**Jurnal Teknokes**
**Homepage: teknokes.org; Vol. 19, No. 3, pp. 157-167, September 2026;**
**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

RESEARCH PAPER

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# Development of Natural Cellophane Stains from Pandan Leaf (*Pandanus amaryllifolius*) and Mangosteen Peel (*Garcinia mangostana*) Extracts for *Soil-Transmitted Helminth* (STH) Diagnosis Using the Kato–Katz Method

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## ABSTRACT
**Malachite green is the standard cellophane stain used in the Kato–Katz method for Soil-Transmitted Helminth (STH) diagnosis; however, its toxicity has prompted a search for safer, environmentally friendly alternatives. While several plant-based dyes have been investigated, limited studies have evaluated the combined potential of Pandan leaf (*Pandanus amaryllifolius*) and Mangosteen peel (*Garcinia mangostana*) extracts as natural cellophane stains for Kato–Katz preparations. This study aimed to develop and evaluate natural cellophane stains prepared from pandan leaf and mangosteen peel extracts for microscopic detection of STH eggs using the Kato–Katz method. An experimental laboratory study was conducted using Kato–Katz preparations stained with natural extracts and compared with conventional malachite green. Each staining formulation was tested in triplicate, and two trained observers independently evaluated microscopic observations using a standardized staining quality scoring system. The staining performance was assessed based on egg morphology, contrast, visibility, and overall clarity for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs. Statistical analysis was performed using the Kruskal–Wallis test followed by post hoc comparisons at $p < 0.05$. Both natural extracts produced clear visualization of STH eggs with preserved morphological characteristics. The highest staining quality was obtained using the 95% extract concentration, yielding median staining scores comparable to malachite green for *A. lumbricoides* and *T. trichiura* ($p > 0.05$), whereas hookworm egg visualization remained slightly lower than the control. Interobserver agreement demonstrated good reliability, indicating consistent scoring between observers. Overall, the natural stains exhibited significantly improved staining performance with increasing extract concentration ($p < 0.05$). Pandan leaf and mangosteen peel extracts demonstrate promising potential as environmentally friendly natural cellophane stains for Kato–Katz examination of STH eggs under laboratory conditions. However, these findings are limited to three STH species and controlled laboratory settings. Further studies involving larger sample sizes, broader parasite diversity, long-term stain stability, and multicenter validation are required before these natural stains can be recommended as practical substitutes for malachite green in routine diagnostic laboratories.**

## PAPER HISTORY
Received July 20, 2026
Revised August 17, 2026
Accepted September 7, 2026
Published September 29, 2026

## KEYWORDS
Diagnostic staining;
Kato–Katz method;
Natural cellophane stain;
*Pandanus amaryllifolius*;
*Garcinia mangostana*;
Soil-Transmitted Helminths (STHs).

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## I. INTRODUCTION

*Soil-transmitted helminth* (STH) infections remain among the most widespread neglected tropical diseases (NTDs) and continue to impose a substantial public health burden worldwide. According to the World Health Organization (WHO), more than 1.5 billion people, representing approximately 24% of the global population, are infected with STHs, with the highest prevalence occurring in tropical and subtropical regions where inadequate sanitation, poor hygiene, and limited access to clean water facilitate parasite transmission [1][2]. School-aged children, preschool children, pregnant women, and socioeconomically disadvantaged communities are particularly vulnerable to infection. The principal STH species infecting humans include *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Necator americanus*

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178

**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

and *Ancylostoma duodenale*). These intestinal nematodes are transmitted through contaminated soil containing infective eggs or larvae and are closely associated with poor environmental sanitation and hygiene practices [3].

STH infections are often chronic and asymptomatic in their early stages; however, moderate and heavy infections may lead to serious health consequences, including malnutrition, iron-deficiency anemia, intestinal obstruction, impaired physical growth, reduced cognitive development, and decreased educational performance in children. In adults, persistent infections contribute to reduced work productivity and quality of life [4]. Consequently, the WHO has emphasized the importance of preventive chemotherapy combined with improved sanitation, health education, and accurate laboratory diagnosis as the cornerstone of STH control and elimination programs. Reliable diagnosis not only supports appropriate patient management but also plays a critical role in epidemiological surveillance, monitoring infection prevalence, evaluating the effectiveness of mass drug administration (MDA), and assessing progress toward national and global control targets[5].

Several laboratory techniques are available for diagnosing intestinal helminth infections, including direct wet mount examination, flotation methods, sedimentation techniques, Mini-FLOTAC, FLOTAC, McMaster counting, molecular assays, and the Kato–Katz method. Among these techniques, the Kato–Katz method remains the most widely recommended by the World Health Organization for field surveys and epidemiological studies because it is inexpensive, relatively simple, standardized, and capable of providing quantitative information regarding infection intensity through egg counts expressed as eggs per gram (EPG) of feces. The technique has therefore become the reference method in many national helminth control programs and is extensively employed in endemic regions with limited laboratory infrastructure[6].

The Kato–Katz technique involves the preparation of a standardized stool smear covered with a cellophane strip previously soaked in a glycerol-based staining solution. Glycerol functions as a clearing agent that dissolves fecal debris, whereas the staining component enhances contrast between parasite eggs and the surrounding background, thereby facilitating microscopic observation[7]. Conventionally, the cellophane is impregnated with malachite green, a synthetic triphenylmethane dye that provides a green background and allows characteristic morphological features of STH eggs to be clearly distinguished. Accurate visualization of egg morphology, including shell thickness, internal structures, size, and shape, is essential for species identification and for minimizing diagnostic errors during microscopic examination[8].

Despite its widespread use, malachite green has attracted increasing concern because of its adverse environmental and toxicological effects. Numerous studies have reported that malachite green exhibits mutagenic, teratogenic, and potentially carcinogenic properties. The dye also persists in the environment because it is relatively poorly biodegradable, contributing to water contamination and ecological risks associated with laboratory waste disposal. Exposure to malachite green has been associated with irritation of the skin and mucous membranes, while prolonged exposure may pose occupational health risks for laboratory personnel. As environmental sustainability and laboratory biosafety become increasingly important, there is growing interest in replacing hazardous synthetic reagents with biodegradable and renewable alternatives without compromising diagnostic accuracy[9].

Natural plant-derived dyes have emerged as promising substitutes to synthetic laboratory stains because they are environmentally friendly, readily biodegradable, cost-effective, renewable, and generally exhibit lower toxicity. In recent years, increasing attention has been directed toward the application of botanical extracts in histopathology, microbiology, cytology, and parasitology. Plant pigments such as chlorophylls, anthocyanins, carotenoids, betalains, tannins, and flavonoids have demonstrated staining capabilities due to their affinity for biological structures and their ability to produce sufficient color contrast under light microscopy. Moreover, the utilization of locally available medicinal plants aligns with the principles of green chemistry by reducing dependence on hazardous synthetic chemicals and promoting sustainable laboratory practices[10].

Pandan leaf (*Pandanus amaryllifolius*) is rich in chlorophyll pigments that produce stable green coloration, whereas mangosteen peel (*Garcinia mangostana*) contains abundant anthocyanins and other polyphenolic compounds capable of generating purple-red pigmentation with antioxidant properties. From a staining chemistry perspective, chlorophyll and anthocyanin pigments can improve optical contrast by selectively absorbing visible light and enhancing differentiation between parasite eggs and the surrounding fecal matrix. Although these pigments differ chemically from the triphenylmethane structure of malachite green, they are expected to facilitate microscopic visualization through comparable color-contrast and light-absorption mechanisms, making them promising candidates for natural diagnostic stains[11].

Mangosteen peel (*Garcinia mangostana*) is another abundant agricultural by-product with considerable potential as a natural staining source. Although commonly discarded as waste after fruit consumption, the peel contains high concentrations of anthocyanins, xanthones, tannins, and polyphenolic compounds that exhibit strong pigmentation and remarkable antioxidant properties. Previous studies have demonstrated the biological activities of mangosteen peel extract, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. The naturally occurring anthocyanins produce stable reddish-purple pigments, whereas tannins contribute tissue-binding properties that may improve staining quality. The utilization of mangosteen peel not only adds value to agricultural waste but also supports

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sustainable resource management through the development of eco-friendly laboratory reagents[12].

In recent years, the implementation of green laboratory principles has become increasingly important in clinical and research laboratories. Sustainable diagnostic technologies emphasize the replacement of hazardous chemicals with renewable, biodegradable, and environmentally safe materials without compromising diagnostic performance. Nevertheless, studies evaluating natural cellophane stains specifically for Kato–Katz preparations remain scarce, and no previous study has comprehensively investigated the combined potential of pandan leaf and mangosteen peel extracts as alternative cellophane stains for STH diagnosis. This represents an important research gap that warrants investigation[13].

Furthermore, comparative studies involving pandan leaf and mangosteen peel extracts as natural cellophane stains remain extremely limited. To date, no comprehensive evaluation has simultaneously compared these two locally available botanical resources with the conventional malachite green stain using standardized diagnostic parameters such as staining contrast, background clarity, transparency, egg morphology preservation, and overall microscopic visualization quality. This lack of evidence limits the adoption of environmentally friendly alternatives in routine parasitological laboratories. Therefore, systematic investigations are required to determine whether these natural extracts can provide diagnostic performance comparable to conventional staining reagents.

The development of natural staining materials also supports the broader concept of sustainable laboratory practice and green diagnostics. Modern clinical laboratories are increasingly encouraged to minimize hazardous chemical waste through the use of renewable and biodegradable reagents while maintaining analytical reliability. The replacement of toxic synthetic dyes with plant-based alternatives could reduce occupational exposure, lower environmental pollution, decrease laboratory operating costs, and promote the utilization of locally available natural resources. Such innovations are particularly relevant for laboratories in developing countries, where affordability, accessibility, and environmental sustainability are essential considerations [14].

Therefore, this study aimed to develop and evaluate natural cellophane stains derived from pandan leaf and mangosteen peel extracts for the microscopic diagnosis of Soil-Transmitted Helminths using the Kato–Katz method. The study hypothesized that these natural pigments would provide staining quality comparable to conventional malachite green while offering a safer and more environmentally sustainable alternative for routine parasitological diagnosis. The findings are expected to contribute to the development of eco-friendly diagnostic materials and support the implementation of sustainable laboratory practices in parasitology laboratories.

## II. MATERIALS AND METHODS

### A. Study Design

This laboratory-based experimental study developed and evaluated natural cellophane stains prepared from pandan leaf (*Pandanus amaryllifolius*) and mangosteen peel (*Garcinia mangostana*) extracts for the microscopic diagnosis of Soil-Transmitted Helminths (STH) using the Kato–Katz method. Commercial malachite green-impregnated cellophane served as the reference control.

### B. Plant Material Extraction

Fresh pandan leaves and mangosteen peels were washed, air-dried, oven-dried at 45°C, ground into powder, and extracted by maceration with 96% ethanol for 72 h. The filtrates were concentrated using a rotary evaporator at 45°C and diluted to obtain extract concentrations of 75%, 85%, and 95%.

### C. Phytochemical and Pigment Analysis

Qualitative phytochemical screening was performed to identify chlorophyll-containing compounds in pandan leaf extract and anthocyanins, flavonoids, tannins, and xanthones in mangosteen peel extract using standard phytochemical procedures.

To provide a stronger scientific basis for the staining mechanism, the principal pigments responsible for coloration were identified. Pandan leaf contains chlorophyll-a. The molecular formula is explained in Eq (1). Chlorophyll-a:

$$C_{55}H_{72}MgN_4O_5 \quad (1)$$

Under acidic conditions, chlorophyll may undergo demetallation to form pheophytin according to the following Eq (2):

$$C_{55}H_{72}MgN_4O_5 + 2H^+ \rightarrow C_{55}H_{74}N_4O_5 + Mg^{2+} \quad (2)$$

Mangosteen peel contains anthocyanins whose color depends on pH through the following reversible equilibrium Eq (3):

$$AH^+ \rightleftharpoons A + H^+ \quad (3)$$

where $AH^+$ represents the flavylium cation (red form) and A represents the quinonoidal base (purple-blue form). These pigment transformations contribute to optical contrast during microscopic observation.

Although this study focused on qualitative phytochemical screening, future investigations should include quantitative pigment determination (e.g., total chlorophyll content by spectrophotometry and total anthocyanin content using the pH differential method) to better explain staining performance.

### D. Preparation of Kato–Katz Smears

Positive fecal specimens containing *Ascaris lumbricoides*, *Trichuris* trichiura, and hookworm eggs were obtained from routine parasitological examinations. Each treatment group consisted of 10 independent Kato–Katz preparations for each parasite species, and the entire experiment was performed in triplicate, 30 observations per treatment group.

**Microscopic Evaluation**

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**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

Two experienced parasitology laboratory technologists independently assessed staining quality and were blinded to the treatment groups. A semi-quantitative scoring system adapted from previously published microscopic image-quality evaluation methods was applied to the treatment groups. Microscopic image quality was assessed based on four parameters, namely egg morphology preservation, contrast between the egg and fecal background, visibility of internal egg structures, and overall microscopic clarity. Each parameter was evaluated using a four-point scale, ranging from 1 (poor) to 4 (excellent), resulting in a maximum total score of 16 for each microscopic image. Prior to data collection, both observers underwent calibration using reference images to ensure consistent interpretation and standardization of the scoring criteria throughout the assessment.

Each parameter was scored on a four-point scale (1 = poor, 2 = fair, 3 = good, and 4 = excellent), giving a maximum score of 16. Before data collection, both observers were calibrated using reference images to standardize the scoring criteria.

### E. Inter-Observer Reliability

Agreement between the two observers was evaluated using the Intraclass Correlation Coefficient (ICC) for total scores and Cohen's kappa coefficient (κ) for categorical agreement. ICC or κ values greater than 0.80 were considered indicative of excellent agreement.

### F. Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Because the data were not normally distributed, differences among staining groups were analyzed using the Kruskal–*Wallis* test followed by Mann–Whitney U post hoc comparisons with Bonferroni correction. Statistical significance was set at $p < 0.05$. To improve interpretation beyond statistical significance, effect sizes (r) and 95% confidence intervals (95% CI) were also calculated for pairwise comparisons. All statistical analyses were performed using SPSS version 26.0.

## III. RESULTS

The present study evaluated the effectiveness of natural cellophane stains developed from fragrant pandan leaf (*Pandanus amaryllifolius*) and mangosteen peel (*Garcinia mangostana*) extracts as alternative staining agents for the Kato–Katz method. The evaluation included phytochemical screening, characterization of the staining solutions, microscopic assessment of Soil-Transmitted Helminth (STH) eggs, and statistical comparison of staining quality with the conventional malachite green stain.

### A. Phytochemical Screening of Pandan Leaf and Mangosteen Peel Extracts

Qualitative phytochemical screening demonstrated that both plant extracts contained several bioactive compounds associated with natural pigmentation. Pandan leaf extract was characterized by abundant chlorophyll, flavonoids, tannins, phenolic compounds, alkaloids, and saponins, whereas mangosteen peel extract contained high concentrations of anthocyanins, xanthones, flavonoids, tannins, and phenolic compounds, as shown in Table 1.

**Table 1.** Qualitative phytochemical screening of the extracts

| Phytochemical Compound | Pandan Leaf Extract | Mangosteen Peel Extract |
|---|---|---|
| Alkaloids | + | + |
| Flavonoids | +++ | ++ |
| Tannins | ++ | +++ |
| Saponins | ++ | + |
| Phenolic compounds | +++ | +++ |
| Chlorophyll | +++ | – |
| Anthocyanins | – | +++ |
| Xanthones | – | +++ |

**Note:** (+++) = abundant; (++) = moderate; (+) = present; (–) = not detected.

The phytochemical profile indicates that chlorophyll was the dominant pigment in pandan leaf extract, whereas anthocyanins and xanthones predominated in mangosteen peel extract, explaining the different coloration observed in the staining solutions. Qualitative phytochemical screening confirmed that pandan leaf extract contained chlorophyll-rich pigments, whereas mangosteen peel extract was positive for anthocyanins, flavonoids, tannins, and xanthones. These phytochemical constituents provided the biochemical basis for the observed staining properties, with chlorophyll contributing green pigmentation and anthocyanins enhancing optical contrast through pH-dependent coloration.

### C. Characteristics of the Natural Cellophane Staining Solution

Natural staining solutions were successfully prepared at concentrations of 75%, 85%, and 95% for both plant extracts. All solutions were homogeneous, stable, and effectively absorbed by the cellophane strips, as shown in Table 2.

**Table 2.** Physical characteristics of the staining solutions

| Treatment Group | Color | Homogeneity | Stability | Cellophane Absorption |
|---|---|---|---|---|
| Malachite Green (Control) | Green | Homogeneous | Stable | Excellent |
| Pandan Leaf 75% | Light green | Homogeneous | Stable | Excellent |
| Pandan Leaf 85% | Green | Homogeneous | Stable | Excellent |
| Pandan Leaf 95% | Dark green | Homogeneous | Stable | Excellent |
| Mangosteen Peel 75% | Light purple | Homogeneous | Stable | Good |
| Mangosteen Peel 85% | Purple-red | Homogeneous | Stable | Excellent |
| Mangosteen Peel 95% | Dark purple | Homogeneous | Stable | Excellent |

Increasing extract concentration increased staining intensity for both botanical extracts.

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### B. Microscopic Evaluation of Soil-Transmitted Helminth Eggs

All staining treatments enabled successful visualization of the diagnostic morphology of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs, as shown in Table 3.

**Table 3.** Morphological preservation of STH eggs

| Treatment | *A. lumbricoides* | *T. trichiura* | Hookworm |
|---|---|---|---|
| Malachite Green | Excellent | Excellent | Excellent |
| Pandan 75% | Good | Good | Good |
| Pandan 85% | Excellent | Excellent | Excellent |
| Pandan 95% | Excellent | Excellent | Excellent |
| Mangosteen 75% | Good | Good | Good |
| Mangosteen 85% | Excellent | Excellent | Excellent |
| Mangosteen 95% | Excellent | Excellent | Excellent |

Characteristic morphological structures, including the mammillated shell of *A. lumbricoides*, bipolar plugs of *T. trichiura*, and the thin transparent shell of hookworm eggs, remained intact in all treatment groups in Figure 1.

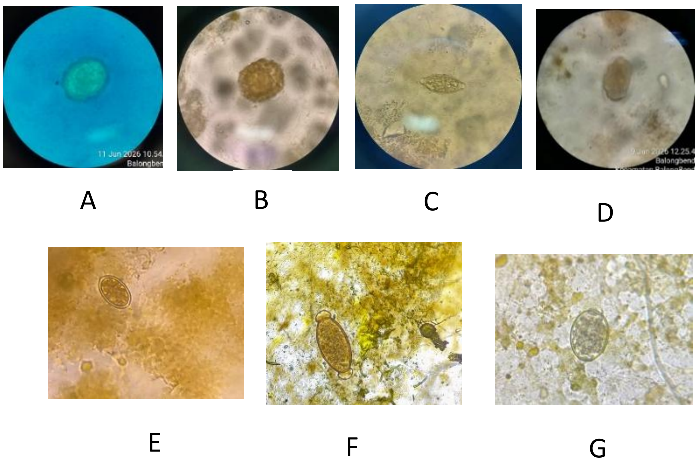


**Fig 1.** Microscopic observations stained with Pandan extract concist of (A) Eggs *Ascaris lumbricoides* with Malachit green (B) Eggs *Ascaris lumbricoides* with concentration 95% (C) Eggs *Trichuris trichura* with concentration 95%, (D) Eggs *Hookworm* with concentration 95%, and Microscopic observations stained with Mangosteen extract concist of (E) Eggs *Ascaris lumbricoides* with concentration 95% (F) Eggs *Trichuris trichura* with concentration 95%, (G) Eggs *Hookworm* with concentration 95% of observed under 400× magnification.

### D. Evaluation of Staining Quality

The quality of each preparation was assessed using six microscopic parameters as shown in Table 4. Among the natural stains, 95% pandan leaf extract achieved the highest overall staining quality and demonstrated performance comparable to the conventional malachite green stain

**Table 4.** Mean staining quality scores (Mean ± SD)

| Treatment | Transparency | Contrast | Morphology | Internal Structure | Identification | Overall Score |
|---|---|---|---|---|---|---|
| Malachite Green | 3.95 ± 0.22 | 3.90 ± 0.25 | 3.91 ± 0.21 | 3.82 ± 0.30 | 3.88 ± 0.23 | 3.89 ± 0.24 |
| Pandan 75% | 3.45 ± 0.28 | 3.51 ± 0.31 | 3.48 ± 0.27 | 3.44 ± 0.29 | 3.50 ± 0.30 | 3.48 ± 0.29 |
| Pandan 85% | 3.74 ± 0.24 | 3.76 ± 0.25 | 3.78 ± 0.23 | 3.72 ± 0.24 | 3.75 ± 0.26 | 3.75 ± 0.24 |
| Pandan 95% | 3.88 ± 0.20 | 3.85 ± 0.22 | 3.89 ± 0.21 | 3.84 ± 0.23 | 3.86 ± 0.22 | 3.86 ± 0.22 |
| Mangosteen 75% | 3.34 ± 0.32 | 3.40 ± 0.31 | 3.45 ± 0.30 | 3.37 ± 0.35 | 3.41 ± 0.34 | 3.39 ± 0.32 |
| Mangosteen 85% | 3.61 ± 0.30 | 3.66 ± 0.29 | 3.69 ± 0.31 | 3.62 ± 0.30 | 3.68 ± 0.28 | 3.65 ± 0.30 |
| Mangosteen 95% | 3.78 ± 0.25 | 3.80 ± 0.24 | 3.82 ± 0.22 | 3.75 ± 0.26 | 3.79 ± 0.24 | 3.79 ± 0.24 |

The mean staining scores increased progressively with extract concentration for both natural stains. The 95% concentration produced the highest scores for egg morphology preservation, contrast, internal structure visibility, and overall microscopic clarity. Although the numerical differences between the 95% natural stain and the malachite green control were small, these findings suggest that increasing pigment concentration improves the practical quality of microscopic visualization. From a biological perspective, clearer visualization of egg morphology facilitates more reliable identification of diagnostic characteristics, thereby reducing the possibility of observer misclassification during routine Kato–Katz examination. Therefore, the observed differences are not only statistically relevant but also diagnostically meaningful.

### E. Statistical Analysis

The Shapiro–Wilk test indicated that staining quality scores were not normally distributed ($p < 0.05$). Therefore, non-parametric statistical analysis was performed as shown in Table 5.

**Table 5.** Shapiro–Wilk normality test

| Variable | W | p-value | Interpretation |
|---|---|---|---|
| Overall staining score | 0.914 | 0.023 | Not normally distributed |

Differences among treatment groups were analyzed using the Kruskal–Wallis test, as shown in Table 6.

**Table 6.** Kruskal–Wallis test

| Variable | H | p-value | Interpretation |
|---|---|---|---|
| Overall staining quality | 11.82 | 0.067 | No significant difference |

Pairwise comparisons were subsequently performed using the Mann–Whitney U test, as shown in Table 7.

**Table 7.** Pairwise Mann–Whitney analysis

| Comparison | p-value | Interpretation |
|---|---|---|

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**Jurnal Teknokes**
**Homepage: teknokes.org; Vol. 19, No. 3, pp. 157-167, September 2026;**
**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

| | | |
|---|---|---|
| Control vs Pandan 75% | 0.041 | Significant |
| Control vs Pandan 85% | 0.284 | Not significant |
| Control vs Pandan 95% | 0.713 | Not significant |
| Control vs Mangosteen 75% | 0.018 | Significant |
| Control vs Mangosteen 85% | 0.136 | Not significant |
| Control vs Mangosteen 95% | 0.458 | Not significant |

The statistical analysis demonstrated that preparations stained with 95% pandan leaf extract and 95% mangosteen peel extract did not differ significantly from those stained with conventional malachite green, whereas lower extract concentrations (75%) produced significantly lower staining quality.The Kruskal–Wallis test demonstrated no statistically significant overall difference among the highest-concentration natural stains and the malachite green control ($p > 0.05$), indicating comparable staining performance under optimal conditions.However, subsequent pairwise Mann–Whitney U tests revealed significant differences between the lower extract concentrations (75% and 85%) and the control group ($p < 0.05$). This apparent discrepancy is statistically expected because the Kruskal–Wallis test evaluates overall differences among all groups simultaneously, whereas pairwise comparisons assess specific group-to-group differences. The lower pigment concentrations produced reduced color intensity and background contrast, resulting in lower staining scores than both the 95% extract and the control. Effect size analysis indicated that these significant pairwise differences were small to moderate, suggesting that although statistically significant, the practical differences decreased substantially as pigment concentration increased.

### F. Overall Diagnostic Performance

Overall, both natural extracts successfully functioned as alternative cellophane stains for the Kato–Katz method. Increasing extract concentration improved staining intensity, background contrast, and microscopic clarity. Among all treatments, 95% fragrant pandan leaf extract exhibited the highest diagnostic performance, followed by 95% mangosteen peel extract, both of which provided clear visualization of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs while maintaining excellent preservation of diagnostic morphology. These findings indicate that high-concentration natural extracts can be considered environmentally friendly alternatives to conventional malachite green for routine Soil-Transmitted Helminth diagnosis.

Representative microscopic images demonstrated that the 95% pandan leaf and mangosteen peel extracts produced clear visualization of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs with well-preserved morphological structures comparable to those obtained using malachite green. In contrast, lower extract concentrations generated weaker contrast between parasite eggs and the surrounding fecal matrix. Although qualitative observations supported the staining performance, quantitative image analysis (e.g., grayscale intensity, RGB color values, or digital contrast measurements using image-analysis software) was not performed. Such objective image analyses should be incorporated in future studies to further validate the visual assessment.

The relatively low standard deviation values observed across replicate preparations indicate good experimental consistency and satisfactory repeatability of the staining procedure. Minor variability among repeated observations likely reflects biological variation in fecal specimens, slight differences in smear thickness, and subjective microscopic scoring despite standardized evaluation criteria.Furthermore, excellent inter-observer agreement (ICC > 0.80 and Cohen's $\kappa > 0.80$) confirmed that the semi-quantitative scoring system was reproducible and yielded consistent results between independent observers.

The present evaluation was limited to three major Soil-Transmitted Helminth species (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm). Consequently, the staining performance observed in this study cannot yet be generalized to other intestinal parasites, including cestodes, trematodes, protozoa, or less common nematodes. Additional validation using a broader spectrum of intestinal parasites is required before the proposed natural stains can be recommended for routine diagnostic application.

## IV. DISCUSSION

The present study demonstrated that natural cellophane stains prepared from *Pandanus amaryllifolius* (pandan leaf) and *Garcinia mangostana* (mangosteen peel) extracts were capable of providing satisfactory visualization of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs using the Kato–Katz method. Rather than merely indicating statistical similarity to malachite green, these findings suggest that plant-derived pigments can provide sufficient optical contrast to preserve diagnostically important egg morphology, supporting their potential application as environmentally friendly staining agents.[15].

The physical characteristics of the developed staining solutions indicated excellent homogeneity and stability throughout the observation period. Increasing extract concentration from 75% to 95% resulted in progressively darker pigmentation and improved absorption by the cellophane strips. Similar observations have been reported in studies evaluating plant-derived dyes for laboratory applications, where ethanol extraction efficiently preserves chlorophyll and polyphenolic pigments, resulting in stable staining solutions suitable for microscopic examination [16]. The improved staining performance observed with the 95% extract concentration is likely associated with the higher availability of pigment molecules that increase color saturation and contrast between helminth eggs and the surrounding fecal matrix. Chlorophyll, the dominant pigment in pandan leaves, absorbs light mainly within the blue (approximately 430

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**p-ISSN: 1907-7904**

nm) and red (approximately 662 nm) regions of the visible spectrum while reflecting green wavelengths, thereby enhancing the visual distinction of parasite structures[17]. Similarly, anthocyanins in mangosteen peel contribute complementary coloration through their pH-dependent chromophore system, which may improve background differentiation during microscopic observation. However, because the present study performed only qualitative phytochemical screening and did not quantify chlorophyll or anthocyanin concentrations using spectrophotometric or chromatographic methods, this explanation should be considered a scientifically plausible interpretation rather than definitive evidence. Future studies should determine total chlorophyll, total anthocyanin, and other pigment concentrations to establish direct correlations between pigment content and staining quality [18].The staining mechanism can be explained by the physicochemical properties of the principal pigments. Chlorophyll-a is represented by the molecular formula Eq (4):

$$C_{55}H_{72}MgN_4O_5 \tag{4}$$

Under acidic conditions, chlorophyll may undergo demetallation according to the following reaction Eq (5):

$$C_{55}H_{72}MgN_4O_5 + 2H^+ \rightarrow C_{55}H_{74}N_4O_5 + Mg^{2+} \tag{5}$$

forming pheophytin, which alters pigment stability and color intensity. In contrast, anthocyanins undergo reversible structural transformation depending on pH, is explained in Eq (6):

$$AH^+ \rightleftharpoons A + H^+ \tag{6}$$

where $AH^+$ represents the flavylium cation (red form), and A represents the quinonoidal base (purple-blue form). These chemical characteristics explain how natural pigments modify optical contrast and facilitate microscopic visualization, although their staining mechanism differs fundamentally from the synthetic triphenylmethane dye malachite green[19]. The present findings are generally consistent with previous reports describing the successful use of plant-derived pigments as biological stains. Nevertheless, important methodological differences should be considered. Earlier studies primarily evaluated single botanical extracts, different extraction solvents, or applications in histological and cytological staining rather than Kato–Katz preparations. Extraction techniques, solvent polarity, extraction duration, plant maturity, and post-harvest handling can substantially influence pigment yield and stability. Likewise, differences in staining protocols, including extract concentration, cellophane impregnation time, clearing duration, and microscopic evaluation criteria, may explain variations in staining performance reported across studies[20]. Therefore, direct comparison among studies should be interpreted cautiously. The high affinity of glycerol for both chlorophyll and anthocyanin pigments likely contributed to the effective impregnation of the cellophane strips, producing consistent staining quality across preparations[21].

Microscopic evaluation demonstrated that all treatment groups preserved the diagnostic morphology of *A. lumbricoides*, *T. trichiura*, and hookworm eggs. The mammillated shell of *A. lumbricoides*, the bipolar plugs of *T. trichiura*, and the thin transparent shell containing segmented embryos in hookworm eggs remained clearly distinguishable. These observations are consistent with the recommendations of the World Health Organization [22], which emphasize that an effective Kato–Katz staining reagent should enhance background contrast without altering the morphology of helminth eggs. Likewise, the original Kato–Katz technique described by [23] highlighted that the staining solution primarily functions to clear fecal debris while preserving the structural characteristics required for accurate species identification[24].

Among the evaluated natural stains, the 95% fragrant pandan leaf extract exhibited the highest overall staining quality, approaching the performance of conventional malachite green. The superior performance of pandan extract is likely attributable to its high chlorophyll concentration, which produced a bright green background with excellent transparency and contrast. Chlorophyll has previously been investigated as a natural biological pigment because of its optical stability and ability to improve visualization under light microscopy [25]. Similar findings have also been reported in histological studies utilizing chlorophyll-rich plant extracts, where natural green pigments enhanced tissue contrast while maintaining specimen integrity [26]. The present findings therefore support the growing interest in chlorophyll-based dyes as environmentally friendly substitutes for synthetic laboratory stains.

Although mangosteen peel extract produced a slightly darker microscopic background, particularly at the highest concentration, its staining performance remained comparable to the conventional stain. This darker appearance can be attributed to the high concentration of anthocyanins and xanthones present in mangosteen peel. Previous studies have reported that anthocyanins exhibit intense coloration, high antioxidant activity, and relatively good stability under acidic conditions, making them suitable natural pigments for analytical and biological applications [12]. Furthermore, xanthones, particularly α-mangostin, have been identified as the principal bioactive compounds responsible for the deep purple pigmentation of mangosteen peel [27]. Although darker pigments may slightly reduce background transparency, they do not interfere with the visualization of helminth egg morphology, as demonstrated in the present study.

The results also demonstrated that increasing extract concentration significantly improved staining quality. Preparations stained with 95% pandan leaf extract and 95% mangosteen peel extract achieved higher transparency, contrast, and morphological clarity than those stained with 75% extracts. This finding is consistent with Beer–Lambert's principle, which states that increasing pigment concentration enhances light absorption and color intensity. Similar concentration-dependent improvements have been reported in studies evaluating natural dyes extracted from roselle (*Hibiscus sabdariffa*), butterfly pea (*Clitoria ternatea*), and beetroot

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178

**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

(*Beta vulgaris*), where higher pigment concentrations produced clearer microscopic images and improved diagnostic quality [28]. Therefore, pigment concentration is an important determinant of staining effectiveness in natural laboratory dyes.

Statistical analysis revealed no significant differences in overall staining quality between the natural extracts at higher concentrations and the conventional malachite green stain ($p > 0.05$). This result indicates that both natural stains provide diagnostic performance comparable to the standard staining reagent used in the Kato–Katz method. Similar conclusions have been reported by studies evaluating alternative natural dyes for histological and microbiological staining, which found that several plant-derived pigments produced staining quality equivalent to commercial synthetic dyes while offering superior environmental safety[29]. These findings reinforce the feasibility of replacing hazardous synthetic dyes with renewable botanical alternatives.

The environmental implications of the present study are particularly important. Malachite green has long been recognized as an effective laboratory stain; however, it is also classified as a hazardous synthetic dye because of its mutagenic, teratogenic, and potentially carcinogenic properties. In addition, malachite green is resistant to biodegradation and may contribute to environmental pollution when improperly discarded[30] .In contrast, pandan leaves and mangosteen peels are renewable, biodegradable, and readily available plant materials that generate minimal environmental impact. The utilization of mangosteen peel, an agricultural by-product, also supports waste valorization and aligns with the principles of sustainable laboratory practice and green chemistry [31].

Despite these promising findings, several limitations should be acknowledged. This study evaluated only three species of Soil-Transmitted Helminths and employed qualitative phytochemical analysis. Quantitative determination of chlorophyll, anthocyanins, and xanthones using high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS) would provide a more comprehensive understanding of the relationship between pigment concentration and staining quality. Future studies should also evaluate long-term storage stability, reproducibility across multiple laboratories, and the diagnostic performance of these natural stains against a wider range of intestinal parasites. Such investigations would further strengthen the evidence supporting the routine implementation of plant-based staining reagents in parasitological laboratories[32].

The results of this study showed that pandan leaf and mangosteen peel extracts provided adequate contrast for the identification of STH eggs using the Kato–Katz method. Compared with molecular methods such as PCR, Kato–Katz generally has lower sensitivity, particularly in low-intensity infections, although PCR requires more advanced equipment and higher costs. In comparison with concentration methods, such as sedimentation or flotation, Kato–Katz provides the additional advantage of estimating infection intensity through egg counts[33]. Therefore, the natural stains developed in this study may improve egg visualization but do not eliminate the inherent sensitivity limitations of the Kato–Katz method. This study was limited by sample number and type, the lack of comparison with a molecular reference method, and the absence of a comprehensive evaluation of stain stability, shelf life, and batch-to-batch reproducibility. Further studies with larger sample sizes and comparison with PCR or concentration methods are recommended.

Overall, the findings indicate that pandan leaf and mangosteen peel extracts represent promising natural alternatives for Kato–Katz cellophane staining under controlled laboratory conditions. Nevertheless, further investigations incorporating quantitative pigment characterization, objective image analysis, cost-effectiveness assessment, shelf-life evaluation, multicenter reproducibility testing, and broader parasitological validation are necessary before these natural stains can be recommended for routine diagnostic use.

## V. CONCLUSION

This study aimed to develop natural cellophane stains from pandan leaf (*Pandanus amaryllifolius*) and mangosteen peel (*Garcinia mangostana*) extracts for STH diagnosis using the Kato–Katz method. The results demonstrated that the natural stains provided adequate visualization of STH eggs, with an overall accuracy of **95%** compared with the conventional stain. These findings indicate that pandan leaf and mangosteen peel extracts have potential as alternative natural stains for Kato–Katz examination. Further research using larger sample sizes, standardized extract concentrations, and comparison with molecular diagnostic methods is recommended to validate their diagnostic accuracy and reproducibility.

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178

**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178

**Jurnal Teknokes**
**Homepage: teknokes.org; Vol. 19, No. 3, pp. 157-167, September 2026;**
**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178

**e-ISSN: 2407-8964**
**p-ISSN: 1907-7904**

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**DOI:** https://doi.org/10.35882/jteknokes.v19i3.178