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Histomorphological Staining of Selected Organs by Iron-Roselle Nuclear Technique and Natural Counter Stains

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAB conceptualized and designed the study, managed the experimental studies, managed the literature search, performed the laboratory analysis and interpretation of the result, the statistical analysis and wrote the first draft of the manuscript. Author TDA contributed to the concept and design of this work, contributed to the analyses and interpretation of the result. Authors JKB and JOO contributed to the definition of intellectual content. All authors read and approved the final manuscript.

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Method Article

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ABSTRACT

Background: Roselle extract is proving to be a staining alternative to haematoxylin in histopathological studies. Early studies concentrated on organs like lymph nodes, appendix, liver, kidneys and brain. The present study aims at expanding the frontiers of knowledge on the histological application of natural dyes from *H. sabdariffa* on the histo-morphology of some connective tissue rich organs viz uterus, ovary, breast and skin. Electrostatic compatibility with

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natural counter stains such as Sorghum bicolour, Baphia nitida (Camwood) and comparative reactions with standard counter stains like eosin, Masson Trichrome and Van Gieson was also explored.

Methods: Dry roselle calyces, Sorghum bicolour stalk, Camwood (Baphia nitida) powder were purchased at local markets in Ilorin, capital of Kwara State, Nigeria and identified at the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria. Staining solutions were prepared as recommended. Blocks of tissues from the Skin, Ovary, Breast and Uterus were retrieved from the archive of the Pathology Department, University of Ilorin Teaching Hospital, Ilorin. Six sections were cut at 4 microns from each organ for each of the following Hibiscus based techniques-{Hib-Eosin (A), Hib-Trichrome(B), Hib-Van Gieson(C), Hib-Sorg (D), Hib-Camwood(E)} and haematoxylin based staining techniques { H&E(F), MT(G), HVG(H), Hx-Sorg(L) and Hx-Camwood (J)}as parallel controls. Stained sections were blindly evaluated by three independent Histopathology experts for nucleus-cytoplasm staining, connective tissue demonstration and morphological preservation. Statistical evaluation was done using Near test, Kruskal-Wallis test and Kappa statistics with p-value set at 0.05.

Results: Histological observations show a satisfactory morphological demonstration of all the organs. Statistical analysis shows no significance difference between the Hibiscus *sabdariffa* based staining techniques and standard haematoxylin based staining techniques.

Conclusion: The histo-morphological demonstration of skin, ovary, uterus and breast was well preserved by Hibiscus sabdarifa solution (Iron-Roselle) nuclear staining and showed satisfactory electrostatic combinations with eosin, trichrome, Sorghum bicolour, Van Gieson and Camwood counter stains.

Keywords: Roselle; natural dye; histology; stain; hibiscus.

1. INTRODUCTION

Staining techniques originated in the second half of the eighteenth century and have been found very useful in the accurate microscopic descriptions of structures of tissues [1,2]. Plants with colouring and dyeing effects have found a place in histological staining [1]. Haematoxylin is the most important and most used dye in histopathology and histochemistry laboratories. Different formulations of haematoxylin exist for the demonstration of cytoplasmic and nuclear components of tissues [3].

The use of haematoxylin hasn't been without challenges. Notable among these is the periodic scarcity witnessed over the decades [4]. As a result, the scientific community has made efforts at finding applicable alternatives to haematoxylin [4,5].

The economic situations in developing countries, the continuous devaluations of local currencies, the need to explore available local dyes that are safe, cheaper and eco-friendly has all combined to stimulate interest in finding local alternatives to haematoxylin. Prominent among these is roselle (Hibiscus *sabdariffa*) extract [1,2,4,5,6,7].

Hibiscus extract has been used both as a cytoplasmic stain and nuclear stain in histology research [6]. As a cytoplasmic stain, it has been found useful as histological counter stain for kidney, artery and muscle hence its recommendation as a potential substitute foreosin [8].

Its application on renal histology as a dye was explored too and found to be a cheap and natural substitute for eosin in histological sections [9].

Hibiscus watery extract was investigated for its proprietary as a cytoplasmic histological stain in skin using formalin fixed, paraffin embedded tissue sections and found to be suitable as a replacement for standard eosin [10].

Hibsicus staining was applied on the intestine and found to be useful in demonstrating certain components of the organ thereby being recommended as a cheap, safe and natural dye for histological section [11].

The application of Hibiscus for the demonstration of sperm cell morphology has also been reported by Bassey and colleagues [12].

Works on the use of roselle aqueous extract as a nuclear stain have also sparsely graced the pages of scientific publications. The extract believed to contain anthocyanin as colouring the

matter was mordanted with ferric chloride solution ($FeCl_3.6H_20$) and applied as a nuclear stain as substitute to haematoxylin in formalin fixed, paraffin embedded sections of lymph node and appendix [5].

The use of Roselle (*H. sabdariffa*) extract mordanted with iron alum and potash alum at various temperatures and pH as a nuclear stain on the histological section of rat testis yielded successful outcome at low pH and acidified milieu. Iron mordanting gave impressive results [2].

The nucleus of 10% formalin fixed and paraffin embedded liver and kidney was found to stain well with aqueous extract of *H. sabdariffa* with Sorghum bicolour alcoholic extract as a counter stain [13].

The cerebrum, cerebellum and pons, all brain tissues fixed in 10% formalin and embedded in paraffin gave satisfactory nuclear staining with *H. sabdariffa* extract-iron solution when counter stained with eosin [14].

Other reports have shown that the extract-iron solution demonstrates the nucleus of the hippocampus and skin with Sorghum and eosin as counter stains respectively [1,6].

Early studies concentrated on organs like lymph nodes, appendix, liver, kidney and brain. This study attempts to expand the frontiers of knowledge on the histological application of natural dyes from *H. sabdariffa* on the histo-morphology of some connective tissue rich organs viz uterus, ovary, breast and skin. Electrostatic compatibility with natural counter stains such as Sorghum bicolor, baphia nitida (Camwood) and comparative staining with standard counter stains like eosin, Masson Trichrome, Van Gieson was also considered.

2. METHODOLOGY

2.1 Sourcing of Local Dyes

H. sabdariffa dry calyx, Sorghum bicolour stalk, and camwood powder were purchased at local markets in Ilorin, the capital city of Kwara State, North Central region of Nigeria and identified at the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria.

2.2 Sample Collection

Blocks of tissues from 10% buffered formalin fixed, paraffin embedded skin, uterus, ovary and breast were retrieved from the archive of the Pathology Department, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria and sectioned on a Leica microtome at 4 microns. Six sections were taken from each organ for each of haematoxylin and eosin, Hibiscus-eosin, Masson Trichrome, Hib-Trichrome, HVG, Hib-VG, Haematoxylin-Sorghum, Hib-Sorghum, Haematoxylin-Camwood and Hib-Camwood staining techniques labelled A-J.

2.3 Preparation of Roselle Extract and Formula

The dry calyces of *H. sabdariffa* were ground using a binatone blender to a fairly powdery form. To 10 g of the ground red calyces of *H. sabdariffa* in a conical flask, 200 ml distilled water was added and brought to boil to give the brilliant red coloured extract which was immediately allowed to cool and filtered using a Whatman filter paper to give a clear *H. sabdariffa* extract. To compound the staining formula, 100 ml of clear *H. sabdariffa* extract was mixed with 2g NaCl, 1.2 ml of 10% ferric chloride solution and 3 ml of glacial acetic acid.

2.4 Preparation of Harris Haematoxlin Solution

Haematoxylin (2.5 g), absolute alcohol (50 ml), potassium alum (50 g), distilled water (500 ml), mercuric oxide (1.5 g) and glacial acetic acid (20 ml) were weighed and measured respectively. The haematoxylin was dissolved in absolute alcohol, and the potassium alum was dissolved in distilled water. The heat was applied to dissolve the dye after which the two solutions were mixed. The solution was boiled in a large flask, mercuric oxide added and subsequently mixed. Cooling was done in cold water and glacial acetic acid added [3].

2.5 Preparation of 1% Camwood Alcoholic Extract

2 g of the camwood powder was weighed on Ohaus digital weighing balance, poured into a 250 ml reagent bottle and dissolved in 200 ml of 70% alcohol.

2.6 Preparation of 10% Sorghum Bicolour Alcoholic Extract

S. bicolour stalk was ground into a powdery form with a Binatone blender. 10 g of the ground

powder was weighed using a sensitive balance (Ohaus) and dissolved in a conical flask containing 100 ml of absolute alcohol. The solution was allowed to stay for 24 hrs at room temperature (25±2ºC) after which it was filtered into a staining jar [6].

Preparation of Weigert's Haematoxylin 1% alcoholic haematoxylin (Weigert's solution A) was mixed with equal volume of Weigert's solution B (30% aqueous ferric chloride (4 ml), concentrated hydrochloric acid (1 ml) and 95 ml of distilled water [3].

2.7 Preparation of Ponceau 2R fuchsin

1 g of Ponceau 2R was dissolved in 100 ml of 1% acetic acid and 0.5 g of acid fuchsin and dissolved in 50 ml of acetic acid. The solutions were thereafter be mixed together [3].

2.8 Preparation of Phosphomolybdic Acid

1 g of Phosphomolybdic acid was dissolved in 100 ml of distilled water [3].

2.9 Preparation of Acidified Light Green

2 g of light green was dissolved in 100 ml of 2% acetic acid [3].

2.10 H&E Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Harris haematoxylin for 15 minutes, washed in running tap for 2 minutes, differentiated in 1% acid alcohol, blued in running tap water for 10 minutes, counter stained in 1% alcoholic eosin for 30 seconds and mounted in DPX [3].

2.11 Hib-Eosin Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in hibiscus extract-iron solution for 5 minutes, rinsed and blued in running tap water for 10 minutes, counter stained in 1% alcoholic eosin for 30 seconds, dehydrated, cleared in xylene and mounted with DPX [13].

2.12 HVG Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in equal volumes of Weigert's A and B, rinsed in water, differentiated in 1% acid alcohol (10 seconds), blued in running tap water for 10 minutes, counter stained in van Gieson for 3 minutes, dehydrated in 95% and absolute alcohol, cleared in xylene and mounted in DPX [3].

2.13 Hib-VG Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Hibiscus extract solution for 5 minutes, blued in running tap water for 10 minutes, counterstained in Van Gieson for 3 minutes, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

2.14 Masson Trichrome Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in equal volumes of Weigert's A and B for 20 minutes, differentiated in 1% acid alcohol, washed in water for 10 minutes, stained in acidified Ponceau 2R for 5 minutes, rinsed in distilled water, differentiated and mordanted in 1% phosphomolybdic acid for 5 minutes, drained and put in acidified light Green for 5 minutes, differentiated in 1% acetic acid for 1 minute, blotted with filter paper, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX [3].

2.15 Hib-Trichrome Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Hibiscus extract solution for 5 minutes, blued in running tap water for 10 minutes, stained in acidified ponceau 2R for 5 minutes, rinsed in distilled water, differentiated and mordanted in 1% phosphomolybdic acid for 5 minutes, drained and put in acidified light Green for 5 minutes, differentiated in 1% acetic acid for 1 minute, blotted with filter paper, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX.

2.16 Haematoxylin-Sorghum Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Harris haematoxylin for 15 minutes, rinsed in water, differentiated in 1% acid alcohol, blued in running tap water for 10 minutes, counter stained in 10% alcoholic

sorghum for 3 minutes, dehydrated in ascending grades of alcohol, cleared in xylene and mounted with DPX [6].

2.17 Hib-Sorghum Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Hibiscus extract solution for 5 minutes, blued in running tap water for 10 minutes, counterstained in 10% alcoholic sorghum extract for 3 minutes, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX [6].

2.18 Haematoxylin-Camwood Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Harris haematoxylin for 15 minutes, rinsed in water, differentiated in 1% acid alcohol, blued in running tap water for 10 minutes, counterstained in 1% alcoholic camwood for 60 seconds, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

2.19 Hib-Camwood Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Hibiscus extract solution for 5 minutes, blued in running tap water for 10 minutes, counterstained in 1% alcoholic camwood for 60 seconds, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

2.20 Statistical Analysis

Near test, Kruskal-Wallis test and Kappa statistics were done and P value set at 0.05. Six sections each from the control and Hibiscus group were selected for each organ and assessed for staining efficiency, intensity and inter-observer reliability.

2.21 Staining Evaluation

Stained slides were coded and assessed by three histopathology experts for staining intensity and efficacy based on set parameters such as epithelial cellular contrast, stratification details, keratin demonstration, basement membrane presentation and melanin pigmentation. Connective tissue cells versus background contrast (tissue contrast), nucleus versus cytoplasm, vasculature, nerve cells, muscle cells, salivary gland and adipose tissue features shall also be evaluated [15].

3. RESULTS

3.1 Histological observations

3.1.1 Skin

The epidermis, dermis, stratification details, connective tissues, red blood cells and sweat glands were well demonstrated by the Hibiscus-Eosin technique. The nucleus was stained black-violet {Fig.1A}. The Hibiscus-Trichrome method for connective tissue stained collagen green while nucleus was stained brownish-black {Fig.1B}. Hibiscus-Van Gieson method stained nucleus brownish-black and collagen fibres, red {Fig.1C}. The Hibiscus-Sorghum technique stained nucleus dark-brown and collagen fibres, light brown {Fig.1D}. The nucleus was stained brownish-black by the Hibiscus-Camwood technique, collagen fibres, brown and keratin, golden brown {Fig.1E}.

3.1.2 Breast

The breast lobules, ducts, connective tissue and cellular elements were well demonstrated and nucleus stained dark-violet by the Hibiscus-Eosin technique {Fig.2A}. Connective tissue collagen was stained green by the Hibiscus-trichrome technique while nucleus was stained brownish-black. Red blood cells were stained red {Fig.2B}. With the Hibiscus-Van Gieson method, collagen was stained red and nucleus brown-black {Fig.2C}. Collagen was stained golden-brown and nucleus black by the Hibiscus-Camwood technique{Fig.2D} The Hibiscus-Sorghum technique stained collagen golden-yellow and nucleus black {Fig.2E}.

3.1.3 Ovary

The ovarian cortex, medulla, corpus luteum, corpus albicans and connective tissue stroma were well demonstrated by the Hibiscus-Eosin technique while nucleus was stained dark-violet {Fig.3A}. Connective tissue collagen was stained green by the Hibiscus-trichrome technique while nucleus was stained brownish-black {Fig.3B}. With the Hibiscus-Van Gieson technique, collagen was stained red while the nucleus appears black {Fig.3C}. Collagen was stained light-brown and nucleus black by the Hibiscus-Camwood technique {Fig.3D}. The Hibiscus-Sorghum technique stained collagen light brown and nucleus black {Fig.3E}.

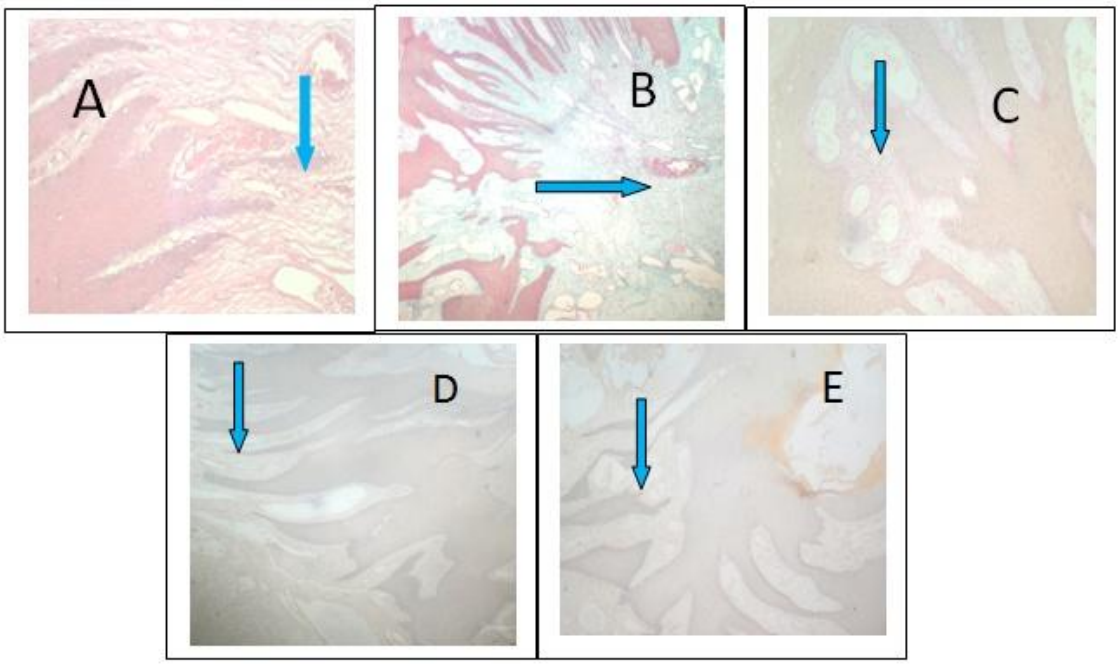

Fig. 3.1.1. Skin-showing morphological preservation with the nuclear background (blue arrow) by Hibiscus-eosin (A), Hibiscus-trichrome (B), Hibiscus-van Gieson (C), Hibiscus-sorghum (D) and Hibiscus-camwood (E)respectively. Mag. X 200

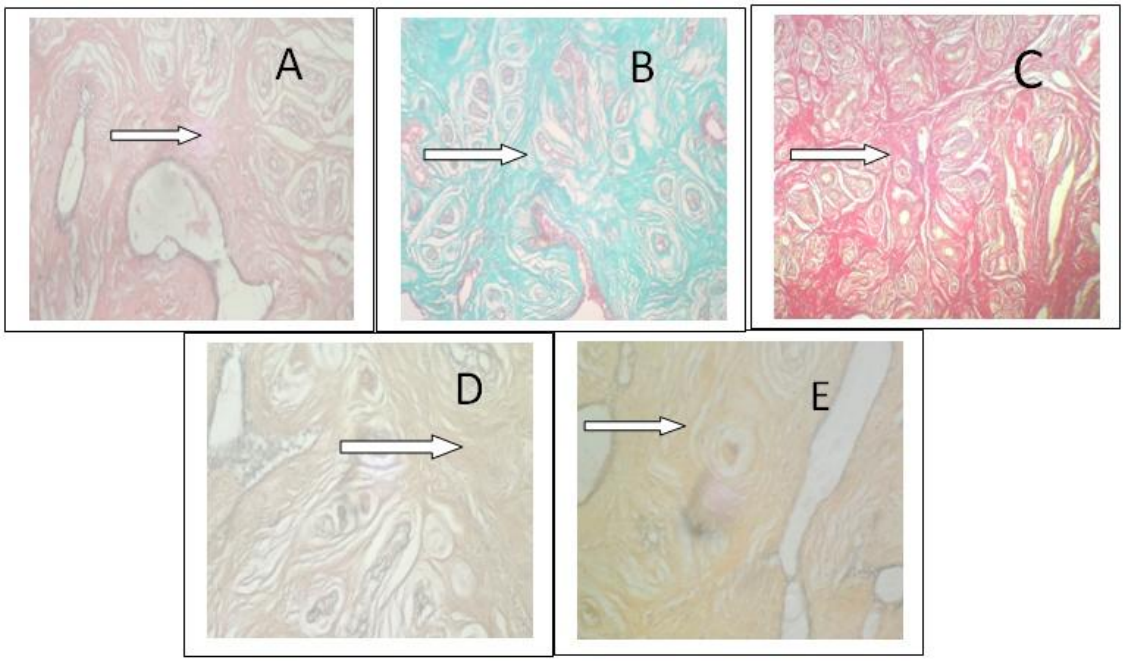

Fig. 3.1.2 Breast-showing morphological preservation and differential staining of connective tissue collagen (white arrow) by Hibiscus-eosin (A), Hibiscus-trichrome (B), Hibiscus-van Gieson (C), Hibiscus-sorghum (D) and Hibiscus-camwood (E) respectively. Mag. X 200

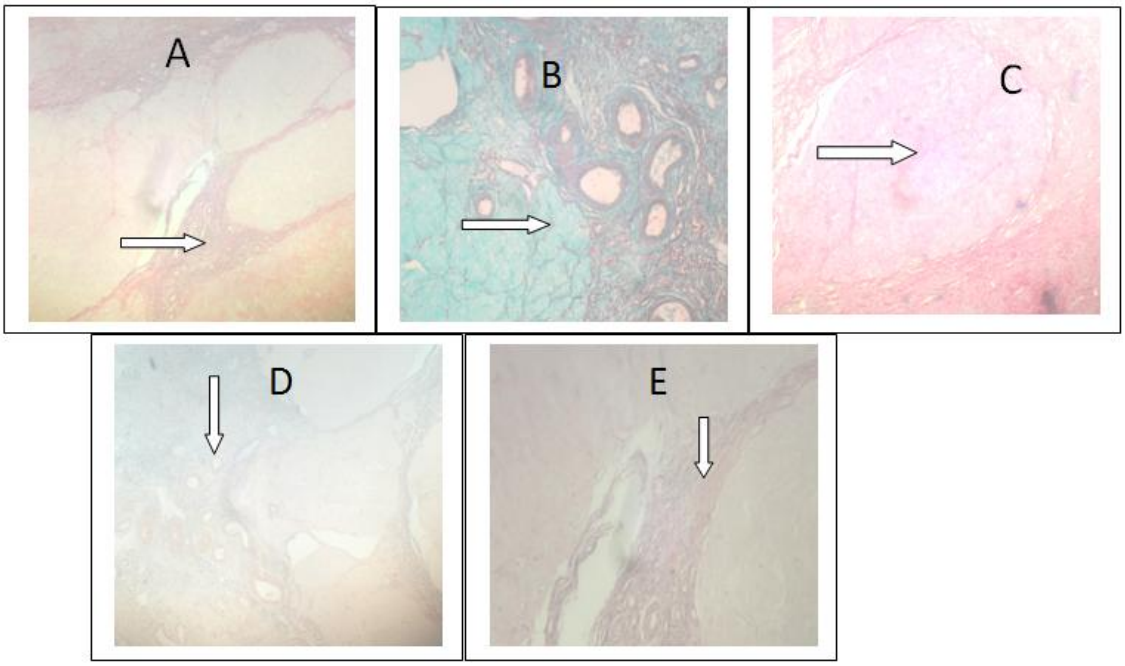

Fig. 3.1.3 Ovary showing morphological preservation and differential staining of connective tissue collagen (white arrow) by Hibiscus-eosin (A), Hibiscus-trichrome (B), Hibiscus-van Gieson (C), Hibiscus-sorghum (D) and Hibiscus-camwood (E) respectively.Mag. X 200

3.1.4 Uterus

The myometrium, endometrium, connective tissues, fibroblasts and nucleus were well demonstrated by the Hibiscus-Eosin technique. Nuclei appeared purplish-blue while red blood cells and cytoplasm were stained pink {Fig.4A} The Hibiscus-Trichrome techniques stained collagen fibres, green while the nucleus stained brownish-black {Fig.4B}. With the Hibiscus-Van Gieson technique, collagen was stained red while nucleus appeared black{Fig.4C}. Collagen was stained golden yellow by the Hibiscus-Camwood technique while nucleus was stained black {Fig.4D}. The nucleus was stained black by the Hibiscus-Sorghum technique while collagen appeared yellowish-brown {Fig.4E}.

3.2 Evaluation

Independent assessment by the three histopathology experts rated the quality of staining by the Hibiscus techniques as satisfactory {Table 1}. Morphological parameters such as epithelial cellular contrast, stratification details, keratin demonstration, basement membrane presentation, melanin pigmentation, connective tissue cells versus background contrast (tissue contrast), nucleus versus cytoplasm clarity, vasculature, nerve cells, muscle cells, salivary gland and adipose tissue as it affects respective organs as appropriate were well demonstrated.

3.3 Statistical Analysis

Statistical analysis showed no significant difference between the Hibiscus techniques and the standard haematoxylin based techniques for morphological staining {Table 2}.

Npar test, Kruskal-Wallis test and Kappa statistics done. * Statistical significance $P< 0.05$. No statistical significant difference between haematoxylin based staining and Hibiscus solution based staining of connective tissue and nuclei in all the organs investigated.

4. DISCUSSION

The haematoxylin and eosin {H&E} staining treatment is often required for every specimen processed in the histopathology laboratory worldwide thanks to the decades old effort of Wissowsky in 1876 [16]. Just on the basis of the H&E technique, many and variable histopathological conditions can be studied and diagnosed [17].

Recent economic realities in developing countries, concern for safety, clamour for the eco-friendly environment and the repeated episodes of haematoxylin scarcity has made the search for haematoxylin substitutes a compelling necessity in histopathology research [5,6]. Natural plants began to gain attention as more workers continue to explore the histological application of natural dyes [18].

This study attempts to expand the frontiers of knowledge on the histological application of natural dyes from *H. sabdariffa* extract on the histo-morphology of some connective tissue rich organs such as uterus, ovary, breast and skin. Electrostatic compatibility with natural counter stains such as Sorghum bicolour and baphia nitida (Camwood) coupled with comparative assessment with standard counter stains like eosin, Masson Trichrome and Van Gieson were also considered.

In this study, the histomorphological architecture of the selected organs was well preserved and satisfactorily demonstrated {Fig. 1-4}.

For the skin, the dermis, epidermis, connective tissues, stratification detail, sweat glands, sebaceous glands, connective tissue cells and adipose tissue were all distinctly demonstrated by the Hibiscus-iron nuclear staining technique with eosin counter stain. This compared well with outcome observed in standard H&E. Staining and morphological appearance of the skin's connective tissue collagen compares favourably with the outcome of haematoxylin based Masson Trichrome technique. Similar comparative outcomes were observed for Hibiscus-Van Gieson and standard HVG, where collagen was stained red, red blood cells yellow and muscle fibres red. The morphological staining of the skin by the Hibiscus-Camwood technique is similar to that obtained by the Haematoxylin-Camwood technique. Comparative assessment of the morphological staining of the skin by the Hibiscus-Sorghum and Haematoxylin-Sorghum revealed similar outcomes.

Breast staining by the Hibiscus-eosin technique revealed histomorphological components like the glands, dense connective tissue and red blood cells. This compares well with the outcome for standard H&E. The histomorphological staining of collagen by the Hibiscus-trichrome technique was similar to the results obtained by the standard Masson Trichrome staining technique. Other Hibiscus techniques, Hibiscus-Van Gieson, Hibiscus-Camwood and Hibiscus-Sorghum yielded similar outcomes as for standard HVG, Haematoxylin-Camwood and Haematoxylin-Sorghum.

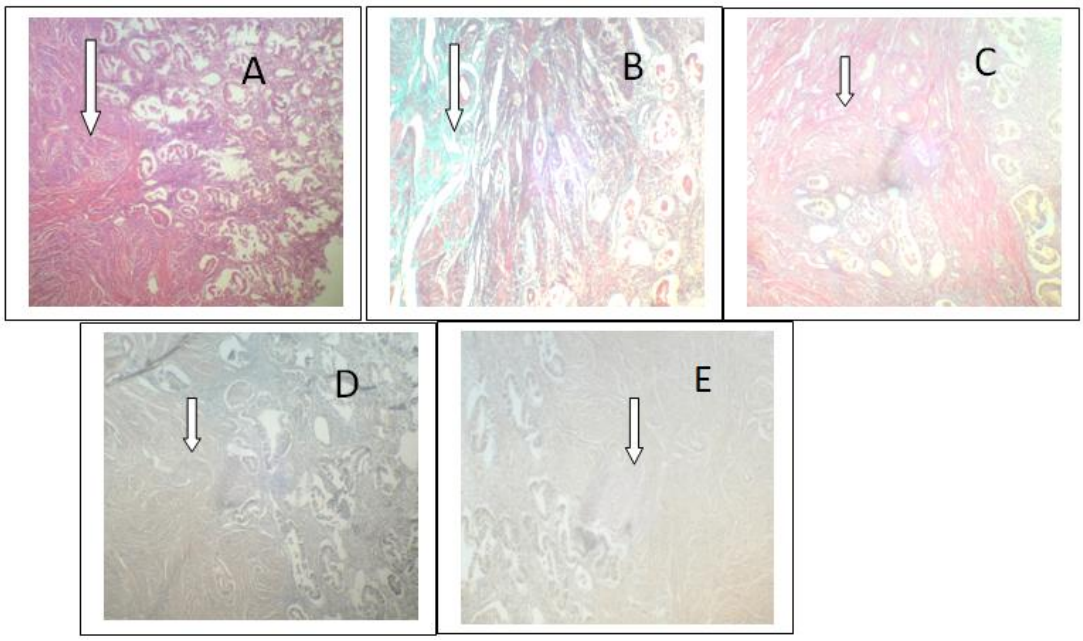

Fig. 3.1.4. Uterus showing morphological preservation and differential staining of connective tissue collagen (white arrow) by Hibiscus-eosin (A), Hibiscus-trichrome (B), Hibiscus-van Gieson (C), Hibiscus-sorghum (D) and Hibiscus-camwood (E) respectively. Mag. X 200

Table 1. Showing the evaluation of connective tissue and validated tissue components by Histopathology Experts.

Organ	Epithelial Contrast	Stratification Details	Keratin Demonstration	Basement membrane	Melanin Pigmentation	Connective Tissues/cellular Background	Nucleus/ Cytoplasm contrast	RBC	Muscle	Salivary Gland	Adipose Cells
Skin	+	+	+	+	+	+	+	+	+	+	+
Ovary	N/A	N/A	N/A	N/A	N/A	+	+	+	+	NA	N/A
Breast	+	+	+	+	+	+	+	+	+	+	+
Uterus	N/A	N/A	N/A	N/A	N/A	+	+	+	+	N/A	N/A

+= Satisfactory N/A= Not applicable

Table 2. Results of statistical analysis of connective tissue staining of skin, ovary, uterus and breast

Parameters	n	Mean	SD	Chi-square	P value
Cells versus background (Tissue contrast)	240	1.1667	1.00722	0.000	1.000
Nucleus versus cytoplasm	240	1.1667	1.00722	0.000	1.000
Vasculature	240	0.7500	0.98907	0.170	0.680
Muscle	240	0.7917	1.06237	0.057	0.812
Nerve	240	0.1667	1.00722	0.000	1.000
Salivary gland	240	0.0667	0.96309	0.000	1.000
Others	240	0.0667	0.96309	0.000	1.000

(Control-120, Hibiscus-120)

P<0.05, SD: Standard deviation n=number of stained slides

Histomorphologically, the corpus albicans, corpus luteum, the medulla and blood vessels of the ovary were well demonstrated by the Hibiscus-eosin technique in a comparable manner with standard H&E. Collagen fibres, red blood cells were well stained by the Hibiscus-trichrome method in a similar manner to standard Masson Trichrome. Hibiscus-Van Gieson technique also stained the collagen fibres histochemically in a comparable manner to the standard HVG. The histochemical staining of collagen fibres by the Hibiscus-Camwood technique is impressive and comparable to the haematoxylin based Haematoxylin-Camwood technique. In the same manner, the Hibiscus-Sorghum technique stained the collagen component of the ovary well in a comparable manner to the Haematoxylin-Sorghum technique which served as a parallel control.

Histomorphological components of the uterus such as the myometrium, dense connective tissue stroma and fibroblasts were well demonstrated by the Hibiscus-eosin technique comparable with the standard H&E. The collagen fibres were histochemically stained green by the Hibiscus-trichrome technique comparable with standard Masson Trichrome. The Hibiscus-Van Gieson stain coloured collagen fibre red histochemically comparable to standard HVG. Similar satisfactory comparative staining was observed for the Hibiscus-Camwood technique and Haematoxylin-Camwood technique which stains collagen fibres histochemically with black nuclei background. The light light-brown staining of connective tissue collagen and nuclei black by the Hibiscus-Sorghum method attests to its histochemical staining ability and electrostatic compatibility of the dyes.

In this study, the electrostatic compatibility of the dye extract, iron-Roselle with the counter stains experimented i.e. eosin, trichrome, Van Gieson, Camwood and Sorghum bicolour is impressive. This agrees with the findings of earlier researchers who combined the dye with eosin for histological staining in lymphnode and appendix [5], Sorghum bicolour for collagen [19] and intestinal tissue [20], Van Gieson for the demonstration of brain tissues [21] and Camwood for blood smear [22].

Preliminary investigation shows that the Hibiscus-iron stain lasts for more than 3 months in the refrigerator. The addition of few thymol crystals has been reported to keep Hibiscus aqueous extract for four months at room temperature [23].

The nuclei staining observed in this study is made possible by the mordanting effect of iron alum ($FeCl_3.6H_20$) which makes the hibiscus dye nuclei-specific. The pH of the solution is acidic (2.0). The staining pigment in Hibiscus is anthocyanin (Cyanidin-3-glucoside) which has been reported to be stable at acidic pH [24]. Staining was achieved using 5% concentration of the dye extract with optimal staining time of five minutes which confirms its use as a progressive stain. The Hibiscus stain resists fading and independent observers rarely distinguish it from standard haematoxylin stain. This is consistent with an earlier observation made by Altakriti and colleague [25].

5. CONCLUSION

Results from this investigation show that roselle extract mordanted with Ferric Chloride solution demonstrated the histomorphology of skin, breast, ovary and uterus. The stain is also electrostatically compatible with counterstains such as eosin, trichrome, Van Gieson, Camwood alcoholic extract and Sorghum bicolour alcoholic extract. Further studies are however still required to find stable preservatives for the stain and also better manipulations for high-quality photomicrographs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard was written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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