

# Evaluation of Hibiscus Sabdariffa L. Flower Aqueous Extract as a Natural Histological Stain for Tissue Analysis

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## ABSTRACT

**Background:** Natural colorants are widely used in various industries, including textiles, cosmetics, and medicine. In histopathology, synthetic dyes such as hematoxylin and eosin (H&E) are commonly used for tissue staining, but concerns regarding their availability, cost, and potential toxicity have led to increased interest in natural alternatives. **Objective:** This study aimed to evaluate the efficacy of Hibiscus sabdariffa L. (roselle) aqueous extract as a natural histological stain and its potential as a substitute for eosin in routine hematoxylin and eosin (H&E) staining. **Methods:** A concentrated extract of Hibiscus sabdariffa flowers was prepared at a 50% (w/v) ratio. The extract was used to stain rabbit tissue sections, replacing eosin in conventional H&E staining protocols. Microscopic evaluation was performed to assess the staining characteristics and histological differentiation of various tissue components. **Results:** The Hibiscus sabdariffa extract effectively stained hepatocytes, central veins, hepatic sinusoids, endothelial cells, and nuclear/cytoplasmic structures, producing a reddish to orange coloration. The staining was comparable to conventional eosin in highlighting tissue morphology and cellular components. **Conclusion:** The findings suggest that Hibiscus sabdariffa L. flower extract is a viable, natural alternative to synthetic eosin for histological staining. As a readily available and eco-friendly botanical dye, it offers a potential substitute for conventional staining agents in histopathology, reducing reliance on synthetic chemicals while maintaining staining quality. Further studies are recommended to standardize staining protocols and evaluate long-term stability.

**Keywords:** Kujarat, Hibiscus Sabdariffa, Histologic Section, Testis, Liver

## INTRODUCTION

The hematoxylin-eosin stain is widely regarded as the principal histological stain employed in most laboratories. This stain is a combination of the naturally occurring hematoxylin stain and the synthetic eosin stain. Hematoxylin, being a basic dye, selectively stains the acidic components of the cell, while eosin, an acidic dye, stains the basic cytoplasmic components of the cells. Consequently, the nuclei of the cells take on the hematoxylin dye, resulting in a dark violet or blue color, while the cytoplasm of certain epithelial cells and erythrocytes are stained pink by the eosin dye [1]. The family Malvaceae, commonly referred to as roselle, encompasses the hibiscus sabdariffa flowers native to Gujarat. Roselle is cultivated in various regions, including India, Malaysia, the Tropics, subtropics, and Central America [2, 3]. The ancient Egyptians consumed extracts from the Gujarat flowers, known as karkadae, while in Iraq, it is referred to as red tea. Studies conducted in Iraq have proposed the utilization of kujarat extract in the food industry, particularly as a syrup and coloring agent [4]. Additionally, kujarat has been identified as a natural source of pectin, a substance that contributes to the solidification of jelly and ice cream preparations [5, 6], whereas seeds undergo fermentation and are utilized as a substance for thickening or enhancing the taste. Aqueous extracts of calyces are extensively employed as consumable goods or beverages due to their inherent antioxidant properties [7]. Phenolic compounds, notably anthocyanins, have been intriguingly identified as the primary origins of antioxidants found in Roselle extracts [8]. The calyces also contained other significant phenolic compounds, such as protocatechuic acid [9, 10]. As has been indicated for various polyphenols, they exhibit a wide range of therapeutic properties, including antispasmodic, anthelmintic, anti-leukemia, bactericidal, antihypertensive effects, and cardioprotective properties [11-14].

Phenolic compounds extracted from natural sources, such as anthocyanins, are currently utilized in the formulation of dietary supplements, functional food ingredients, food additives, pharmaceuticals, and cosmetic products, among others. However, the utilization of roselle for beverage production is constrained by the extraction conditions and the instability of anthocyanins caused by various physicochemical factors [15]. The rationale behind initiating this study stems from the straightforward retrieval of the pigment from kujarat flowers through the process of boiling them in water. Another contributing motive is the extensive cultivation of these flowers within the borders of Iraq. The procurement of dried flowers from local markets is a highly feasible and economical endeavor. Furthermore, it is worth noting that the acidic flavor of the kujarat extract serves as an additional impetus when compared to the acidic eosin stain.

## MATERIALS & METHODS

Hibiscus sabdariffa flowers and cups were obtained from the local markets of Diyala province -Baqubah, available in the apothecary shops. They were washed with water to clean them from dust, The Hibiscus sabdariffa that had undergone the process of dehydration was subjected to grinding through the utilization of manual grinding machines (specifically, Waring and commercial machines). Subsequently, the resulting dried powder of the aforementioned plant weighed 50 grams as confirmed by the Shimadzu bl 3200 apparatus. To initiate the extraction process, the powder was thoroughly boiled in a beaker containing 100 milliliters of distilled water, with a ratio of 1 gram of powder to 2 milliliters of water, for a duration of thirty minutes. Following the cooling of the mixture, a 50% (w/v) extract was produced and subsequently filtered twice through Whatman No.1 filter paper. In order to maintain the stability and hinder any form of

degradation, the filtrate was then stored within a refrigerator at a temperature of 4°C, until it was required for staining procedures. Two types of tissue were select the rabbit Liver and testis tissue for histology, fixed the samples by natural buffer formalin, processed for paraffin embedding technique, sections of tissues at 5 ums in thickness. The selection was made based on the basophilic elements present in this tissue and this potential to accept the kujarat stain. Sections from the Liver and testis of a rabbit were prepared using standard histological procedures.

The slides were then subjected to staining using hematoxylin kujarat stain, following the proposed steps: 1- The slides were treated with two consecutive steps of xylene for a duration of 5 minutes each. 2- Dehydration of the slides was carried out using ethyl alcohol, progressing from absolute to 95%, then to 70%, and finally to 50% concentration. 3- The slides were stained with hematoxylin for a period of 10 minutes. 4- Subsequently, the slides were rinsed under running water for 10 minutes. 5- An immersion in acid alcohol was performed. 6- The slides were then rinsed under running water for another 10 minutes. 7- Staining with kujarat extract was performed for 10 minutes. 8- The slides were washed with running water. 9- Finally, a

dip in xylene was carried out. 10- The slides were examined by covering the tissue with D.P and observing the results. The stained slides that had been prepared were scrutinized by means of a light microscope in order to assess and construe the outcomes.

## Result

The PH value of the aqueous extract of Kujarat powder was calculated and was 2.39. Other results proved that animal tissues reacted with Kujarat dye when it was used instead of eosin dye. The findings of the microscopic analysis of histological sections of the liver indicate that the liver tissue is composed of multiple lobules, each of which harbors a central vein positioned at its core.

The lobule and the hepatocytes are arranged in a radial fashion around the central vein, forming hepatic cords in an orderly manner. The hepatocytes, which possess a polygonal shape, contain a solitary nucleus, sometimes circular in appearance. Additionally, there were light-colored regions observed between hepatic cells, which are localized within the hepatic sinusoids and are lined by internal endothelial cells (Figure 1 A, B to 4 F).

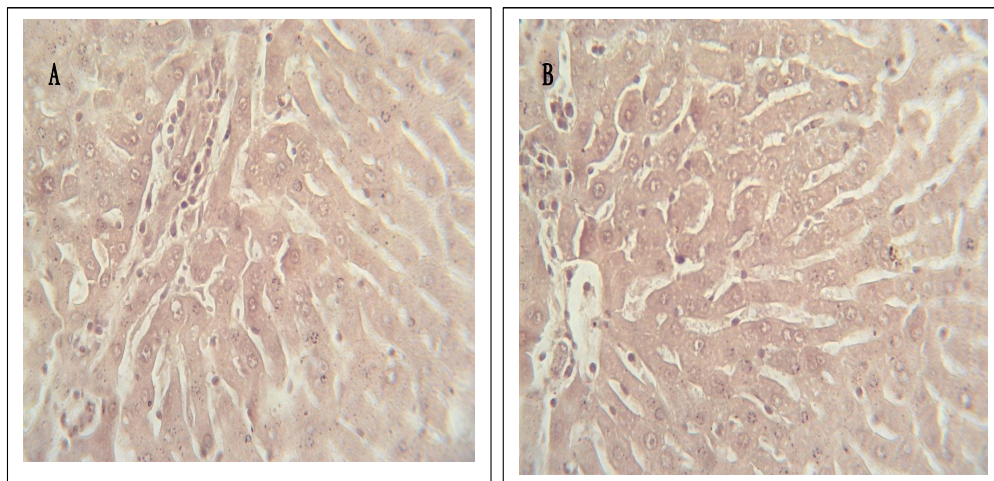


**FIGURE 1(A, B):** A Cross-Section of a Rabbit's Liver Showing the Histological Structure of the Liver, Where We Notice CV Central Vein, HC Hepatocyte, N Nucleus, BN Binucleate Cell, S Sineuses. Hematoxylin & Kujarat Stain X40.

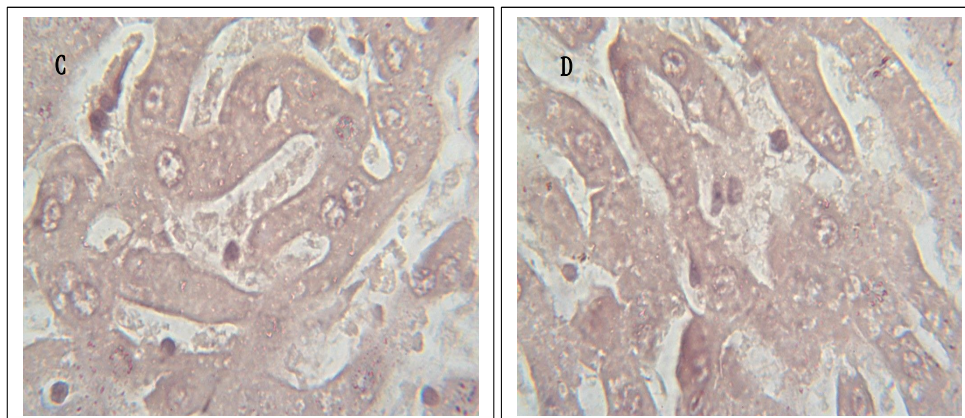


**FIGURE 2(C, D):** A Cross-Section of a Rabbit's Liver Showing the Histological Structure of the Liver, Where We Notice CV Central Vein, HC Hepatocyte, N Nucleus, BN Binucleate Cell, S Sineuses.

Hematoxylin&Kujarat Stain X100. The findings further demonstrated the existence of the triad portal at a singular extremity or periphery of each entity. The lobule encompasses three primary constituents: the portal vein, which is situated towards the middle,

possesses a larger magnitude compared to the other blood vessels within the space. Additionally, the hepatic artery and bile duct can be found in this courtyard. The bile duct is accompanied by somber, circular cells.

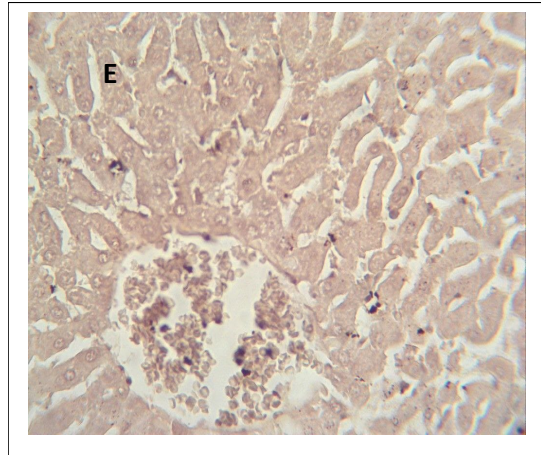


**Figure 3 (E):** A Cross-Section of the Rabbit Liver Showing the Histological Structure of the Liver and the Components of the Portal Area, Where We Notice the PV Portal Vein, PA Portal Artery, Bdbile Duct, S Sineuses, HC Hepatocytes, BN Binucleate Cell. Hematoxylin&Kujarat Stain X40.

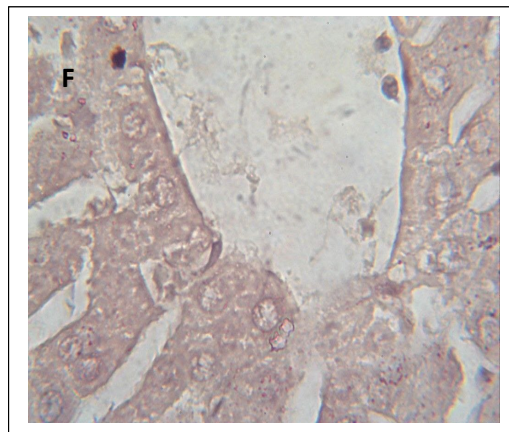


**Figure 4 (F):** - A Cross-Section of the Rabbit Liver Showing the Histological Structure of the Liver and the Components of the Portal Area, Where We Notice the PV Portal Vein, PA Portal Artery, BDbile duct, S Sineuses, HC hepatocytes, BN Binucleate cell. Hematoxylin & Kujarat Stain X100.

The findings of the research revealed that the utilization of an acidic dye for histological staining of the aqueous extract derived from hibiscus flowers. The histological analysis of the testicular tissue (Figure 5 G, H) displayed a deeply stained reddish outer fibrous layer known as the tunica albuginea. Below this layer, one can observe a multitude of seminiferous tubules that have been sectioned in various planes. In the interstitial spaces between the tubules, there are connective tissues that house both blood vessels and clusters of

interstitial cells. Each seminiferous tubule is lined by multiple layers of cells, which, when observed under high magnification, exhibit an unclear delineation between cells and prominently visible nuclei. The nuclei found in the outermost rows pertain to sustentacular cells and spermatogonia. The nuclear/cytoplasmic differentiation is not as good as in haematoxylin and eosin method staining of the testis (Figure 6 I, Figure 7 J, K) the observation revealed a profound crimson-stained outer fibrous layer, known as the tunica albuginea..

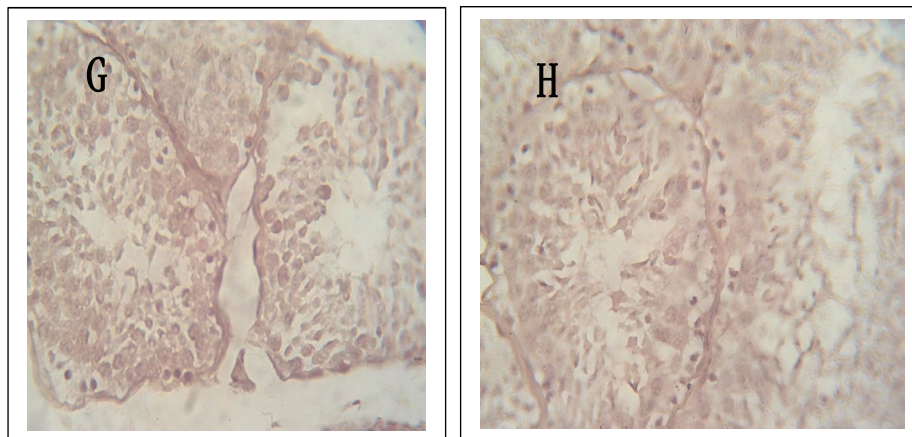


**Figure 5 (G, H):** Across-Section of the Rabbit Testis Showing the Histological Structure of the Testes Where the Cross-Section Showed Clear Nuclear /Cytoplasmic Differentiation with Numerous Seminiferous Tubules and the Outer Tinica Albuginea Hematoxyline & Kujarat Stain X40.

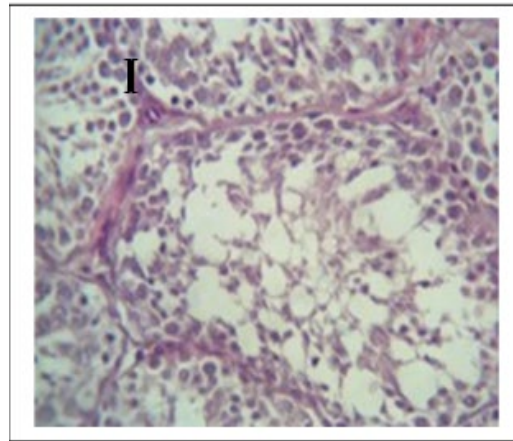


**FIGURE 6(I):** Across-Section of the Rabbit Testis Showing the Histological Structure of the Testes where the Cross-Section Showed the Sperm Transporting Tubule, Interstitial Tissue, Germinal Layer, Basement Membrane and Sertoli Cells. Hematoxylin & Eosin stainX40

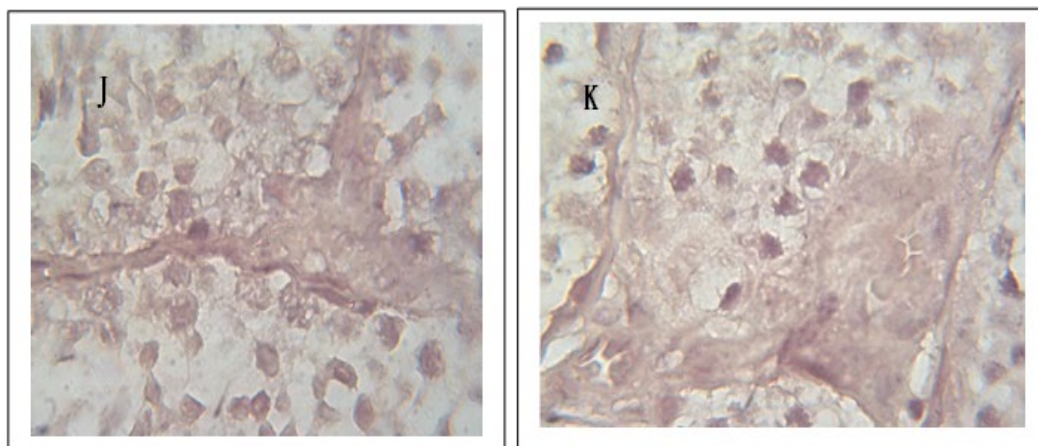


**FIGURE 7 (J, K):** Across-Section of the Rabbit Testis Showing the Histological Structure of the Testes Where the Cross-Section Showed the Sperm Mother, Sperms, Spermatoblast, Secondary Spermatocytes. Hematoxylin & Kujarat stainX100.

## Discussion

The outcome of the pH calculation conducted on the kujarat extract yielded a pH value of 2.39. This finding substantiates our conjecture regarding the substitution of eosin with kujarat as an acidic stain. Previous research has suggested the utilization of a 20% concentration of aqueous extract derived from kujarat for the purpose of staining blood cells, fungi, and plant cells [16]. In our study, the concentration of Kujarat extract utilized was 50% weight/volume. It is worth noting that prior studies did not address the potential risks associated with the utilization of concentrations exceeding 20% of watery extract of Kujarat this is consistent with a study conducted by the researcher [17]. Our study aligns with this observation as we employed a concentration higher than 20%. Previous studies suggested the use of a 20% concentration of water extract of Kujarate [18]. We

observed that the extract will undergo solidification as the concentration increases during the process of boiling. This observation is supported by the presence of pectin, as indicated 3. The outcomes of the study reveal variations in coloration between the pigment found in the kujarat extract and the observable coloration in slides following the staining procedure. The aqueous solution of kujarat exhibits a deep red and lucid shade of orange within the tissues post-staining this is due to the presence of chemical components that lead to this change in color after dyeing, and this explains the color of aqueous extract of Kujarat from red to orang.

The solution, whether it is watery or alcoholic, exhibits a vivid and lucid shade of orange. This is consistent with our study. The staining technique is capable of coloring the cytoplasm of hepatocytes and binucleate cells, as well as the sinusoids, central vein, and portal area, resulting in a red to orang appearance.

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