Journal of Indonesian Medical Laboratory and Science
pISSN 2775-0108 || eISSN 2774-2504

Evaluation of mangosteen peel extract in the kato-katz technique for enhanced visualization of helminth eggs

Asbar Tanjung[1*], Sharfina Maulidayanti[1], Ing Mayfa Br. Sitomorang[1]

[1] Department of Medical Laboratory Technology, STIKes Prima Indonesia, Bekasi, Indonesia.

Correspondence
Asbar Tanjung
Bekasi, West Jawa - 17610, Indonesia
Email: asbartanjung@gmail.com

Received: 2025-08-03
Revised: 2025-09-28
Accepted: 2025-09-29
Available online: 2025-10-08

DOI: https://doi.org/10.53699/joimedlabs.v5i2.235

Citation
Tanjung, A., Maulidayanti, S., & Sitomorang, I. M. B. (2025). Evaluation of mangosteen peel extract in the Kato-Katz technique for enhanced visualization of helminth eggs. Journal of Indonesian Medical Laboratory and Science, 6(2), 83-91. https://doi.org/10.53699/joimedlabs.v5i2.25

Abstract
Background: Mangosteen rind (*Garcinia mangostana*) contains anthocyanins, which have potential as natural dyes. One promising application is as a substitute for malachite green in the Kato-Katz technique, a fecal examination method used to detect helminth eggs such as *Ascaris lumbricoides*. *Objectives*: To evaluate the effectiveness of mangosteen peel extract at various concentrations (25%, 50%, 75%, and 100%) as an alternative staining agent in the Kato-Katz method. *Materials and Methods*: This laboratory-based experimental study utilized fecal samples positive for *A. lumbricoides*, which were examined using the Kato-Katz method with selophane tape soaked in mangosteen rind extract. Observed parameters included egg count per gram of feces (epg), clarity of egg morphology, and background contrast under the microscope. *Results*: The 75% concentration yielded the best performance, with optimal background staining, clear egg morphology, and the highest average egg count (853 eggs per gram). Concentrations of 25%, 50%, and 100% showed lower effectiveness. *Conclusions*: A 75% concentration of mangosteen rind extract is effective as a natural dye substitute for malachite green in the Kato-Katz method, providing good visual contrast and supporting accurate identification of helminth eggs

Keywords
Anthocyanins, *Ascaris lumbricoides*, Helminthiasis, Kato-Katz technique, Mangosteen peel extract.

1. Introduction

Soil-transmitted helminth (STH) infections are among the most prevalent parasitic diseases globally, primarily transmitted through contact with contaminated soil (Chan et al. 2023; Tembo et al. 2019). The major species responsible for these infections include *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura* (Tinkitina et al. 2024). According to the World Health Organization (WHO), more than 1.5 billion people—approximately 24% of the global population—are affected by STH infections, with *A. lumbricoides* being the most prevalent causative agent (Tembo et al. 2019; Tinkitina et al. 2024). Ascariasis is particularly widespread due to the

high fecundity of female *A. lumbricoides*, which can produce up to 200,000 eggs per day under favorable environmental conditions such as warm, humid, and shaded soil. Children are especially vulnerable to infection due to frequent contact with contaminated soil (Meliance Bria and Honey Donuarta 2024), inadequate hygiene practices, poor sanitation, low socioeconomic status, and limited access to clean water and proper toilet facilities (Nikmatullah et al. 2023).

The gold standard for diagnosing STH infections is microscopic examination of stool samples to detect helminth eggs (Charisma, Rahayu, and Anwari 2024). Among the recommended techniques, Kato-Katz method stands out for its simplicity, cost-effectiveness, and ability to quantify egg burden, this technique involves placing a fecal smear under a cellophane strip previously soaked in malachite green solution, which stains the background and enhances the visibility of helminth eggs under the microscope (Bosch et al. 2021).

Malachite green, a synthetic triphenylmethane dye, is widely used in laboratory diagnostics and the aquaculture industry due to its antimicrobial properties. However, it is associated with toxic and carcinogenic effects, particularly when disposed of in water systems. Its toxicity increases with higher concentrations, prolonged exposure, and elevated temperatures (Ahamed et al. 2024). Due to these concerns, malachite green has been banned in several countries, including those in the European Union and North America, although it remains in use in many developing regions because of its accessibility and low cost (Ahamed et al. 2024; Gu et al. 2024; Yang et al. 2023). Mangosteen (*Garcinia mangostana*), a tropical fruit native to Southeast Asia, contains numerous bioactive compounds in its rind, including xanthones, catechins, proanthocyanidins, and anthocyanins (Basri et al. 2021; Kurinjimalar et al. 2022). Notably, the rind has been reported to contain high levels of anthocyanins and have been utilized to remove violet dye in biological stain (Samrot et al. 2022).

A previous study reported *Garcinia megostana* extract reported having potential in staining *Trichuris suis* and *Strongyloides ransomis* eggs (Rose Bremner et al. 2023), furthermore (Ni'ma Azis and Harwani 2020) examine 50% mangosteen rind solution on the feces of individuals with helminth infections to determine the intensity level of infection using the modified Kato-Katz method. The results showed that the 50% mangosteen rind extract can be used as an alternative staining agent in the modified Kato-Katz method. However, there remains a lack of studies investigating the optimal concentration of this natural extract for routine diagnostic use. Therefore, this study aims to evaluate the effectiveness of mangosteen peel extract at concentrations of 25%, 50%, 75%, and 100% as a natural and safer alternative to malachite green in the Kato-Katz technique for helminth egg identification.

2. Materials and Methods

2.1. Study Design

This study was a laboratory-based experimental research aimed at evaluating the potential use of *Garcinia mangostana* (mangosteen) peel extract as a natural staining alternative in the Kato-Katz technique for the identification of *A. lumbricoides* eggs.

2.2. Materials and Equipment

The materials used included mangosteen peel (fresh, dark purple rind), aquadest (Ikapharmindo, Jakarta, Indonesia), glycerin (Brataco, Jakarta, Indonesia), 3% malachite green solution (Sigma-Aldrich, USA), and stool samples confirmed to be positive for *A. lumbricoides* eggs.

Instruments and equipment used including Juicer (Philips HR1832, Philips Electronics, Jakarta, Indonesia), Analytical balance (Ohaus PA214C, Parsippany, NJ, USA), Fine mesh filter (stainless steel, 100 mesh), Measuring glass (Pyrex®, Corning, NY, USA), Beaker glass (Iwaki, Tokyo, Japan), Light microscope (Olympus CX23, Olympus Corporation, Tokyo, Japan), Object glass slides (Citotest, Jiangsu, China), Cellophane tape (3M™, St. Paul, MN, USA), Cardboard templates (custom made), Wire mesh, paraffin paper, gloves (Sensi, Medisafe, Indonesia), surgical masks (Sensi), and standard laboratory coat.

2.3. Preparation of Mangosteen Peel Extract

Fresh mangosteen peels were washed thoroughly and separated from the pulp. A total of 100 grams of peel was blended with 100 mL of aquadest. The mixture was filtered using a fine mesh to obtain a clear filtrate. This filtrate was diluted with aquadest to obtain four concentrations: 25%, 50%, 75%, and 100% (Ni'ma Azis and Harwani 2020; Odongo-Aginya et al. 2007).

2.4. Preparation of Control and Test Solutions

The control solution was prepared by mixing 100 mL of Aquadest, 100 mL of glycerin, and 1 mL of 3% malachite green, then homogenizing. For the test solutions, 1 mL of mangosteen peel extract at each concentration (25%, 50%, 75%, and 100%) was mixed with 100 mL of Aquadest and 100 mL of glycerin. The pH of each test solution was measured using a calibrated digital pH meter before use (Odongo-Aginya et al. 2007).

2.5. Staining of Cellophane Tape

Cellophane tape was cut into pieces measuring 2.5 × 3 cm and soaked in both control and

test solutions for 18 hours to allow dye absorption.

2.6. Microscopic Examination

One gram of fecal sample positive for *A. lumbricoides* was placed on paraffin paper and pressed through a wire mesh to obtain a fine fraction. The filtered sample was placed into a perforated cardboard frame set on an object glass. After removing the frame, the sample was covered with cellophane tape that had been pre-soaked in the test or control solution. The smear was flattened gently and allowed to stand for 20–30 minutes.

Microscopic observation was performed using a light microscope under 100× and 400× magnifications. Observed parameters included the number of *A. lumbricoides* eggs per gram of feces, the clarity of the microscopic field, and the visibility of the egg's morphological layers (albuminoid, hyaline, and vitelline)(Bosch et al. 2021).

2.7. Data Analysis

Descriptive analysis was conducted to assess the distribution of egg counts and the visual quality of microscopic fields stained using different concentrations of mangosteen peel extract (25%, 50%, 75%, and 100%). Observations were presented in both narrative and tabulated formats.

3. Results and Discussion

3.1. Results

Examination of *Ascaris lumbricoides* eggs was carried out using the Kato-Katz method with two types of staining agents: malachite green as the control and mangosteen peel extract at various concentrations, continuous by microscope. Results are present in the Table 1.

Table 1 presents the number of *Ascaris lumbricoides* eggs found in each treatment per gram of feces (eggs per gram/EPG). The control using malachite green produced a uniform green field of view with clearly visible egg morphology, with a total of 820 EPG. Meanwhile, in the treatment using mangosteen peel extract, the 25% and 100% concentrations produced suboptimal field-of-view colours—too pale at 25% and too dark at 100%—which affected the visibility of egg morphology and resulted in fewer observable eggs (240 and 220 EPG, respectively). The 50% concentration showed an increased egg count of 813 EPG, though the egg morphology was still not as clear as the

control. The 75% concentration gave the best results in terms of both morphology visibility and egg count, with 853 EPG observed.

Figure 1 shows a composite visual of microscopic observations from all treatments. Differences in colour intensity and egg morphology clarity can be seen at each concentration. In the control and 75% concentration treatments, characteristic egg structures such as the albuminoid layer, hyaline layer, vitelline layer, and the embryo inside the egg were clearly visible. In contrast, the 25% and 100% concentrations exhibited decreased morphology clarity due to unsuitable colour intensity.

Table 1. Number of *Ascaris lumbricoides* Eggs Observed Using the Kato-Katz Method with Malachite Green and Mangosteen Peel Extract

Treatment	Field-of-View Colour	Egg Count (EPG)	Description
Malachite green (control)	Uniform green	820	Egg morphology very clear
Mangosteen peel extract 25 %	Pale	240	Egg morphology not clearly visible
Mangosteen peel extract 50 %	Slightly pale	813	Egg morphology fairly visible
Mangosteen peel extract 75 %	Neither too dark nor pale	853	Egg morphology very clearly visible
Mangosteen peel extract 100 %	Too dark	220	Egg morphology difficult to observe

Visualization of each *Ascaris lumbricoides* eggs in 400x magnification among all treatment showed variation in morphology clarity for each treatment. Morphology clarity of each eggs structure of different treatment present in figure 1.

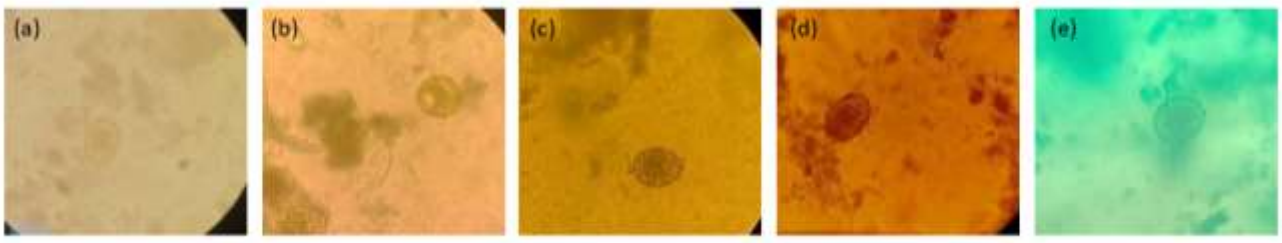

Figure 1. Microscopic Observation of *Ascaris lumbricoides* Eggs Using the Kato-Katz Method with Mangosteen Peel Extract Staining at 25% (a), 50% (b), 75% (c), 100% (d), and Malachite Green as Control (e).

3.2. Discussion

Mangosteen fruit is known to contain anthocyanins in its peel, which give it a purple colour (Basri et al. 2021; Kurinjimalar et al. 2022). Anthocyanins are natural pigments that can appear red, purple, or blue depending on pH, making mangosteen peel extract a potential natural alternative to synthetic dyes such as malachite green. In this study, mangosteen peel extract was tested as a substitute dye in the Kato-Katz method—a fecal examination technique in which samples are visualized under a microscope after being flattened with dye-soaked cellophane.

Table 1 presents the number of *Ascaris lumbricoides* eggs observed per gram of feces (EPG) and describes the field-of-view characteristics under each treatment. The control using malachite green yielded 820 EPG, serving as a benchmark for effective staining and quantification. The 25% mangosteen peel extract produced the lowest egg count (240 EPG), suggesting weak staining capacity. The 50% concentration yielded 813 EPG, nearly equivalent to the control in quantity, but lower in clarity. The 75% concentration resulted in the highest egg count (853 EPG), indicating its effectiveness in highlighting parasite eggs. However, the 100% concentration—despite having the highest extract strength—produced only 220 EPG, likely due to over-darkening that hindered egg visibility and counting. These findings indicate that egg detection is not solely dependent on anthocyanin concentration but also on the staining balance that affects visibility.

Figure 1 illustrates the microscopic appearance of *Ascaris lumbricoides* eggs stained with different treatments. In the control (Figure 1e) and 75% extract group (Figure 1c), egg morphology was clearly visible—including the albuminoid layer, hyaline layer, vitelline layer, and embryonic structures. In contrast, the 25% concentration (Figure 1a) resulted in a pale background, making eggs appear faint and difficult to distinguish from debris. The 50% concentration (Figure 1b) improved visualization slightly but still lacked sharp contrast. The 100% extract (Figure 1d) produced a field that was too dark, obscuring internal structures and making egg identification difficult. These microscopic visuals confirm that 75% mangosteen peel extract offers optimal coloration—providing both visibility and morphological clarity comparable to malachite green.

A previous study (Ni'ma Azis and Harwani 2020) also demonstrated the potential of mangosteen peel extract as a natural stain in the Kato-Katz method. Their research, using 50% extract, showed that anthocyanins could provide red coloration, although not always

optimal for structure visualization. They also tested other fruits such as red fruit and beetroot with promising results. Our current findings suggest that a higher concentration—specifically 75%—is more effective both in egg recovery and morphological visualization.

The pH values of the mangosteen peel extracts, ranging from 3.4 to 4.0, also played a role in colour development. According to (Mattioli et al. 2020) anthocyanins appear red in acidic pH and blue in alkaline conditions. The extracts in this study all had acidic pH values, which affected the colour outcome in the field of view. However, according to (Maya et al. 2012) viability and microscopic clarity of helminth eggs may affected by several condition including temperature, pH and dryness. Furthermore according to (Wisetmora et al. 2014) formalin fixed stool improve the Kato-Katz method performance.

In summary, based on both quantitative results in Table 1 and microscopic observations in Figure 1, the 75% mangosteen peel extract is the most effective natural alternative to malachite green in the Kato-Katz method.

4. Conclusions

Mangosteen peel extract demonstrates strong potential as a natural staining agent in the Kato-Katz method for visualizing *Ascaris lumbricoides* eggs. Among all tested concentrations, the 75% extract provided the best balance of colour intensity and morphological clarity, even outperforming malachite green in egg count. This finding supports the use of 75% mangosteen peel extract as an eco-friendly and effective alternative to synthetic dyes in parasitological diagnostics.

Acknowledgments: The authors would like to express their gratitude to the Research and Community Service Institute (LPPM) of STIKes Prima Indonesia for facilitating this research. We also thank the staff of the Microbiology Laboratory at STIKes Prima Indonesia for their technical support throughout the study.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest: The authors declare that there are no conflicts of interest related to this research.

Author Contributions: AT: Conceptualization, Methodology, Data curation, Writing-Original draft preparation. SM: Investigation, Project administration, Writing- Reviewing and Editing. IMBS: Investigation, Resources, Writing-Reviewing and Editing.

5. References

Ahamed, H. N., Ismail, Y., Navabshan, I., S, M. Z., Ts, S., Jaleel, I., Lh, T. A. (2024). Investigating the toxicity of malachite green and copper sulfate in brine shrimp: In-vivo and computational study. *Toxicology reports,* 13(13), 101811. https://doi.org/10.1016/j.toxrep.2024.101811

Basri, M. S. M., Ren, B. L. M., Rosnita A. Talib, R. A., and Kamarudin, S. H. (2021). Novel mangosteen-leaves-based marker ink: Color lightness, viscosity, optimized composition, and microstructural analysis. *Polymers,* 13(10), 1581. https://doi.org/10.3390/polym13101581

Bosch, F., Palmeirim, M. S., Ali, S. M., Ame, S. M., Hattendorf, J., & Keiser, J. (2021). Diagnosis of soil-transmitted helminths using the Kato-Katz technique: What is the influence of stirring, storage time and storage temperature on stool sample egg counts?. *PLoS neglected tropical diseases*, 15(1), e0009032. https://doi.org/10.1371/journal.pntd.0009032

Chan, H. G., Flores, M. J. C., Maghiran, E. S. V., Arellano, B. C., Chan J. M. P. (2023). A report of neglected zoonotic helminth infections among house rats in selected areas of metro manila and region IV-A calabarzon, Philippines. *Epidemiology and Public Health,* 1(3), https://www.jpublichealth.org/articles/1022.pdf

Charisma, A. M., Rahayu, E. O., & Anwari, F. (2024). The comparison of the effectiveness of shoe flower (*Hibiscus rosa-sinensis* L.) and roselle flower (*Hibiscus sabdariffa* L.) infusions as alternative reagents for the examination of soil transmitted helminths (STH) eggs. *Indonesian Journal of Medical Laboratory Science and Technology,* 6(1), 65–74. https://doi.org/10.33086/ijmlst.v6i1.5409

Gu, J., Han, T., Peng, X., Kang, H., & Dong, L. (2024). Highly sensitive fluorescent probe and portable test strip based on polyacrylic acid functionalized quantum dots for rapid visual detection of malachite green. *Talanta,* 268(Pt 2), 125359. https://doi.org/10.1016/j.talanta.2023.125359

Kurinjimalar, C., Nagarajan Usharani, Balaji Kanimozhi, Gladstone Christopher Jayakumar, and Swarna V. Kanth. (2022). Extraction and optimization of natural colorant from *garcinia mangostana* linn peels for leather dyeing. *Cleaner Engineering and Technology,* 7, 100423. https://doi.org/10.1016/j.clet.2022.100423

Mattioli, R., Francioso, A., Mosca, L., & Silva, P. (2020). Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. *Molecules* 25(17), 3809. https://doi.org/10.3390/molecules25173809

Maya, C., F. J. Torner-Morales, E. S. Lucario, E. Hernández, and B. Jiménez. (2012). Viability of Six species of larval and non-larval helminth eggs for different conditions of temperature, PH and dryness. *Water Research,* 46(15), 4770–82. https://doi.org/10.1016/j.watres.2012.06.014

Bria, M., Donuarta, H. (2024). Analysis of *Ascaris lumbricoides* infection with total ig e levels in elementary school children of lifuleo village, East Kupang District. *International Journal of Health and Social Behavior,* 1(4), 160–69. https://doi.org/10.62951/ijhsb.v1i4.190

Nikmatullah, Nurul Azmah, Irmawati Masyhuda, Etin Diah Permanasari, and Chintya Rahmadilla. (2023). Correlation Between nutritional status, age, and gender on the incidence of soil-transmitted helminth infections. *Jurnal Profesi Medika : Jurnal Kedokteran dan Kesehatan,* 17(2), 190–95. https://doi.org/10.33533/jpm.v17i2.6896

Nikmatullah, N. A., Masyuda, I., Permanasari, E. D., Rahmadilla, C. (2020). Modifikasi metode kato katz dengan perasan kulit buah manggis (*Garcinia Mangostana* L.) in *SNPPM-2 (Seminar Nasional Penelitian Dan Pengabdian Kepada Masyarakat) Tahun 2020 ISBN 978-623-90328-5-2,* Universitas Muhammadiyah Metro. https://repository.ummetro.ac.id/files/artikel/f2c9b631e7f74dad4f54dda6436b6084.pdf

Odongo-Aginya, Emmanuel I., Narcis Kabatereine, Siefert Ludwig, Henry Wabinga, Alan Fenwick, and Antonio Montresor. (2007). Substitution of malachite green with nigrosin - eosin yellow stain in the kato-katz method: microscopical appearance of the helminth eggs. *African Health Sciences,* 7(1), 33. https://doi.org/10.5555/afhs.2007.7.1.33

Doria, A. J. E., Dalusong, C. J. S., De Castro, R. A. M., Dioneda, T. N. M., Estefanio, K. Z. B., Galves, L. A. P., Gregorio, M. F. B., Bremner, P. R. F. (2023). Comparative evaluation on the staining potential of *Garcinia Mangostana* (Mangosteen) and selenicereus guatemalensis (dragon fruit) for ova of *Trichuris suis* and *Strongyloides ansom*i. *Asian Journal of Biological and Life Sciences,* 12(2). http://dx.doi.org/10.5530/ajbls.2023.12.56

Samot, A. V., Michael, E. N., Anand, D. E., Mercy, J. L., Sabesan, G. S., Mohanty, B. K., Visvanathan, S., Saigeetha, S. (2022). The utilization of *Garcinia mangostana* fibers for the removal of crystal violet dye. *Materials Today: Proceedings,* 59(2), 1550–54. https://doi.org/10.1016/j.matpr.2022.02.232

Tembo, S., Mubita, P., Sitali, L, Zgambo, J. (2019). Prevalence, intensity, and factors associated with soil-transmitted helminths infection among children in zambia: A cross-sectional study. *The Open Public Health Journal,* 12(1), 284–93. http://dx.doi.org/10.2174/1874944501912010284

Tinkitina, B., Beinamaryo, P., Kyarisiima, H., Nabatte, B., Arinaitwe, M., Mubangizi, A., Emerson, P., & M Sullivan, K. (2024). Prevalence and intensity of soil-transmitted helminth infections among school-aged children in Five Districts in Uganda. *PLoS Neglected Tropical Diseases,* 18(8), e0012324. https://doi.org/10.1371/journal.pntd.0012324

Wisetmora, A., Artchayasawat, A., Laummaunwai, P., Pitaksakulrat, O., Wattanawong, O., & Boonmars, T. (2014). Formalin-fixed stool improves the performance of the Kato-Katz method. *Veterinary World,* 17(1), 99–107. https://www.veterinaryworld.org/Vol.17/January-2024/11.pdf (August 3, 2025)

Yang, G., Zhang, J., Gu, L., Tang, Y., Zhang, X., Huang, X., Shen, X., Zhai, W., Fodjo, E. K., & Kong, C. (2023). Ratiometric fluorescence immunoassay based on carbon quantum dots for sensitive detection of malachite green in fish. *Biosensors,* 13(1), 38. https://doi.org/10.3390/bios13010038