FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 6, December, (Special Issue) 2024, pp 239 - 241
DOI: https://doi.org/10.33003/fjs-2024-0806-2897

BUCCAL CELL STAINING EFFECT OF *LAWSONIA INERMIS* AQUEOUS LEAF EXTRACT

Salomi, S. S., *Nathan, I. D., Sani, H. G., Joseph, V. Z., Zainab, M. G. and Sunday, J. M.

Department of Human Anatomy, Faculty of Basic Sciences, University of Maiduguri, P.M.B 1069, Nigeria.

*Corresponding authors' email: salomi.simon85@gmail.com Phone: +2347066762191

ABSTRACT

Lawsonia inermis (henna) is a perennial shrub or plant belonging to the *Lythraceae* family. Hands, nails, fingers, and even hair can be dyed using henna plants, which are a natural colour. The study aims to examine the use of *Lawsonia inermis* aqueous extract (local stain) as a substitute for methylene blue in the demonstration of buccal swabs in people. Twelve volunteers offered their time to help gather buccal mucosa samples. The volunteers used tap water and normal saline to rinse their mouths. A wooden spatula was used to scrape the cheeks' buccal surface. Each was applied on a glass slide after being diluted with regular saline. 70% alcohol was used to fix the smear, and it was then let to dry. Methylene blue and *Lawsonia inermis* aqueous extract were used to stain the smears. Squamous epithelial cells' cytoplasm and nucleus were visible on methylene blue-stained slides. There was no nuclear staining in the squamous epithelial cell stained with *Lawsonia inermis* aqueous extract. Nevertheless, it stained the nucleus when counterstained with hematoxylin. *Lawsonia inermis* plus hematoxylin-stained buccal smear present a better result compared to the *Lawsonia inermis* plus eosin-stained slides. Hence, it can be counterstained with hematoxylin to demonstrate buccal cells.

Keywords: *Lawsonia inermis,* Buccal smear, Methylene blue, Hematoxylin, Eosin

INTRODUCTION

The complexity of tissue was highlighted by the range of cell sizes and configurations; staining cells has several benefits, including enabling visualization, identification, contrast, diagnosis, and other uses (Chrischelle & Romnick 2022). Taking a sample of cells from the mouth for research is known as a buccal smear, or cheek cell (Eslam *et al.,* 2021). Methylene blue is the most effective dye for check cells (Chrischelle & Romnick 2022). Potential sources of natural dyes with varying colour intensities, ranging from yellow to red to black, include plant and fruit extracts. Furthermore, because they are organic, herbal, and environmentally friendly, they pose no threat to the environment and their users (Chrischelle & Omnic, 2022). *Lawsonia inermis* (*Lythraceae*) includes the perennial plant or shrub known as henna (Barde et al., 2021). Common names for the plant include Laali in Yoruba, Lalle in Hausa, Nchanwu in Igbo, and Nalle in Kanuri. It can be found in sub-tropical and tropical regions of the Middle East, Africa, Southern Asia, and Northern Australia (Odigie *et al.,* 2022). Lawsone, also known as hennotannic acid, is an aromatic naphthoquinone that gives henna its staining properties. Lawsone is the source of the reddish-brown dye used as a cosmetic agent and is present in considerable amounts in the dried leaves (Barde *et al.,* 2021). Hands, nails, fingers, and even hair can be dyed using henna plants, which are a natural colour. It is applied to the hands and feet as a fertility symbol in Indian traditional medicine (Fareha, 2016). Additionally, it is used in the textile industry to dye wool and nylon and has long been used to cure many diseases (Alawa *et al.,* 2015). Natural dyes such as *Lawsonia inermis* are vital because synthetic dyes are expensive and pose environmental risks. Applying a natural dye, such as *Lawsonia inermis* to stain different biological tissues will be cheap and have a smaller impact on people and the environment (James *et al.,* 2017). The study aims to explore the use of *Lawsonia inermis* aqueous extract (local stain) as a substitute for methylene blue in buccal cell staining.

Figure 1: *Lawsonia inermis* plant (Source: Varghese *et al.,* 2010)

MATERIALS AND METHODS

Materials

Lawsonia inermis, containers, weighing scales, knives, sieves, trays, an oven machine, glass slides, coverslips, kits, a dissecting set, needles, syringes, pipettes, masking tape, surgical hand gloves, surgical blades, slide racks, a stopwatch, plain bottles, pins, distilled water, forceps, normal saline, buccal smears, hydrogen peroxide, alcohol, methylene blue stain, and microscopes are among the materials used in the study.

Authentication and collection of plant materials

We bought *Lawsonia inermis* in the Monday market in Maiduguri, Borno state. A taxonomist from the University of Maiduguri's Faculty of Pharmacy identified it (UMM/FPH/LYR/001).

Preparation of the plant extract

The extraction was conducted using the procedure described by Raju *et al.* (2018), which involved soaking 100 g of dried powdered *Lawsonia inermis* leaf in 1000 ml of distilled water. The solution was left to stand for twenty-four hours while being stirred periodically. After filtering the mixture, the filtrate was evaporated at 45°C in an air oven. Before usage, the dried residue was scraped off and kept in an airtight, dry container.

Buccal smear collection

Twelve volunteers offered their time to help collect buccal mucosae. The volunteers rinsed their mouths with water and normal saline before smear collection. A wooden spatula was used to scrape the cheeks' buccal surface and smeared on a glass slide after being diluted with normal saline. After fixing the smear with 70% alcohol, it was left to dry for ten minutes.

Staining

Four groups of three each were created from the twelve glass slides. For ten minutes each, the groups were stained with methylene blue, *Lawsonia inermis* (LI) aqueous extract, LI and eosin, and LI and hematoxylin, respectively. They were examined under a microscope after being coated with dibutyl phthalate polystyrene xylene (DPX). AmScope was used to take photomicrographs at a magnification of x400.

RESULTS AND DISCUSSION

Results

Squamous epithelial cells' cytoplasm and nucleus were clearly distinguished on methylene blue stain slides, with the latter staining dark blue and the former pale blue (Figure 2A). The cytoplasm of the squamous epithelial cell stained with *Lawsonia inermis* aqueous extract was pale yellow, but the cell's nucleus did not exhibit any staining (Figure 2B). When the cytoplasm was dyed pale pink, the LI-stained squamous epithelial cells counterstained with eosin also displayed deep pink nuclear staining (Figure 2C). On the other hand, hematoxylin counterstaining revealed a purple-stained nucleus and pale-yellow cytoplasm (Figure 2D).

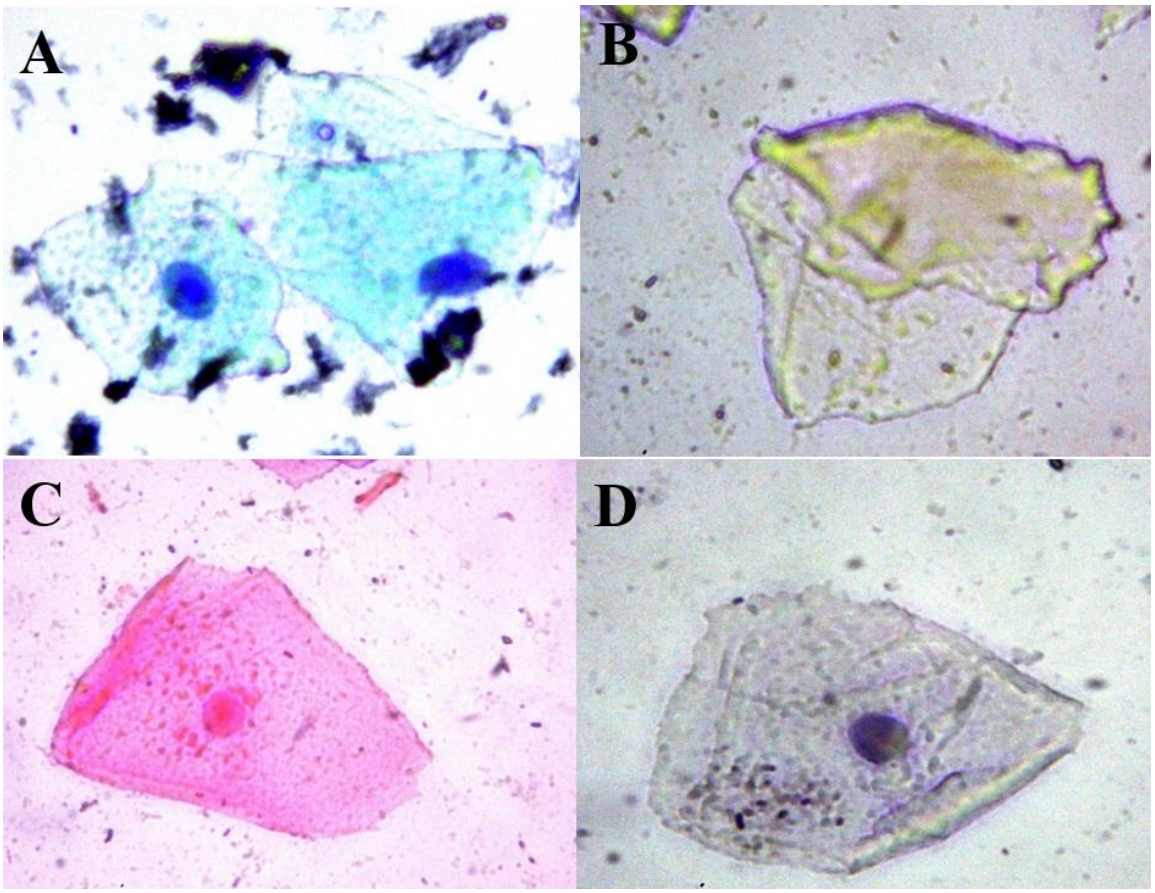

Figure 2: Methylene blue-stained photomicrograph of a buccal smear displaying deep blue nucleus and pale blue squamous epithelial cell cytoplasm in A. *Lawsonia inermis* aqueous extract displaying squamous epithelial cells that are pale yellow and devoid of nuclear, B. The cytoplasm of the squamous epithelial cells is stained pale pink by *Lawsonia inermis* counterstain with eosin and the nucleus-stained deep pink, C. Hematoxylin counterstaining reveals a purple-stained nucleus and pale yellow-stained cytoplasm of the squamous epithelial cells, D (x400 magnification).

Discussion

Staining tissue samples with histological stains for microscopic examinations is one of the technical procedures involved in histological staining (Alturkistani *et al.,* 2016). Staining has grown in importance due to its relevance in medical education and disease diagnosis (Dibal *et al.,* 2020). Cells are stained blue by the cationic dye methylene blue. Since the positively charged, dye is drawn to negatively charged particles like DNA and RNA, the staining phenomena are brought on by the presence of negatively charged molecules in the cell (Lillie, 1991). To assist the economy and lessen the cost burden on citizens seeking health care and medical personnel by lowering the hazards associated with synthetic dye, this study uses local stain (*Lawsonia inermis* aqueous extract) as an alternative to methylene blue in demonstrating buccal smear. *Lawsonia inermis* is a common ornamental stain that gives skin, hair, and nails a reddish-orange to brown hue. It is a biological stain for oral tissues, gastrointestinal specimens, liver and kidney tissues, tonsils, lungs, epithelial cells and cancer cells (Chukwu *et al.,* 2011; Raju *et al.,* 2018). Natural dyes, such as *Lawsonia inermis*, are vital because manufactured dyes are expensive and pose environmental risks. When used as a primary stain, *Lawsonia inermis* (henna) was observed to stain only the cytoplasm but not the nucleus. This is consistent with the fact that *Lawsonia inermis* is an acidic stain and, therefore, could only stain the alkaline cytoplasm and not the acidic nucleic component of the tissue (Okolie *et al.,* 2021). Because *Lawsonia inermis* stain does not obscure hematoxylin colour, there is a good contrast between the two, allowing for the morphologic identification of epithelial and connective tissue elements. Compared to hematoxylin and eosin staining, the staining time for *Lawsonia inermis* and hematoxylin is short (Chukwu *et al.,* 2011). The *Lawsonia inermis* plus hematoxylin-stained buccal smear presents a better result compared to the *Lawsonia inermis* plus eosin-stained slides, suggesting that *Lawsonia inermis* can be counter-stained with hematoxylin for demonstrating buccal smear. Because *Lawsonia inermis* is cheap and readily available with little or no environmental hazard, it can be counterstained with hematoxylin to demonstrate oral tissues.

CONCLUSION

Lawsonia inermis is a natural dye that can be used to stain tissues. Because it does not obscure the hematoxylin colour, there is a good contrast between the two stains. As a result, it can be counterstained with hematoxylin to demonstrate oral tissues. Natural dyes, such as *Lawsonia inermis*, are vital because manufactured dyes are expensive and pose environmental risks.

REFERENCES

Alawa, J.N., Gideon, G.O., Adetiba, B. and Alawa, C.B. (2015). Comparative tissue stainability of Lawsonia inermis (Henna) and eosin as counterstains to hematoxylin in brain tissues. *Microscopy and Microanalysis,* 21(2), 343-350.

Alturkistani, H.A., Tashkandi, F. M. and Mohammedsaleh, Z.M. (2016). Histological Stains. A Literature Review and Case Study. *Global Journal Health Science*,8(3),72-79.

Barde, I. J., Kamdip, S.N., Gushe, D. D. and Dashe, Y. G. (2021). Assessing the Use of *Lawsonia inermis* and *Hibiscus sabdariffa* Aqueous Extracts as a Possible Substitute for Eosin Stain in Paraffin-embedded Tissues. Internal. *Journal of Medical Science and Academic Research,* 2(2), 5-12.

Chrischelle, F.B. and Romnick, Z. (2022). Screening of Lipote (*Syzigium currannii*) pure fruit extract for use as cheek cell and onion cell stain. *Journal of Biodiversity and Environmental Sciences,* 20 (5), 52-56.

Chukwu, O.O., C. Odu, C. E., Chukwu, D.I., Hafiz, N., Chidozie, V.N. and Onyimba, I.A. (2011). Application of extracts of Henna (*Lawsonia inermis*) leaves as a counter stain. *African Journal of Microbiology Resource,* 5(21),3351–3356.

Dibal, N.I., Garba, S.H. and Jacks, T.W. (2020). Morphological Assessment of Epididymal Sperm in Wistar Rats Using Different Histological Stains. *Acta Veterinaria Eurasia,* 46, 132-136.

Eslam, M. I., Aisha, A.A., Anfal, E. and Sahar, A.Z. (2021). Assessment of the ability of extracts from *Lawsonia inermis* (Henna) as a stain for cytology smears. *International Journal of Academic Health and Medical Research,* 5(8),45-55.

Fareha, M. (2016). Effect of Aqueous Extract of *Lawsonia Inermis* Leaves on The Liver Following Acute Ethanol-Induced Hepatic Damage in Wistar Rats. Ph.D. Thesis, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria Nigeria.

James, O.A., Khadijah, K. M., Ejike, C. E., Ijeoma, M. U. (2017). A study of various modifications of *Lawsonia inermis* (Henna) leaf extract as a cytoplasmic stain in liver biopsies. *Journal of Research in Medical Sciences,* 5(3),1058-1065.

Lillie, R.D. (1991). H.J. Conn's Biological Stains, 9th ed. Williams & Wilkins Company, Baltimore, MD. Reprint by Sigma Chemical Company.

Odigie, E.B., Ehimigbai, R.O.A. and Aliu, H.S. (2022). *Lawsonia inermis* (Henna) Lyophilized extracts as alternate stains may deter staining efficacy in histology sections. *Journal of Pure and Applied Science*, 8(3a),1-7.

Okolie, N., Ngokere, A.A. and Enweani, I.B. (2021). Staining potentials of Indigenous *Hibiscus saddariffa calyx*, *Lawsonia intermis* leaves and *Vitex doniana* stem Barks on liver and kidney tissues. *Journal of Cytology and Histology,* 12(3),1-9.

Raju, L., Nambiar, S., Augustine, D. S.V., Haragannavar, V. C .and Babu, A. (2018). *Lawsonia inermis* (Henna) extract: A possible natural substitute to Eosin stain. *Journal of Interdisciplinary Histopathology,* 6(2),54.

Varghese, J.K., Silvipriya, K.S., Resmi, S. and Jolly, C.I. (2010). *Lawsonia inermis* (Henna): A natural dye of various therapeutic uses - A Review. *Cosmeceuticals,* 1(1), 1-5.