International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 06 Issue 01 January 2023
DOI: 10.47191/ijcsrr/V6-i1-61, Impact Factor: 5.995
IJCSRR @ 2023
www.ijcsrr.org

Hibiscus Sabdariffa as Nuclear Stain for Cytological Buccal and Cervical Smear

Sahar Elderdiri Gafar Osman[1], Nusaiba Mohammed Almobarak Alsmmani[2], Eiman Mahmoud Elsharif Agabien[3], Abrar Salim Basheir[4], Mohamed Ibrahim Adam Ali[4], Yousef Breir Yousef[4]

[1] Department of Histopathology and Cytology, Program of Medical Laboratory Science/Alfajr College for Science and Technology, Khartoum, Sudan

[2] Department of Histopathology and Cytology, Program of Medical Laboratory Science/Alfajr College for Science and Technology, Khartoum, Sudan

[3] Department of Histopathology and Cytology, Soba University Hospital of Khartoum, Khartoum, Sudan

[4] Department of Histopathology and Cytology, Program of Medical Laboratory Science/Alfajr College for Science and Technology, Khartoum, Sudan

ABSTRACT

Introduction: Sudan is one of the biggest countries that produces and exports hibiscus Sabdariffa in Africa. The possibility of HS staining cytological specimens is not well explored. Therefore, the present study aimed to evaluate the staining quality of Hibiscus Sabdariffa on cytological buccal and cervical smear as a cheap viable nuclear stain alternative to hematoxylin.

Methods: This study was an experimental descriptive study conducted at Alfajr College for Science and Technology from (June to September 2022). A total of 30 smears, 15 buccal smears collected from students and 15 cervical smears were retrieved from Alfajr's histopathology lab.

Results: All (30) slides showed good staining quality with 20% concentration Hibiscus Sabdariffa water extract when mordanted with Iron, and acidified with acetic acid.

Conclusion: Hibiscus Sabdariffa is good as nuclear staining for a buccal and cervical pap smear and can replace hematoxylin by adjustment of concentration, time and pH.

KEYWORDS: Buccal, Cervical, Hibiscus Sabdariffa, Nuclear Stain, Sudan

INTRODUCTION

Hibiscus Sabdariffa (HS) is a plant belonging to the Malvaceae family which is a vascular flowering plant, popularly known as (Roselle) or Red Sorrel in English and composed of over a hundred species (Ananthalakshmi et al., 2016; Joshua et al., 2021; Konar et al., 2019; Saxena et al., 2021). The colour of calyces varies from white-yellow to dark red due to anthocyanin (El-Hashash et al., 2022). Hibiscus sabdariffa is cultivated in many countries around the world and Sudan is one of these countries and known as (Karkade) (Abd-alhafeez et al., 2014; Kuşçulu, 2021). Hibiscus Sabdariffa plants contain anthocyanin, organic acids, proteins, flavonoids, vitamins, and polysaccharides (S. Benard et al., 2015; N Mahadevan, 2009), and thus have a variety of industrial, nutritional, and medical uses (Agbede et al., 2017). Hibiscus is used to lower blood pressure, stimulate diuretic effects, and reduce high cholesterol, cosmetics, and food colourants. Anthocyanins are responsible for colour and extract from different plant materials and Hibiscus Sabdariffa is the most important one (S. A. Benard et al., 2015; El-Hashash et al., 2022; Pattanittum et al., 2021). HS extract has a colour ranging from red, and purple to blue, and the optimum pH for staining range from 2.5 to 4.0 (Solomon A. Benard, 2008). The deep red colour of Hibiscus Sabdariffa extract is soluble in water and convert into pink by the addition of HCL, and bluish to green by alkali (Maaza, 2014).

Sudan is one of the biggest countries that produces and exports hibiscus Sabdariffa in Africa (Abd-alhafeez et al., 2014). Synthetic dyes, hematoxylin of them are well known to expose laboratory workers to adverse health effect as allergic and toxicity (Hartika et al., 2021), moreover the shortage of hematoxylin reported by several studies (Dapson et al., 2010). Therefore, is necessary to find a natural, safe, less costly and harmful, and easy-to-obtain stain. In the current literature, several studies reported the possibility of Hibiscus Sabdariffa staining histological tissue sections, parasites, and fungi (Omorodion & Achukwu, 2017; Saxena

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et al., 2021), however, the possibility of staining cytological specimens is not covered well. Benard 2008 has established the capability of Hibiscus Sabdariffa as nuclear staining. Benard has reported that the combination of Hibiscus Sabdariffa with ferric chloride, sodium chloride, and glacial acetic acid followed by eosin produces a similar result to hematoxylin& eosin for histological section (Solomon A. Benard, 2008). In the current study, we used the above formula with slight modification and we proposed that Hibiscus Sabdariffa when followed by orangeG6, and eosin Y produce a comparable result to conventional Pap for the cytological smear. Therefore, the present study aimed to evaluate the staining quality of Hibiscus Sabdariffa on cytological buccal and cervical smear as a cheap viable nuclear stain alternative to hematoxylin.

METHODS

Study design:

This study was an experimental descriptive study to evaluate the staining quality of Hibiscus Sabdariffa on cytological buccal and cervical smear at Alfajr College for Science and Technology from (June to September 2022). A total of 15 buccal smears were obtained from medical laboratory science students according to voluntary base and regardless of gender and age, and 15 cervical smears were retrieved from the Alfajr histopathology lab.

Preparation of Hibiscus Sabdariffa extract solution:

Dry red calyces of Hibiscus Sabdariffa were obtained at the local market in Khartoum, the capital of Sudan. washed with water, dried with sun and subsequently finely grounded using a Moulinex homogenizer. 40g of the powdered Hibiscus Sabdariffa was added to 200 ml of distilled water, and then 10g of sodium chloride was added in a conical flask to prepare a 20% concentration. The solution was brought to boiling to give it a brilliant red colour. Then 2.5g of 10% ferric chloride solution and 6.0 mL of glacial acetic acid were added with shaking. The extract was immediately allowed to cool at room temperature and filtered through gauze followed by Whitman's filtered paper to give a clear Hibiscus Sabdariffa extract. The solution was protected from light by covering the conical flask with aluminium foil and stored at 4°C until further used. The pH of the extract was 2.8 measured by a pH meter.

Sample Collection:

Samples were collected from clinically healthy buccal mucosa. The participants were asked to rinse their mouths using tap water twice. The buccal surface was scraped using a spatula, the material was smeared on glass slides and fixed with 95% ethyl alcohol for 15 minutes.

Staining Method:

Cervical and buccal smears were brought in water, followed by hibiscus extract for 30 minutes, followed by bluing in running tap water for 3minute, followed by orange G-6 stain for 3 minutes, followed by 10 dips in 95% Ethanol, then eosin Azure for 3 minutes. Then all smears were rinsed in 70%, 95%, and 100% Ethanol for 2 minutes, cleared in xylene for 2 minutes and mounted with a permanent mounting medium (DPX).

Slide examination:

The staining quality of Hibiscus Sabdariffa was examined by two observers. The slides were evaluated according to microscopic criteria of the nuclear stain, cytoplasm stain, stain intensity, and contrast. Each one of the criteria was given a score of 0 or 1. According to the sum of the scoring, the slide was then graded into 4 grades; 0= poor, 1= satisfied, 2= good and 3= excellent. Poor is referred to as the absence of stain on the nucleus/cytoplasm, and no nuclear details/ difficult assessment on the cytoplasm. Satisfy is referred to the presence a pale of stain on the nucleus/ cytoplasm and no nuclear details/ difficult assessment of cytoplasm. Good is referred to the presence of stain on the nucleus/cytoplasm comparable to hematoxylin, and the presence of nuclear details but chromatin not clearly defined/ difficult assessment on the cytoplasm. Excellent is referred to the presence of stain on the nucleus comparable to hematoxylin, and the presence of nuclear details clearly defined (Zaimy, 2017).

RESULTS

Hibiscus Sabdariffa solution stained the nucleus of superficial and intermediate cells dark violet, whereas the cytoplasm of superficial stained with pink and intermediate with blue. The granules of neutrophils in the cervical smear stained blue-black with pink cytoplasm, and Red blood cells stained red. All (30) slides showed good staining quality with 20% concentration Hibiscus Sabdariffa water extract when mordanted with Iron, and acidified with acetic acid.

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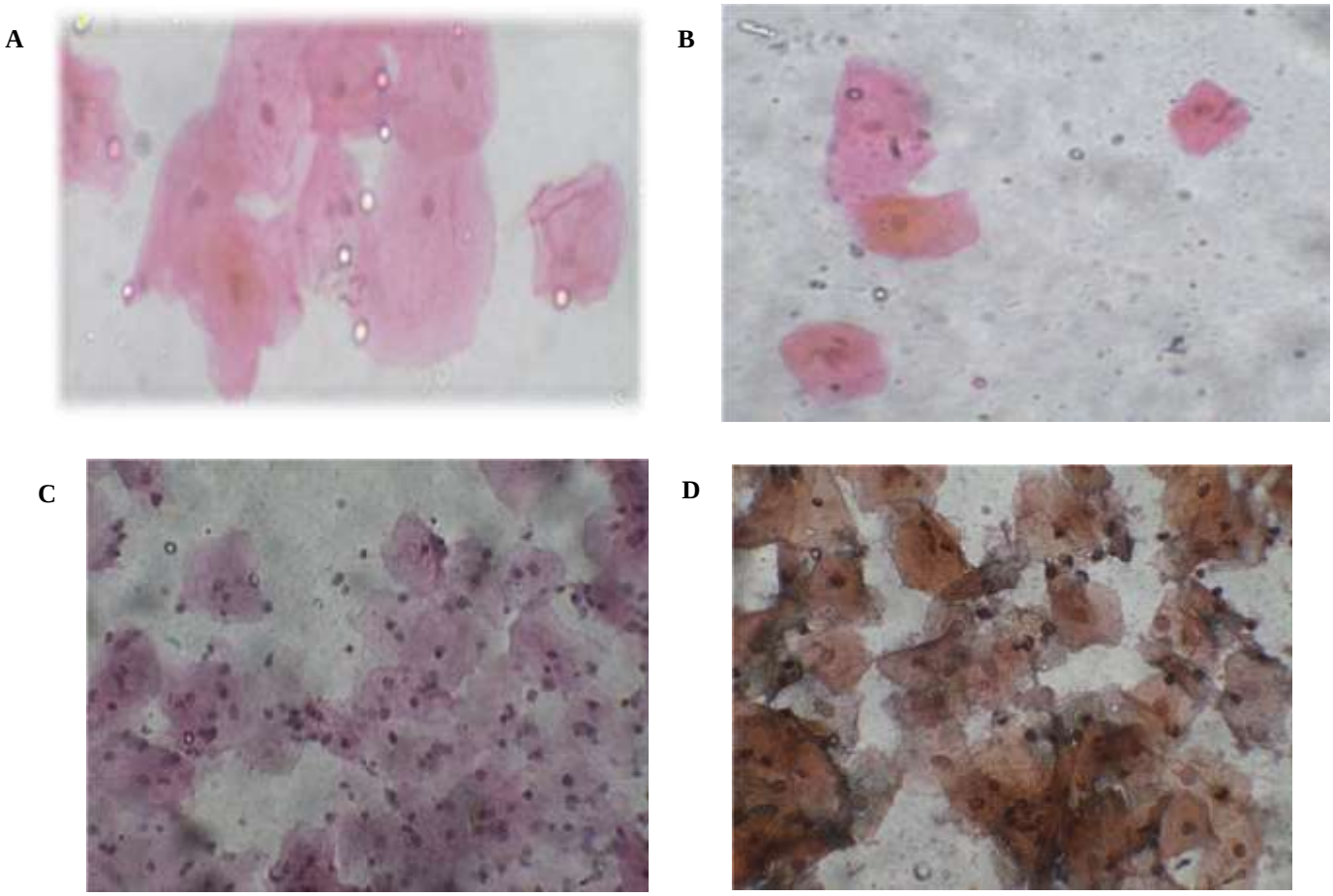

Figure 1: (A) Buccal smear stained with Hibiscus Sabdariffa X40; (B) Buccal smear stained with Pap stain; (C) Cervical smear stained with Hibiscus Sabdariffa X40; (D) Cervical smear stained with Pap stain X40.

DISCUSSION

Perhaps the present study is the first study done in Sudan to evaluate HS as a nuclear stain alternative to hematoxylin in cervical and buccal cytological smears. There is a new trend to use Hibiscus Sabdariffa as a histological stain, either an alternative to hematoxylin or eosin. Previous studies done in Sudan by Ibnouf and his colleagues aimed to explore the efficacy of HS with different concentrations and times as a cytoplasmic stain alternative to eosin. They found that the best concentration and time for skin, renal, and appendicular tissue are 5% concentration for 60 minutes (A Raheem et al., 2015; Abd-alhafeez et al., 2014; Abd-Alhafeez Osman Ibnouf, 2020). In contrast, Benard and his colleagues in Nigeria reported that a 5% concentration of hibiscus Sabdariffa is suitable as nuclear staining alternative to hematoxylin (Agbede et al., 2017; S. Benard et al., 2015, 2017; S. A. Benard et al., 2015; Solomon A. Benard, 2008; Wicaksana, 2016). Our results are superior to Ibnouf and his colleagues probably due to the addition of ferric chloride as mordant and the nucleus stained well at 20% concentration for 20 minutes. Previous studies reported the importance of the addition of Ferric Chloride and Glacial acetic Acid (Alshamar & Dapson, 2021b; Bassey et al., 2012; Bose et al., 2022; Okolie et al., 2021; Ola et al., 2016). We noticed that when using a 25% concentration of HS, the extract solidified and this is probably due to pectin, a previous study reported a such finding (Hashim, 2006). Moreover, the nucleus stained blue-black with running tap water for 10 minutes (bluing), whereas stained red-purple with water washing for 1 minute as well as hematoxylin, such findings reported by (Alshamar & Dapson, 2021a). In the present study HS failed to stain the bar body and this result comparable to (Omorodion & Achukwu, 2017). In contrast, a previous study done by (Saxena et al., 2021) on buccal smear to evaluate Hibiscus as a counter stain for hematoxylin comparable to Pap stain, found that Hibiscus gave the poorest result and failed to stain the cytoplasm when ammonia was used to alkalify the pH. Several previous studies focused on HS as a substitution for hematoxylin

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and eosin as a counterstain and found good results (Agbede et al., 2017; S. Benard et al., 2017; S. A. Benard et al., 2015; Solomon A. Benard, 2008; Sridhara et al., 2016), On the other hand, other studies focused on HS as a substitution for eosin and the results were controversial (Bose et al., 2022; Egbujo et al., 2008; Surendra et al., 2018).

Based on the fact that acidic dye stains basic structure and Vic versa, Hibiscus Sabdariffa can be used either as a cytoplasmic or nuclear stain by adjustment of pH. Several factors contributed to tissue stains such as dye concentration, type of mordant, either aqueous or alcoholic extract, and time of staining. Further research is needed in this context to optimize these factors and to answer the question that whether Hibiscus Sabdariffa gives better results when used either as a cytoplasmic or nuclear stain.

CONCLUSION

In conclusion, Hibiscus Sabdariffa is good as nuclear staining for a buccal and cervical pap smear and can replace hematoxylin by adjustment of concentration, time and pH and iron mordant is recommended for better results.

Ethical Statement: Alfajr College Research Ethical Committee reviewed and approved the study. All participants were assigned informed written consent after being oriented with the study objectives and sampling technique. All methods were carried out following relevant guidelines and regulations.

Funding: Authors declare no fund.

Acknowledgements: All authors would like to thank the participants.

Competing interests: None.

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Cite this Article: Sahar Elderdiri Gafar Osman, Nusaiba Mohammed Almobarak Alsmmani, Eiman Mahmoud Elsharif Agabien, Abrar Salim Basheir, Mohamed Ibrahim Adam Ali, Yousef Breir Yousef (2023). Hibiscus Sabdariffa as Nuclear Stain for Cytological Buccal and Cervical Smear. International Journal of Current Science Research and Review, 6(1), 561-566