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USING OF HIBISCUS SABDARIFFA IN STAINING OF APPENDICULAR TISSUE SECTIONS

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ABSTRACT
Objectives: this was a retrospective descriptive study performed on formalin-fixed paraffin-embedded tissue sections prepared from healthy and/or inflamed Appendix and aimed to assess the quality of staining by Roselle (Hibiscus Sabdariffa) natural extract compared to the routine H&E staining method. **Methods:** the appendicular tissue sections were grouped and stained by different concentrations of **Hibiscus Sabdariffa solution** with parallel H&E staining as control. **Results:** about 20% of sections showed first degree staining quality in 5% solution, more than half of them showed the best staining quality within 60 minutes time duration. **Conclusions:** Hibiscus Sabdariffa solution can be used effectively in staining of appendicular tissues instead of H&E staining method.

KEYWORDS: Hibiscus Sabdariffa, Appendix, Tissue Staining.

INTRODUCTION
Detection and insertion of a new histological stain into routine laboratory practice remains justified especially if the new stain is harmless, easy to use, cheap and easily available.[1] Although most of the dyes in current use in histology laboratories are of synthetic origin, natural dyes are showing encouraging results.[2]

Hibiscus Sabdariffa is an annual herbaceous shrub belonging to the family Malvaceae.[3] Sudan is considered as the country in which Roselle originated, particularly in Kordofan and Darfur areas.[4] Roselle is known as karkade in the Sudan and other Arab countries. It is mainly grown for its fleshy calyx (sepals), which is the commercially valuable part of the plant. The color of the calyx plays an important role in determining the quality of karkade. The plant has some medicinal uses[5,6,and7]; in Europe, it is used in food preparation in sauces, jams, juices, jellies, syrups and flavoring, and as coloring agent for food and drinks.[8, 9]

The tissue staining potentials of this plant were rarely explored previously [10- 13]; the current study represents an additional step in the using of Hibiscus Sabdariffa as an aqueous extract in staining of different histological tissue sections.

MATERIA AND METHODS
This study was conducted within three months duration. Healthy-appeared tissues were selected from appendicular tissues removed from patients with acute appendicitis. These selected tissues were sliced in the histopathology laboratory, fixed in 10% formalin, and prepared into several paraffin-embedded tissue blocks. Four blocks (4 groups) were randomly selected; from each block, 40 sections of 3-5 µm-thicknesses were cut by using a rotary microtome. The first group of sections was stained by 1% Hibiscus solution, the second group was stained by 5% solution, the third group was stained by 10% solution, and the fourth group was stained by 100% solution. Within each group, 10 sections were stained for 1-2 minutes, 10 sections for 10 minutes, 10 sections for 30 minutes, and 10 sections for 60 minutes. All staining procedures were done at room temperature. Stained slides were then put ready for microscopic evaluation. Another few slides were prepared and stained by routine hematoxylin and eosin method and considered as control.

Preparation of Roselle solution
To prepare 1% solution, 1 g of Karkade powder was added to 100 ml distilled water and the mixture was boiled, cooled, filtered, and kept into suitable container. To prepare 5% solution, 10% solution, and 100%

solution, 5 g, 10 g, and 100 g, respectively were added to the 100 ml water.

The obtained data was analyzed by using SPSS software version 16 and Ethical Clearance was obtained from the Ethical Committee of Faculty of Medical Laboratory **S**ciences at Neelain University.

RESULTS AND DISCUSSION

Quality of staining by Roselle was graded into four grades, first grade (very good to excellent), second grade (good), third grade (poor), and fourth grade (bad); that was according to the microscopic appearance of cell membrane, nuclear membrane, cytoplasm transparency, and extracellular matrix. If all were clearly seen, quality was put as very good to excellent; if three out of four were clearly seen, it was of good quality; if two, it was poor; if only one out of the four parameters was clearly seen, then quality was considered bad.

A lot of research discussed the nutritional and therapeutic benefits of Hibiscus Sabdariffa.[14,15,and16] However, few researchers reported about using and applying extracts of the plant in diagnostic medical laboratory procedures. In previous work of the authors, Hibiscus solution has given encouraging results with intestinal tissue[10], renal tissue[11], and skin.[12]

When using **1% solution** in the **current study**, most of the stained sections (70 %) showed poor and bad quality of staining. The highest quality of staining obtained in this concentration was good; it was noticed in about 30% of cases. No very good to excellent quality was noticed (table No 1).

With renal tissue[11], 1% solution resulted in 95% poor and bad quality and only 5% showed good quality. With intestinal tissue[10], 5% were excellent, 30% were good, and 65% were poor and bad in quality. With skin tissue[12], all sections showed poor and bad quality.

It seems that 1% Hibiscus solution has encouraging results with intestinal tissue and, to some extent, with appendicular and renal tissue.

Table No 1 shows the staining quality of 1% Hibiscus solution on appendicular tissues

NO	Time	Very good to excellent	Good	Poor	Bad	Total
1.	1-2 min	**0**	**2**	**1**	**7**	10
2.	10 min	0	**3**	2	5	10
3.	30 min	0	**3**	1	6	10
4.	60 min	0	**4**	**1**	5	10
Total		0	**12**	5	23	40

With using 5% solution in this study, 42.5% of sections showed good to excellent quality of staining and 57.5% showed poor and bad quality (table No 2). With renal tissue, 15% were good to excellent and 85% were poor and bad. With intestinal tissue, 47.5% were good to excellent and 52.5% were poor and bad quality. With skin tissue, only 10% were good to excellent and 90% were poor and bad in quality. It is evident that this concentration (5% solution) is very encouraging with intestinal and appendicular tissues and, to some extent, with renal and skin tissues.

Table No 2 shows the staining quality of 5% Hibiscus solution on appendicular tissues

NO	Time	Very good to excellent	Good	Poor	Bad	Total
1.	1-2 min	**0**	**1**	2	7	10
2.	10 min	**1**	**2**	3	4	10
3.	30 min	**2**	3	3	2	10
4.	60 min	5	3	**1**	1	10
Total		8	9	9	14	40

With 10% solution, only 10% of sections showed good to excellent quality of staining and 90% showed poor and bad quality (table No 3). With renal and intestinal tissues, 10% were good to excellent and 90% were poor and bad. With skin tissue, 2.5% were good, 97.5% were poor and bad, and no very good to excellent results. It seems that this concentration (10% solution) is not suitable for such tissues.

Table No 3 shows the staining quality of 10 % Hibiscus solution on appendicular tissues

NO	Time	Very good to excellent	Good	Poor	Bad	Total
1.	1-2 min	**0**	**0**	**2**	8	10
2.	10 min	**0**	**0**	**3**	**7**	10
3.	30 min	**0**	**1**	**4**	5	10
4.	60 min	**1**	**2**	**3**	4	10
Total		**1**	**3**	**12**	24	40

With **100% solution**, 27.5% of sections showed good to excellent quality of staining and 72.5% showed poor and bad quality (table No 4). **With renal tissue**, 12.5% were good, 87.5% were poor and bad, and no very good to excellent results. With **intestinal tissue**, 27.5% were good to excellent and 72.5% were of poor and bad quality. With **skin tissue**, 5% showed good quality, 95% showed poor and bad quality, and very good to excellent results were noticed. It is evident that this concentration (100% solution) is, to some extent, encouraging with intestinal and appendicular tissues.

Table No 4 shows the staining quality of 100% Hibiscus solution on appendicular tissues.

NO	Time	Very good to excellent	Good	Poor	Bad	Total
1.	1-2 min	**0**	**0**	**4**	6	10
2.	10 min	**1**	**1**	2	6	10
3.	30 min	**2**	**2**	**3**	3	10
4.	60 min	**3**	**2**	**3**	2	10
Total		**6**	**5**	12	17	40

With all concentrations, the best time duration for staining was 60 minutes, the same as in previous studies.

CONCLUSION

Hibiscus Sabdariffa 5 % solutions can be used effectively in staining of appendicular tissues instead of eosin in H&E staining method. The best time duration for staining is 60 minutes. However, further work is still needed to try to get better staining in as minimum time as possible.

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