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Preliminary Evaluation of Guava (*Psidium guajava*) Leaves Extract as an Alternative Eosin Stain in Hematoxylin and Eosin Staining of Rat Liver

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ABSTRACT: Hematoxylin and Eosin are the standard histological staining dyes for routine histopathologic specimens due to its ability to differentiate a normal cell and its components from an abnormal one. However, due to the classification of Eosin stain as a Group 3 carcinogen by the International Agency for Research on Cancer (IARC), it poses a health risk to health care professionals, particularly the Medical Technologist and Histotechnologist who handle this stain on a daily basis. The histological staining capabilities of Guava leaves (*Psidium guavaja*) ethanolic extract as an alternative stain for Eosin in the Hematoxylin and Eosin staining of rat liver were examined. This study used an experimental research design that primarily focused on evaluating the effectiveness of different concentrations of guava leaf extract (15 g with 1:5 ratio of powdered guava leaves and 70% alcohol, 25 g with 1:10, and 35 g with 1:15) as an alternative to eosin in the standard hematoxylin and eosin (H&E) stain by examining the cytoplasmic staining and staining clarity of rat liver samples. Modified scoring sheets were used for data collection, which included three trials for the three different concentrations of guava leaf extract, with three replicates for each concentration. The study uses mean, ANOVA, Games Howell post hoc test, and Welch's T-test as statistical tools. The results indicated no significant differences in cytoplasmic staining among the three concentrations (15 g, 25 g, and 35 g) of ethanolic guava leaf extract ($F = 0.853$, $p = 0.445$). However, in the comparison between these concentrations in the criteria of clarity of staining, the 15 g concentration combined with hematoxylin produced the clearest staining, allowing for distinct visualization of rat liver tissue structures ($F = 5.928$, $p = 0.012$) ($M = 2.22$). When compared with the standard hematoxylin and eosin (H&E) stain, the best concentration (15 g with a 1:5 ratio) produced comparable results in cytoplasmic staining; however, a significant difference in staining clarity was still observed. Although guava leaf extract demonstrates potential as an alternative staining agent, the standard H&E stain may still provide clearer and more well-defined staining results.

KEYWORDS: Hematoxylin and Eosin, *Psidium guavaja*, Cytoplasmic staining, Clarity of Staining

I. INTRODUCTION

Hematoxylin & Eosin are one of the most common staining chemicals in the laboratory due to their ability to distinguish nuclei and cytoplasm and their relative ease of use. However, Eosin is classified as a Group 3 Carcinogen by the International Agency for Research on Cancer (IARC), therefore posing a significant hazard and risk for Medical Technologists and Histotechnologists who handle it in the laboratory (Yeti & Hariyanto, 2020). Based on these findings, an alternative staining dye would help reduce the hazard and risk faced by laboratory personnel. A study of Deepali et al. (2014, as cited in Hartika et al., 2021) indicated that natural dyes that come from plants may have staining abilities that are inexpensive, non-toxic, and non-allergenic, which may offer an alternative to Eosin. This was further supported by the study of Saraswati & Rahmawati (2023), in which their findings state that natural dyes should be investigated further as an alternative histopathological stain, as exposure to prolonged Hematoxylin and Eosin may have negative effects on laboratory personnel.

Despite the presence of various studies tackling alternative plant-based dyes to replace eosin, there are still plant species without adequate investigation of their staining capabilities for histological specimens. This is despite studies highlighting that they contain phytochemicals that have the potential to stain. This list of plant species includes the guava. The guava tree is

a fruit-bearing tropical tree that naturally grows in tropical regions, including the Philippines (Kumar et al., 2021). Flavonoids and tannins in particular are the two metabolites found in this plant that are capable of staining (Asra et al., 2021). However, the lack of studies focusing on *Psidium guavaja*, particularly on its leaves, which also possess the same metabolites that possess staining characteristics, was identified by the researchers as a possible gap to study. Along with the wide usability of rats, particularly in the medical field (Bryda, 2013, as cited by Chamorro-Cevallos et al., 2022), the researchers focused on the preliminary evaluation of *Psidium guavaja* leaves extract, as a possible alternative to eosin in the hematoxylin and eosin routine staining using a rat liver specimen.

The researchers aimed to demonstrate through this study the possible cost-effective plant-based dye for cytoplasmic stain that reduces and mitigates the risk associated with the use of Eosin in the laboratory by medical technologists and histopathologists, while still providing the cytoplasmic staining and clarity that is comparable to that of Eosin. The use of rat liver in this study allowed the researchers to simulate human histological specimens being subjected to the control and experimental variables of the study.

II. METHODOLOGY

Research Design

In this study, an experimental research design was employed to achieve the research objectives and collect the necessary data. Experimental research was used to determine the relationship and correlation between the dependent and independent variables. It consisted of a hypothesis and measurable variables that could be calculated and compared under controlled conditions. The data gathered in this research design were measured and quantifiable (Ghanad, 2023). The experimental research design employed in this study allowed the researchers to evaluate the quality of the guava leaf extract in terms of clarity and cytoplasmic staining compared to the standard hematoxylin and eosin staining. It also aimed to determine which quantity of powdered guava leaves (15 g, 25 g, 35 g) provided the best staining quality of rat liver tissue when stained. Through experimentation, the researchers assessed whether the guava leaf extract could serve as a comparable or effective alternative to the conventional eosin stain.

Sources of Data

The primary data source was the experimentation of the guava leaves extract staining, where the data was gathered after experimentation at the Perpetual Help Medical Center in Biñan, Laguna. The secondary sources of the study were the studies cited in the study, as well as related studies published about natural dyes that contained phenolic compounds as staining properties, and Hematoxylin and Eosin studies.

Instrumentation and Validation

After acquiring the rat liver from the Mots Animal House Laboratory and Research, the tissue underwent tissue processing. From fixation to infiltration, the main equipment used is an automatic tissue processor. The embedding portion used a heated paraffin reservoir, a cold plate (for rapid solidification of blocks), embedding molds, and labeling materials. Next was sectioning, where a microtome was used to cut thin slices from the paraffin block for microscopic examination. The tissue sections were put in a water bath to float and flatten tissue ribbons; these were mounted on glass slides. After that, the slides were placed on a slide dryer to adhere sections and remove any excess wax. Then, some slides were stained with the prepared 70% ethanolic guava leaves extract and hematoxylin stain, and the others were stained with the standard H&E stain. After this, the stained tissue sections were mounted using a mounting medium and coverslips. Lastly, the slides were evaluated using a microscope to assess the clarity and cytoplasmic staining of the prepared ethanolic guava leaves extract and hematoxylin stain. One (1) histotechnologist and two (2) medical technologists conducted the validation of the stained tissue slides. The reported values were based on the scoring criteria for ethanolic guava leaves extract staining.

Material Collection and Preparation

The rat liver was obtained from the Mots Animal House Laboratory and Research in Sta. Rosa City, Laguna, along with the certification needed to verify the organ extracted from the rat. The rats were maintained under controlled laboratory conditions to ensure proper care prior to liver extraction. Cages were cleaned weekly with detergent, bedding was changed every other day, and environmental conditions were regulated at 20–24 °C, 40–60% humidity, and a 12-hour light/dark cycle. The animals were fed standardized rat feed with scheduled feeding times and given ad libitum access to distilled water to maintain optimal health and minimize experimental variability. About 3kg of guava leaves were gathered from a garden in Sinalhan, Sta. Rosa City, Laguna. The leaves were sent to the Bureau of Plant Industry (BPI) to issue certificates on the identification, authentication, and verification

of the leaves. The researchers obtained the physical and digital copies of the necessary documents given by the aforementioned organizations. The ethyl alcohol and distilled water to be used for the maceration process were bought from an online shop.

A. ***Plant Preparation and Extraction.*** Fresh and mature guava (*Psidium guajava*) leaves were collected from healthy trees to ensure that the leaves were pest-free and free from physical damage. The collected leaves were first thoroughly washed with tap water, followed by distilled water, to remove any dust, debris, and other contaminants. The leaves were then air-dried at room temperature for several days. After air drying, the leaves were ground into a fine powder using a milling machine and were stored in an airtight container at room temperature until extraction. This process ensured the preservation of the bioactive compounds essential for preparing the ethanolic extract used in staining procedures.

B. ***Preparation of Ethanolic Extract.*** The researchers prepared the ethanolic extract using 100 ml of 99% Ethanol from an online retail store and 100ml distilled water sourced from the local convenience store of UPHS-DJGTMU. The 70% ethanol solution was prepared from 99% ethanol using the dilution formula C1V1 = C2V2, where C1 represented the initial concentration of ethanol, V1 was the required volume of the stock solution, C2 was the desired concentration, and V2 was the final total volume. To prepare 100 mL of 70% ethanol, 70.7 mL of 99% ethanol was measured and diluted with 29.3 mL of distilled water. During preparation, ethanol was added gradually to distilled water to minimize heat generation and vapor release. The resulting solutions were mixed thoroughly, properly labeled, and stored in tightly sealed containers away from heat, sunlight, and open flames to ensure safety and maintain concentration stability. To start the maceration process, the guava leaves, obtained from Sinalhan, Sta. Rosa City, were washed and air-dried. After this, the air-dried leaves were milled using a milling machine. Three bottles were used for the preparation of the extract; each contained 15g, 25g, and 35g of *Psidium guajava* powder. The powder was dissolved in 70% ethyl alcohol concentrations. The 1st beaker contained 15 grams of powdered guava leaves and 75 ml of 70% alcohol, with the ratio of 1:5; the 2nd contained 25 grams of powdered guava leaves and 250 ml 70% alcohol, with the ratio of 1:10; and the 3rd contained 35 grams of powdered guava leaves and 525 ml of 70% alcohol, with the ratio of 1:15. With a total volume of 1.5 liters of ethanol used. The ratio provided was recommended by the University of the Philippines, Los Banos - Central Analytical Laboratory. All prepared solutions were shaken by mechanical shaking and were left for 24 hours. Then, the three solutions underwent filtration using Whatman's filter paper No. 1; this was done to filter the macerated guava leaves. To collect the fluid, a micropipette was used, and it was stored in coupling jars. This process was performed by the University of the Philippines, Los Banos - Central Analytical Laboratory.

C. ***Tissue Processing.*** The tissue processing was carried out by the histotechnologist at Perpetual Help Medical Center, Binan. After harvesting the rat livers, they were immediately placed in 10% neutral buffered formalin for fixation for approximately 24-48 hours to preserve tissue morphology. Then, the liver samples were grossed and trimmed to appropriate sizes to ensure uniform processing. Dehydration was performed using a series of ascending grade alcohols (70%, 80%, 95%, and 100% ethanol) to remove water from the tissues, followed by the clearing process using a xylene solution to make the tissue receptive to paraffin infiltration. The cleared tissues were then infiltrated with molten paraffin wax at 56-60°C until complete saturation was achieved. The infiltrated tissues were then embedded in paraffin molds with proper orientation, cooled, and allowed to solidify before tissue sectioning. The resulting paraffin blocks were trimmed and sectioned using a microtome at 4–5 µm thickness. Ribboned sections were floated on a warm water bath and carefully mounted onto clean, labeled glass slides, which were then dried on a hot plate to ensure proper adhesion. Prior to staining, slides were deparaffinized in xylene and rehydrated through descending grades of ethanol to distilled water (100%, 95%, 80%, 70% ethanol, and water). The prepared slides were then ready for staining.

Evaluation and Criteria Grading

The study utilized the standardized scoring criterion adapted from a previous study by Ma et al. (2024), which evaluated the histological staining (See Table 1). Part I focused on the nuclear staining, which evaluated the sharpness, uniformity, and intensity of the hematoxylin-stained nuclei, determining whether nuclear details such as chromatin and nucleoli were distinctly visible. Part II assessed the cytoplasmic coloration produced by the guava leaf extract compared to eosin, focusing on staining intensity, uniformity, and contrast with the nucleus. Part III examined the overall clarity, background cleanliness, and differentiation between cellular components, indicating how well the tissue morphology was preserved and visualized under the microscope. To ensure content validity, either one pathologist, a licensed histological technologist, or a medical technologist with extensive experience, blindly assessed the staining quality of the guava leaf extract to reduce subjective bias. In the study, the researchers

only used the Part II and Part III scoring criteria, as the focus of the study was an alternative to eosin, which only stained the cytoplasmic part of the cell.

Table I. Scoring criteria for hematoxylin and eosin staining quality

Score	Nuclear staining	Cytoplasmic Staining	Clarity of staining
3	Blue-purple	Light pink	Distinct
2	Navy Blue	Dark peach or red	Inconspicuous
1	Gray-Blue	Deep red or purplish red	Ambiguous

Cytoplasmic Staining

- **Deep red or purplish red** - Indicates excessive staining intensity, which may obscure cytoplasmic details and reduce clarity of tissue structures.
- **Dark peach or red** - Indicates moderate staining intensity, showing acceptable cytoplasmic visualization but not optimal contrast.
- **Light pink** - This mimics the traditional Eosin Y result. Indicates optimal staining quality, demonstrating clear, well-defined cytoplasmic structures with appropriate contrast and detail.

Clarity of Staining

- **Ambiguous** - Unclear, uncertain, or difficult to interpret. The staining may be uneven, faint, patchy, or overlapping with the background stain.
- **Inconspicuous** - Present but very faint or subtle. The structure does not stand out clearly against the background.
- **Distinct** - Clearly visible, well-defined, and sharply differentiated from surrounding structures. The stained structure stands out clearly against the background.

Table 2. Scoring criteria for Ethanolic Guava Leaves extract Staining

Score	Cytoplasmic Staining	Clarity of staining
3	Light pink	Distinct
2	Dark peach or red	Inconspicuous
1	Deep red or purplish red	Ambiguous

Derived from Table 1

Waste Disposal

All waste materials generated from the study were disposed of in accordance with the established waste management protocols of Perpetual Help Medical Center, Biñan. Biological wastes, including rat liver tissues and other organic materials, were collected in properly labeled biohazard containers and disposed of through the hospital's designated biomedical waste disposal system to ensure biosafety and environmental protection. Chemical wastes such as ethanol, xylene, and other reagents were placed in appropriate chemical waste containers and managed following the hospital's chemical waste segregation and disposal guidelines. Sharps, including used glass slides, were disposed of in puncture-proof containers. All procedures were strictly adhered to the hospital's safety and infection control policies to prevent contamination and ensure compliance with institutional and environmental standards.

Data Gathering and Procedure

The researchers first submitted a letter to conduct a study to the University of Perpetual Help, Dr. Jose G. Tamayo Medical Center, College of Medical Technology department. Then we started to gather data by experimenting with rat liver slides that are ready for staining. The rat liver was first grossed in the University of Perpetual Help - Medical Center laboratory. The sections were deparaffinized in xylene, followed by rehydration through 95% ethanol. Then, be washed briefly in running tap water for several dips. Hematoxylin staining was performed for 5–6 minutes, after which the slides were rinsed in tap water for 5 dips. Excess dye was removed by a quick dip in 1% acid alcohol, followed by another rinse in tap water. The sections were then treated with ammonia water for 8 dips until it will develop into blue color, and rinsed again in tap water. For counterstaining, the control group used eosin, while the experimental groups used guava leaf extract. Staining continued with 10 dips in eosin or experimental stains,

followed by dehydration through graded ethanol concentrations (95% and 100%) for 5 minutes and clearing in xylene for 2 minutes. Finally, the sections were mounted for microscopic examination.

The staining of rat liver tissue was conducted in three trials for the experimental groups, which consisted of three different quantities of powdered guava leaves, each having three replicates. Meanwhile, for the standard eosin in the H&E stain, one slide was prepared. This resulted in a total of 28 rat liver tissue slides needed for analysis. The results were used to compute the one-way Analysis of Variance (ANOVA), Games–Howell post hoc test, and Welch's t-test for the researchers to be able to compare the experimental and control groups with each other.

Statistical Treatment of Data

The statistical tools used in this study after data collection were expressed through the use of mean, one-way ANOVA, and Games-Howell post hoc test, and Welch's T-test. Mean score was conducted to determine which among the three different weights of guava leaves (15 g, 25 g, and 35 g) contributed the most in terms of cytoplasmic staining and clarity of staining. A one-way Analysis of Variance (ANOVA) was conducted to determine whether there were significant differences in the cytoplasmic staining and clarity of staining using guava leaf extract when grouped according to the different weights (15 g, 25 g, and 35 g). If a significant difference was found between the experimental groups and the control group, the Games–Howell post hoc test was performed to identify which specific group differences contributed to the significant effect found in the clarity and cytoplasmic staining. Welch's T-test was used to identify whether there was a significant difference between the best-performing concentration of ethanolic guava leaf extract and the standard Hematoxylin and Eosin (H&E) stain in terms of cytoplasmic staining and clarity of staining.

III. RESULTS AND DISCUSSIONS

Table III. Determination of Best Concentration of Guava Leaves Extract in terms of Cytoplasmic Staining based on Mean Differences between Experimental Groups

	Guava Leaves Extract	Mean
Cytoplasmic Staining	15g	2.11
	25g	2.56
	35g	2.56

Identification of the best concentration of ethanolic guava leaves with hematoxylin in terms of cytoplasmic staining

Table III showed that the mean scores for the 25 g (M = 2.56) and 35 g (M = 2.56) groups were slightly higher than those of the 15 g group (M = 2.11), the variation among the groups was not large enough to be considered statistically meaningful. Since this was the result, this suggests that increasing or decreasing the weight of guava leaf extract did not significantly affect the cytoplasmic staining of the tissue samples. This suggests that the staining capability of the extract remained relatively consistent despite differences in the amount of extract used. The guava leaves extract was able to stain the rat liver due to the presence of phenolic acids, flavonoids, and tannins, which was supported by the findings of Asra et al. (2021). The use of 70% ethyl alcohol in the maceration process was able to extract the phenolic contents of the guava leaves, as this concentration allows the highest total phenolic content (TPC) (Monnoubie, 2023). The result indicated that a change in the amount of guava leaves in the extract does not indicate a change in the quality of the cytoplasmic staining, which shows a pink coloration of the cytoplasm, a similarity to the results observed by Okelie et al. (2021). Although there is no variation in the quality of the cytoplasmic staining between different amounts, the staining ability of the guava leaves was supported by the findings of the study of Krampah et al. (2021) due to the similarity in the results with great millet (*Sorghum bicolor*) in the ability to stain the cytoplasm. This indicates that the guava leaves extract was able to stain the rat liver specimen regardless of the amount of the guava leaves due to the presence of necessary biochemical compounds that allow it to stain, and compared to the results obtained on similar alternative staining dyes.

Table IV. Test of Significant Differences in the Cytoplasmic Staining and Clarity of Staining using Guava Leaf Extract when grouped according to the Different Weights

	Gram	Mean	p-value	Conclusion
Clarity of Staining	15g	2.22		
	25g	1.44	0.012	Significant
	35g	2.11		

Identification of the best concentration of ethanolic guava leaf extract with hematoxylin in terms of clarity of staining

Table IV showed a statistically significant difference in the clarity of staining among the three extract weights, F = 5.928, p = 0.012. The mean clarity scores indicated that 15 g (M = 2.22) achieved the highest clarity, 35 g (M = 2.11) was slightly lower, and 25 g (M = 1.44) had the lowest clarity. With a p-value below 0.05, the null hypothesis was rejected, confirming that the weight of guava leaf extract significantly influences the clarity of cytoplasmic staining. These results imply that increasing extract weight in moderation does not significantly improve staining clarity and that certain extract concentrations may produce clearer histological results than others. The guava leaves extract possesses a variety of phytochemicals, such as flavonoids and tannins, that allow for staining that is stable and resistant to fading (Butt et al., 2025). This allows for the rat liver slides to be visualized clearly for microscopic viewing of its cells in detail due to the stain's clarity (Lee et al., 2025). The 15 g of guava leaves extract has the best staining clarity among the different concentrations of guava leaves with a similarity to the findings of Bondoc (2018) as cited by Rubina, et. al. (2023), the lowest concentration has the highest clarity due to its large ratio of 1:5 when compared to the ratio of other concentrations, allowing it to penetrate and stain the rat liver specimen. Clarity of staining is an important criterion for assessing a staining component as it enables one to clearly distinguish the different components and structures that can be found in tissues (Ma et al., 2024). In these results, it allowed for the distinction of the different components of the rat liver tissue sample, similar to the results of Karline et. al. (2025) with the use of *Syzygium cumini*.

Table V. Games-Howell Post-Hoc Test on Clarity of Staining

		15g	25g	35g
15g	Mean difference	—	0.778	0.111
	p-value	—	0.010	0.896
25g	Mean difference		—	-0.667
	p-value		—	0.059
35g	Mean difference			—
	p-value			—

Determination of the presence of a significant difference between the best concentration of ethanolic guava leaf extract with hematoxylin and the standard hematoxylin and eosin stain in terms of cytoplasmic staining

Table V revealed a significant difference between the 15 g and 25 g extract groups, with a mean difference of 0.778 and a p-value of 0.010. This indicates that 15 g of extract produced a significantly higher clarity of staining compared to 25 g of extract, suggesting that lower extract weight produced better clarity of staining under experimental conditions. In contrast, the comparison between 15 g and 35 g did not show a statistically significant difference, with a mean difference of 0.111 and a p-value of 0.896. This suggests that these two extract weights produced a similar clarity of staining. Similarly, the comparison between 25 g and 35 g also did not reach statistical significance, with a mean difference of -0.667 and a p-value of 0.059. Although this value was close to the 0.05 significance level, it still indicates that the difference between these two groups was not strong enough to be considered statistically significant. Overall, these findings suggest that 15 g of guava leaf extract produced significantly clearer staining compared to 25 g, while the other pairwise comparisons do not show meaningful differences. With this, lower concentrations of guava leaf extract may provide better staining clarity than higher concentrations, implying that increasing the amount of extract does not necessarily improve staining quality and may even reduce clarity under the specific experimental conditions of the study. Guava leaf extraction produces a dark green, viscous semisolid that is rich in flavonoids and phenolic compounds (Ruksiriwanich et al., 2022). Hassanpour and Doroudi (2023) explained that the hydroxyl groups present in most flavonoids make them acidic, allowing them to interact well with the basic components of the cell cytoplasm. Kumar et al. (2021) also reported that guava leaves are naturally acidic because of compounds such as oleanolic acid, suggesting that guava leaves may serve as an alternative to eosin, an acidic dye commonly used in histological staining. Results are supported by the study of Prajapati et al. (2024), which used 15 grams of *Zingiber officinale* and *Curcuma longa* as alternative eosin stains. Findings showed that *Z. officinale* demonstrated better staining efficiency than *C. longa* when compared with eosin. This indicates that a lower concentration with a smaller ratio (15 g with a 1:5 ratio of guava leaves to alcohol) produces clearer staining than higher concentrations, such as 25 g with a 1:10 ratio and 35 g with a 1:15 ratio.

Table VI. Test of Significant Difference between the Best Concentration of Ethanolic Guava Leaf Extract with Hematoxylin and the Standard Hematoxylin and Eosin Stain

	Weight	Mean	Welch's t	df	p-value	Mean Difference	Decision	Conclusion
Cytoplasmic Staining	15g	2.11	-0.31	2.64	0.779	-0.22	Failed to reject Ho	Not significant
	H&E Stain							
Clarity of Staining			-5.292	8	<.001	-0.78	Reject Ho	Significant
	H&E Stain							

Note. $H_a\ \mu_{15g} \neq \mu_{H\&E\ stain\ (control)}$

Determination of the presence of a significant difference between the best concentration of ethanolic guava leaf extract with hematoxylin and the standard hematoxylin and eosin stain in terms of clarity of staining.

Table VI indicated that no statistically significant difference was observed in cytoplasmic staining between the 15 g guava leaf extract and the standard H&E stain, $t(2.64) = -0.31$, $p = 0.779$. The mean difference of −0.22 showed only a minimal variation between the two staining methods. Since the p-value is greater than the 0.05 level of significance, the null hypothesis was not rejected, indicating that the cytoplasmic staining produced by the guava leaf extract is comparable to that produced by the conventional H&E stain. This suggests that the guava leaf extract has the potential to perform similarly to the standard stain in highlighting the cytoplasmic components of the tissue. In contrast, the analysis showed a statistically significant difference in staining clarity, $t(8) = -5.292$, $p < 0.001$, with a mean difference of −0.78. Because the p-value is far below the 0.05 significance threshold, the null hypothesis was rejected, suggesting that the staining clarity from the guava leaf extract significantly varies from that of the standard H&E stain. Overall, these findings suggest that while the 15 g concentration of ethanolic guava leaf extract can produce cytoplasmic staining comparable to the conventional H&E stain, there remains a significant difference in staining clarity. This suggests that while guava leaf extract shows promise as an alternative staining agent, the standard H&E stain might still yield clearer and more distinct staining outcomes under the study's experimental conditions. Future research might delve deeper into enhancements of extraction techniques, staining procedures, or concentration modifications. Standard Hematoxylin and eosin (H&E) stain is the most widely used staining method in histology because it is cost-effective and effective in staining tissue samples (De Haan et al., 2021). Studies showed that guava leaf extract could act as a possible alternative stain for the cytoplasmic components in rat liver tissues. Results also revealed that the lowest concentration of guava leaf extract (15 g) produced cytoplasmic staining that was nearly similar to the staining quality of standard eosin in H&E staining. Nonetheless, H&E staining remains more widely utilized as it offers clearer and more detailed staining than the 15 g guava leaf extract. Aiswarya et al. (2025) stated that H&E staining continues to be the standard in histopathology. The ability to clearly show cell structure and cytoplasmic features helps support accurate tissue diagnosis. This shows that guava leaf extract could serve as a natural dye that offers a non-toxic and environmentally friendly alternative for histological staining (KS, 2024).

IV. CONCLUSIONS

Based on the findings of the study, the following conclusions are presented:

1. The results showed no significant difference in cytoplasmic staining among the different concentrations of guava leaf extract. This implies that the extract can effectively produce pink cytoplasmic staining regardless of concentration.
2. The lowest concentration of guava leaf ethanolic extract combined with hematoxylin produced the clearest staining of rat liver tissues. This suggests that its phytochemical components may enhance staining stability and tissue dye retention.
3. The lowest concentration provided better staining clarity than the higher concentration tested. This implies that lower concentrations may offer a more detailed microscopic view and serve as an effective plant-based staining alternative.
4. The findings showed that guava leaf extract can function as a natural alternative to eosin for cytoplasmic staining. However, conventional H&E staining remains superior in clarity and detail, supporting its continued use as the standard staining method.

V. RECOMMENDATION

Based on the findings and analysis presented in this study, the following recommendations are proposed for the possible actions:

1. Future studies should involve a pathologist in evaluating histopathological slides. This can improve the accuracy and reliability of staining quality assessments.
2. Future researchers should examine the shelf life of guava leaf extract under different storage conditions. This will help determine whether its staining effectiveness can be maintained over time.
3. Future studies should test lower concentrations of guava leaf extract to identify the optimal level for staining. This may improve staining consistency and tissue visualization.
4. Future researchers can investigate other parts of the guava plant, such as the peel, seeds, and bark, as natural dyes. These parts may also possess effective staining properties for histological applications.
5. Future studies should evaluate other factors that affect staining quality, including extraction methods, staining time, pH, and preparation techniques. Understanding these variables may improve staining clarity and accuracy.
6. Future researchers should conduct phytochemical analyses to identify the compounds responsible for staining properties. Determining the optimal levels of these compounds may enhance staining performance.
7. Future studies should test guava leaf extract on different animal and human tissues. This will help assess its broader applicability as an alternative histological stain.
8. Standardized preparation procedures should be established for future studies. This will ensure consistency, reliability, and reproducibility of results.
9. Future researchers should compare different extraction solvents, such as ethanol, methanol, and water. This may identify the solvent that produces the most effective staining properties.
10. Future studies should explore combining guava leaf extract with other natural or conventional dyes. Such combinations may improve staining clarity and overall performance.
11. Future researchers should evaluate guava leaf extract without adding hematoxylin. This will allow a more accurate assessment of its ability to replicate eosin's cytoplasmic staining properties.

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