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*Full Length Research*

# Evaluation of *Lawsonia inermis* (Henna) and *Hibiscus sabdariffa* (Roselle) ethanol extracts as substitute for Eosin stain in Histology using Albino rats

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**ABSTRACT:** Staining techniques have significantly advanced the identification of tissue structures under the microscope, facilitating precise diagnostic processes. Stains, which are dyes modified for biological use, are essential in histological studies. This study aims to assess the staining efficacy of ethanol extracts from *Lawsonia inermis* (Henna) and *Hibiscus sabdariffa* (Roselle) compared to the traditional eosin stain. Various ethanol extract solutions of *L. inermis* and *H. sabdariffa* leaves were prepared at concentrations of 20 and 50%. These solutions were further modified by incorporating aluminium sulphate as a mordant, applied at intervals of 5 minutes (20%) and 10 minutes (50%). The acidity of these solutions, including those with mordants, was confirmed. Histological sections of the lungs, liver, kidney, intestine, and heart were stained using Hematoxylin and Eosin as a control, alongside the modified ethanol extract solutions as counterstains. The extracts stained the cytoplasm, yielding a brownish hue, with no significant differences observed between the photomicrographs of cells stained with pure and mordant solutions when compared with the control group. These findings showed that Henna and Roselle extracts could serve as viable substitutes for eosin stain due to their acidic properties, appropriate staining times, of 5 and 10 minutes, and lower toxicity. Further research that will address the clarity of the stains was recommended.

**Keywords:** Eosin substitute, *Hibiscus sabdariffa* extract, histology stains, *Lawsonia inermis* extract.

## INTRODUCTION

*Lawsonia inermis* Linn, commonly known as Henna, is a shrub belonging to the Lythraceae family. It is a perennial shrub prevalent in subtropical and tropical regions of the Middle East, Africa, Southern Asia, Northern Australia, and other semi-arid areas. Henna is not only widely utilized both for its beauty-enhancing properties such as dyeing hair and nails but also serves as a commercial crop (Hafiz *et al*., 2012; Jan *et al*., 2011). The primary active ingredient in Henna is lawsone, which is a naphthoquinone derivative. Lawsone is a reddish-orange dye that binds to keratin, making it an effective hair and nail dye. In addition to lawsone, Henna contains other compounds such as tannins, flavonoids, and volatile oils (Raju *et al*., 2018). These compounds contribute to the overall staining properties of Henna, but lawsone is the main compound responsible for its red-orange colour. This dye has been

studied for its potential use in histology as a substitute for eosin stain (Umar, 2020). Several studies have investigated the potential of Henna as a substitute for eosin stain in histology. The Henna extract has been reported to show good staining properties when used on animal tissue sections (Barde *et al*., 2021).

Similarly, *Hibiscus sabdariffa* L. (Roselle) (HS), also known as Red Sorrel in English, is a vascular flowering plant. It has multiple uses, particularly its thick, red, fleshy cup-shaped leaves, which are used in food production and consumed worldwide as both a cold beverage and a hot (sour tea) drink (Ibnouf *et al*., 2014). The calyces of the Roselle plant contain various compounds such as anthocyanins, flavonoids, and polyphenols, which give it its colour and antioxidant properties. These compounds have also been studied for their potential use in histology as a substitute for eosin stain (Umar, 2020). The primary active ingredients in Roselle are anthocyanins, which are water-soluble vacuolar pigments that belong to the flavylium salt group. Anthocyanins are responsible for the red-purple colour of Roselle calyces (Alawa *et al*., 2015; Umar, 2020). In addition to anthocyanins, Roselle contains other compounds such as flavonoids, polyphenols, and organic acids. These compounds contribute to the overall staining properties of Roselle and have been studied for their potential use in histology as a substitute for eosin stain. Several studies have investigated the potential of Roselle as a substitute for eosin stain in histology (Alawa *et al*., 2015; Barde *et al.,* 2021; Raju *et al*., 2018).

The evolution of histopathology can be significantly attributed to the availability of a diverse range of stains, which facilitate the identification of various tissue structures under the microscope, aiding in accurate diagnosis (Barde *et al*., 2021). Stains are dyes modified for biological use, which impart colour to tissues based on their affinity to specific substrates. Some stains require mordants to improve their adherence to specific tissues. Historically, dyes were sourced from natural materials, primarily vegetables and some animals (Chukwu *et al*., 2011).

Staining is crucial in histopathology for visualizing histological sections under a microscope. Without staining, tissue sections appear dull and undifferentiated, making morphological analysis challenging (James *et al*., 2017). There are natural dyes from organic sources and synthetic dyes produced through chemical processes. The Hematoxylin and Eosin (H & E) stain is the most commonly used in histopathology, colouring nuclei dark blue or purple and staining cytoplasm and connective tissue in shades of pink. Using natural dyes for staining can be cost-effective and environmentally friendly. Henna, a naturally occurring plant, produces a red-brown dye from its leaves, widely used for cosmetic purposes (James *et al.,* 2017).

Aqueous extracts of *Hibiscus sabdariffa* are red and acidic. Previous studies have used these extracts in medical research and the food industry, as well as for staining blood films, fungi, and plants. Egbujo *et al*. (2008) noted the recent use of these extracts for staining lymph node and kidney biopsies, though the trials lacked clearly defined treatment protocols.

Synthetic stains used in medical laboratories, typically imported, can be replaced with local natural products. Natural dyes, such as those derived from plants, can serve as effective alternatives. For instance, hematoxylin, a commonly used histopathological dye, is obtained from the South American tree *Hematoxylin campechianum*. Studies in Nigeria have shown that red dyestuffs from the Pterocarpus osun species effectively stain tissue sections for histopathological diagnosis (Uchejeso *et al*., 2021). However, synthetic dyes are expensive and hazardous to human health, with some components being carcinogenic or strongly allergenic (Uchejeso *et al*., 2021).

Despite the dyeing potential of Henna extracts, they have not been reported as histological stains, particularly as an alternative counterstain in H&E staining techniques. Eosin, a widely used synthetic counterstain to hematoxylin, poses ecological and health risks due to its toxicity and non-biodegradability (Barde *et al*., 2021). Therefore, developing natural histological stains that are harmless, cheaper, and readily available is justified. This study aims to examine the use of *Lawsonia inermis* and *Hibiscus sabdariffa* ethanol extracts as substitutes for eosin stain in histology.

This study will evaluate whether *Lawsonia inermis* and *Hibiscus sabdariffa* ethanol extracts can serve as cost-effective substitutes for eosin stains in histology. The findings may provide empirical data on using these natural extracts in histology and serve as a reference for future research. The study's results are expected to advance knowledge and inform policy planning and developmental processes in histological techniques.

## MATERIALS AND METHODS

### Study area

The research was conducted at the Central Diagnostic Laboratory of the National Veterinary Research Institute, situated in Vom, Jos South Local Government Area of Plateau State, Nigeria. Jos South Local Government Area spans between latitude 9°30' to 10°N and longitude 8°30'E, located in the southern part of Plateau State. Its administrative centre, Bukuru, is approximately 15 kilometres from Jos, the state capital. Jos South is positioned near the geographical centre of Nigeria, about 244 kilometres (111 miles) from Abuja, the national capital (https://en.m.wikipedia.org/wiki/jos-south).

### Plant collection and identification

Fresh leaves of *Lawsonia inermis* (Henna) and flowers of

*Hibiscus sabdariffa* (Roselle) were collected from Jos Relax Garden, Jos. Plant samples were identified and authenticated using the methodology outlined by James *et al*. (2024).

### Source of animal tissues

Ten albino rats were sourced from the experimental animal facility at the National Veterinary Research Institute, Vom. Tissue samples including intestine, heart, lung, liver, and kidney were excised and utilized for histological analysis.

### Preparation of plant materials

Following the protocol described by Avwioro *et al.* (2005), plant materials were meticulously washed with sterile water and subsequently dried in an oven at 40±2˚C for 5 days. The dried plant materials were then ground into coarse granules to facilitate extraction processes.

### Extraction and preparation of staining solutions from Henna and Roselle powders

Following the method outlined by Avwioro *et al.* (2005), exactly 100 g of pulverised Henna and Roselle leaves were each macerated in 1000 ml of ethanol for 24 hours. After which the extract was filtered using Whatman No. 1 filter paper. The filtrate was dried under cold streams of air in pre-weighed vials. The extracts were each stored at 4˚C in the refrigerator until further use.

The staining solutions of Henna and Roselle powder were prepared following the technique described by Alawa *et al.* (2014). Specifically, 10 and 5 g of the ethanol extracts of Henna and Roselle were dissolved in 100 ml of distilled water in four separate bottles. These solutions consisted of 10 g and 5 g of the extracts, both with and without the mordant (aluminium sulphate). The pH of the various solutions was determined using a pH meter.

### Preparation of tissue sections

The tissue section techniques described by Raheem *et al.* (2015) were meticulously followed. The procedure is summarized as follows: Specimens were fixed in 10% formalin for 24-48 hours. Subsequently, they were processed in the histopathology laboratory to produce thinly sliced sections from paraffin-embedded tissue blocks of the heart, lung, intestine, kidney, and liver.

### Experimental trial of Henna staining solutions

The routine hematoxylin-eosin staining procedure, as described by Horobion and Kiernan (2002), was utilized with slight modifications to assess the efficacy of Henna and Roselle extract solutions separately as a counterstain replacement for Eosin. Solutions were prepared at concentrations of 20 and 50%, respectively, both with and without mordant, to determine their relative effectiveness. The counterstaining was performed for intervals of 5 and 10 minutes at each concentration level.

**Table 1.** Hydrogen ion concentration of various solution of Henna and Roselle extracts.

| Solutions | pH Values |
|---|---|
| 20% Henna solution | 3.4 |
| 20% Henna solution with aluminum sulphate | 3.1 |
| 50%Henna solution | 3.3 |
| 50% Henna solution with aluminum sulphate | 3.1 |
| 20% Roselle solution | 2.1 |
| 20% Roselle solution with aluminum sulphate | 1.8 |
| 50% Roselle solution | 2.3 |
| 50% Roselle solution with aluminum sulphate | 2.1 |

### Staining of control slides

Some of the tissue sections were stained using the routine Hematoxylin and Eosin (H&E) staining protocol to serve as positive controls for the study.

## RESULTS

### pH values of Henna and Roselle solutions

The hydrogen ion concentrations of various Henna and Roselle extract solutions are presented in Table 1. The results indicate that solutions of Henna and Roselle, without any additives and at room temperature, are acidic. However, the addition of aluminium sulphate lowers the pH of these solutions.

### Histological analysis

The photomicrographs presented in Figure 1 display: (A) the alveoli of the lung, (B) the parenchyma and stroma of the liver, (C) the glomeruli and renal tubules of the kidney, (D) the villi projections of the intestine, and (E) the myocardium of the heart. In the control group stained with Hematoxylin and Eosin (H&E), the cytoplasm appears pink-red.

### Henna staining

Figures 2 and 3 illustrate the results of counterstaining with 50 and 20% Henna solutions, respectively, without

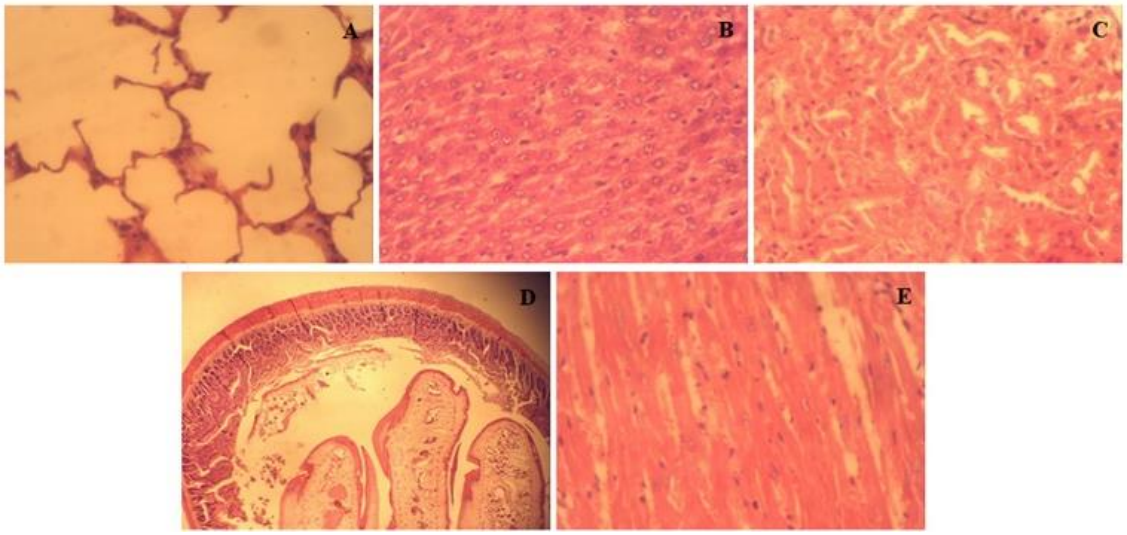


**Figure 1.** Photomicrographs of various tissues stained with Hematoxylin and Eosin Stains as control showing: A - Lung x 400; B - Liver x 400; C - Kidney x 400; D - Intestine x 400; E - Heart x 400.

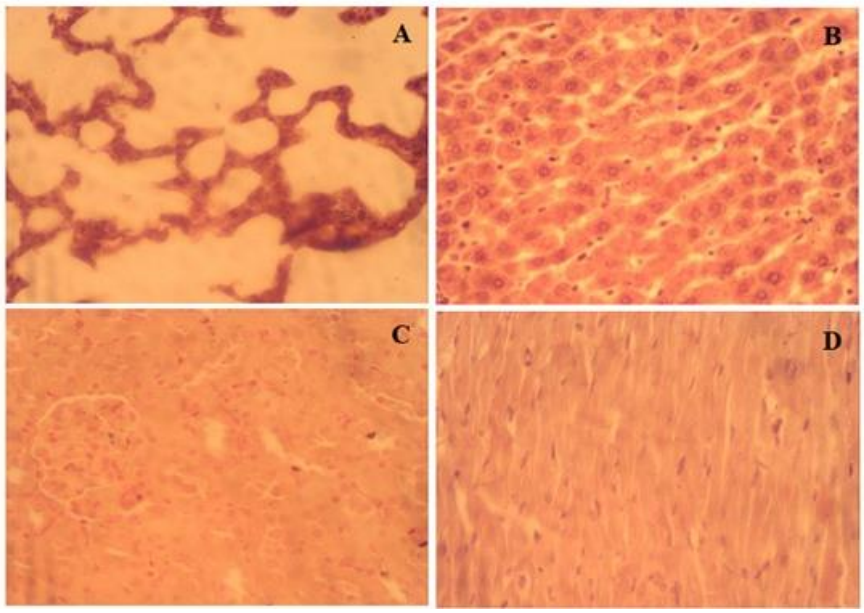


**Figure 2.** Photomicrographs of various tissues counterstained with 50% Henna solution & Hematoxylin for 10 minutes without mordant showing: A - Lung x 400; B - Liver x 100; C - Kidney - Roselle & Henna without mordant x 400; D - Heart-Roselle & Henna without mordant x 400.

mordant. Figures 4 and 5 demonstrate the results of counterstaining with 50 and 20% Henna solutions, respectively, with mordant.

### Roselle staining

Figure 6 shows the photomicrograph of a 20% Roselle solution without mordant. Figures 7 and 8 display the results of counterstaining with 20 and 50% Roselle solutions, respectively, with mordant. The photomicrographs in Figures 2-8 revealed that both Henna and Roselle solutions impart a brownish hue to the cytoplasm, in contrast to the pink-red colouration observed in the control (Figure 1).

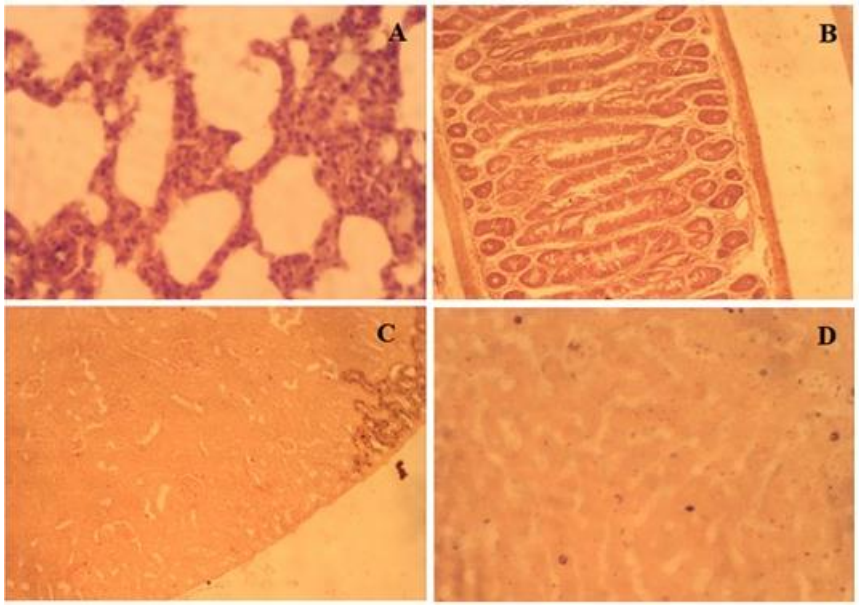


**Figure 3.** Photomicrographs of various tissues counterstained with 20% Henna solution & Hematoxylin for 5 minutes without mordant showing: A - Lung x 400; B - Intestine x 100; C - Kidney - Roselle & Henna 10 g without mordant x 100 and D - Liver - Roselle & Henna 10 g without mordant x 400.

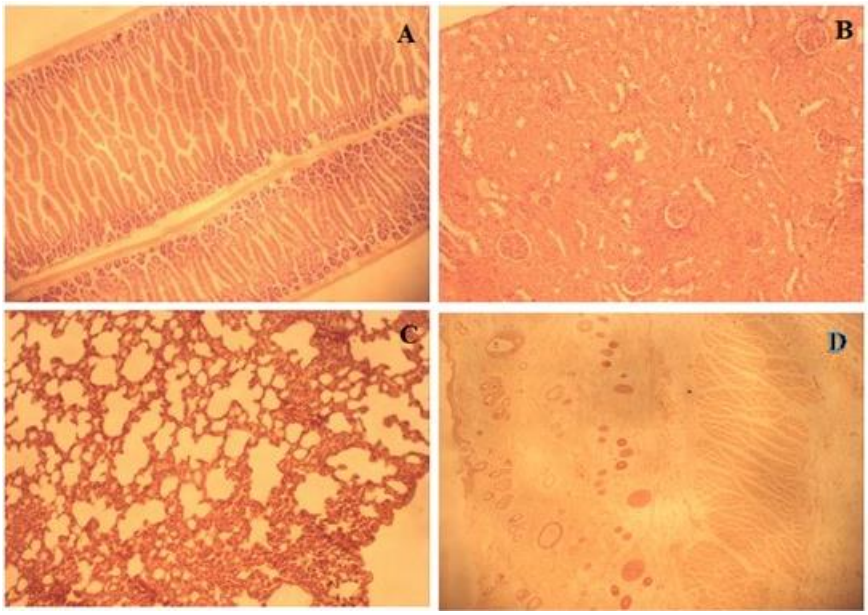


**Figure 4.** Photomicrographs of various tissues counterstained with 50% Henna solution & Hematoxylin for 10 minutes with mordant showing: A - Intestine x 40; B - Kidney x100; C - Lung - Hematoxylin x 100; D - Skin - Hematoxylin x 100.

## DISCUSSION

Figure 1 shows photomicrographs of various tissues stained with the commonly used Hematoxylin and Eosin stain, providing a clear view of the tissues. Globally, staining techniques have significantly improved, particularly in replacing eosin with local pigments such as Henna and Roselle. This study focuses on evaluating the

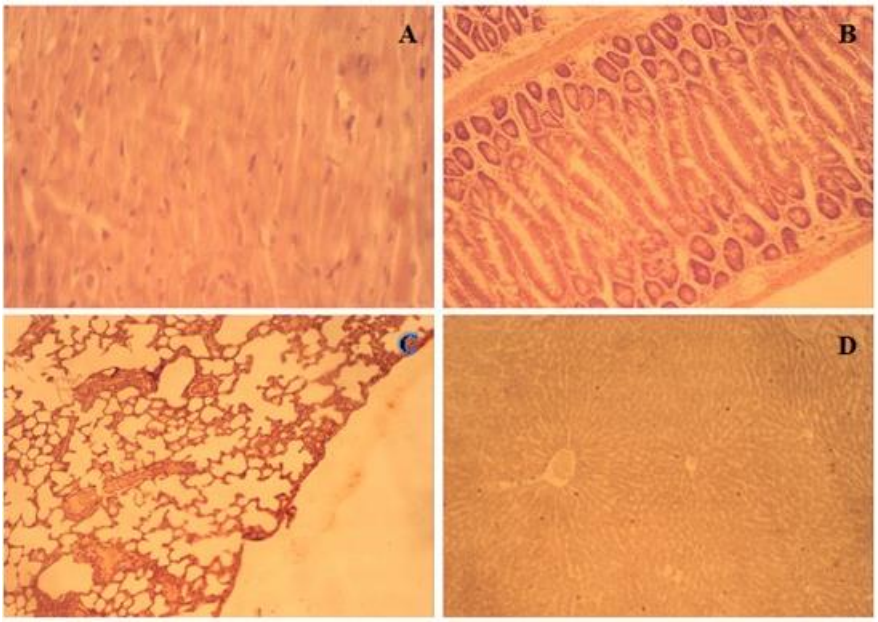


**Figure 5.** Photomicrographs of various tissues counterstained with 20% Henna solution & Hematoxylin for 5 minutes with mordant showing: A - Heart x 400; B - Intestine x 100; C - Lung - Roselle & Henna 10 g with mordant x 40; D - Liver - Roselle & Henna 10 g with mordant x 100.

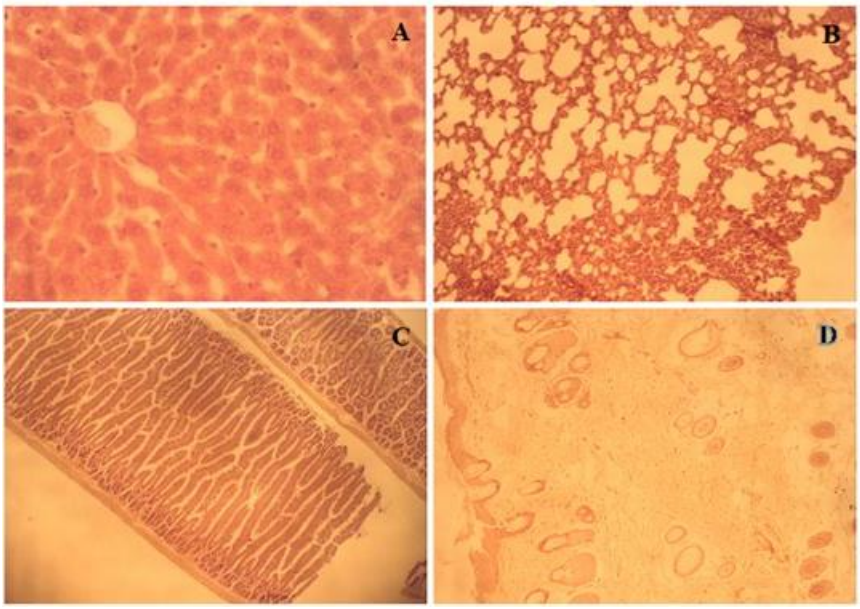


**Figure 6.** Photomicrographs of various tissues counterstained with 20% Roselle solution & Hematoxylin for 5 minutes without mordant showing: A - Liver x 40; B: Lung x 100; C: Intestine x 100; D: Skin x 100.

efficacy of these local pigments as counterstains in histopathology compared to eosin at different solution concentrations (Figures 2-8). This aligns with Barde *et al*. (2021), who used aqueous extracts, while this study employed ethanolic extracts.

The tissue staining was completed within 5 minutes

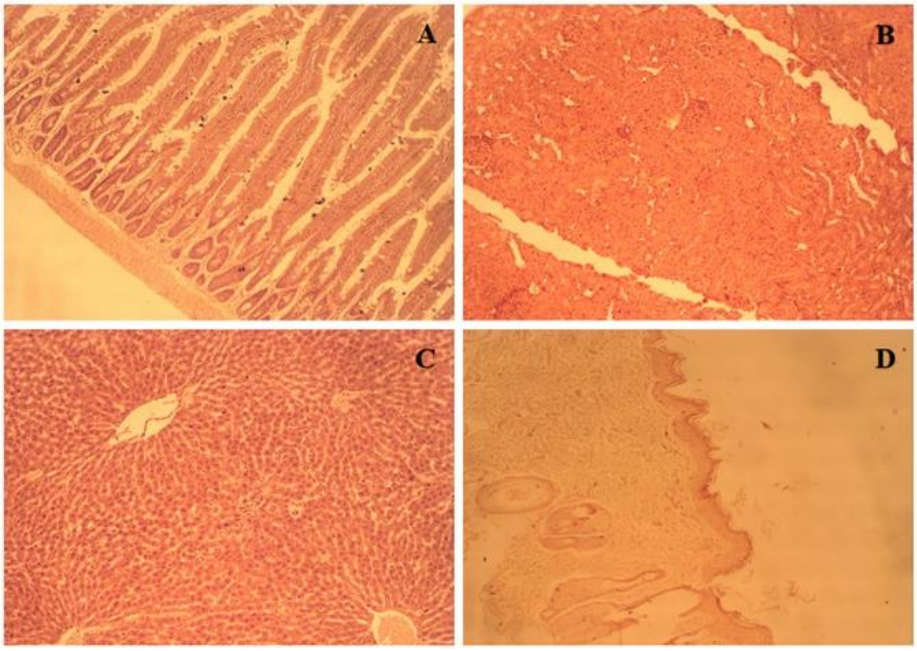


**Figure 7.** Photomicrographs of various tissues counterstained with 50% Roselle solution & Hematoxylin for 10 minutes with mordant showing: A - Intestine x 400; B - Kidney x 100; C - Liver x 100; D: Skin x 400.

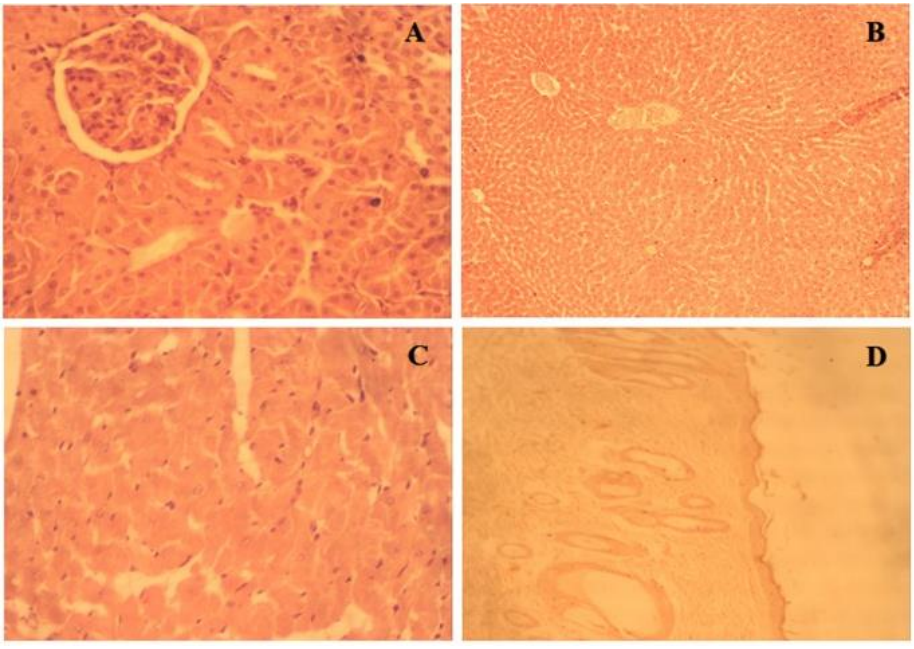


**Figure 8.** Photomicrographs of various tissues counterstained with 20% Roselle solution & Hematoxylin for 5 minutes with mordant showing: A- Kidney x 400; B - Liver x 40; C - Heart x 400; D: Skin x100.

using a 20% concentration of Henna and Roselle and 10 minutes using a 50% concentration, with or without mordant (Figures 2-8). This represents an improved staining time compared to Barde *et al*. (2021), who reported a staining time of 30 minutes, similar to the 5-minute staining time of Hematoxylin and Eosin. Thus, the

ethanolic extracts of Henna and Roselle can substitute eosin in tissue staining.

Figures 2B, 3A, 3B, 4A, and 5B show tissues counterstained with Henna, consistent with Chukwu *et al*. (2011) and Omorodion and Achukwu (2017), who found that ethanol extracts of Henna and *H. sabdariffa* are effective staining agents. They also noted that these extracts are less toxic compared to conventional Eosin.

The study's results indicate that 20 and 50% Henna and Roselle solutions stain the cytoplasm brown, while the control H&E stain renders the cytoplasm pink-red (Figures 1-8). It was also observed that the pH of the Roselle extract solution was more acidic than that of Henna, aligning with Barde *et al*. (2021), who reported that extracts with mordant are more acidic than single solutions without mordant (Table 1).

Given that the acidity of Eosin enables cytoplasm staining, it is expected that the acidic nature of the extracts also makes them effective in staining cytoplasm (Figures 1-8). However, no increase in cell clarity was observed when Henna or Roselle were used as primary and counterstains, respectively (Figures 2C, 3C, 3D, 5C, 5D), even with mordant which increases their acidity.

## Conclusion

With the advancement of ethno-veterinary medicine and increased attention to the ecosystem, there is a need to replace conventional synthetic dyes with natural, readily available, easily accessible, less toxic, and eco-friendly alternatives for eosin tissue staining. This study achieved 5 and 10-minute staining times, similar to the 5-minute staining time of Hematoxylin and Eosin. Therefore, ethanolic extracts of Henna and Roselle can substitute eosin in tissue staining. Additionally, Henna and Roselle can be used based on their acidic nature and lower toxicity.

## Recommendation

Based on the findings of this study, further research with different extract concentrations and suitable mordants is suggested to improve clarity and reduce staining time. Ethanol extracts of Henna and Roselle should be considered as alternatives if there is a shortage or absence of eosin in tissue staining. Henna and Roselle should be considered in histopathological tissue staining as they reduce occupational hazards compared to synthetic eosin. They are good alternatives because they are cost-effective, non-toxic, and offer short staining times.

## CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

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