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Evaluating the Staining Potential of Dragon Fruit (*Hylocereus polyrhizus*) Extract as an Alternative for Eosin Stain

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Abstract

Histological staining, specifically hematoxylin and eosin (H&E), is the gold standard in diagnostic pathology; however, eosin and other synthetic dyes have health and environmental risks. The extract from dragon fruit (Hylocereus polyrhizus) was examined in this study as a natural substitute stain for histological preparations. Using ethanol maceration and rotary evaporation, peel and pulp extracts from dragon fruits harvested 38 and 42 days after anthesis were made. These extracts were subsequently diluted to 50%, 80%, and 100% concentrations. Pigs' hearts and livers are employed as the tissue sample, and they undergo both tissue processing and physical evaluation. Using a Likert-scale, one pathologist and three medical technologists evaluated the intensity, clarity, and contrast and overall staining quality of the dragon fruit extract. ANOVA with post hoc tests, independent-sample t-tests, and one-sample t-tests were used to examine the data. While 42-day fruits did not exhibit any significant changes among concentrations ($p = .129$), the staining performance of 38-day fruits was significantly influenced by extract concentration ($F(2,33) = 6.247$, $p = .005$), with 100% concentration producing color intensity most comparable to eosin. At 50% concentration, 42-day extracts showed greater staining, according to independent t-tests; at higher concentrations, no significant changes were seen. Overall, 100% concentration immature fruits (38 days) produced excellent staining quality, allowing for a satisfactory view of the pig's liver and heart tissues. Therefore, at 100% concentration from 38-day fruits, dragon fruit extract shows promise staining potential as an economical and environmentally friendly alternative to eosin; nevertheless, more research on long-term stability and broader tissue applications is recommended.

Keywords: *Dragon fruit extract, Hylocereus polyrhizus, Eosin, Pigs' Heart, Pigs' Liver*

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Introduction

Histological staining is an essential technique in pathology and scientific research. Currently acknowledged as being important in clinical diagnosis and therapy in almost every field of medicine and is the principal way of analyzing tissue samples. Staining is an essential component of histological examination, which is the gold standard for diagnosing many clinical disorders. It entails the use of various dyes and stains to improve the microscope visibility of particular tissues, cells, and structures. These stains aid in the proper identification and examination of cellular and tissue structures by aiding in the differentiation of various tissue constituents such as nuclei, cytoplasm, connective tissue, and cellular organelles.

In histopathology and diagnostics, staining is widely employed to reveal abnormalities in cell count and structure under the microscope. Stains used in histology include colors, metals, and tagged antibodies (Jhonson, D., 2022). Hematoxylin and eosin (H&E), which offer good contrast and specificity, have historically been the primary synthetic dyes used in histological staining. However, concerns about the toxicity and environmental impact of these synthetic colors, which may influence human health by causing skin allergies, digestive problems, and respiratory problems, have been raised. (Hartika et al, 2021). The prevalence of eosin-related skin and ocular irritation is difficult to measure due to underreporting and variation in exposure levels. Direct contact with eosin can produce skin irritation, redness, and itching. If it gets into your eyes, it can cause irritation and conjunctivitis. adverse reactions: Although uncommon, some people may experience adverse reactions to eosin, including skin rashes, hives, and even breathing issues. The hazards and benefits of employing eosin must be carefully considered in each unique situation; other dyes with fewer potential for negative effects may be better for certain applications (Kar et al, 2024).

Histopathology, vital for medical diagnostics and research, relies on diverse staining techniques for visualizing cellular structures and pathology. Eosin, a key counterstain with hematoxylin, selectively binds to acidic structures, imparting a pink hue under a microscope. It differentiates cellular elements, highlights pathology, and, combined with hematoxylin, produces detailed images crucial for interpreting histological specimens. Eosin's unique staining enhances accuracy in histopathological analyses (Roy et al, 2019).

Furthermore, in the course of the study, tissue samples are prepared and stained using various concentrations of dragon fruit (*Hylocereus polyrhizus)* flesh/pulp and peel/mesocarp extracts. The intensity, specificity, and contrast of the staining will be assessed under a microscope on the stained tissue samples. This study will compare the qualities of dragon fruit (*Hylocereus polyrhizus)* stain in addition to highlighting their advantages over synthetic alternatives.

This study, evaluates the staining potential of Dragon Fruit (*Hylocereus polyrhizus*) extract as an alternative to eosin stain, utilizing pig liver and heart as our histologic smear substrate. The escalating demand for nontoxic, cost-effective, and readily available stains underscores the need for a comprehensive investigation into the factors influencing the staining efficacy on selected tissues. Therefore, this research is centered on determining the staining effectiveness of Dragon Fruit extract compared to eosin dye. Statistical analysis plays a pivotal role in revealing patterns, relationships, and trends within the collected data. Employing Independent Sample T-Test, One-Sample T-Test, and ANOVA to compare the ability of different concentrations dragon fruit extract as a stain through examining the different parts of the dragon fruit while considering the factors of age brackets on phytochemicals and pH levels across various concentrations based on color intensity with different concentrations to identify the most effective concentration and part of the dragon fruit as an alternative to Eosin stain. This research is pivotal in the quest for a cost-effective and organic substitute for eosin in histologic smears. In the subsequent sections, we expound on our research design and the application of statistical methods to unravel these crucial dynamics. Thus, if this study is successful, its findings could contribute to the creation of safe, non-toxic staining techniques, eliminating the need for synthetic dyes and encouraging the use of environmentally friendly procedures in pathology and biomedical research.

Methods

This experimental and comparative study evaluated the staining potential of *Hylocereus polyrhizus* (dragon fruit) extract as an alternative to eosin in histological preparations using pig liver and heart tissues. The study examined the effect of different variables—fruit part (peel and flesh), fruit age (38 and 42 days post-anthesis), and extract concentration (50%, 80%, and 100%)—on staining quality, assessed via pH and color intensity. Pig liver and heart tissues served as samples.

Dragon fruit samples were collected from Arcan Dragon Fruit Farm in Davao City and authenticated by a taxonomist. Extracts were prepared through ethanol maceration and rotary evaporation. One kilogram of peel or pulp was macerated in 95% ethanol (2:1 ethanol-to-sample ratio) for 48 hours, with periodic agitation. The solution was concentrated using a rotary evaporator at 50 °c under reduced pressure until ethanol was removed. The resulting solutions were diluted with buffer into three concentrations— 100%, 80%, and 50% working solutions, which were stored and labeled airtight vials at 4°c until use.

Histological tissue samples underwent standard processing, fixed in 10% neutral-buffered formalin for 24 hours then processed via standard paraffin embedding. Sections were then prepared with 5µm thickness. Slides were rehydrated using graduated ethanol solutions to distilled water after being deparaffinized in xylene. After 15 minutes of hematoxylin application, the samples were differentiated in acid alcohol, bluened in ammonia water, and rinsed. Sections were counterstained with dragon fruit extract (1–3 dips; 50%, 80%, or 100% concentration). For positive control, the slides underwent to the same procedure but instead of dragon fruit extract as counterstain, eosin was used. Before mounting with a coverslip, the sample was dehydrated using graded ethanol and xylene clearing.

A total of 3 slides per group were prepared. Staining quality was independently assessed by one pathologist and three licensed medical technologists. Evaluation was based on a 5-point Likert scale assessing (1) color intensity, (2) contrast between tissue components, and (3) clarity of structural details developed by the researchers.

Data were statistically analyzed using ANOVA to compare mean staining intensities among extract concentrations, one-sample t-test to compare dragon fruit extracts against eosin, and independent sample t-test to compare groups by fruit age; a significance level of $p < 0.05$ was adopted. Post hoc analysis was conducted to identify significant differences among the groups.

The study followed ethical and biosafety protocols in handling biological materials and disposing of waste. The scope was limited to dragon fruit extract's use in liver and heart sections and does not cover other histological tissues or stains.

Results and Discussion

This chapter presents the data collected, tables, analyses, and interpretation on Evaluating the Staining Potential of Dragon Fruit (*Hylocereus polyrhizus*) Extract As An Alternative For Eosin Stain.

Table 1. Statement of the problem 1: What is the pH level of the different concentrations of the Dragon Fruit extract

Sample 1 Immature sample (Day 38)	**pH**
50 percent concentration immature sample (Day 38)	5.05
80 percent concentration immature sample (Day 38)	4.97
100 percent concentration immature sample (Day 38)	4.86

Sample 2 Mature Sample (Day 42)	**pH**
50 percent concentration mature sample (Day 42)	4.82
80 percent concentration mature sample (Day 42)	4.84
100 percent concentration mature sample (Day 42)	4.69

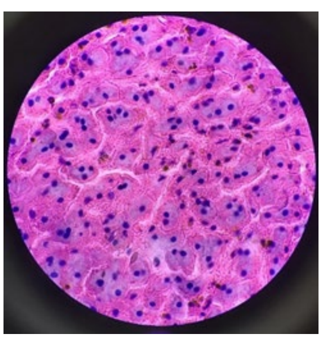
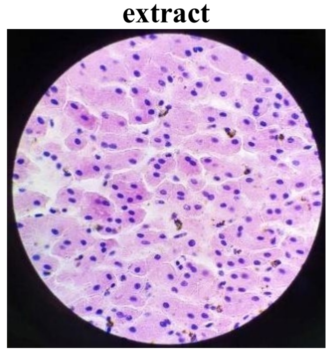
Table 2. Statement of the problem 2: What is the total amount of flavonoids present in the different concentrations of the Dragon Fruit extract

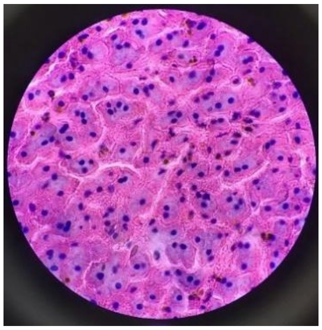
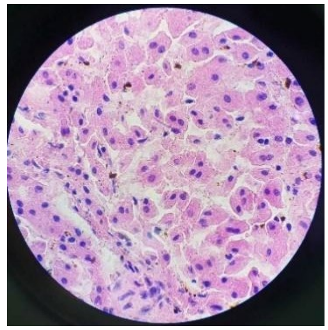
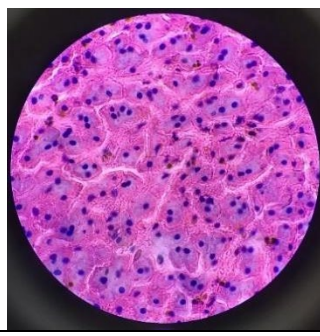
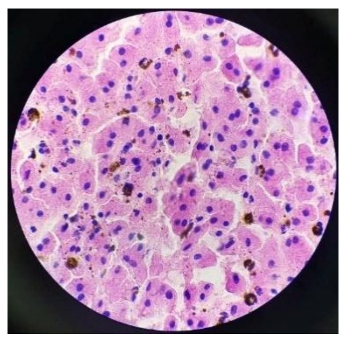
Sample 1 Immature sample (Day 38)	**Flavonoids, ppm (as Quercetin)**
50 percent concentration immature sample (Day 38)	44.1
80 percent concentration immature sample (Day 38)	44.8

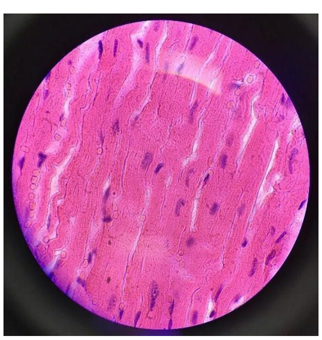
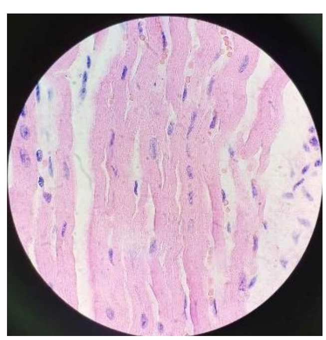
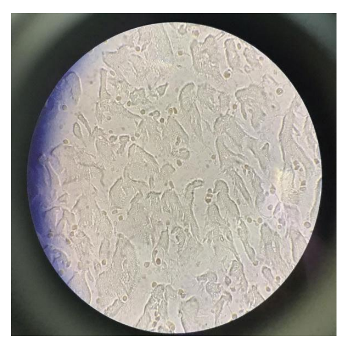
Sample 1 Immature sample (Day 38)	**Flavonoids, ppm (as Quercetin)**
100 percent concentration immature sample (Day 38)	44.6

Sample 2 Mature Sample (Day 42)	**Flavonoids, ppm (as Quercetin)**
50 percent concentration mature sample (Day 42)	24.33
80 percent concentration mature sample (Day 42)	36.00
100 percent concentration mature sample (Day 42)	47.67

Table 3. Statement of the problem 3: What is the level of visibility of the tissue sample according to color intensity, with the following concentrations of Dragon Fruit extract

Sample 1: Immature (Day 38) Liver Sample	**Concentration**	**Mean Score**
	50%	**3.111**
POSITIVE CONTROL (EOSIN)	**50% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	80%	**3.750**

POSITIVE CONTROL (EOSIN)	**80% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	100%	**3.667**
POSITIVE CONTROL (EOSIN)	**100% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	

Sample 1: Immature (Day 38) Heart Sample	**Concentration**	**Mean Score**
	50%	**3.111**
POSITIVE CONTROL (EOSIN)	**50% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		

DDC Professional Journal
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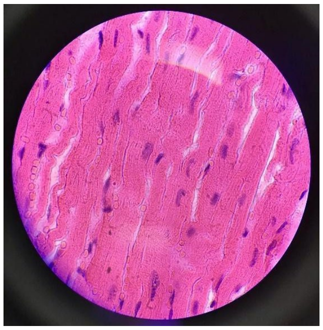
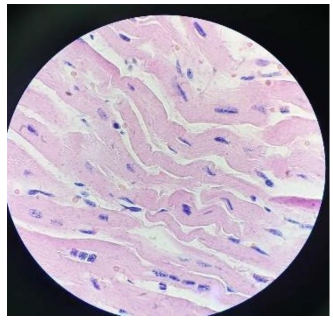
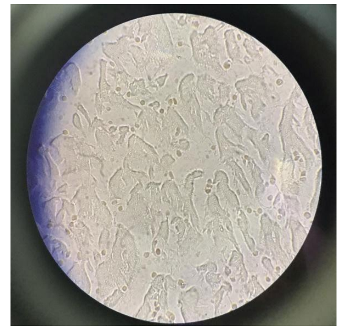
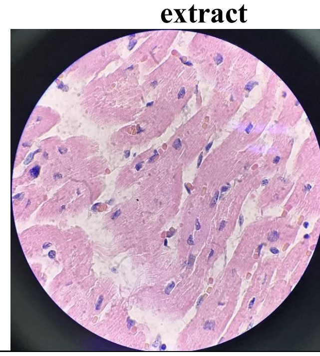
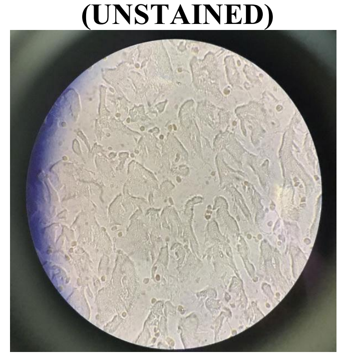
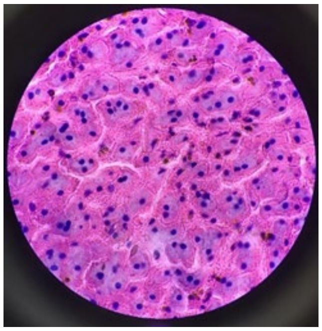
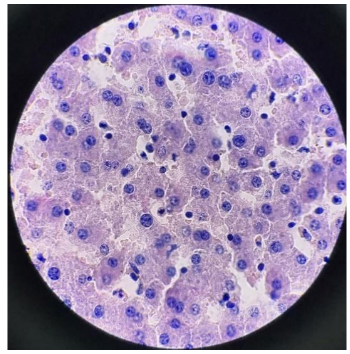
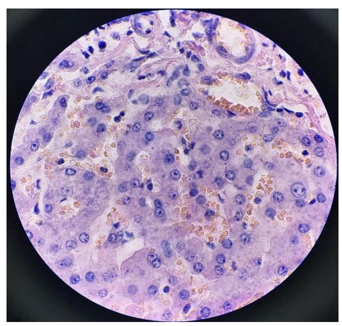
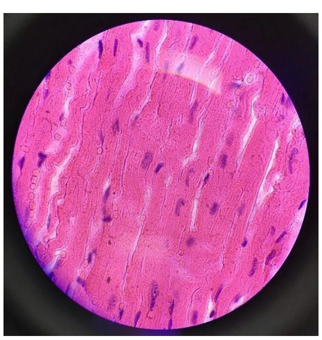
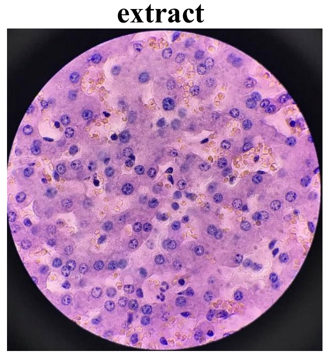
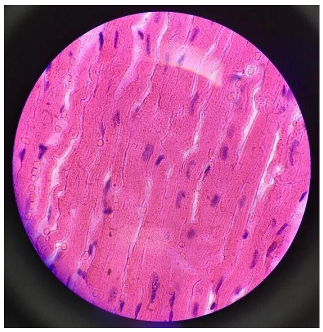
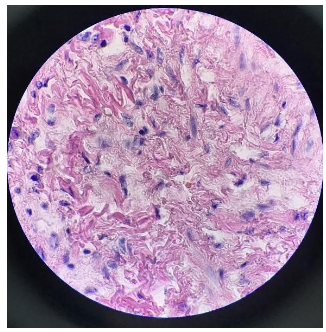
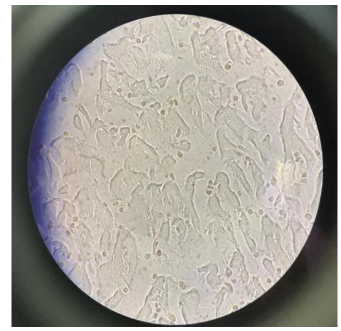
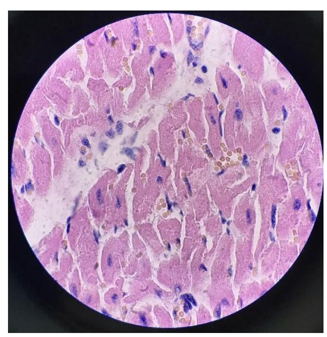
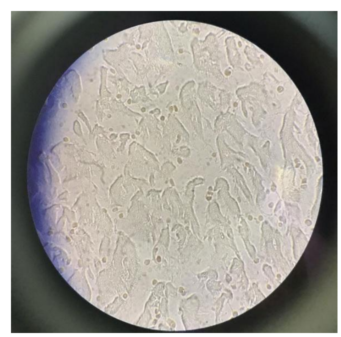
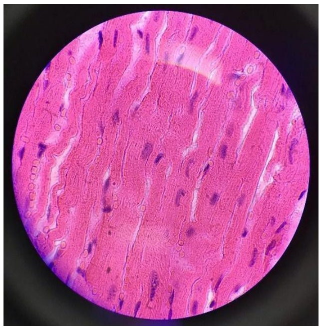
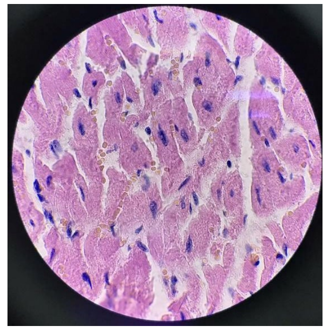
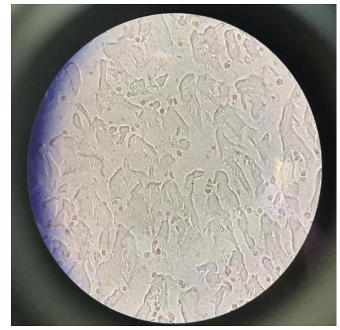
	Under Oil Immersion Objective (1000x)	
	80%	**3.750**
POSITIVE CONTROL (EOSIN)	**80% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	100%	**3.667**
POSITIVE CONTROL (EOSIN)	**100% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	

Sample 2: Mature (Day 42) Liver Sample	**Concentration**	**Mean Score**
	50%	3.444

POSITIVE CONTROL (EOSIN)	**50% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	80%	3.751
POSITIVE CONTROL (EOSIN)	**80% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	100%	3.750
POSITIVE CONTROL (EOSIN)	**100% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	

Sample 2: Mature (Day 42) Heart Sample	Concentration	Mean Score
	50%	3.444
POSITIVE CONTROL (EOSIN)	**50% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	80%	3.751
POSITIVE CONTROL (EOSIN)	**80% concentration of 38 days after anthesis dragon fruit extract**	**NEGATIVE CONTROL (UNSTAINED)**
		
	Under Oil Immersion Objective (1000x)	
	100%	3.750

POSITIVE CONTROL (EOSIN)	100% concentration of 38 days after anthesis dragon fruit extract	NEGATIVE CONTROL (UNSTAINED)
		

Under Oil Immersion Objective (1000x)

The data shows that there were significant differences in the intensity of the stain depending on the concentration of the stain used. In relation to the data gathered which shows concentration and fruit age does indeed cause drastic difference in tissue staining capabilities, elevated levels of stain concentration typically result in heightened staining intensity. Augmenting the concentration of the staining solution enhances the quantity of dye molecules accessible for binding to the targeted structures or molecules within the sample, thereby yielding a more pronounced coloration as stated by Smith (2018).

Table 4.1 Statement of the problem 4: Significant Difference Between the pH and Color Intensity of the Eosin Stain and the Different Concentrations of Dragon Fruit extract

One Sample T-Test for Sample after day 38 of Anthesis 50%

One Sample T-Test

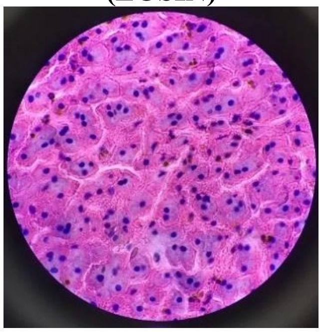
					95% CI for Mean Difference	
	t	df	p	Mean Difference	Lower	Upper
50percent38days	-30.917	11	< .001	-1.939	-2.077	-1.801

Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from 5.05.

One sample t-test was used to compare the color intensity of the 50 percent concentration of dragon fruit extract against the pH value of 5.05 for eosin stain. The 50 percent concentration for immature sample (38 days) is lesser than 1.939, with 95% CI [-2.007, -1.801]. The **difference was found to be statistically significant** $t(11)=-30.917$ and $p<0.001$.

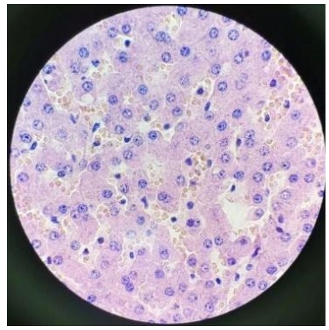
LIVER SAMPLE

POSITIVE CONTROL (EOSIN)



Under Oil Immersion Objective (1000x)

50% concentration of 38 days after anthesis dragon fruit extract



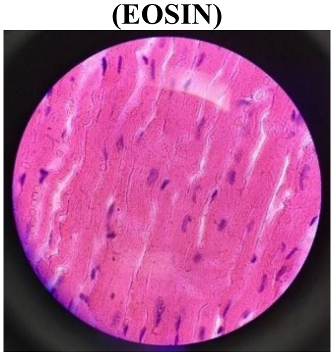
Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)

Under Oil Immersion Objective (1000x)

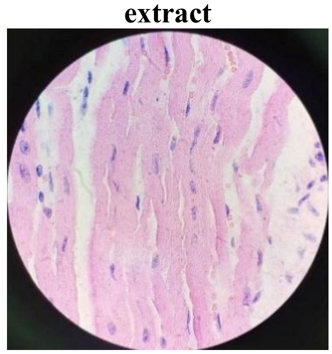
HEART SAMPLE

POSITIVE CONTROL (EOSIN)



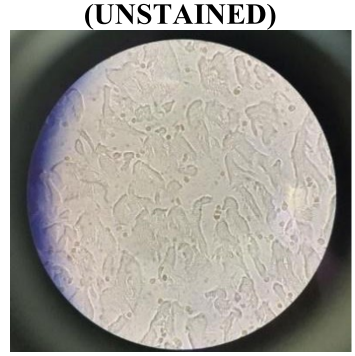
Under Oil Immersion Objective (1000x)

50% concentration of 38 days after anthesis dragon fruit extract



Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)



Under Oil Immersion Objective (1000x)

Table 4.2 Statement of the problem 4: Is there a significant difference between the pH and color intensity of the Eosin stain and the different concentrations of dragon fruit extract

One Sample T-Test for Sample after day 38 of Anthesis 80%

One Sample T-Test

					95% CI for Mean Difference	
	t	**df**	**p**	**Mean Difference**	**Lower**	**Upper**
80percent Immature sample (38days)	-9.344	11	< .001	-1.220	-1.507	-0.933

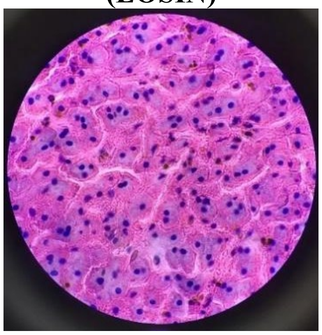
Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from **4.97.**

DDC Professional Journal
Vol.6, No.3, September 2025
ISSN 1908-3130

One sample t-test was used to compare the color intensity of the 80 percent concentration of dragon fruit extract against the pH value of 4.97 for eosin stain. The 80 percent concentration for immature sample (38 days) is lesser than 1.220, with 95% CI [-1.507, -0.933]. The **difference was found to be statistically significant** t(11)=-9.344 and p<0.001.

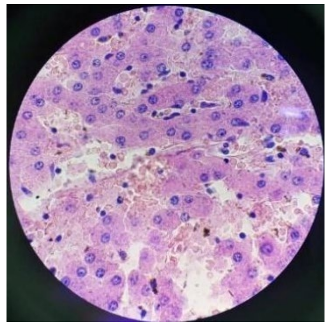
LIVER SAMPLE

POSITIVE CONTROL (EOSIN)



Under Oil Immersion Objective (1000x)

80% concentration of 38 days after anthesis dragon fruit extract



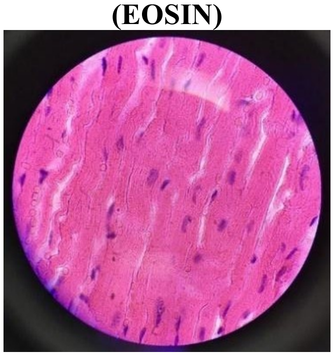
Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)

Under Oil Immersion Objective (1000x)

HEART SAMPLE

POSITIVE CONTROL (EOSIN)

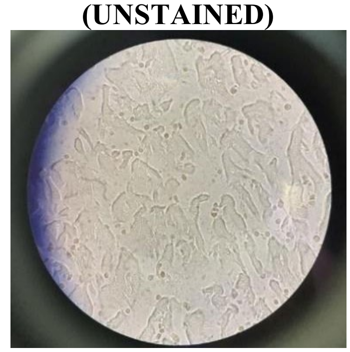


Under Oil Immersion Objective (1000x)

80% concentration of 38 days after anthesis dragon fruit extract

Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)



Under Oil Immersion Objective (1000x)

Table 4.3: Is there a significant difference between the pH and color intensity of the Eosin stain and the different concentrations of dragon fruit extract

One Sample T-Test for Sample after day 38 of Anthesis 100%

One Sample T-Test

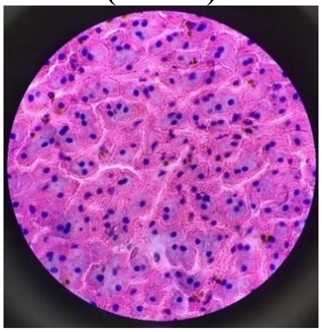
	t	df	p	Mean Difference	95% CI for Mean Difference	
					Lower	Upper
100percent Immature sample (38days)	-6.204	11	< .001	-1.193	-1.617	-0.770

Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from **4.86.**

One sample t-test was used to compare the color intensity of the 100 percent concentration of dragon fruit extract against the pH value of 4.86 for eosin stain. The 100 percent concentration for immature sample (38 days) is lesser than 1.193, with 95% CI [-1.617, -0.770]. The **difference was found to be statistically significant** t(11)=-6.204 and p<0.001.

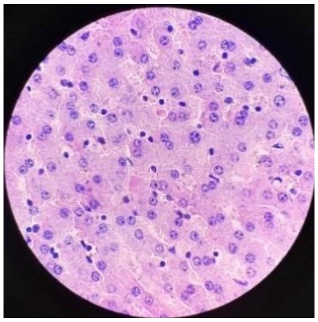
LIVER SAMPLE

POSITIVE CONTROL (EOSIN)



Under Oil Immersion Objective (1000x)

100% concentration of 38 days after anthesis dragon fruit extract



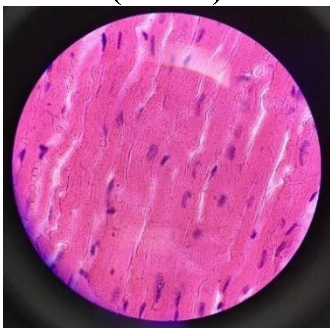
Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)

Under Oil Immersion Objective (1000x)

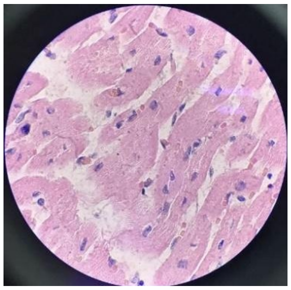
HEART SAMPLE

POSITIVE CONTROL (EOSIN)



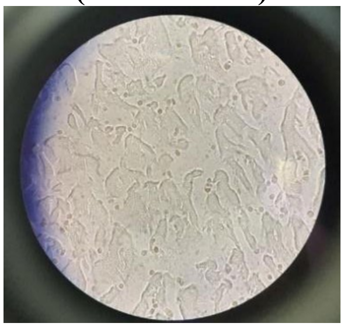
Under Oil Immersion Objective (1000x)

100% concentration of 38 days after anthesis dragon fruit extract



Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)



Under Oil Immersion Objective (1000x)

Table 4.4 Statement of the problem 4: One Sample T-Test for Sample after day 42 of Anthesis 50%

One Sample T-Test

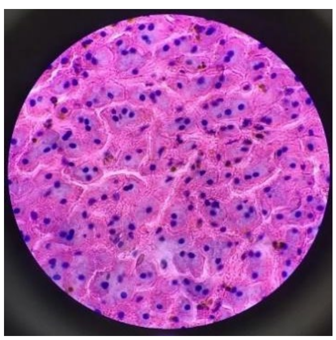
	t	df	p	Mean Difference	95% CI for Mean Difference	
					Lower	Upper
50percent42days	-9.547	11	< .001	-1.376	-1.693	-1.059

Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from **4.82.**

One sample t-test was used to compare the color intensity of the 50 percent concentration of dragon fruit extract against the pH value of 4.82 for eosin stain. The 50 percent concentration for the mature sample (42 days) is less than 1.376, with 95% CI [-1.693, -1.059]. The **difference was found to be statistically significant** t(11)=-9.547 and p<0.001

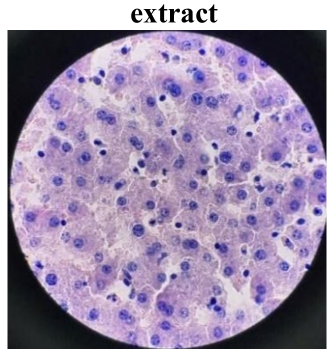
NEW LIVER SAMPLE

POSITIVE CONTROL (EOSIN)



Under Oil Immersion Objective (1000x)

50% concentration of 42 days after anthesis dragon fruit extract



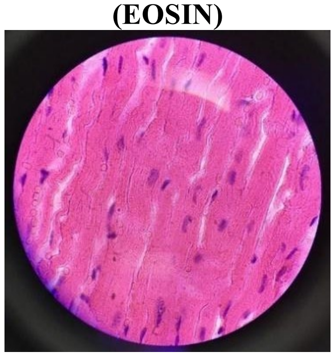
Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)

Under Oil Immersion Objective (1000x)

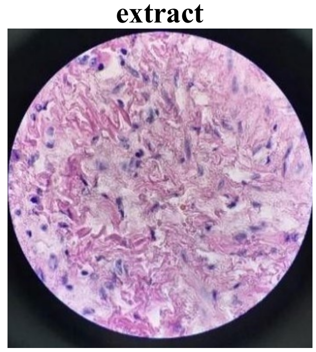
HEART SAMPLE

POSITIVE CONTROL (EOSIN)



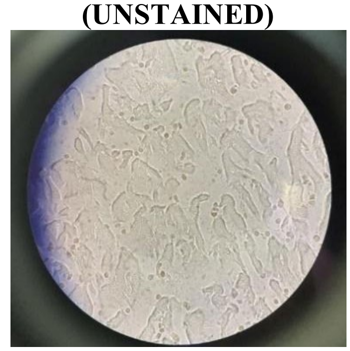
Under Oil Immersion Objective (1000x)

50% concentration of 38 days after anthesis dragon fruit extract



Under Oil Immersion Objective (1000x)

NEGATIVE CONTROL (UNSTAINED)



Under Oil Immersion Objective (1000x)

Table 4.5 Statement of the problem 4: Is there a significant difference between the pH and color intensity of the Eosin stain and the different concentrations of dragon fruit extract

One Sample T-Test for Sample after day 42 of Anthesis 80%

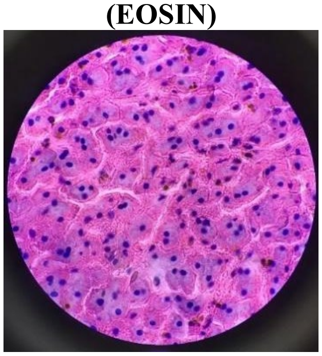
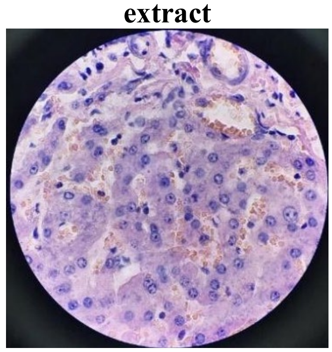
One Sample T-Test

	t	df	p	Mean Difference	95% CI for Mean Difference Lower	95% CI for Mean Difference Upper
80percent42days	-9.950	11	< .001	-1.089	-1.330	-0.848

Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from 4.84.

One sample t-test was used to compare the color intensity of the 80 percent concentration of dragon fruit extract against the pH value of 4.84 for eosin stain. The 80 percent concentration for the mature sample (42 days) is lesser than 1.330 units, with 95% CI [-1.330, -0.848]. The **difference was found to be statistically significant** t(11)=-9.950 and p<0.001.

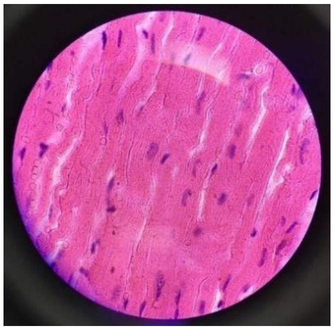
NEW LIVER SAMPLE

POSITIVE CONTROL (EOSIN)	80% concentration of 42 days after anthesis dragon fruit extract	NEGATIVE CONTROL (UNSTAINED)
 *Under Oil Immersion Objective (1000x)*	 *Under Oil Immersion Objective (1000x)*	 *Under Oil Immersion Objective (1000x)*

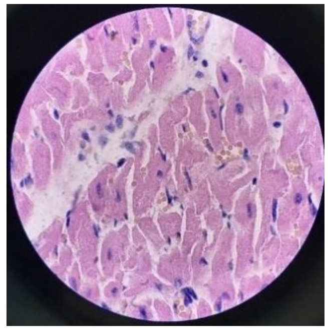
HEART SAMPLE

POSITIVE CONTROL	50% concentration of 38 days after anthesis dragon fruit extract	NEGATIVE CONTROL (UNSTAINED)

(EOSIN)



Under Oil Immersion Objective (1000x)



Under Oil Immersion Objective (1000x)

Under Oil Immersion Objective (1000x)

Table 4.6 Statement of the problem 4: Is there a significant difference between the pH and color intensity of the Eosin stain and the different concentrations of dragon fruit extract

One Sample T-Test for Sample after day 42 of Anthesis 100%

One Sample T-Test

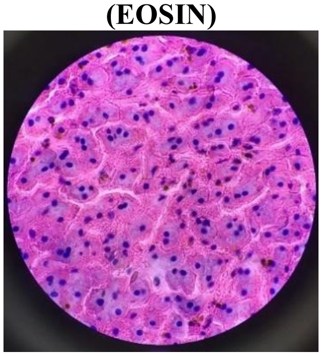
	t	df	p	Mean Difference	95% CI for Mean Difference	
					Lower	Upper
100percent42days	-9.242	11	< .001	-0.940	-1.164	-0.716

Note. For the Student t-test, the alternative hypothesis specifies that the mean is different from **4.69.**

One sample t-test was used to compare the color intensity of the 100 percent concentration of dragon fruit extract against the pH value of 4.69 for eosin stain. The 100 percent concentration for 42 days is lesser than 1.164 units, with 95% CI [-1.330, -0.848]. The **difference was found to be statistically significant** t(11)=-9.242 and p<0.001.

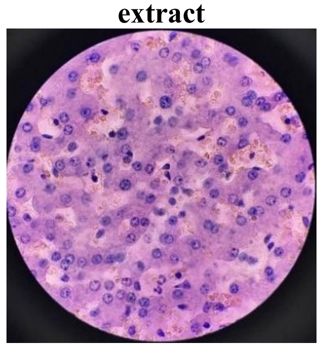
LIVER SAMPLE

POSITIVE CONTROL (EOSIN)



Under Oil Immersion Objective (1000x)

100% concentration of 42 days after anthesis dragon fruit extract

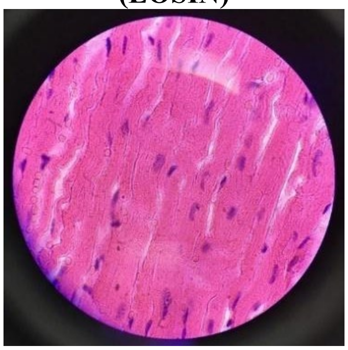
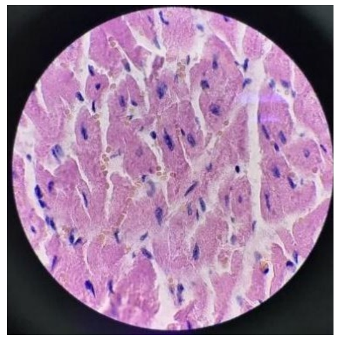
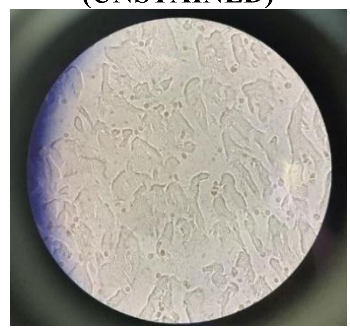


NEGATIVE CONTROL (UNSTAINED)

Under Oil Immersion Objective (1000x)

Under Oil Immersion Objective (1000x)

HEART SAMPLE

POSITIVE CONTROL (EOSIN)	100% concentration of 38 days after anthesis dragon fruit extract	NEGATIVE CONTROL (UNSTAINED)
		
Under Oil Immersion Objective (1000x)	*Under Oil Immersion Objective (1000x)*	*Under Oil Immersion Objective (1000x)*

Different levels of staining intensity were observed when pH levels were altered. Notably, variations in stain retention were detected upon staining, reflecting tissue staining responses to pH changes. Hairston (2019), stated that altering the hydrogen ion concentrations in dye solutions results in varying degrees of stain retention. Furthermore, staining conducted outside the optimal pH range of eosin is either excessively heavy or light, and occasionally exhibited signs of varying color intensity. Various cellular components possess distinct isoelectric points, leading to differing affinities for dyes depending on the pH of the environment (Hairston, 2019).

Table 5.1 Statement of the problem 5: Which among the different concentrations of dragon fruit is most effective as an alternative for Eosin stain

Concentrations are compared according to days of sample: ANOVA and Descriptives-Color Intensity for Sample after day 38 of Anthesis

ANOVA - color intensity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Concentrations immature sample (38 days)	2.898	2	1.449	6.247	0.005	0.275
Residuals	7.654	33	0.232			

ANOVA - color intensity

Cases	Sum of Squares	df	Mean Square	F	p	η²

Descriptive - color intensity

Concentrations 38 days	N	Mean	SD	SE	Coefficient of variation
100 percent concentration immature sample (38 days)	12	3.667	0.666	0.192	0.182
50 percent concentration immature sample (38 days)	12	3.111	0.217	0.063	0.070
80percent concentration immature sample (38 days)	12	3.750	0.452	0.131	0.121

The 36 samples from different concentration (50%, 80%, 100%) were compared using analysis of variance (ANOVA) to see if there a significant difference among the concentration of dragon fruit. The 50 % concentration has a **moderate effect** 3.111 (SD=0.217), while 80 % concentration has **high effect** with 3.750 (SD=0.452) and lastly 100 % concentration is **highly effective** as well at 3.667 (SD=0.666). The effect these concentrations, therefore, was significant, $F(2, 33) = 6.247$, $p=.005$.

Table 5.2: Statement of the problem 5: Which among the different concentrations of dragon fruit is most effective as an alternative for Eosin stain

ANOVA and Descriptives-Color Intensity for Sample after day 42 of Anthesis

ANOVA - color intensity 2

Cases	Sum of Squares	df	Mean Square	F	p	η²
Concentrations 42 days	0.750	2	0.375	2.176	0.129	0.117
Residuals	5.689	33	0.172			

Note. Type III Sum of Squares

Descriptives - color intensity 2

Concentrations 42 days	N	Mean	SD	SE	Coefficient of variation
100 percent concentration mature sample (42 days)	12	3.750	0.352	0.102	0.094
50 percent concentration mature sample (42 days)	12	3.444	0.499	0.144	0.145
80 percent concentration mature sample (42 days)	12	3.751	0.379	0.109	0.101

The 36 samples from different concentrations (50%, 80%, 100%) were compared using analysis of variance (ANOVA) to see if there was a significant difference in the concentration of dragon fruit after 42 days. As shown in the table, there is no significant difference between the three groups compared, $F(2, 33) = 2.178$, $p=0.129$. Hence the null hypothesis is accepted. In simpler words, we cannot identify which group is more effective since the groups compared are not significantly different.

Asra et al. (2021) demonstrated that betacyanin extracted from the pulp of red dragon fruit can serve as an effective substitute for eosin dye in hematoxylin counterstaining at a concentration of 100% (v/v). The results revealed that the dye extract from red dragon fruit pulp, when used at a concentration of 100% (v/v), displayed sinusoids, central veins, cytoplasm, and a single hepatocyte cell. In comparison, the positive control group treated with Hematoxylin-Eosin dye exhibited sinusoids, main veins, cytoplasm, a hepatocyte cell, cell nucleus, and evidence of hepatocyte damage including necrosis, degeneration, inflammation, and bleeding. However, the standard betacyanin dye showed sinusoids and central veins without observable hepatocyte damage. Moreover, the staining of red dragon fruit pulp extract at 100% (v/v) concentration displayed great results among the concentrations.

Table 6.1: Statement of the problem 6: Which among the different age brackets of dragon fruit is most effective as an alternative for Eosin stain?

50% concentration

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper
50per cent	-2.121	22	0.045	-0.333	0.157	-0.659	-0.007

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
50percent	38 days	12	3.111	0.217	0.063	0.070
	42 days	12	3.444	0.499	0.144	0.145

Independent sample t-test was used to determine which group of concentration according to days is better substitute as eosin stain. Immature sample (38 days) of 50 percent concentration (M =3.11, SD =0.217) has **significantly** lesser color intensity compared to the mature sample (42 days) concentration (M = 3.44, SD = 0.499), t(24) =-2.121, p = 0.045. Thus, the immature sample (38 days) has better color intensity compared to the mature sample (42 days).

Table 6.2: Statement of the problem 6: 80% concentration

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper
80 percent	-0.005	22	0.996	-8.333×10^{-4}	0.170	-0.354	0.353

Note. Student's t-test.

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
80 percent	38 days	12	3.750	0.452	0.131	0.121
	42 days	12	3.751	0.379	0.109	0.101

Independent sample t-test was used to determine which group of concentration according to days is better substitute as eosin stain. 38 days concentration of 80 percent concentration (M =3.750, SD =0.452) is not significantly different compared to the mature sample (42 days) concentration (M = 3.751, SD = 0.379), t(24) =-0.005, p = 0.045. Thus, neither of the samples is better for 80 percent concentration.

Table 6.2: Statement of the problem 6: Which among the different age brackets of dragon fruit is most effective as an alternative for Eosin stain?

80% concentration

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper
100 percent	-0.383	22	0.705	-0.083	0.218	-0.535	0.368

Note. Student's t-test.

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
100 percent	38 days	12	3.667	0.666	0.192	0.182
	42 days	12	3.750	0.352	0.102	0.094

Independent sample t-test was used to determine which group of concentration according to days is better substitute as eosin stain. 38 days concentration of 80 percent concentration (M =3.750, SD =0.452) is not significantly different compared to the mature sample (42 days) concentration (M = 3.751, SD = 0.379), t(24) =-0.005, p = 0.045. Thus neither of the samples is better for 80 percent concentration.

Table 6.3: Statement of the problem 6: Which among the different age brackets of dragon fruit is most effective as an alternative for Eosin stain?

100% Concentration

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper
100 percent	-0.383	22	0.705	-0.083	0.218	-0.535	0.368

Independent Samples T-Test

	t	df	p	Mean Difference	SE Difference	95% CI for Mean Difference	
						Lower	Upper

Note. Student's t-test.

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
100 percent	38 days	12	3.667	0.666	0.192	0.182
	42 days	12	3.750	0.352	0.102	0.094

Independent sample t-test was used to determine whether which group of concentration according to days is better substitute as eosin stain. Immature (38 days) concentration of 100 percent concentration (M =3.667, SD =0.666) is not significantly different from compared to the mature sample (42 days) concentration (M = 3.750, SD = 0.352), t(24) =-0.383, p = 0.705. Thus neither of the samples is better for 100 percent concentration.

In relation to the data gathered which shows concentration and fruit age does indeed cause drastic difference in tissue staining capabilities, elevated levels of stain concentration typically result in heightened staining intensity. Augmenting the concentration of the staining solution enhances the quantity of dye molecules accessible for binding to the targeted structures or molecules within the sample, thereby yielding a more pronounced coloration as stated by Smith (2018).

Discussion

The results of this study show that dragon fruit (Hylocereus polyrhizus) extract has the potential to replace eosin in histological staining. Both concentration and fruit maturity influenced staining results across all testing conditions. The idea that more pigment availability improves tissue binding is supported by the fact that higher concentrations typically resulted in enhanced staining intensity (Smith, 2018). While the 42-day samples displayed less variance among concentrations, the 38-day samples at 80% and 100% concentration produced staining quality most equal to eosin among the groups. According to findings that betalain levels peak before full maturation, this implies that younger fruits may have more persistent pigments, especially betacyanins (Magalhães et al., 2019).

The staining performance was also influenced by the observed variations in pH throughout concentrations. The slightly higher pH levels found in extracts from immature fruits could indicate stronger pigment-tissue interactions. As Hairston (2019) stated, the affinity of dyes for tissue components is pH-dependent, and changes from ideal pH might lead to weaker or inconsistent staining. According to Siow and Wong (2016), the findings are consistent with the biochemical behavior of betalains, which are sensitive to both pH and fruit developmental stage.

Samples of pig liver and heart stained with dragon fruit extract demonstrated adequate contrast between structural elements in terms of tissue visibility, especially at higher concentrations. These results are consistent with other research utilizing plant-based stains like hibiscus and curcumin, which showed tissue visibility comparable to eosin while providing safety and environmental benefits (Kumar et al., 2014; Surendra et al., 2019). Dragon fruit extract's potential use in diagnostic histology is further supported by its capacity to draw attention to cytoplasmic and connective tissue components, particularly in situations where availability to synthetic dyes is restricted.

These results were subsequently validated using statistical analyses. The pigment concentration is a stronger predictor of staining efficacy in immature fruits, as demonstrated by the ANOVA that revealed significant differences among concentrations in the 38-day group ($F(2,33) = 6.247$, $p = .005$) but not in the 42-day group ($p = .129$). The 42-day samples staining was marginally better than the 38-day samples for 50% concentrations, according to independent sample t-tests, but there were no discernible differences at 80% and 100%. Together, these findings imply that extract content, rather than fruit age, consistently affects staining quality, while immaturity may increase pigment availability.

Overall, the findings support the potential of dragon fruit extract as a non-toxic, biodegradable, and cost-effective alternative to eosin. Its staining capacity, especially at high concentrations, is comparable to that of traditional eosin without the hazards to human health and the environment that come with synthetic dyes. Nevertheless, more research is needed to evaluate the extract's long-term stability, repeatability across various tissue types, and potential for widespread laboratory use.

Conclusion

This study demonstrates the promising potential of Dragon Fruit (*Hylocereus polyrhizus*) Ethanolic Extract as an alternative to Eosin stain for histological staining. The research explored the staining efficacy of Dragon Fruit extract at various concentrations (50%, 80%, and 100%) and from different age brackets, utilizing pig liver and heart tissue samples due to their structural similarity to human organs. The results indicate that the 100% concentration is the most effective for histological staining, with factors such as pH, color intensity, and phytochemicals influencing the outcome.

While the study contributes to the understanding of Dragon Fruit extract as a histological stain, it is essential to acknowledge the limitations of the research, including the need for more longitudinal and experimental studies to establish causality and identify optimal methods for strengthening the stain and preserving the extract. Despite these limitations, the current evidence highlights the importance of developing safe and cost-effective alternative stains for histological sections.

As the medical field continues to evolve, the discovery of effective and affordable alternatives remains crucial. This study aims to inform individual choices and guide future research in this dynamic field, ultimately contributing to the advancement of medical practices.

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