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The Use of Hibiscus Sabdariffa Extract as Nuclear Stain for Skin Morphology and Connective Tissue with Eosin Counterstain

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Abstract

This study was carried out to investigate the suitability of *Hibiscus sabdariffa* aqueous extract solution as nuclear stain substitute for haematoxylin in the demonstration of skin morphology and connective tissue. In this study, 10% neutral buffered formalin fixed, paraffin embedded tissue blocks of skin were retrieved from the tissue block archive of the Pathology Department, Unilorin Teaching Hospital. Two blocks were randomly selected. Serial sections were cut at 3 μ from each and stained with 5% concentration of *Hibiscus sabdariffa* solution and H&E as parallel control. Results show distinct bluish purple- nuclear staining and light-pink cytoplasmic appearance comparable with H&E. *Hibiscus sabdariffa* extract solution is a suitable nuclear stain substitute to H&E for skin morphology and connective tissue.

Keywords: *Hibiscus sabdariffa*, natural dye, skin, Connective tissue

Introduction

Staining techniques originated from the second half of the last century (Kolliker, 1852). Stains have been used to enhance accurate descriptions of the microscopic structure of tissues, which is

necessary for histopathologic diagnosis (Egbujo *et al.*, 2008).

Plant and insect parts have found place in histological staining due to their colouring and dying effect. *Haematoxylon campechianum*, from which haematoxylin is obtained (Bohmer, 1865) and *Dactylopius cacti*, from which carmine stain is obtained (Goppert and Cohn, 1849).

The most common and popular stain in histopathology laboratories is Haematoxylin-Eosin stain (H&E). The Haematoxylin stains the acidic components of the cell (nucleus), giving them dark violet or blue color. The Eosin stains the basic components of the cell (cytoplasm), giving them pink color (Avwioro, 2011).

Hibiscus sabdariffa belongs to the family Malvaceae and is an important annual crop grown successfully in tropical and sub-tropical climates. The commercially important part of the plant is the fleshy calyx (sepals) surrounding



the fruit (capsules). It is used for making wine, juice, jelly, syrup etc. The main area of product in Nigeria are Niger state, Plateau state and Oyo state.

The plant is also widely grown in Kogi, Kwara, Kebbi, Sokoto, Zamfara, Katsina, Borno, Kaduna, Bauchi and Kano states (Ojigbo, 2013). Aqueous extract of *Hibiscus sabdariffa* is red in color and acidic in taste. The plant has several well-known industrial, medical, and nutritional uses (Ahmed *et al.*, 1993). *Hibiscus sabdariffa* extract used as local drink is medicinal and have antioxidant effect (Tseng *et al.*, 1997).

Little research explored the staining potentials of *Hibiscus sabdariffa* as a substitution of eosin in H&E staining method (Abd-Alhafeez *et al.*, 2014). Elastic tissues such as the skin require a strong and resilient structural framework. This framework is called the extracellular matrix, or connective tissue. The orientation of the connective tissues adipose (fat cells), cartilage, bone, tendons, and ligaments found beneath the skin are also key for tissue appearance and function. All connective tissue is composed of three major classes of biomolecules: structural proteins (collagen and elastin), specialized proteins (fibrillin, fibronectin, and laminin), and proteoglycans (Larkin *et al.*, 1992).

Development of new histological staining method remains justified especially if the new stain is harmless, easy to use, cheap, commercially favorable, and gives the desired practical results (Penny *et al.*, 2002).

The objective of this work is to introduce a natural dye for histological diagnosis in comparison to the commonly used haematoxylin and eosin. This research effort is done to investigate the suitability of *Hibiscus*

sabdariffa aqueous extract solution as a nuclear stain substitute to haematoxylin in the demonstration of skin morphology and connective tissue.

This dye is readily available and also very affordable because it can be cultivated seasonally on any land and does not need so much care (Tseng *et al.*, 1997).

Materials and Methods

Dry leaves of *Hibiscus sabdariffa* were purchased in a local market in Ilorin, Kwara State, Nigeria and processed as recommended by Benard (2008). The dry calyces were ground using a blender to a fairly powdery form.

Preparation of Hibiscus Extract Solution

To 10g of the ground red calyces of *H. sabdariffa* in a conical flask, 200ml of distilled water was added and brought to boil to give the brilliant red coloured extract which was immediately allowed to cool and filtered to give a clear extract. The staining formulae was compounded as follows:

H. sabdariffa extract	100ml
NaCl	5g
10% ferric chloride solution	1.2ml
Glacial acetic acid	3.0ml

Blocks of tissues retrieved from the archive were sectioned at 3 microns and stained with the extract solution. Parallel sections were stained with H&E.

Hibiscus-Eosin Staining Procedure

Sections were dewaxed in Xylene and hydrated through 100%, 90%, 70% and 50% alcohol to water. They were subsequently stained in Hibiscus extract solution for 5 minutes, washed in running water for 2 minutes, counterstained in 1% alcoholic eosin for 1 minute, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

Haematoxylin/Eosin Procedure

Sections were dewaxed in Xylene and hydrated through 100%, 90%, 70% and 50% alcohol to water. They were subsequently stained in Harris haematoxylin for 15 minutes, washed in running water for 2 minutes, differentiated in 1% acid alcohol, blued in running tap water for 10 minutes, counterstained in 1% alcoholic eosin for 1 minute, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

Results

The nucleus was stained bluish-purple comparable with that of the H&E control. The cytoplasm was light pink as compared with H&E control.

Discussion

Hibiscus has long been used as a natural dye in food and beverages, in the cosmetic industry as a hair dye, and in dyeing fabrics and textiles (Abou-Arab *et al.*, 2011). It renders vibrant colors owing to the presence of anthocyanins and is well-known for the color stability and fastness (Abou-Arab *et al.*, 2011; Benard, 2008). Researchers have applied this property of *Hibiscus* dyes and have used it for staining fungal species, parasites, neural tissue, and sperm cells and found the staining properties to be satisfactory (Ihuma *et al.*, 2012). Eman (2006) reported that the purified acidic part of *Hibiscus sabdariffa* could be used to replace eosin since it has similar physical and chemical characteristics to the eosin stain. Later, Egbujo and Colleagues (2008) prepared Roselle (*Hibiscus sabdariffa*) aqueous extracts with various modifications, and used it for the differential staining of rabbit testicular tissue sections. Various levels of differentiation of

nuclear and cytoplasmic structures were reported with 1% eosin as counter stain.

Reports from other researchers (Benard *et al.*, 2016; Muhammed *et al.*, 2016; Benard *et al.*, 2015 Benard, 2008) who used the extract of *hibiscus sabdariffa* (Iron-Roselle) has shown promising outcomes for nuclear staining. In these regard, it was used on appendix, lymph node, liver, kidney, brain and hippocampus respectively.

This current research investigated the nuclear staining of *H. sabdariffa* extract-iron solution on the morphology of skin. It has by so doing successfully reproduced the method of Benard, (2008). The results gotten from this research is similar but not better compared to that of standard haematoxylin and eosin. The dermis, epidermis and connective tissue collagen of skin were well demonstrated (Fig A & B). *Hibiscus sabdariffa* is easily available and economical and hence could become a cost effective readily available attractive alternative particularly in resource-limited settings to haematoxylin in the histological demonstration of skin morphology and connective tissue with 1% eosin as counter stain.

Conclusion and Recommendation

Hibiscus extract aqueous solution is a suitable substitute to haematoxylin in the histological demonstration of connective tissue of skin and its morphology with eosin as a counter stain. However, long-term retention of *Hibiscus* stain and its use as a special stain have to be further evaluated.

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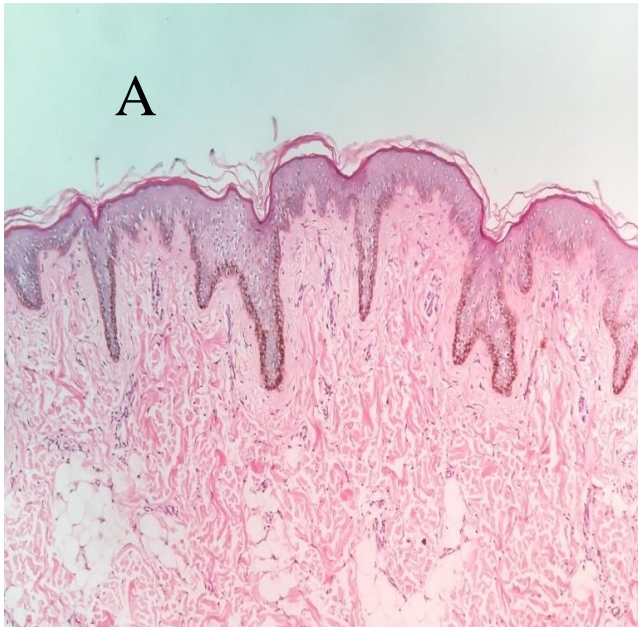


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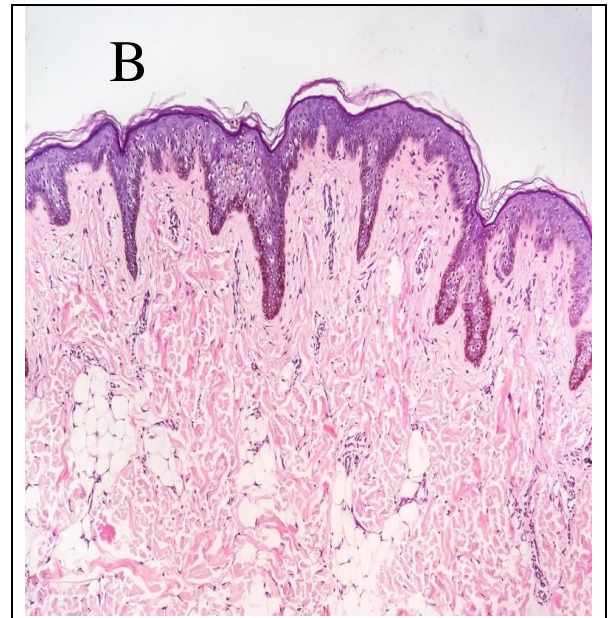
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Skin section showing Hibiscus sabdariffa staining with distinct nucleus and pink cytoplasm. Mag x10



Control showing haematoxylin and eosin stained skin section with purplish nucleus and pink cytoplasm. Mag x10

Figure 1: Skin section stained with Hibiscus sabdariffa and H&E stain with distinct nucleus and pink cytoplasm

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