eosin.

REFERENCES

Artanti, D., Sari, Y. E. S., & Ariana, D. (2020). Perbedaan Kualitas Preparat Telur Cacing Gelang (Ascaris lumbricoides, Linn) Menggunakan Rendaman Batang Pohon Jati Dan Kuncup Daun Jati. *Seminar Nasional Kesehatan Prodi DIII Teknologi Laboratorium Medik STIKES Rumah Sakit Anwar Medika, 1,* 21-30. Retrieved from: *https://repository.um-surabaya.ac.id/7328/*

Bukhari, A., Ijaz, I., Zain, H., Gilani, E., Nazir, A., Bukhari, A., ... & Iram, S. (2022). Removal of Eosin dye from simulated media onto lemon peel-based low cost biosorbent. *Arabian Journal of Chemistry*, 15(7), 103873. https://doi.org/10.1016/j.arabjc.2022.103873

Dhalimi, A. (2006). Permasalahan gambir (Uncaria gambir L.) di Sumatera Barat dan

alternatif pemecahannya. *Perspektif: Review Penelitian Tanaman Industri*, 5(1), 46-59.
Fauziah, N., Aviani, J. K., Agrianfanny, Y. N., & Fatimah, S. N. (2022). Intestinal parasitic infection and nutritional status in children under five years old: a systematic review. Tropical Medicine and Infectious Disease, 7(11), 371. https://doi.org/10.3390/tropicalmed7110371
Firdausni, F., Yeni, G., Failisnur, F., & Kamsina, K. (2019). Karakteristik pewarna alam gambir (Uncaria gambir Roxb) untuk produk pangan. *Indonesian Journal of Industrial Research,* 9(2), 89-96.
Gabain, I. L., Ramsteijn, A. S., & Webster, J. P. (2023). Parasites and childhood stunting–a mechanistic interplay with nutrition, anaemia, gut health, microbiota, and epigenetics. Trends in Parasitology, 39(3), 167-180. https://doi.org/10.1016/j.pt.2022.12.004
Masturoh, I., & Anggita, N. (2018). *Metodologi penelitian kesehatan*. Jakarta: Pusat Pendidikan Sumber Daya Manusia Kesehatan.
Latirah, L., Indrawati, T., & Purba, A. V. (2015). Hair Coloring Development of Extract Gambier (Uncaria gambir Roxb.) in Viscous Liquid. *Jurnal Ilmu Kefarmasian Indonesia,* 13(1), 89-93. Retrieved from: http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/130
Khasanah, N. A. H., Husen, F., Yuniati, N. I., & Rudatiningtyas, U. F. (2023). Kualitas Rendaman Simplisia Rimpang Kunyit (Curcuma longa) Sebagai Pewarna Alternatif Telur Ascaris lumbricoides. *Jurnal Bina Cipta Husada: Jurnal Kesehatan Dan Science*, 19(2), 54-62. Retrieved from: http://jurnal.stikesbch.ac.id/index.php/jurnal/article/view/109
Masangcay, D. U., Amado, A. J. Y., Bulalas, A. R., Ciudadano, S. R., Fernandez, J. D., Sastrillo, S. M., & Mabaggu, R. M. (2021). Association of soil-transmitted helminth infection and micronutrient malnutrition: A narrative review. Asian Journal of Biological and Life Sciences, 10(2), 317-324.
Maulida, A. (2016). Perbedaan Kualitas Sediaan Telur Cacing Gelang (Ascaris Lumbricoides, Linnaeus 1758) Menggunakan Pewarnaan Eosin dan Pewarnaan Giemsa. *Skripsi.* Universitas Muhammadiyah Semarang.
Ramayanti, I. (2018). Prevalensi Infeksi Soil Transmitted Helminths pada Siswa Madrasah Ibtidaiyah Ittihadiyah Kecamatan Gandus Kota Palembang. *Syifa'Medika*, 8(2), 102-107.
Nurfadillah, N., Ridwan, A., & Arwie, D. (2021). Identifikasi Soil Transmitted Helminth (STH) Anak Usia 7-10 Tahun Menggunakan Sampel Feses Metode Natif. *Jurnal TLM Blood Smear*, 2(2), 54-59. Retrieved from: http://ojs.stikespanritahusada.ac.id/index.php/tlmbs/article/view/280
Sihombing, J. R., & Gultom, E. (2018). Analisa telur cacing ascaris lumbricoides pada faeces anak usia 4-6 tahun di tk nurul hasanah walbarokah â¬(nhw) marelan tahun 2018. *Jurnal Analis Laboratorium Medik*, 3(1). Retrieved from: https://e-journal.sari-mutiara.ac.id/index.php/ALM/article/view/807
Sofyan, S., & Failisnur, F. (2016). Gambir (Uncaria gambir Roxb) sebagai pewarna alam kain batik sutera, katun, dan rayon. *Jurnal Litbang Industri*, 6(2), 89-98.
Sugiyono, D. (2013). *Metode penelitian pendidikan pendekatan kuantitatif, kualitatif dan R&D*. Bandung: CV. Alfabeta.
Suharman, S. (2018). *GAMBIR; Peluang Pasar, Budidaya, dan Pengolahannya*. Yogyakarta: CV. Budi Utama
Suraini, S., & Sophia, A. S. (2022). Optimasi air perasan ubi jalar ungu (Ipomea batatas L.) pada pemeriksaan telur cacing. *Bioma: Jurnal Biologi Makassar,* 7(2), 8-13. Retrieved from: http://journal.unhas.ac.id/index.php/bioma/article/view/21212
Supraningsih, A., Maria, A., Hendra, L., & Gandasari, A. (2024). Kearifan Lokal Pemanfaatan

Gambir Dalam Pengobatan Tradisional Pada Masyarakat Desa Seluan. JPPM: Jurnal Pelayanan dan Pemberdayaan Masyarakat, 3(1), 11-23. https://doi.org/10.31932/jppm.v3i1.3443

Walidah, I., Supriyanta, B., & Sujono, S. (2014). Daya Bunuh Hand Sanitizer Berbahan Aktif Alkohol 59% dalam Kemasan Setelah Penggunaan Berulang terhadap Angka Lempeng Total (ALT). *Jurnal Teknologi Laboratorium*, 3(1), 7-12. Retrieved from: https://teknolabjournal.com/index.php/Jtl/article/view/52

Wulansari, A., Prasetyo, D. B., Lejaringtyas, M., Hidayat, A., & Anggarini, S. (2012). Aplikasi dan analisis kelayakan pewarna bubuk merah alami berantioksidan dari ekstrak biji buah pinang (Areca catechu) sebagai bahan pengganti pewarna sintetik pada produk pangan. *Industria: Jurnal Teknologi dan Manajemen Agroindustri,* 1(1), 1-9. Retrieved from: https://industria.ub.ac.id/index.php/industri/article/view/95

Zainal, A., Ferita, I., Gustian, G., & Warnita, W. (2022). *Kajian Karakterisasi Terkait Potensi Kadar Katekin pada Tanaman Gambir (Uncaria gambir (Hunt) Roxb).* Bandung: Media Sains Indonesia.