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Potential of Hibiscus sabdariffa Extracts as Alternative to Hematoxylin and Eosin in Histological Staining

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Abstract

Hibiscus sabdariffa, a plant grown in tropical and subtropical regions of Africa. It contains natural pigments that may work as an alternative to synthetic dyes that are expensive and harmful to the environment. The goal of this study was to assess how well aqueous and ethanolic extracts of Hibiscus sabdariffa can stain normal and abnormal tissue compared to H&E in terms of stain quality, clarity, and contrast. Dried Hibiscus sabdariffa leaves were used to prepare both aqueous and ethanolic extracts. The extracts were applied to tissue samples, and the results were compared to the traditional (H&E) method. Tissue staining was evaluated for clarity, intensity, contrast in a scale of 1-10. A team of five pathologist reviewed the samples and scored them based on these qualities. The comparison of normal and abnormal tissue staining with aqueous and ethanolic extracts of Hibiscus sabdariffa revealed that aqueous extract generally provided superior results in terms of better nuclei ***staining quality****,* ***nucleus clarity****,* ***contrast****,* ***clarity of cellular details****, and* ***background staining*** *(P < 0.05). Aqueous and ethanolic extracts of Hibiscus sabdariffa can serve as effective alternatives to the traditional Hematoxylin and Eosin (H&E) staining method for tissue morphology.*

Keywords: Hibiscus sabdariffa, suitable alternative.

Introduction:

Histological staining with dyes plays a crucial role in visualizing cellular structures and tissue morphology in biological research and pathological diagnostics. Hematoxylin and Eosin (H&E) staining is one of the most widely used methods, considered as the gold standard in tissue analysis. Hematoxylin stains the

cell nuclei blue or purple hue, while Eosin imparts a pink or red color to the cytoplasm and extracellular matrix, enabling clear differentiation between various tissue components [1]. However, the chemicals involved in H&E staining, pose significant health and environmental risks. Eosin, specifically, is classified as a carcinogen by the International Agency for Research on Cancer (IARC) and can lead to skin irritation and other health problems with prolonged exposure. These concerns have driven the need for safer, more environmentally friendly alternatives to traditional staining methods [2].

One promising alternative is the use of natural dyes derived from plants. Hibiscus sabdariffa, commonly known as roselle, is rich in anthocyanins, flavonoids, and other bioactive compounds, which have demonstrated potential as natural dyes. Anthocyanins, a diverse group of over 600 water-soluble phenolic pigments, are responsible for the blue, purple, and red coloration in various fruits, flowers, and vegetables, making them an attractive candidate for use in histological staining[3-5].These pigments are typically tasteless and odorless, offering substantial color stability, which makes them ideal for use in staining applications. Their natural origin and ability to maintain color over time further enhance their potential as a viable alternative to synthetic dyes in histological procedure[5]. These pigments have been widely promoted as safe-to-consume coloring agents in foods and beverages, further supporting their non-toxic nature and suitability for various applications. Their widespread acceptance in the food industry highlights their potential as safe and effective alternatives for use in scientific and medical contexts, such as histological staining [4,6]. Furthermore, various researchers have successfully demonstrated that anthocyanins obtained from Hibiscus sabdariffa (roselle), R. indica (rose), Bougainvillea glabra (bougainvillea), and beetroot can be effectively used as natural dyes for histological staining. These studies have shown that the anthocyanins from these plants possess significant staining properties, offering promising alternatives to synthetic dyes like hematoxylin and Eosin in the visualization of tissue morphology[7-9].Studies have indicated that anthocyanins, the pigments responsible for the red color in Hibiscus sabdariffa flowers, share similar fluorescence properties with Eosin, making them a potential substitute for tissue staining. These natural compounds not only exhibit promising staining characteristics but also possess the advantage of being non-toxic, readily available, and affordable. As a result, hibiscus extracts present an attractive alternative, particularly in resource-limited settings where synthetic dyes may be either inaccessible or prohibitively expensive. The use of Hibiscus sabdariffa extracts in histological staining could therefore offer an environmentally friendly and cost-effective solution for tissue analysis[10].

The study aimed at exploreing the potential of aqueous and ethanolic extracts of **Hibiscus sabdariffa** as substitutes for Hematoxylin and Eosin (H&E) in demonstrating tissue morphology. The primary objective is to assess the staining efficiency, clarity, contrast, and overall performance of these natural extracts when applied to both normal and abnormal tissue samples.

By comparing these plant-based extracts to the traditional H&E staining method, the study aims to highlight their effectiveness in histological practices. The findings of this research could provide valuable insights into the viability of using Hibiscus-based dyes as an eco-friendly and cost-effective

alternative, potentially promoting a shift toward more sustainable and accessible staining methods in biomedical research and clinical diagnostics

Methodology

Dried leaves of Hibiscus sabdariffa were purchased from a local Sudanese market in Ishaka, located in the Western region of Uganda. These leaves were used to prepare both aqueous and ethanolic extracts for tissue staining.

Preparation of Hibiscus sabdariffa Staining Solutions:

Nucleus Stain: Fifty grams of dried H. sabdariffa leaves were soaked in 100 mL of distilled water (DW) and incubated at room temperature for 48 hours. After incubation, the solution was filtered through filter paper to remove plant debris, yielding a filtrate that was divided into two equal portions. One portion was designated for use as a nucleus stain. Another 50g of dried H. sabdariffa leaves were soaked in 100 mL of 90% ethanol and incubated for 48 hours at room temperature. After filtering to remove solid material, the resulting alcoholic extract was divided into two 50 mL portions, with one designated as a nucleus stain.

Cytoplasm Stain: The remaining 50 mL of both the distilled water and 90% ethanol Hibiscus solutions were adjusted to an alkaline pH using sodium bicarbonate ($NaHCO_3$), ensuring compatibility with cytoplasmic structures. These alkaline solutions were then used to stain the cytoplasm of tissue samples.

The prepared Hibiscus solutions was applied to 5um(thin) tissue sections for 10minutes each. The aqueous and alcoholic extracts used for nucleus staining and the alkaline solutions used for cytoplasm staining. Staining was conducted under standardized conditions to ensure consistent exposure times to each solution with suitable controls. The effectiveness of the Hibiscus extracts was compared to traditional synthetic stains (H&E) across the following parameters: Cytoplasm and nucleus staining quality, Nucleus and cytoplasm clarity , Cell membrane, Specificity of stain (ability to selectively target), Contrast, cellular components background staining, Penetration and Uniformity. A total of five histopathology experimenters scored the staining performance on a scale from 0 to 10 for each parameter, allowing for a comprehensive comparison. To provide a baseline comparison, the standard Harris regressive hematoxylin and eosin (H&E) method was employed using 20 slides. Tissue sections were deparaffinized, hydrated, stained with hematoxylin, differentiated with acid alcohol, counter-stained with Eosin, and mounted for microscopic evaluation as per standard protocols

Results and Discussion

The Aqueous extract showed notable advantages in nucleus staining quality, nucleus clarity, cytoplasm clarity, cell membrane, contrast, clarity of cellular details, and background staining when compared to control(standard Hematoxylin and Eosin staining) and Alcohol. Alcohol extract performed well in background staining and penetration and uniformity. Aqueous extract generally showed the best overall performance in normal tissue staining quality, particularly in nucleus and cytoplasm clarity, clarity of cellular details, and contrast, while the Alcohol extract excelled in Background Staining and Penetration and Uniformity as shown in table table 4.1

Table 4.1. Staining qualities of Aqueous and Ethanolic Hibiscus sabdariffa leaves extract.

Parameters	N	Mean ± SD			P. value	F. value
		Control	Aqueous	Ethanolic		
Cytoplasm staining quality	5	7.80 ± 0.83	7.40 ± 0.89	8.00 ± 1.00	.585	0.560
Nucleus staining quality	5	7.40 ± 0.54	8.60 ± 0.89	7.40 ± 0.89	.053	3.789
Nucleus clearity	5	7.40 ± 0.89	8.60 ± 0.54	7.80 ± 0.83	.082	3.111
Cytoplasm clearity	5	7.20 ± 0.44	8.00 ± 0.70	8.00 ± 0.70	.110	2.667
Cell membrane	5	7.20 ± 0.83	8.40 ± 0.89	7.60 ± 0.54	.082	3.111
Specifity	5	7.40 ± 0.54	8.20± 1.09	7.60 ± 0.54	.274	1.444
Contrast	5	8.40 ± 0.54	8.80 ± 0.44	7.60± 0.54	.010	7.000
Clearity of cellular details	5	7.80 ± 0.44	8.80 ± 0.44	7.80 ± 0.83	.034	4.545
Backgraound staining	5	6.60 ± 0.89	7.80 ± 0.44	8.00 ± 0.70	.018	5.733
Penetration and Uniformity	5	7.40 ± 0.89	7.60 ± 0.54	8.20 ± 0.83	.274	1.444

For abnormal tissue, the study showed that the three groups (Control, DW, and Alcohol) provided similar results in most parameters. However, **Contrast** was significantly better in both control and aqueous compared to Alcohol. While the aqueous group showed some improvement in clarity of cellular details and background staining, these differences were not statistically significant. See Table 4.2 below.

Table 4.2. Staining qualities of Aqueous and Ethanolic *Hibiscus sabdariffa* leaves extract in abnormal and normal tissues

Parameters	N	Mean ± SD			P. value	F. value
		Control	Aqueous	Alcohol		
Cytoplasm staining quality	5	7.80 ± 0.83	7.60 ± 0.54	7.80 ± 0.83	.890	0.118
Nucleus staining quality	5	7.40 ± 0.54	7.40 ±0.89	7.60 ± 0.54	.868	0.143
Nucleus clarity	5	7.40 ± 0.89	7.00 ± 0.70	7.80 ± 0.83	.335	1.200
Cytoplasm clarity	5	7.20 ± 0.44	7.00 ± 1.22	7.20 ± 0.83	.921	0.083
Cell membrane	5	7.20 ± 0.83	7.40 ± 0.89	7.20 ± 0.83	.914	0.091
Specificity	5	7.40 ± 0.54	8.00 ± 1.22	7.20 ±0.83	.383	1.040
Contrast	5	8.40 ± 0.54	8.40 ± 0.89	7.20 ± 0.44	.020	5.538
Clarity of cellular details	5	7.80 ± 0.44	8.40 ± 1.34	7.00 ± 0.70	.090	2.960
Backgraound staining	5	6.60 ± 0.89	8.00 ± 1.73	7.00 ± 0.70	.205	1.814
Penetration and Uniformity	5	7.40 ± 0.89	7.60 ± 0.89	7.00 ± 0.70	.531	0.667

Nucleus clarity was significantly better in normal tissue compared to abnormal tissue. Nucleus staining quality showed a marginal improvement in normal tissue, although this difference did not reach full statistical significance. The rest of the parameters (cytoplasm staining quality, clarity, cell membrane definition, specificity, contrast, clarity of cellular details, background staining, and penetration/uniformity) showed no significant differences between normal and abnormal tissues. Overall, the aqueous extract of Hibiscus sabdariffa performed similarly for both normal and abnormal tissues, with slight advantages in staining quality and clarity for normal tissue, particularly in nucleus clarity as represented table 4.3

Table 4.3. Comparison of staining quality of aqueous leaves extract of *Hibiscus sabdariffa* in staining of normal and abnormal tissue sections

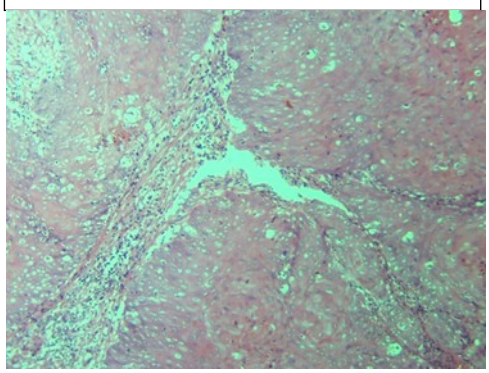
Parameters	N	Mean ± SD		P. value	F. value
		Normal	Abnormal		
Cytoplasm staining quality	5	7.40 ± 0.89	7.60 ± 0.54	.683	1.756
Nucleus staining quality	5	8.60 ± 0.89	7.40 ±0.89	.067	0.000
Nucleus clarity	5	8.60 ± 0.54	7.00 ± 0.70	.004	0.103
Cytoplasm clarity	5	8.00 ± 0.70	7.00 ± 1.22	.162	0.800
Cell membrane	5	8.40 ± 0.89	7.40 ± 0.89	.115	0.000
Specificity	5	8.20± 1.09	8.00 ± 1.22	.792	0.171
Contrast	5	8.80 ± 0.44	8.40 ± 0.89	.406	3.571
Clarity of cellular details	5	8.80 ± 0.44	8.40 ± 1.34	.556	2.844
Backgraound staining	5	7.80 ± 0.44	8.00 ± 1.73	.814	3.044
Penetration and Uniformity	5	7.60 ± 0.54	7.60 ± 0.89	1.000	1.756

Penetration and Uniformity showed a significant difference, with normal tissue exhibiting better penetration and uniformity than abnormal tissue. Background Staining showed a marginally significant improvement for normal tissue compared to abnormal tissue, although this was not statistically significant. Other parameters, such as Cytoplasm Staining Quality, Nucleus Staining Quality, Nucleus Clarity, Cytoplasm Clarity, Cell Membrane Definition, Specificity, Contrast, and Clarity of Cellular Details, showed no significant differences between normal and abnormal tissue. Overall, while most parameters were similar for normal and abnormal tissues, normal tissue demonstrated slight advantages in background staining and penetration/uniformity. See table 4.4

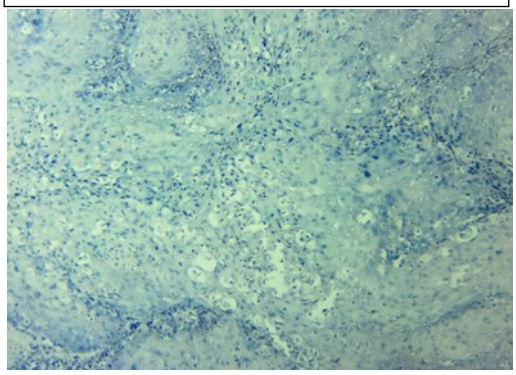
Table 4.4. Comparison of staining quality of ethanolic leaves extract of Hibiscus sabdariffa in staining of normal and abnormal tissue sections

Parameters	N	Mean ± SD		P. value	F. value
		Normal	Abnormal		
Cytoplasm staining quality	5	8.00 ± 1.00	7.80 ± 0.83	.741	0.330
Nucleus staining quality	5	7.40 ± 0.89	7.60 ± 0.54	.683	1.756
Nucleus clarity	5	7.80 ± 0.83	7.80 ± 0.83	1.000	0.000
Cytoplasm clarity	5	8.00 ± 0.70	7.20 ± 0.83	.142	0.590
Cell membrane	5	7.60 ± 0.54	7.20 ± 0.83	.401	0.640
Specificity	5	7.60 ± 0.54	7.20 ±0.83	.401	0.640
Contrast	5	7.60± 0.54	7.20 ± 0.44	.243	1.524
Clarity of cellular details	5	7.80 ± 0.83	7.00 ± 0.70	.142	0.590
Backgraound staining	5	8.00 ± 0.70	7.00 ± 0.70	.056	0.000
Penetration and Uniformity	5	8.20 ± 0.83	7.00 ± 0.70	.041	0.590

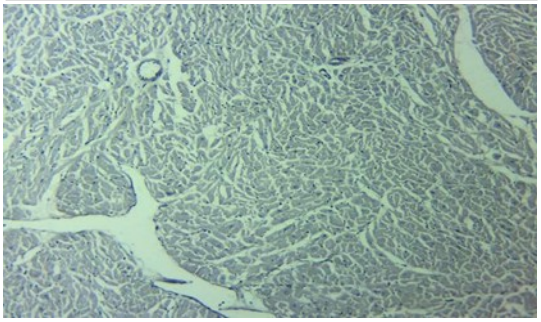
Standard Haematoxylin and Eosin stain x100mag



Alcoholic extract stain x100mag



Aqueous extract stain x100mag



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