Research Article

Comparative Evaluation on the Staining Potential of *Garcinia mangostana* (Mangosteen) and *Selenicereus guatemalensis* (Dragon Fruit) for Ova of *Trichuris suis* and *Strongyloides ransomi*

Anne Jolina Eustaquio Doria, Crislyn Joy Saballa Dalusong, Roma Alheia Mendoza De Castro, Tricia Nicole Malabanan Dioneda, Kyzha Zen Buencuchillo Estefanio, Liv Andrea Perez Galvez, Maria Fe Bago Gregorio, Pamela Rose F. Bremner

Department of Medical Technology, Far Eastern University, Manila, PHILIPPINES.

Submission Date: 14-06-2023; Revision Date: 19-08-2023; Accepted Date: 23-09-2023.

Correspondence:
Ms. Pamela Rose F. Bremner, RMT MSMT Bachelor of Science in Medical Technology, Far Eastern University, Manila, PHILIPPINES.

Email: pbremner@feu.edu.ph

ABSTRACT

The conventional stains used for the detection of parasitic helminths are hazardous to laboratory scientists, who are exposed to iodine. A potential alternative stain to detect helminth ova is found in this study. This investigates the possibility of a natural dye between *Garcinia mangostana* (Mangosteen) and *Selenicereus guatamalensis* (Dragon Fruit) extracts. These fruits contain anthocyanin and betacyanin pigments that stain from purplish to brownish colour. The experiment utilizes distilled water and ethanol with two different concentrations to assess the effectivity of the colour of the pigments in helminth detection. The concentrations tested included 50% ethanol extract of mangosteen, 50% ethanol extract of dragon fruit, 50% distilled water extract of mangosteen, and 50% distilled water extract of dragon fruit. Statistical analysis revealed no significant difference in staining potential between the stain extracts and Lugol's Iodine. However, further analysis indicated that 50% ethanol extract of mangosteen and 50% ethanol extract of dragon fruit exhibited better staining compared to their distilled water counterparts. The pH values of the stains were also measured, with Lugol's Iodine having a pH value of 6.0, and the ethanol extracts having a pH value of 5.0, while the distilled water extracts had a pH value of 4.0. Based on the results, it can be concluded that ethanol extracts possess the best staining capacity against distilled water extracts.

Keywords: Anthocyanin, Betacyanin, *Garcinia mangostana,* Lugol's Iodine, *Selenicereus guatamalensis, Trichuris suis, Strongyloides stercoralis.*

INTRODUCTION

Schistosomiasis and soil-transmitted helminthiasis are common parasitic illnesses in tropical and subtropical nations like the Philippines.[1] In most cases, soil-transmitted helminth infection occurs in warm and humid settings with inadequate hygiene and sanitation.

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DOI: 10.5530/ajbls.2023.12.56

Whereas the spread of contaminated soil can infect humans and eventually develop as contagious upon exposure to oral-fecal contact from contaminated feces of infectious parasite eggs.[2]

Trichuris suis and *Strongyloides ransomi* are known parasites found in pigs, are known to infect humans through the oral-fecal route and in contact with pigs, as well the unhealthy use of pig manure fertilizers.[3] The associated conditions of acquiring *Trichuris suis* infection, such as anorexia, mucoid to hemorrhagic diarrhea, dehydration, and worst possible infection, death in the transmission of infection through ingestion of eggs.[4]

Strongyloidiasis is one of the neglected diseases in tropical countries. There is a lack of high sensitivity tests in diagnosing strongyloidiasis. It was noted that strongyloidiasis has become prevalent and neglected.[5] It was also mentioned that strongyloidiasis is caused by a nematode parasite *Strongyloides stercoralis*.

Strongyloides stercoralis is a nematode parasite that infects humans.[6] Moreover, *Strongyloides ransomi* is a nematode parasite most often seen infecting a live pig. The morphology of both species has the same appearance when examined microscopically.

Furthermore, detecting soil-transmitted helminths and other parasites is done through different laboratory testing techniques. The stool samples are stained using one of the commonly used stains in the laboratory to look for helminth eggs, larvae, and occasional adult worms or some segments in stools that can be visible under the microscope.[7] A known standard technique in viewing parasites under the microscope is the Egg-counting Kato-Katz Method, wherein a drop of regular saline or iodine is placed in a slide with an assumed infected stool specimen, then, placing a 22 mm square coverslip over the uniformly thin suspension, just enough to undergo a thorough examination for detection of the presence of a protozoan cyst, helminth ova, and larvae.[8]

The researchers experimented with alternative stains using mangosteen and dragon fruit peel extracts in comparison to the standard control, Lugol's Iodine. This study aims to determine the efficacy of 50% ethanol and 50% distilled water extracts from mangosteen and dragon fruit peels as stains for *Trichuris suis* and *Strongyloides ransomi* ova, as an alternative to lugol's iodine commonly used in laboratories.

MATERIALS AND METHODS

The study focuses on the comparison of the staining potential of the mangosteen and dragon fruit in different concentrations such as ethanol and distilled water for staining the Ova of *Trichuris suis* and *Strongyloides ransomi*. In this study, the stain is formulated using the peels of the dragon fruit and mangosteen. In this study the peels were left to dry in the oven overnight at 65°C which was modified by the study of Chua *et al.* 2020, where researchers dried the peels for 65°C but not dried overnight.[9] Afterwards, the peels that are dried overnight were left again for another 8 hr for mangosteen peels and 12 hr for Dragon Fruit. After drying the peels are powdered using the electronic grinder.

Following the drying of the fruit materials, two extractions are formulated (Ethanol and Distilled Water) The researchers obtain ten grams of the powdered dragon fruit and mangosteen and mix with 100 mL of 50% Distilled water in a beaker and heated at 70-80°C in a water bath for 3 hr and afterward let the beakers cool down. This procedure is derived from the study of Abdullah *et al.* 2014.[10] Subsequently, for the ethanol extraction the researchers obtained 10 g of powdered peels and in a separate beaker containing 100 mL of 50% ethanol and mixed the powdered peels with the ethanol. Consequently, for 3 hr heat the beaker in a water bath at 70-80°C. Afterwards, cool down the beaker. For the purification of the extracts, it was subjected to centrifugation for 15 min at 3000 revolutions per minute (rpm) and transferred into a bottle.

In the staining process, researchers used the Formalin Ethanol Acetate Technique for wet mount preparations. They applied 50% ethanol dragon fruit extract to slides, emulsified stool samples, covered them with slips, and observed them under LPO and HPO magnification. This procedure was repeated for four different concentrations. ANOVA, Post Hoc Analysis, and Paired T-Test assessed differences among Ethanol, Distilled, and Lugol's concentrations.

RESULTS

As shown in Figure 1, 50% ethanol concentrations provided the best staining results. Researchers easily distinguished *Trichuris suis* and *Strongyloides ransomi* ova. In Figure 1, the extracts from mangosteen and dragon fruit concentrations effectively captured the albuminous coating of parasite eggs. Notably, Figures 1-b to d distinctly reveal the mucoid plugs at both ends of *Trichuris suis* and clearly differentiate the coating, unlike Figure 1-e. Additionally, Figures 1-g to j highlight the well-defined hyaline shell of *Strongyloides ransomi*, making the egg's morphology readily identifiable.

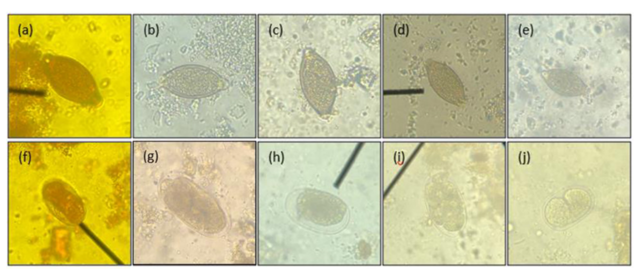

Figure 1: Stained ova of Soil Transmitted-Helminths (a) to (e) Demonstrated the egg of *Trichuris suis* and (f) to (j) demonstrated the egg of *Strongyloides ransomi*. (a) Lugol's iodine. (b) Ethanol extract of Mangosteen. (c) Distilled water extract of Mangosteen. (d) Ethanol extract of Dragon fruit. (e) Distilled water extract of dragon fruit. (g) Ethanol extract of Mangosteen. (h) Distilled water extract of Mangosteen. (i) Ethanol extract of Dragon fruit. (j) Distilled water extract of dragon fruit.

This implies that the extracts can stain the ova's external and internal external and internal structures. It is worth noting that this can be used to study the eggs' overall morphology.

As indicated in Table 1, 50% ethanol mangosteen effectively stained *Trichuris suis* and *Strongyloides ransomi*, whereas 50% distilled water mangosteen lacked this staining ability. The researchers noticed that the mangosteen sediments did not fully dissolve in 50% distilled water, in contrast to the better dissolution observed in 50% ethanol. Ethanol has a strong, medium polarity solvent, it will extract a wider variety of compounds than you intend.[11]

Additionally, the 50% ethanol dragon fruit has stained the *Trichuris suis* and *Strongyloides ransomi*. When compared to mangosteen, the researchers identified that the isolated components of dragon fruit have a better staining potential. To add more, the researchers also observed that the use of ethanol has a huge impact on the staining ability of each component. Moreover, the optimum solvents for extracting substances with antibacterial action were ethanol and acetone.[12]

Based on the findings in Table 2, it indicates that the null hypothesis is rejected because the p-value of 0.000 is smaller than the value. This suggests that the properties of the eggs dyed with mangosteen and dragon fruit against Lugol's iodine significantly differ statistically (*p*-value 0.000).

Table 3 illustrates multiple comparisons of the control against the four concentrations as well as four concentrations against each other. To test their significant difference, Post Hoc analysis was utilized. The null hypothesis, which states that there is no significant difference, will be accepted if the p-value is greater than the alpha threshold of 0.05 because the hypothesis is not rejected at this level.

In this table, the results indicate that both mangosteen and dragon fruit extracts with 50% ethanol show no significant difference compared to Lugol's Iodine (p-values = 0.488 and 0.983, respectively, both > α = 0.05). This suggests that the ethanol extracts effectively stain ova, similar to Lugol's iodine. Respectively, our experiments demonstrate that ethanol concentrations offer superior staining capacity compared to Lugol's Iodine, as seen in Figure 1. *Trichuris Suis* mucoid plugs and *Strongyloides ransomi* egg cells are clearly distinguishable under microscopic examination, supported by our research findings. Ethanol is the best way to extract the dye from fruits because it prevents fungal growth, and the volatile nature of the ethanol increased the staining capacity, therefore it produced a great staining affinity to the parasites' ova.[10]

Table 4 displays the correlation and significance of various mangosteen and dragon fruit extract concentrations. The results indicate that 50% ethanol

Table 1: Morphological Comparison of Each Stains.

Concentration	Reagent	Component	Staining performance
50%	Ethanol	Mangosteen	4.7600
50%	Distilled water	Mangosteen	3.7600
50%	Ethanol	Dragon fruit	4.9200
50%	Distilled water	Dragon fruit	4.0000

Table 2: Summary of the ANOVA for the Concentrations of Mangosteen and Dragon fruit against the Control.

	Sum of Squares	d_f	Mean Square	F	Sig.
Between groups	32.272	4	8.068	29.374	0.000
Within groups	32.960	120	0.275		
Total	65.232	124			

Table 3: Tukey's Test.

Dependent Variables		Mean Difference	Standard Error	Significance Lower Bound	95% Confidence Interval	
					Upper Bound	
Lugol's Iodine (Control).	50% Ethanol Extract of Mangosteen.	0.24	0.148234049	0.488254449	-0.170562838	0.650562838
	50% Ethanol Extract of Dragon Fruit.	-0.24000	0.14823	0.488	-0.6506	0.1706
50% Ethanol Extract of Mangosteen.	Lugol's Iodine.	-0.24000	0.14823	0.488	-0.6506	0.1706
	50% Ethanol Extract of Dragon Fruit.	-0.16000	0.14823	0.817	-0.5706	0.2506
50% Ethanol Extract of Dragon Fruit.	Lugol's Iodine	-0.08000	0.14823	0.983	-0.4906	0.3306
	50% Ethanol Extract of Mangosteen.	0.16000	0.14823	0.817	-0.2506	0.5706

Table 4: Paired Sample Correlation of Concentrations of 50% Ethanol and 50% Distilled Water.

		N	Correlation	Significance
Pair 1	Mangosteen 50% ethanol extract and Dragon fruit 50% ethanol extract.	25	0.180	0.391
Pair 2	Mangosteen 50% distilled water extract and Dragon Fruit 50% distilled water extract.	25	0.000	1.000

Table 5: pH value of extracts and Lugol's Iodine.

Extracts	pH value
Lugol's Iodine.	6.0
Ethanol 50% *Selenicereus guatemalensis* (Dragon fruit).	5.0
Distilled water 50% *Selenicereus guatemalensis* (Dragon fruit).	4.0
Ethanol 50% *Garcinia mangostana* (Mangosteen).	5.0
Distilled water 50% *Garcinia mangostana* (Mangosteen).	4.0

solutions of mangosteen and dragon fruit exhibit no significant difference (*p*-value: 0.391), while 50% distilled water solutions also show no significant difference (*p*-value: 1.000). In the experiment, 50% ethanol concentrations in mangosteen and dragon fruit yielded superior staining, as illustrated in Figure 1.

As seen from Table 5, the pH values of different stains were compared to Lugol's Iodine (pH 6.0). Results show that both mangosteen and dragon fruit concentrations effectively stained *Trichuris suis* and *Strongyloides ransomi* ova, preserving their morphology and viability under microscopic observation despite differences in pH.

DISCUSSION

Dragon fruit and mangosteen are both recognized for their remarkable coloration capabilities attributed to anthocyanin, a natural pigment with various applications, including food dye.[13] These fruits share the common feature of anthocyanin content, contributing to the distinctive purplish-to-black color observed in mangosteen peel.[14] Our study involved two statistical analyses, comparing different concentrations of dragon fruit and mangosteen extracts. The primary objective was to assess their effectiveness as staining agents for parasitic helminths, specifically focusing on their impact on helminth ova morphology.

In line with previous research by Okolie 2008, which highlighted the enhanced intensity of ethanol-based fruit extract for staining purposes, our findings reinforced this notion.[15] Our research demonstrated that ethanol concentrations indeed produce more intense coloration compared to distilled water concentrations (Figure 1).

Other studies have found that natural stains composed of ethanol have enhanced the absorptive ability of natural pigment.[10] Interestingly, our statistical analysis indicated no significant difference between the staining capabilities of dragon fruit and mangosteen extracts, regardless of whether ethanol or distilled water was employed. Both extracts exhibited the same capacity to effectively stain parasitic helminths, providing clear morphology of helminth ova.

Moreover, our experimentation extended to determine the pH of each stain using pH indicator strips. Notably, the pH levels of the ethanol concentrations of each fruit extract closely resembled that of lugol's iodine, a commonly used stain in microbiology. Furthermore, our study contributes to the body of knowledge supporting the morphological similarities in helminth species such as *T. suis* and *T. trichuria*, despite their genetic differences.[16] Similarly, the staining results for *S. ransomi* and *S. stercoralis* were consistent with previous findings.[6] Our research underscores the potential applications of dragon fruit and mangosteen extracts, their staining capabilities, and their relevance to helminth research. The findings of dragon fruit and mangosteen's staining capabilities carry practical implications for various fields, particularly in clinical diagnostics involving parasitic helminths. The research revealed that mangosteen and dragon fruit extracts are important components for making an alternative stain which can be used in investigating parasites and their ova, specifically *Trichuris suis* and *Strongyloides ransomi*. Future researchers should utilize other staining procedures as it may increase effectiveness.

The future researchers must continue to dig deep into the effectiveness of their stains and the problems that may occur. Also, they should examine the extracts and their storage conditions to ensure their utmost effectiveness. This is to avoid circumstances, such as appearance of molds in the stains as it may interfere and may impede the experiment procedures. Second, it is advisable to observe the preservation of stains and how long it will take before expiration. Also, It is important to determine the quality and shelf life of the stains to determine signs of spoilage and to identify indicators of spoiling, it's critical to assess the stain quality and shelf life. Third, examining different concentrations and how it affects the staining capacity of the extracts should also be done. Researchers may observe how different concentrations, higher or lower, influence the lightness or darkness of the stain for easier observation.

Moreover, the current study was able to stain a hookworm in motion inside an ovum. In line with this, we also recommend the next studies to use mangosteen and dragon fruit extracts on trophozoites since the Lugol's Iodine cannot be used because the iodine kills the trophozoite.[17]

CONCLUSION

The researchers examined morphological characteristics using different mangosteen and dragon fruit concentrations (50% ethanol and 50% distilled water). Results show 50% ethanol mangosteen and dragon fruit offer superior staining for *Trichuris suis* and *Strongyloides ransomi* ova compared to their distilled water counterparts. Statistical analysis highlights significant differences from lugol's iodine. Notably, 50% ethanol extracts match lugol's iodine's staining capabilities. Despite pH variations, all concentrations-maintained ova viability and stability.

ACKNOWLEDGEMENT

We thank the laboratory staff in the Medical Technology Laboratory for assisting and directing us as we used the various tools and materials for our research and, to Ms. Pamela Rose Bremner, our research adviser, for sharing her time, effort, and expertise with the researchers and providing them with advice and knowledge throughout the creation of this thesis paper.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RPM: Revolutions per minute; **LPO:** Low Power Objective; **HPO:** High Power Objective; **ANOVA:** Analysis of Variance; **STH:** Soil-Transmitted Helminths; **MDA:** Mass Drug Administration; **CDC:** Centers for Disease Control.

SUMMARY

This study aims to compare the staining potential of *Garcinia mangostana* (Mangosteen) and *Selenicereus guatemalensis* (Dragon Fruit) for the ova of *Trichuris suis* and *Strongyloides ransomi*. It also evaluates the optimal concentrations of these fruits for staining the ova. The study explores these fruits as alternative stains to toxic synthetic reagents used in clinical laboratories. The fruit peels are dried, powdered, and mixed with distilled water or ethanol. The extracts undergo purification and are used to stain samples prepared from pig's stool using the formalin ethanol acetate technique. Statistical analysis methods such as One-Way ANOVA, Paired T-test, and Post Hoc analysis are employed to assess the differences between fruit extracts and the control (lugol's iodine). The staining results are graded based on the visibility of morphological characteristics of the ova. The study concludes that both mangosteen and dragon fruit extracts show staining capacity comparable to lugol's iodine. The pH values of the fruit extracts and lugol's iodine are also compared.

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Cite this article: Doria AJE, Dalusong CJS, Castro RAMD, Dioneda TNM, Estefanio KZB, Galvez LAP, *et al*. Comparative Evaluation on the Staining Potential of Garcinia *mangostana* (Mangosteen) and S*elenicereus guatemalensis* (Dragon Fruit) for Ova of *Trichuris suis* and *Strongyloides ransomi*. Asian J Biol Life Sci. 2023;12(2):422-7