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Optimizing *Canang* Flower Waste Extract for Staining *Fasciolopsis buski* Eggs: An Enhancement of the Kato-Katz Method

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Abstract. *Fasciolopsis buski* is a parasitic helminth that can infect humans. Diagnosing helminthiasis can be confirmed through fecal testing utilizing the Kato-Katz method. The Kato-Katz method employs methylene blue staining, which poses environmental hazards due to its carcinogenic characteristics. An alternative to staining is the use of a *canang*, a common Balinese item used for prayers. The utilized waste *canang* flowers were *Tagetes erecta*, *Impatiens balsamina* (red), and *Impatiens balsamina* (purple). The research began with an extraction procedure that involved cutting the flowers into small pieces, resulting in approximately 500 grams of fragments. The flower components were macerated in two liters of 96% ethanol for five days. Additionally, cellophane immersion of the extracted findings was performed. The findings indicated that each sample from the three treatment groups (T1, 1%; T2, 2%; and T3, 3%) and the two control groups. Sub-analysis testing evaluated the quantity of helminth eggs, quantified as eggs per gram of feces via field-of-view observation. The T3 (3%) exhibited results that were not statistically significantly different (P>0.05) from the positive control group. The T3 (3%) test provides the most favorable and optimal results as a substitute for methylene blue in microscopic staining evaluations.

Keywords: *Fasciolopsis buski*; Helminthiasis; *Impatiens balsamina*; Methylene blue; *Tagetes erecta*;

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INTRODUCTION

Fasciolopsis buski is a parasitic helminth responsible for Fasciolopsiasis, first identified in Indonesia with a prevalence of 1.2%–7.8% (Khairudin et al., 2012). Recognized as a significant public health concern, the World Health Organization (WHO) has implemented control measures to mitigate its spread (Sehatman, 2015). Transmission occurs via oral ingestion of contaminated food, water, or soil, particularly in areas with poor sanitation (Cicirielli et al., 2022). Helminthiasis contributes to severe morbidity, including anemia, growth retardation, cognitive impairment, and heightened susceptibility to infections (Ezeamama et al., 2018), with a global burden of six million disability-adjusted life years (GBD, 2015). Its severity is determined by infection prevalence and intensity (Carpenter et al., 2017). Risk factors include inadequate sanitation, poor hygiene, overcrowding, and limited healthcare access (WHO, 2020). Diagnosis relies on the Kato-Katz technique, facilitating targeted interventions, while preventative chemotherapy strategies identify high-risk communities and optimize treatment

frequency (Barenbold et al., 2018).

Fecal analysis is the definitive method for diagnosing helminthiasis, with the Kato-Katz technique widely utilized due to its reliability, cost-effectiveness, and ease of implementation (Ardi et al., 2019). Its sensitivity, particularly in double applications, is comparable to the formol-ether method (Barenbold et al., 2017). Due to its accessibility and low-cost reusable materials, Kato-Katz remains the preferred diagnostic tool for soil-transmitted helminths (Cools, 2019). As an intermediate quantification method, it plays a vital role in routine health assessments and research, enabling accurate microscopic evaluation of helminth species, infection intensity, and egg morphology (Barbosa et al., 2017). The Kato-Katz technique utilizes methylene blue, a synthetic thiazine dye that enhances helminth egg visibility by staining cellophane (Oladoye et al., 2022). Despite its cost-effectiveness, methylene blue is carcinogenic and environmentally hazardous, contributing to persistent contamination due to its benzene structure and heavy metal content (Mitra et al., 2022; Christina et al., 2007). In Indonesia, its permissible water concentration is regulated at 5–10 mg/L (Ministerial Decree No. 51/MENLH/10/1995). Extensive use of methylene blue in Kato-Katz diagnostics necessitates eco-friendly alternatives to mitigate environmental risks while maintaining diagnostic efficacy, particularly in regions with high infection prevalence (Okoyo et al., 2018).

This study refines the Kato-Katz diagnostic technique by introducing canang flower waste extract as an alternative staining agent for *Fasciolopsis buski* egg detection. Key experiments included cellophane absorption tests, helminth egg identification, and heavy metal analysis of the extracted dye. Repurposing canang flower waste as a sustainable stain enhances the gold-standard method for helminthiasis diagnosis while providing an eco-friendly alternative to methylene blue. This innovation advances environmentally sustainable parasitological diagnostics. Effective staining for helminth egg detection requires sufficient brightness and visibility. This study investigates canang flower waste as an eco-friendly alternative to methylene blue in the Kato-Katz diagnostic procedure. Traditionally used in Balinese Hindu rituals, *Impatiens balsamina* and *Tagetes erecta* possess natural pigment properties suitable for staining (Wijaya et al., 2021). Despite its potential, canang flower waste remains underutilized. Large quantities—averaging 0.8 kg on weekdays and 2.4 kg on holy days—could contribute to environmental concerns if discarded. Repurposing this waste offers a biodegradable staining solution, aligning with local practices while mitigating synthetic dye-related pollution.

METHODS

This study utilized an experimental laboratory design with test and control groups. *Tagetes erecta* and *Impatiens balsamina* (red and purple) samples were collected from eight Hindu temple sites in Buleleng, Bali. The independent variable was extract concentration, while the dependent variables included cellophane absorption, helminth egg morphology, species identification, pH levels, and heavy metal contamination. Ethical approval was obtained from the Faculty of Medicine, Universitas Pendidikan Ganesha (Approval No. 032/UN48.24.11/LT/2024).

Preparation of *Canang* Flower Waste Extract

This study evaluates the extraction of soluble pigments from canang flower waste for potential use as a staining agent. Waste was collected from eight temples in Buleleng, Bali, and categorized into four groups: *Impatiens balsamina* (purple and red), *Tagetes erecta*, and a mixed sample. Extraction involved macerating 500 g of flower material in two liters of 96% ethanol for five days, followed by filtration and concentration using a rotary evaporator at 70°C (Octora & Waruwu, 2022). The resulting extract was stored in sealed containers and analyzed for lutein and anthocyanin content via UV-Vis spectrophotometry, measuring absorbance at 360–550 nm and 465–560 nm, respectively. Cellophane was submerged in 1%–3% methylene blue reagent with glycerol and distilled water as solvents, maintained for 24 hours in a foil-covered container (Calvopina, 2018). This approach assesses the viability of canang flower waste as a sustainable staining alternative in diagnostic procedures.

Table 1. Group Variation of *Canang* Flower Waste Extract Concentration

Group	T1	T2	T3	C (-)	C (+)
Concentration (%)	1%	2%	3%	Methylene blue reagent with a helminth-negative sample	Methylene blue reagent with a helminth-positive sample

Description:

Concentration (%) = $\frac{Extract\ Volume}{Solvent\ Volume} \times 100\%$

T1: Concentration of 1% of *Canang* flower waste extract

T2: Concentration of 2% of *Canang* flower waste extract

T3: Concentration of 3% of *Canang* flower waste extract

Kato-Katz Methods for Identification and Detection of Helminth Eggs

Helminth egg morphology was analyzed using preserved specimens at the Faculty of Medicine, Universitas Pendidikan Ganesha, following WHO Bench Aids (2019) and Kato-Katz methodology. Stool slides were prepared according to standard protocols (Altman et al., 2025), with egg counts calculated as eggs per gram (EPG) using a multiplication factor of 24, based on a mold weight of 41.7 mg. Detection results included quantification and infection intensity, determined by the staining efficacy of extract-treated cellophane. Identification outcomes were classified as: (1) no findings, (2) eggs with abnormal morphology, and (3) eggs with normal morphology. All morphological assessments were validated by a specialized parasitology laboratory to ensure accuracy.

Cellophane Absorption Test

The absorption capacity of cellophane must be systematically evaluated to ensure optimal staining results. Staining quality, including extraction efficiency, cellophane preparation, and microscopic examination findings, will be assessed using a structured questionnaire. This evaluation will include data on cellophane absorption characteristics and will be conducted by six trained experts from the Biomedical Laboratory, Faculty of Medicine, Universitas Pendidikan Ganesha. The assessment prioritizes objectivity to enhance precision in staining analysis, ensuring reliable and reproducible results for diagnostic applications.

Implementation of Heavy Metal Test and pH Analysis

The acidity of each solution was evaluated using standardized pH paper. The extraction results underwent analytical verification to ensure the absence of heavy metal contamination. The extracted solutions were immersed in cellophane and stored in sealed, foil-lined containers for preservation. Lead (Pb), cadmium (Cd), and chromium (Cr) pose significant risks to human health and environmental sustainability due to their toxicity and persistence in ecosystems (Dewi NK. Et al., 2019). All experiments were conducted at the Faculty of Mathematics and Natural Sciences Laboratory, Universitas Pendidikan Ganesha. Heavy metal analysis followed atomic absorption spectrophotometry (AAS) procedures outlined by Mantra & Widnyana (2022). If contamination was detected, AAS provided quantitative data on heavy metal concentrations within the extracted solutions.

Data Analysis

The cellophane absorbency test results were classified as ordinal and analyzed using the Mann-Whitney test. Detection of helminth eggs was assessed using the Chi-Square method with a positive control for comparison, followed by a paired T-test for sub-analysis of egg findings per gram of sample material. Microscopic morphological analysis of stained helminth eggs provided detailed observations of egg characteristics. Heavy metal contamination levels were quantified in ppm units and evaluated against the quality standards specified in the Regulation of the Minister of Environment of the Republic of Indonesia No. 5 of 2014, Appendix XLIV, item b. The alpha level for statistical analysis was set at 0.05 (two-tailed). pH levels were measured using standardized units for acidity assessment.

RESULTS AND DISCUSSION

Result of *Fasciolopsis buski* Detection Test

The Chi-Square test was deemed not applicable (N/A) in the Kato-Katz detection analysis, as results from cellophane treated with canang flower dye were consistent with the gold standard. The observed decrease in sensitivity may be attributed to limited stool sample size, variability in daily egg excretion, and the uneven distribution of eggs within fecal matter (Casacuberta et al., 2016). While faecal egg detection methods exhibit high specificity, their sensitivity is reduced in cases of light-intensity infections, often leading to overestimating population incidence and disease burden (King, 2015). To enhance diagnostic accuracy, a sub-analysis of *Fasciolopsis buski* egg counts per gram of feces was performed on the 1%, 2%, and 3% dye test groups using a paired t-test (Table 2).

Table 2. Detection test on *Fasciolopsis buski* Identification

Sample		Gold Standard *Fasciolopsis buski*		*Chi-Square* Test
		Positives (N=5)	Negatives (N=5)	
Tagetes erecta 1%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (purple) 1%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (red) 1%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Mixed Flowers 1%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Tagetes erecta 2%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (purple) 2%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (red) 2%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Mixed flowers 2%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Tagetes erecta 3%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (purple) 3%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Impatiens balsamina (red) 3%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*
Mixed flowers 3%	Found	5 (100%)	0 (0%)	
	Not found	0 (0%)	0 (0%)	<0.01*

*Using Chi-Square Fisher's exact test
1. Positive means that *Fasciolopsis buski* eggs were found in the fecal.
2. Negative means no *Fasciolopsis buski* eggs were found in the fecal.

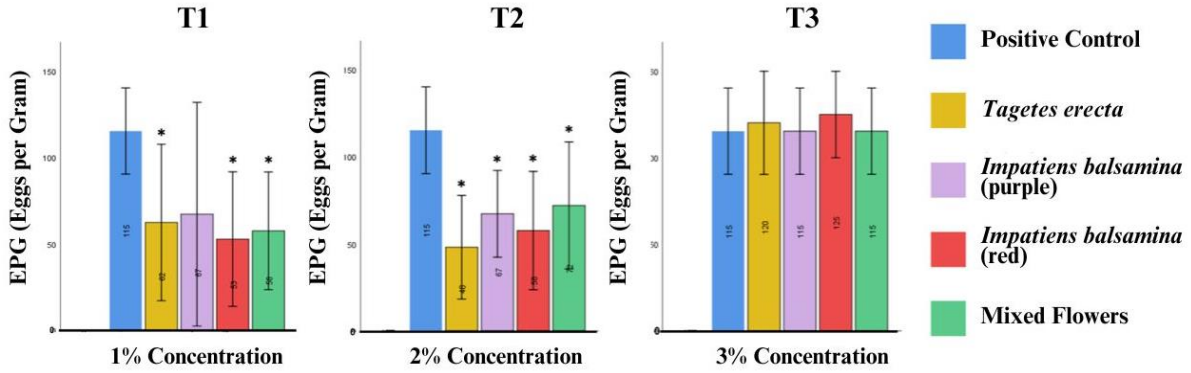


Figure 1. Sub-analysis of the findings on the number of *Fasciolopsis buski* eggs per gram of fecal matter in the 1%, 2%, and 3% dye test groups by paired t-test.

The detection test using the Kato-Katz technique demonstrated that treatments P1 (1%), P2 (2%), and P3 (3%)—derived from *Tagetes erecta* and *Impatiens balsamina* (red and purple) extracts—effectively stained *Fasciolopsis buski* eggs in fecal samples. Each extract produced distinct staining, facilitating helminth egg identification. Sensitivity variation in the Kato-Katz method may be influenced by inter- and intra-specimen differences in helminth egg distribution within stool samples (Bosch et al., 2021).

Stool staining yielded a 100% positive detection rate (n=5). Sub-analysis of helminth egg counts per gram of feces, assessed through field-of-view observation, indicated that staining with P1 and P2 extracts produced significantly different results ($P < 0.05$) compared to the EPG of the positive control. In contrast, P3 (3%) showed no statistically significant difference ($P > 0.05$),

suggesting comparable efficacy to the gold standard.

The specificity of the Kato-Katz method is rarely isolated in diagnostic evaluations, as multiple procedures are often combined to create a composite gold standard (Tarafder, 2010). The P3 group exhibited superior staining performance across all flower extract variations, reinforcing its potential as an alternative staining agent. Sensitivity remains crucial for helminthiasis eradication efforts (Utzinger et al., 2015). Further research is needed to address inter-laboratory variability and assess the cost-effectiveness of stool sample processing in diverse endemic contexts (Kure et al., 2015)

Result of Morphology Identification for *Fasciolopsis buski* Eggs

Morphological identification of *Fasciolopsis buski* eggs was conducted using Figures 2 and 3. Figure 2 illustrates a clear visualization of helminth eggs in the P3 group, while Figure 3 depicts damaged eggs, which were excluded from quantification. A standardized framework for fecal egg count (FEC) estimation ensures precise calculation of population mean FEC across various endemic scenarios, considering individual variability in egg aggregation and diagnostic sensitivity (Levecke et al., 2015). This approach informs sample size requirements for accurate helminthiasis burden assessment

Fasciolopsis buski eggs are broadly ellipsoid, measuring 130–150 µm in length and 60–90 µm in width. They do not undergo embryonation upon excretion and closely resemble *Fasciola hepatica* eggs, though rough abopercular tips aid differentiation (CDC, 2024). Figure A illustrates egg morphology in the positive control group stained with methylene blue, confirming the standard Kato-Katz diagnostic approach. The T3 (3%) sample group exhibited statistically comparable results ($P > 0.05$). Figures B–E depict egg morphology using *Impatiens balsamina* (red and purple) and *Tagetes erecta* (red and purple) extracts, as well as a mixed extract group, demonstrating effective staining and structural visualization. These findings validate the potential of T3 (3%) extract as an alternative staining agent in helminthiasis diagnosis.

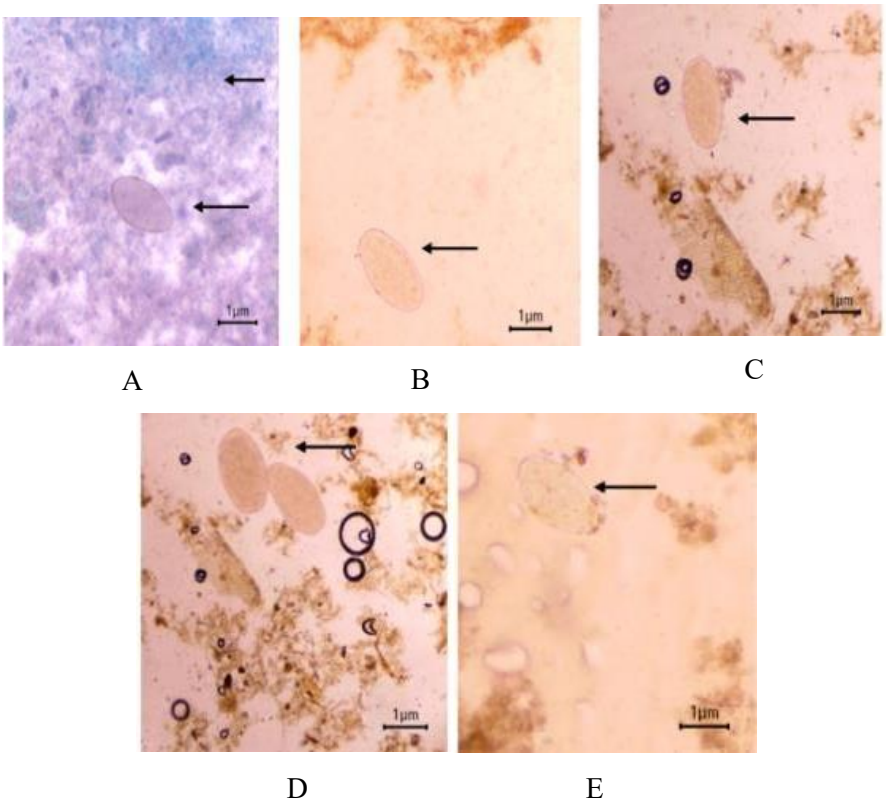
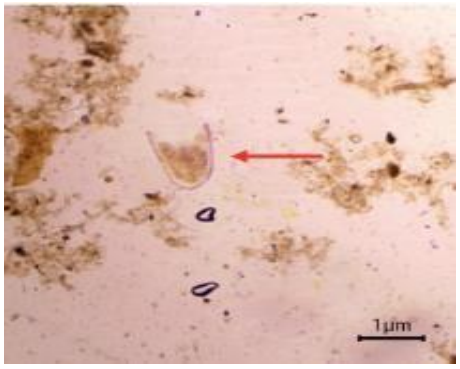


Figure 2. *Fasciolopsis buski* egg finding (black arrow) using the Kato-Katz method in treatment group T3. Left-right microscopic: positive control group, T3 group of *Impatiens balsamina* flowers, T3 group of *Tagetes erecta* (purple) flowers, P3 group of *Tagetes erecta* (red) flowers, T3 group of mixed flowers.

Microscopic examination at a 1µm scale assessed identification outcomes from Figures A–E. Morphological evaluations covered the entire visual field and categorized findings as: (1) No detection, (2) eggs with abnormal morphology, and (3) eggs with normal morphology. Validation was conducted by the parasitology laboratory at the Faculty of Medicine, Universitas Pendidikan Ganesha. Results confirm the effectiveness of the positive control group and T3 (3%) concentration in reliably identifying *Fasciolopsis buski* egg morphology.



F

Figure 3. Findings of *Fasciolopsis buski* eggs with damaged walls (red arrows) in the T1 *Tagetes erecta* flower treatment. Scale bar: 1µm

The picture illustrates further findings from the detection and morphological examination of *Fasciolopsis buski* eggs. The morphological identification results of *Fasciolopsis buski* utilizing the T1 (1%) *Tagetes erecta* group revealed an egg structure exhibiting a damaged egg wall, indicated by the red arrow in Figure F, with a scale bar of 1µm. This indicates that the group can recognise the egg's morphology; nevertheless, the microscopic appearance is unclear, hindering the vision of the egg's morphology with compromised walls.

Cellophane Absorption Test on *Canang* Flower Waste Extract

The Kato-Katz technique exhibits variability in helminth egg detection, particularly in low-infection regions (Kittur et al., 2016). This study assessed the absorption capacity of cellophane treated with canang flower waste extract to enhance staining performance. Microscopic analysis (Table 5) showed that the 3% concentration (T3) produced staining results comparable to the gold standard ($P > 0.05$), whereas 1% (T1) and 2% (T2) exhibited significantly lower effectiveness ($P < 0.01$). The optimal performance of T3 is attributed to increased pigment solubility, enhancing visualization (Mizuno et al., 2024). An ordinal-scale absorption assessment, conducted by biomedical laboratory personnel at Universitas Pendidikan Ganesha, classified samples (1–4) from low (1) to very good (4). These findings support the viability of T3 as a sustainable alternative to methylene blue in helminthiasis diagnostics.

Table 3. The Results of the Absorption Test on the Staining from the Preparation Solution Process.

	Staining of Cellophane Preparation Solution				
Sample	1	2	3	4	Mann-Whitney Test
Control Groups	0 (0%)	0 (0%)	0 (0%)	6 (100%)	N/A
Tagetes erecta 1%	6 (100%)	0 (0%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (purple) 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (red) 1%	4 (66.7%)	2 (33.3%)	0 (0%)	0 (0%)	<0.01
Mixed Flowers 1%	3 (50%)	3 (50%)	0 (0%)	0 (0%)	<0.01
Tagetes erecta 2%	1 (16.7%)	4 (66.7%)	1 (16.7%)	0 (0%)	<0.01
Impatiens balsamina (purple) 2%	1 (16.7%)	4 (66.7%)	1 (16.7%)	0 (0%)	<0.01
Impatiens balsamina (red) 2%	0 (0%)	4 (66.7%)	2 (33.3%)	0 (0%)	<0.01
Mixed Flowers 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	<0.01
Tagetes erecta 3%	0 (0%)	1 (16.7%)	2 (33.3%)	3 (50%)	<0.01
Impatiens balsamina (purple) 3%	0 (0%)	1 (16.7%)	3 (50%)	2 (33.3%)	<0.01
Impatiens balsamina (red) 3%	0 (0%)	1 (16.7%)	3 (50%)	2 (33.3%)	<0.01
Mixed Flowers 3%	0 (0%)	2 (33.3%)	3 (50%)	1 (16.7%)	<0.01

Table 4. The Result of the Absorption Test on the Staining from the Cellophane Slide

Sample	Staining of Cellophane Slide				
	1	2	3	4	Mann-Whitney Test
Control Groups	0 (0%)	0 (0%)	0 (0%)	6 (100%)	N/A
Tagetes erecta 1%	6 (100%)	0 (0%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (purple) 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (red) 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Mixed Flowers 1%	3 (50%)	3 (50%)	0 (0%)	0 (0%)	<0.01
Tagetes erecta 2%	3 (50%)	3 (50%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (purple) 2%	2 (33.3%)	4 (66.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (red) 2%	3 (50%)	3 (50%)	0 (0%)	0 (0%)	<0.01
Mixed Flowers 3%	2 (33.3%)	4 (66.7%)	0 (0%)	0 (0%)	<0.01
Tagetes erecta 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	<0.01
Impatiens balsamina (purple) 3%	0 (0%)	1 (16.7%)	5 (83.3%)	0 (0%)	<0.01
Impatiens balsamina (red) 3%	0 (0%)	1 (16.7%)	5 (83.3%)	0 (0%)	<0.01
Mixed Flowers 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	<0.01

Table 5. The Result of the Absorption Test on the Staining from the Microscopic Checking.

Sample	Staining Produced from the Microscopic Examination				
	1	2	3	4	Mann-Whitney Test
Control	0 (0%)	0 (0%)	4 (66.7%)	2 (33.3%)	N/A
Tagetes erecta 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (purple) 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (red) 1%	4 (66.7%)	2 (33.3%)	0 (0%)	0 (0%)	<0.01
Mixed Flowers 1%	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	<0.01
Tagetes erecta 2%	3 (50%)	3 (50%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (purple) 2%	2 (33.3%)	4 (66.7%)	0 (0%)	0 (0%)	<0.01
Impatiens balsamina (red) 2%	2 (33.3%)	4 (66.7%)	0 (0%)	0 (0%)	<0.01
Mixed Flowers 3%	1 (16.7%)	4 (66.7%)	1 (16.7%)	0 (0%)	<0.01
Tagetes erecta 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.056*
Impatiens balsamina (purple) 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.056*
Impatiens balsamina (red) 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.056*
Mixed Flowers 3%	0 (0%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.056*

The asterisk (*) in the table indicates that the results are not significantly different from or equal to the methylene blue staining capability.

pH Assessment and Heavy Metal Contamination Level Examination

The environmental impact of canang flower waste extract was evaluated through heavy metal content analysis and pH assessment. All test results adhered to the wastewater quality standards for healthcare facilities, as outlined in the Regulation of the Minister of Environment of the Republic of Indonesia No. 5 of 2014, Appendix XLIV, item b. The selection of the 3% concentration (T3) was based on prior findings demonstrating superior staining efficacy. This study sought to confirm whether the T3 concentration met environmental safety criteria. Heavy metal analysis using atomic absorption spectrophotometry (AAS) verified compliance with prescribed standards across all test samples. The 3% mixed floral extract exhibited the highest efficiency, meeting all assessment criteria, including pH and heavy metal content evaluation.

Table 6. Heavy Metal Contamination Level Examination

Sample	pH		Pebble (Pb)		Cadmium (Cd)		Lead (As)		Conclusion
	Test Result (mg/L)	Standard (mg/L)	Test Result (mg/L)	Standard (mg/L)	Test Result (mg/L)	Standard (mg/L)	Test Result (mg/L)	Standard (mg/L)	
Tagetes erecta 3%	7	6-9	-19.14	0.1	-48.24	0.05	-300.84	0.1	Passing Standard
Impatiens balsamina (purple) 3%	7	6-9	-16.98	0.1	-4.29	0.05	-326.94	0.1	Passing Standard
Impatiens balsamina (red) 3%	7	6-9	-13.94	0.1	-59.36	0.05	-369.44	0.1	Passing Standard
Mixed Flowers 3%	6	6-9	-13.84	0.1	-36.41	0.05	-228.62	0.1	Passing Standard

* Assessment of heavy metal contamination levels by atomic absorption spectrophotometry (AAS).

Table 7. pH Assessment Result

Treatment	pH			
	Impatiens balsamina (red)	*Impatiens balsamina* (purple)	*Tagetes Erecta*	Mixed Flowers
P1	6	7	6	6
P2	6	7	7	7
P3	7	7	7	6

* A pH value of 6-9 mg/mL is the gold standard for analysing heavy metal contamination levels, adapted to its use in the health sector.

This study introduces a modified Kato-Katz diagnostic technique, incorporating canang flower waste extract as an alternative staining method for *Fasciolopsis buski* eggs. The conventional Kato-Katz approach relies on methylene blue, a carcinogenic and environmentally harmful dye. This research optimizes natural pigments from *Impatiens balsamina* (red and purple) and *Tagetes erecta* to develop a sustainable staining solution that maintains equivalent diagnostic efficacy. This study advances both scientific innovation and environmental sustainability by repurposing canang flower waste, also providing a biodegradable alternative to methylene blue in helminthiasis diagnosis.

CONCLUSION

Microscopic analysis confirmed clear staining of *Fasciolopsis buski* eggs in fecal samples. Detection testing across 1%, 2%, and 3% dye concentrations found no statistical difference ($P > 0.05$) between the 3% concentration (P3) and the gold standard, validating its diagnostic efficacy. Cellophane absorption testing identified the 3% concentration (T3) as optimal, supporting its viability as a methylene blue alternative. Environmental assessments verified compliance with standardized safety thresholds, with pH levels of 7 for *Impatiens balsamina* (red and purple) and *Tagetes erecta*

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AUTHOR CONTRIBUTION STATEMENT

IKTYW conceptualized and designed the study, performed data analysis, and led manuscript composition. KIM conducted the cellophane test using the Kato-Katz method, detection tests, and morphological identification of *Fasciolopsis buski*. PSAJ managed the preparatory phase, including literature review and resource acquisition. DGPM supervised data collection, analysis, and reporting. MP served as the editor, while MBP was the corresponding author. IR finalized incomplete sections and assisted with manuscript revisions.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest related to the publishing of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors affirm that no artificial intelligence (AI) tools were utilized in the generation, analysis, or writing of this manuscript. All research activities, including data collection, interpretation, and manuscript preparation, were conducted solely by the authors without AI assistance.

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