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Effectiveness of Mangosteen Peel Pressed Water (*Garcinia mangostana* L.) as an Organic Agent for Examination of Feces Confirmed Helminthiasis

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Abstract

Purpose: This study aimed to compare the effectiveness of mangosteen peel pressed water without the addition of other ingredients with mangosteen peel pressed water (*Garcinia mangostana* L.) which has been added with citric acid as an organic agent in fecal examinations confirmed helminthiasis. This study is very relevant considering the global health problem of worms, which is often exacerbated by poor sanitation and traditional diagnostic methods that can pose health risks.
Methodology: An experimental design was conducted with a post-test only group design. The study involved nine treatments and three repetitions, utilizing fecal specimens that tested positive for helminthiasis. The mangosteen peel pressed water was prepared in varying concentrations (10%, 20%, 30%, and 40%), both with and without citric acid. The effectiveness of the dye was assessed through various parameters, including staining quality and visualization of parasitic structures.
Results: The analysis indicated that all concentrations of mangosteen peel pressed water demonstrated consistent effectiveness in staining. Statistical evaluation using the Kruskal Wallis test revealed no significant differences among the treatments, with p-values for effectiveness (0.645), visualization of parasitic structures (0.364), and quality of staining (0.463). This suggests that the different concentrations and conditions did not significantly impact the outcomes, confirming the reliability of mangosteen peel pressed water staining agent for feces examination.
Applications: The findings support the use of mangosteen peel pressed water as a safe and effective alternative dye for detecting helminthiasis, leveraging its anthocyanin content. This research contributes to developing more environmentally friendly diagnostic methods, reducing reliance on potentially harmful chemicals used in traditional staining processes. By enhancing diagnostic accuracy, this study has the potential to improve community health outcomes in areas affected by helminthiasis, particularly in regions with limited access to safe diagnostic tools.

Introduction

Helminthiasis is a global health issue that is often overlooked. This infection is generally caused by intestinal nematodes that fall into the categories of Soil Transmitted Helminths (STH) and non-STH, which can be transmitted when the eggs of the worms are ingested through contaminated food or drink. The STH group consists of *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale,* and *Necator americanus*, while the non-STH group includes *Strongyloides stercoralis and Enterobius vermicularis* (Sutanto *et al.,* 2017).

According to the World Health Organization (WHO) in 2018, more than 1.5 billion people, or approximately 24% of the global population, are infected with various types of parasitic worms, with children under the In Indonesia, the prevalence of helminthiasis infection varies between 2.5% and 62%, particularly in areas with poor sanitation and high population density (Kemenkes RI, 2017). The risk factors for this infection include the consumption of food or drinks contaminated with worm eggs, open defecation practices, and a lack of personal hygiene (Ngwese *et al.,* 2020).

Soil Transmitted Helminths (STH) are species of worms that are transmitted through soil. The eggs of STH can survive in moist soil, where they develop into infectious eggs that can be ingested or penetrate human skin (Lestari 2022). In contrast, non-Soil Transmitted Helminth species do not require soil for their life cycle and can be transmitted in various

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forms. The long-term effects of helminthiasis can disrupt children's growth and development, cause nutritional deficiencies, lower cognitive abilities, and increase the risk of anemia (Panjaitan 2022; Binuko, *et al.,* 2022).

The most commonly utilized method for supporting the diagnosis of helminthiasis involves the application of 2% eosin in the direct slide technique. Synthetic dyes, including eosin, stain the acidophilic components of tissues due to their acidic nature. The cytoplasm and collagen will be colored pink by eosin. Eosin has an acidic composition, which makes the helminth eggs more visible. The use of 2% eosin is intended to help differentiate the worm eggs from the surrounding fecal matter (Sulaeman *et al.,* 2023). However, there are several drawbacks to using eosin, including its tendency to evaporate, the presence of various harmful chemicals, and its classification as a Group 3 carcinogen by the IARC (Wahyuni and Sabban, 2022). Therefore, it is essential to seek safer and more environmentally friendly alternatives.

One alternative to this issue is the use of mangosteen peel pressed water (*Garcinia mangostana* L.) as another option to eosin (Rahmadila *et al.,* 2023). Mangosteen (*Garcinia mangostana L.*) is a tropical plant native to Southeast Asia, including Indonesia. It is considered a functional plant because nearly all parts of it can be utilized. Known as the "Queen of Tropical Fruits," mangosteen is characterized by its unique sweet and sour flavor that sets it apart from other fruits. This seasonal fruit has a dark purple rind and contains 1 to 5 seeds within its white flesh. The rind of the mangosteen exhibits pharmacological activities, functioning as an anti-allergic, anti-inflammatory, antifungal, antioxidant, antibacterial, anticancer agent, and more (Saraswati and Astutik 2022). Mangosteen peel is known to be rich in bioactive compounds, particularly anthocyanins which give the peel its characteristic purple color (Nidyasari *et al.,* 2018). Anthocyanins are natural pigments with strong antioxidant properties, which can help protect the body's cells from damage caused by free radicals (Silalahi 2021). In addition to anthocyanins, mangosteen peel also contains xanthones, which are polyphenolic compounds that have been shown to possess anti-inflammatory and antimicrobial activities (Saraswati & Astutik, 2022). According to previous research by (Suraini and Sophia 2022) the most effective solvent for anthocyanin extraction is water, as water has a polarity level comparable to that of anthocyanins.

The polar nature of anthocyanins necessitates their dissolution in similarly polar substances to prevent degradation, one of which is citric acid. Citric acid plays a crucial role in maintaining the color stability of anthocyanins by creating a low pH environment, thereby increasing acidity and resulting in a deeper red hue. This also enhances the longevity of the preparation due to its ability to inhibit bacterial growth (Akmalia et al. 2021; Sabahannur 2020). Preservation using citric acid has the ability to lower acidity levels (pH), which helps inhibit the growth of microorganisms responsible for spoilage.

In previous research, mangosteen peel juice was used as a substitute for eosin in the examination of worm eggs, producing a purple color due to anthocyanin pigments at a 1:2 ratio, which closely approximated the quality of 2% eosin (Febriyanti, Mulia, and Valencia 2024). Another study by Kartini (2022) utilized red dragon fruit peel and red spinach stems as alternative reagents for examining Ascaris lumbricoides eggs. The results indicated that the juice from red dragon fruit peel at a 1:1 ratio produced contrasting images similar to those obtained using 2% eosin when compared with red spinach stems. Similar research by (Ereskadi and Apriani 2022; Nizar, Hamtini, and Alifah 2023; Rahmadila *et al.,* 2023) found that the use of natural colorants from mangosteen peel could serve as an economical alternative that is easily accessible in tropical countries, representing a new environmentally friendly innovation. Currently, there is no research data regarding the application of mangosteen peel pressed water combined with organic acid, specifically citric acid, for staining feces confirmed to contain helminths. Thus, the researchers were compelled to do a study entitled: "Effectiveness of Mangosteen Peel Pressed Water (*Garcinia mangostana* L.) as an Organic Agent for Examination of Feces Confirmed Helminthiasis."

This study aimed to compare the effectiveness of mangosteen peel juice without the addition of other ingredients with mangosteen peel juice (*Garcinia mangostana* L.) which has been added with citric acid as an organic agent in fecal examinations confirmed helminthiasis. This study is very relevant considering the global health problem of worms, which is often exacerbated by poor sanitation and traditional diagnostic methods that can pose health risks. It is hoped that the results of this research will contribute to the development of safer and more effective diagnostic methods for detecting helminthiasis.

Method

This study employs an experimental design using a post-test only group design, involving nine treatment groups with three repetitions each. The nine treatment groups in this research include:

1. Group 1: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 10% on feces confirmed for helminthiasis.
2. Group 2: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 20% on feces confirmed for helminthiasis.
3. Group 3: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 30% on feces confirmed for helminthiasis.

4. Group 4: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 40% on feces confirmed for helminthiasis.
5. Group 5: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 10% with an addition of citric acid 0.09 grams (9%) on feces confirmed for helminthiasis.
6. Group 6: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 20% with an addition of citric acid 0.09 grams (9%) on feces confirmed for helminthiasis.
7. Group 7: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 30% with an addition of citric acid 0.09 grams (9%) on feces confirmed for helminthiasis.
8. Group 8: Mangosteen peel pressed water (*Garcinia mangostana* L.) with concentration of 40% with an addition of citric acid 0.09 grams (9%) on feces confirmed for helminthiasis.
9. Group 9: 2% Eosin as a comparison to mangosteen peel pressed water (*Garcinia mangostana* L.)

The subjects of the study are feces confirmed for helminthiasis, with the object being mangosteen peel pressed water, using purposive sampling technique. The research was conducted in November 2024 at the Pharmacology Laboratory of Muhammadiyah Surakarta University and the Parasitology Laboratory of FK-KMK Gadjah Mada University, Yogyakarta. Inclusion criteria included feces specimens positive for helminthiasis by STH or non-STH parasites, non-prepared specimens preserved in 10% formalin in good condition. Exclusion criteria included feces preparations in the form of ready-made preparations.

The tools and materials used in this study include a light microscope, object glass, deck glass, feces container, toothpicks, dropper pipettes, digital scale, juicer, basin, tray, knife, spoon, test tubes, beaker glass, filter paper, spatula, funnel, label paper, dark bottles, lakmus paper, gloves, mangosteen peel pressed water, distilled water, citric acid, 2% eosin, and feces specimens of STH and/or non-STH parasites.

The first stage involves the preparation of mangosteen peel pressed water, which begins with washing the fruit, followed by separating the skin from the flesh. Next, 100 grams of the skin are ground with 100 ml of distilled water to create concentrations of 10%, 20%, 30%, and 40%. The resulting pressed water is filtered until clear and can be supplemented with 9% citric acid to enhance its quality (Akmalia *et al.,* 2021). The formulation for preparing the stock solution of mangosteen peel pressed water is derived from the dilution formula as follows:

$$Concentration\ (\frac{b}{v}) = \frac{Mass\ of\ Solute}{Volume\ of\ Solution}\ x\ 100\%$$

The extraction of anthocyanins using water and a mixture of organic acids, such as citric acid, is considered safer compared to using other solvents (Suseno, Surhaini, and Ampitasari 2021). For calculating the addition of citric acid at each concentration, the following formula can be used:

$$Citric\ acid\ addition\ = \frac{Concentration\ of\ Citric\ Acid}{100}\ x\ Volume\ of\ Solution$$

Characterization Test

In the second stage, after the pressed water is prepared, characteristic tests are conducted, including:

1. pH Test

   The purpose is to determine the acidity or alkalinity level of a solution. In this study, pH measurement is performed using litmus paper. Initially, the litmus paper is dipped into the dye solution at each concentration, then left until it changes color according to the pH. The observed pH values are then recorded.

2. Organoleptic Test

   The observation of the mangosteen peel pressed water is conducted by assessing its appearance, consistency, odor, and homogeneity. This test is performed using visual inspection to evaluate the quality of the preparation. These steps ensure thorough evaluation before proceeding with further analyses such as staining feces samples for detecting helminthic eggs.

The examination of worm eggs in feces is conducted by placing drops of mangosteen peel pressed water at each concentration, along with 1-2 drops of eosin, on a microscope slide that has been filled with feces samples. The mixture is then stirred using a wooden stick until homogeneous and covered with a cover slip. The prepared slides are examined under a microscope at magnifications ranging from 10x to 40x. The effectiveness of the staining is assessed based on color intensity, stability, clarity, and contrast using a Likert scale (Rahmadila *et al.,* 2023), as follows:

1. Score (1): If the field of view is not contrasted, worm eggs do not absorb the dye, and the egg structure is not clearly visible.

2. Score (2): If the field of view is less contrasted, worm eggs absorb less dye, and the egg structure is less clearly visible.
3. Score (3): If the field of view is contrasted, worm eggs absorb the dye, and the egg structure is visible.

The morphological examination of STH eggs is carried out by three assessors. The data obtained were analyzed using the Kruskal Wallis test using Jeffreys's Amazing Statistics Program (JASP) statistical software at a significance level of 0,05.

Results and Discussion

Characteristic Test Results

In this study, it was found that mangosteen peel produces a brown color due to the insufficient release of anthocyanins. As a result, the field of view lacks contrast, even though the worm eggs can absorb color adequately. Anthocyanins will produce color changes at specific pH levels, allowing them to impart different colors for each type of cell (Singh et al. 2020). The organoleptic test of mangosteen peel pressed water (*Garcinia mangostana* L.) revealed a brownish color, with an aroma that mixed with the sap from the peel, and a slightly thick liquid consistency (Figure 1).

Figure 1. Mangosteen peel pressed water

This indicates that the visual quality is poor due to the suboptimal anthocyanin content, which affects the color quality of the pressed water. The consistency of the pressed water is thicker compared to eosin and less homogeneous because it is still mixed with sap, while the odor does not have significant meaning. The factors that influence the stability of anthocyanins include modifications to the specific structure of anthocyanins (such as glycosylation and acylation with aliphatic or aromatic acids), pH, temperature, light exposure, and the presence of metal ions (Khatimah, Hasanuddin, and Amirullah 2021).

Organoleptic characteristic test was conducted on days 1, 3, 5, 7, 10 and 14 to assess the preparation of squeezed water, the results of the study showed that the preparation without the addition of citric acid could last for 7 days, while the preparation of squeezed water with the addition of citric acid could last up to 20 days. The assessment was seen based on the absence of changes in terms of color, odor and consistency. This shows that citric acid can be used as an enhancer to stabilize the preparation of squeezed water by maintaining its durability and inhibiting bacterial growth in the preparation.

Table 1. pH test results

Concentration	pH
10% mangosteen peel pressed water	3
20% mangosteen peel pressed water	4
30% mangosteen peel pressed water	4
40% mangosteen peel pressed water	4
10% mangosteen peel pressed water + citric acid	2
20% mangosteen peel pressed water + citric acid	2
30% mangosteen peel pressed water + citric acid	2
40% mangosteen peel pressed water + citric acid	2

The stability of anthocyanin color is influenced by pH, where at high pH levels, anthocyanins tend to appear blue or colorless, while at low pH levels, they turn red (Azis and Harwani 2020). In this study, the solution has a low pH or is acidic, resulting in the absorbed color in the fecal samples appearing red. In Table 1, the lowest pH value obtained was from the mangosteen peel extract with the addition of 9% citric acid, which showed a pH value of 2. All concentrations of the extract can be classified as acidic due to the anthocyanin content within them, making them suitable for identifying the eggs of parasitic worms. The eggs of these worms are basic and positively charged due to their protein content, while

2% eosin and mangosteen peel are acidic and negatively charged. Thus, both can bind together with the positively charged protein molecules in the cytoplasm and connective tissue of the worm eggs, as expected. (Hastuti and Haryatmi 2021).

Microscopic Staining Results

The concentrations of mangosteen peel pressed water obtained were 10%, 20%, 30%, and 40%, each consisting of pressed water with the addition of distilled water and pressed water with both distilled water and citric acid, all of which were repeated three times. Observations were conducted by the researcher and two assessors who are experts in the field of parasitology. The final assessment results were determined by the most frequent mode for each variable and concentration, using a Likert scale (Rahmadila *et al.,* 2023). The following microscopic images show the eggs of Soil Transmitted Helminths:

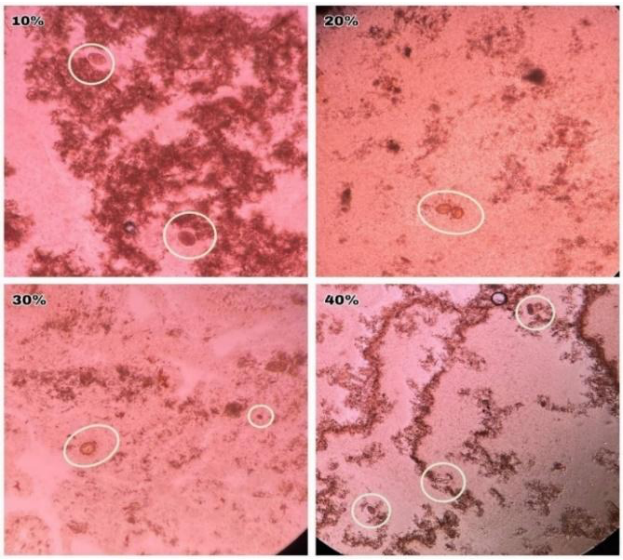

Figure 2. The results of dyeing the mangosteen peel pressed water

Table 2. Microscopic Interpretation of mangosteen pressed water

Mangosteen Peel Pressed Water	STH Egg Types
10%	*Hookworm*
20%	*Ascaris lumbricoides*
30%	*Ascaris lumbricoides*
40%	*Hookworm*

Figure 2 shows the results of staining STH worms using mangosteen peel pressed water (*Garcinia mangostana* L.) along with the concentration and descriptions of the images observed under the microscope in Table 2.

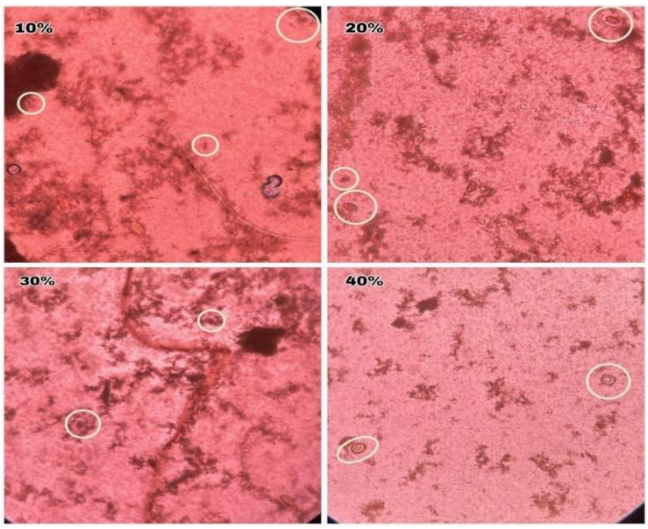

Figure 3. The results of dyeing the mangosteen peel pressed water with citric acid

Table 3. Microscopic Interpretation of mangosteen pressed water with citric acid

Mangosteen Peel Pressed Water With Citric Acid	**STH Egg Types**
10%	*Ascaris lumbricoides*
20%	*Hookworm*
30%	*Hookworm*
40%	*Ascaris lumbricoides*

Figure 3 presents the results of staining STH worms using mangosteen peel pressed water (*Garcinia mangostana* L.) with the addition of 9% citric acid at each concentration, along with the descriptions of the images observed under the microscope in the table 3.

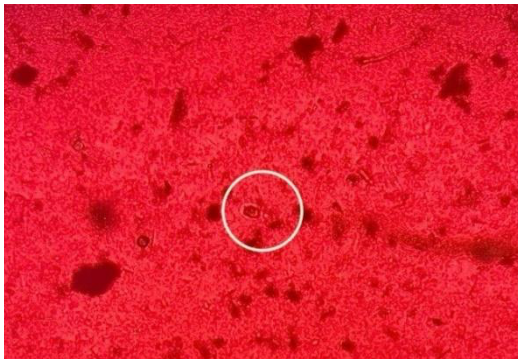

Figure 4. The results of dyeing with eosin 2%

Table 4. Microscopic Interpretation of eosin 2%

Staining Agent	**STH Egg Types**
Eosin 2%	*Hookworm*

Figure 4 shows the results of staining STH worms using 2% eosin, with the image description featuring Hookworm eggs, as observed under the microscope in Table 4.

Statistical Descriptive Test Results

Descriptive analysis is a statistical technique used to describe and summarize the features of a dataset. This method serves as a crucial initial step in more in-depth data analysis, helping researchers or analysts understand the fundamental characteristics of the dataset they possess. In this report, the author discusses the results of descriptive analysis conducted on several samples, including mangosteen peel pressed water (*Garcinia mangostana* L.) and 2% of eosin. The results of this analysis can be utilized to compare the effects of adding citric acid to the mangosteen peel pressed water.

Table 5. Descriptive analysis of mangosteen peel pressed water (*Garcinia mangostana* L.)

Concentration	Median (IQR)		
	Effectiveness of Staining	Visualization of Parasitic Structures	Staining Quality
10%	3 (1)	2 (0.5)	3 (1)
20%	3 (1)	2 (1)	3 (0.5)
30%	3 (0.5)	2 (0)	3 (0.5)
40%	3 (0.5)	2 (0)	3 (0)

Table 6. Descriptive analysis of mangosteen peel pressed water + citric acid (*Garcinia mangostana* L.)

Concentration	Median (IQR)		
	Effectiveness of Staining	Visualization of Parasite Structure	Effectiveness of Staining
10% + citric acid	1 (0.5)	2 (0.5)	3 (0.5)
20% + citric acid	2 (0.5)	2 (1)	1 (1)
30% + citric acid	2 (0.5)	2 (0)	2 (1)
40% + citric acid	3 (0.5)	2 (0.5)	2 (1)

Table 7. Descriptive analysis of eosin 2%

Concentration	Median (IQR)		
	Effectiveness of Staining	Visualization of Parasite Structure	Effectiveness of Staining
Eosin 2%	3 (1)	2 (1)	3 (0)

Descriptive analysis showed the median and interquartile range (IQR) for effectiveness and quality of staining. The median for all concentrations of mangosteen peel pressed water with citric acid (10%, 20%, 30%, and 40%) as well as 2% eosin was consistently 3, while the IQR ranged from 0 to 1, indicating a narrower variation at higher concentrations. In terms of visualization of parasitic structures, a stable median of 2 was obtained for most concentrations, except for the 40% concentration of pressed water with citric acid, which reached 3, with an IQR varying between 0 and 1, reflecting the homogeneity of results across several concentrations. These findings indicate consistent effectiveness and quality of staining across different treatments, despite minor variations in the IQR.

Non-parametric Test Results

The Kruskal-Wallis nonparametric test was conducted following the assessment of homogeneity using the Levene test and the evaluation of data distribution using the Shapiro-Wilk test, which indicated that the data were neither homogeneous nor normally distributed, as the p-value was <0.05.

Table 8. Bivariate analysis (Kruskal Wallis test)

Variable	P value
Effectiveness of Staining	0.645
Visualization of Parasitic Structures	0.364
Staining Quality	0.463

Bivariate analysis using the Kruskal Wallis test (table 8) showed p-values for each analyzed variable. For staining effectiveness, the p-value was 0.645, indicating no significant difference between the compared groups. Similarly, the p-value for the visualization of parasitic structures was 0.364, showing that there was no significant difference, and the

quality of staining had a p-value of 0.463. Overall, the three variables indicated that the applied treatments did not result in statistically significant differences, signifying consistency in staining results across the tested conditions.

During this research process, the researcher encountered several limitations, including the color produced by the mangosteen peel pressed water with only the addition of distilled water, which tended to be browner. Consequently, some assessors and observers of the preparations argued that the color contrast could resemble the feces being studied. This issue arose because the filtering process of the pressed water was not optimal and was still manual. This made it difficult to separate the water content from the sap, requiring multiple filtrations before it could be used for staining. Another limitation is that mangosteen is a fruit that is only available during certain seasons, as noted in the research (Saraswati and Astutik 2022). This makes it relatively difficult to obtain, and its price tends to be quite high during non-harvest days.

Conclusions

This study concluded that mangosteen peel pressed water (*Garcinia mangostana* L.) is effective for staining feces in cases of helminthiasis. The concentration of 40% squeezed water without citric acid showed better staining effectiveness, while 40% with citric acid provided good visualization of parasite structures and consistent color contrast with the background. The addition of citric acid can extend the shelf life of the preparation up to 20 days when compared to mangosteen peel pressed water which is only added with aquadest.

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