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# SUBSTITUTION OF VARIOUS MEDICAL LABORATORY STAINS WITH LOCAL PRODUCTS IS POSSIBLE IN NIGERIA.

**Uchejeso M. Obeta[1]*, Godfrey D. Dajok[1], Obiora Ejinaka[2], Ukpabi Maduka[3], Achagh, J. Iember[1], Muhammad Akram[4]**

1. Department of Medical Laboratory Management, Federal School of Medical Laboratory Science, Jos-Nigeria.
2. Department of Medical Microbiology, Federal School of Medical Laboratory Science, Jos-Nigeria.
3. Department of Medical Parasitology, Federal School of Medical Laboratory Science, Jos-Nigeria.
4. Department of Eastern Medicine, Government College University Faisalabad-Pakistan

**Email id: uchejesoobeta@gmail.com**

## ABSTRACT

100% of all medical laboratory stains used in Nigeria and mostly in West Africa are imported. Such stains used in histology, parasitology, bacteriology and heamatology laboratories such as haematoxylin, eosin, gram stain, field stain, giemsa stain, methylene blue, crystal violet, malachite green, basic fuchsin, and others stains have been imported. This volume of stain importation has consumed a lot of financial resources that could construct the stain manufacturing plants, create employment and save surplus financial resources for medical laboratory business. Local plants and vegetables which can be can be used to replace some medical laboratory stains if adequate mordants, oxidizing agents and solvents are discovered and used may include: Turmeric (*curcuma longua* liun), Henna (*Lawsonia inermis*), Surreal (*Hibiscus sabdariffi*), Beetroot *(Beta vurgaris*), Kolanut (*Cola acuminata*). This calls for further studies, validation, production and registrations so as to provide substitutes for current available stains in commercial quantities.

***Keywords***: Nil

## Introduction

There are several stains ranging from basic, acidic and neutral stains used in medical laboratories for diagnosis. They are synthetic in nature and mostly produced abroad. The synthetic dyes used in Medical laboratories could be replaced with some local products that are around us. The dyestuffs are obtained from a natural source, that is, from plants or vegetables and sometimes animals. Pigments are the specific chemical compounds responsible for the visible colour in plant parts (Green 1995). In histopathology, the most commonly used dye or stain is *haematoxylin*, it is obtained from a South African Tree known as Logwood (*Haematoxylium campechianum*). Studies conducted in Nigeria by Avwioro et al. (2005), showed that, the red dyestuff obtained from *pterocarpus osun* species was used in staining tissue sections for histopathological diagnosis of diseases. Microbial stains are used to impact colour in order to make the cells and tissues more distinct.

Most dyes in current use are chemically synthesized. Besides been expensive, they are also hazardous to human health (Bhuyan & Saikia 2004). Some dye components are carcinogenic or at least strongly allergenic resulting in their withdrawal as their hazard becomes recognized (Bhuyan & Saikia, 2004, Blender, 1982).

At the height of technological advancement and mass production of goods, people nowadays prefer to use natural and organic if given a chance to choose against the commercial, chemically-synthesized products. Aside from reducing if not avoiding skin or body parts irritation, when they are used, the natural organic products are environmental- friendly aside from the fact that they are more economical. The turmeric or *Curcuma longa linn* is an example of the plant that a natural product can be derived. It is a tropical & sub-tropical perennial herb belonging to the family zingiberaceae which is related to ginger.

There are evidence, people have dyed fabrics & woods using locally available materials which produce brilliant permanent colors (Ashis and Adwaita, 2011). Majority of natural dyes are vegetable dye obtained from plant parts such as backs, barriers, leaves, roots and fruits or seeds. (Grubben & Deton, 2004) which have long history of been used traditionally as dye for fabric and woods. Examples are cochineal and leg wood or blackwood or blackwood tree or bluewood campeachy tree or campeachy wood or campeachewood or Jamaica wood or simply logwood tree (*haematoxylum campecianum*). *Hematoxylin* has been imployed in pre-clinical and clinical histological and histopathological routine procedures as stain for tissue to aid disease diagnosis.

**Stains** are generally used to add color to plant and animal tissues, microbes and spores to make them optically distinct (Korade et al.,2014).

**Some Local products that can be used as substitutes for laboratory stains:**

1. **Kolanut** (*Cola acuminata*): The natural extract from kolanut could be used to stain cytoplasm of various tissues with yellowish brown-coloration.The findings made by Shehu et al.,(2012) Kolanut can be used as an alternative dye for histological staining. E.g. Eosin.
2. **Surreal** (*Hibiscus sabdarriffa*): It belongs to Malvaceae family, which is a true Roselle Plant, a very important dye-yielding annual perennial plant variety. The aqueous extract of H. *sabdariffa* is acidic & red in colour. The plant has many industrial, medical, & nutritional uses. (Ihuma et al., 2012). Investigating the potential of methanolic extracts from *H. Sabdariffa* for staining fungal species. Apergillus Niger, rhizopus stonuifer & penecillium notatum were stained with methanolic extracts from *H. sabdariffa* and compared microscopically with lactophenol-in-cotton blue. *H. sabdariffa* preparations appear more contrasted as compared to lacto phenol -in-cotton blue stained preparations. So they concluded that methanolic extracts from *H. sabdariffa* could be used as a mycological stain (Abubarka etal, 2012).
3. **Henna** (*Lawsonia inermis*): It belongs to the lythraceae family, is a small shrub used as a cosmetic for coloring hair and decorating palm of the women (called as "mehandi" in India). Henna gives a red orange dye molecule, "Lawsone" (2-hydroxy-1, 4-napthaquinone), which is known as hennatonnic acid, that has affinity for bonding with protein and also have a fast-dyeing property. Henna leaves extracts oxidized with potassium permanganate can replace counter stains (neutral red or safranin) in gram staining reaction (Hafiz etal., 2012, Ma'aruf etal., 2020). Adisa etal (2017) stated that because of tannin, it can adequately replace safranin. Fresh Leaves of Henna (*L. Inermis*) identified by a taxonomist in the federal college of Forestry Jos, Nigeria, were collected from a farm in Dass town of Dass Local Government Area of Bauchi State, Nigeria. Henna leaves aqueous (hot & cold) extracts oxidized with potassium permanganate impart a better staining reaction with *Lactobacillus species* and *Escherichia coli.* This is an agreement with staining theory by Carletan (1994) that natural dye need to be ripened by oxidation either through natural means or by addition of chemical oxidants.
4. **Turmeric** can also be called *curcuma longa linn*. It is a yellow rooted shrub which is an example of the plant that a natural product can be derived.It is cultivated in Plateau State in Nigeria. Both Turmaric powder and roots are found in Taminus, Feringada and Building materials markets in Plateau State, Jos, Nigeria. Turmeric as the alternative stain is interesting. Its extract could be used in place of Eosin stain since it is acidic and can stain basic parts of the cell like Eosin. The study conducted by Kumar et al. (2014). Revealed that natural tint from turmeric could stain tissues such as collagen muscle fibers. In the investigations made by Bassey et al., (2012) and Inbnouf (2017), they concluded that turmeric has good potential and a promising

histological dye that can excellently replace eosin stain in the Hematoxylin and Eosin routine. In my study, it could be Turmeric and hematoxylin (H and T) (Meenakshi, etal., 2021, Rubina etal., 2020, Kuma etal., 2014,)

5. **Beetroot (***Beta vurgaris*) is a purple root vegetable plant cultivated mostly in the North Eastern parts of Nigeria and also in Plateau State and transported to other parts of the country. Beetroot is cherished for its nutritional & medicinal values thus it is used in cuisines, salads and juices. It produces purple to red color. The essential processes change from purple to red color. The essential process of staining tissues shares certain similarities, in the dyeing process, the dye is placed in a pot of water then the fabric to be dyed is added to the pot & heated with intermittent string until the color is transferred (Seong-il et al., 2001). While histological staining techniques involves stepwise tissue processing stages undertaking in different media & timed to allow tissue/cell components to acquire the specific color (s). mordants such as salts, alum, vinegar and ammonia solution may also be required for some histological stains or natural dye to bind the dye to the textile. The beetroot dyeing process may require alum or vinegar mordant to enhance the color intensity & durability, however, this depends on the material to be dyed (Finbarrs-Bello etal., 2019, Seong-il et al., 2001). Haematoxylin and beetroot extract have similar staining effects by staining tissue. Haematoxylin is a basic dye which stains the nucleus and some parts of the cytoplasm containing the nucleic acid or acidic structures bluish purple or black in some instance. This suggest that beetroot extract exhibited hematoxylin like staining effect. On the contrary, eosin, an acidic dye will stain basic structures deep pink color. The exception to these are neutral cellular and extracellular components that take up neither of these stains and appear relatively clear (Anneh et al., 2006). This implies that the extracts having exhibited hematoxylin property, beetroot extract can be used in combination with eosin (B & E).

Table 1 presents some possible medical laboratory stains substitutes. It is therefore very imperative to study further on their nature, pH, acceptable mordant, solvent and staining colours that adequately replace the synthetic dyes currently in use.

**Some Advantages of local products extracts over synthetic dyes.**

- Extracts from local products are not expensive while, synthetic dyes are expensive (Aguoru et al., 2012).
- Local products extracts are not hazardous to human while, synthetic dyes are hazardous. (Braide et al., 2011) and animal health (Suryawanshi et al., 2017).
- Some chemicals needed have been scientifically proven to be lethal (Alturkistani et al., 2016) while, local products are not. Therefore, natural plant dyes have gained global interest and give promising tissue staining results when tested.

- Synthetic dyes displayed environmental threats while local products extracts are environmentally friendly.
- This evoked the desire of researchers to come up with a biological stain made of eco-friendly and biodegradable materials.
- Besides, as many developing countries cannot afford the expense of synthetic dyes, the use of low-cost, natural colors from plants is observed as the substitute for synthetic dyes. Based on the above-mentioned reasons, the staining potentials of *curcumin* turmeric was examined as a natural dye in the histological application.

Furthermore, Natural dye from *curcuma longa linn* provides a significant alternative as they are safer to use without health hazards, biodegradable, have easy disposability and can be used as compost material for farming purposes after the necessary components have been extracted (Suryawanshi et al., 2017).

**The Qualities of good stains:**

An excellent biological stain must be effective, inexpensive, less poisonous and the source must be accessible. Almost all stains have specific mechanisms which could be used both in plants and animal tissues (Aguora et al., 2015). There are two primary forms of stains based on their origin; synthetic and natural. Synthetic dyes are produced by combining chemicals from petroleum sources (Shehu et al., 2012). It exhibits excellent fastness properties, available in most synthetic dyes and are expensive (Aguoru et al., 2016), desperate to prepare, allergenic, cacinogenic (Bordoloi et al., 2017) and severely detrimental to the environment that can reduce soil fertility (Korade et al., 2014).

A good stain would be either basic, acidic or neutral in nature. Their reactions with mordants, solvents, oxidants or accentuators presents their qualities. The chemicals used in modifying the henna stain solutions either as oxidants or accentuators did not show staining reaction with gram negative bacteria. This may be attributed to their pH and color combination they produced when combined with henna extracts. It was observed that the aqueous extracts (both cold and hot) oxidized with potassium permanganate have a neutral pH as well as better color combination. This might be the reason why it gives a better staining reaction with *lactobacillus species*, because according to chemical staining theory, the coloring matter of dye is contained in the basic part of the compound, while the acidic radical is colorless and vice versa for acidic dyes as reported in Anonymous (2012). It therefore follows that acidic elements will have affinity for basic stains as stressed by Ochei and Kolhatkar (2005). Therefore bacterial cells being rich in nucleic acid have a high affinity to basic dye, hence it was stained by solution with neutral pH which is closer to basic pH and refused to pick the solutions (henna extract) with acidic pH. This is also evident from the pH of usual counter stains used in Gram stain techniques which are highly basic. So, Henna leave extract can be used as a counter stain in Gram staining in place of saffranin or neutral red.

**Conclusion:**

From the brief review of some researches carried out using local products of different types to substitute various medical laboratory stains, beetroot, (*Beta vulgaris*) turmeric(*Curcuma longa Linn*), henna (*Lawsonia inermis*), Surreal (*Hibiscus sabdariffa)* and Kolanut *(Cola acuminata)* are alternatives of Haematoxylin, Eosin, safranin, neutral red & lactophenol-cotton-blue respectively.

**Table 1. Synthetic dyes/stains and their possible replacement with local products.**

| SN | LOCAL PLANT/VEGETABLE | EXISTING STAIN |
|---|---|---|
| 1 | Kolanut (*Cola acuminata)* | Eosin |
| 2 | Beetroot (*Beta Vulgaris)* | Haemotoxylin |
| 3 | Tumeric *(Curcuma longa linn)* | Eosin |
| 4 | Henna *(Lawsonia Inermis)* | Neutral red/Safranin |
| 5 | Surreal *(Hibiscus Sabdariffi)* | Lacto-phenol cotton blue |
| 6 | Osun plant (*Pterocarpus osun)* | Haemotoxylin |
| 7 | Strawberry(*Fragaria ananassa)* | Neutral red |
| 8 | Tomatoes(*Solanum lycopersicum)* | Neutral red |
| 9 | Blackcurrant (*Ribes nigrum*) | Safranin |
| 10 | Onion (*Allium sepa*) | Crystal violet |

**Recommendations:**

1. More local products should be researched into, especially the coloured products such as tomatoes, blackcurrant, onions, black fruits, honey & other colored products should be the targets.
2. More research towards discovery of adequate solvents, oxidizing agents and extraction methods should be carried out massively on noted plants and vegetables.
3. Medical Laboratory Scientists should standardize the use of these local products for wider application and acceptability by the global community.
4. Research authorities should throw their weight behind such areas of research because, it brings about local innovations & discovery in especially developing nations.
5. Government should initiate or support policies towards local content in the aspect of production of medical laboratory stains and other reagents in Nigeria and Africa in general.

**REFERENCES**

Abubakar, S., Usman, A.B., Etim, V., Nandi, O., Alaku, C. (2012); Application of organic dyes from roselle calyx *(Hibiscus sabdariffa*

*linn*) for mycological staining. India Journal of Innovation & Developments.

Adisa JO, Musa KK, Egbujo EC, Uwaeme JM. A study of various modifications of Lawsonia inermis (Henna) leaf extract as a cytoplasmic stain in liver biopsies. Int J Res Med Sci 2017;5:1058-65

Aguoru. C.U., Okelionwa, S., Olasan, J.O. (2016), value of *hibiscus rosa-sinensis* petal extract as biological stain in plant systematic research Journal of chemical, biological & physical sciences.

Alturskistani, H., Tashkandi, F., Mohammed-Saleh, Z. (2016); Histological stains; A literature review case study. Global Journal of Health Sciences.

Anneh, H., Mohammed, J.G., Ghorbani, R, Khazaei, M. (2006). Natural Dye for staining Astrocytes and Neurons, Journal of Neurological sciences.

Anonymous, (2009). Encarta Eneyclopedia. Microsoft Encarta Corporation Inc. Retrieved September 16th (2012).

Ashis KS and Adwaita K. (2011). Dyeing of Textiles with Natural Dyes, Natural Dyes, Dr. Emriye Akcakoca Kumbasar (Ed.), ISBN: 978-953-307-783-3, InTech, Available from: http://www.intechopen.com/books/natural-dyes/dyeing-of-textiles-with-natural-dyes

Avwioro, O.G., Aloamaka, P.C., Ojianya, N.U., Oduola, T and Ekpo, E. (2005). Extracts of prerocarpus osun as a histological stain for collagen fibers. African Journal of Biotechnology.

Bassey, R. Oremosu, A. Osinubi, A. (2012); *Curcuma longa*: *Linn:* staining effects on histological morphology of the testis. Mecedonium journal of medical sciences.

Bhuyan, R., Saikia, C.N., (2004). Isolation of color components from native dye bearing plants in North Eastern India.

Blender, A. E. (1982). Dictionary of Nutrition & Food Technology, 5th Edition, Redwood Burn Limited, London, p 32.

Bordoloi, B., Jaiswal, R., Siddiqui, S., Tandon, A. (2017). Health Hazards of special stains. Saudi Journal of pathology & microbiology.

Braide, W., Akobundu, C, Nwaoguikpe R.N., Njiribaeko, L.C., (2011); The use of extracts from four local Nigerian plants for the staining of microbiology research.

Carleton, H.M., Drummy, RA.B., Willingon, E.A. and Cameron, R. (1996). Histological technique 4th edition. Oxford University press London.

Chairman, K., Jayamala, M., Christy, V.R., Singh, R. (2015); Play to chemical screening & antimicrobial activity of *curcuma longa* natural dye. Gen. Med: An open Access.

Finbarrs-Bello E , Ojo OP , Agu Ck . (2019). A Bioprospective Study on Histological Staining Potential of Beta Vulgaris (Beetroot) Extract. Journal of Experimental Research. 7(4)

Green, C.L. (1995). Nutritional colorants & Dyestuff: A review of production markets & Development potential. Published by the Food & Agricultural Organization of the United Nations, Rome, Italy, pp-1-34.

Grubben, GJ.H., Denton, O.A. (2004) Plant Resources of Tropical Africa Vegetables.

Hafiz, H.1., Chukwu O. O. C., Nura, S. (2012). The potentials of henna (*Lawsonia inamis l*.) Leaves extracts as counter stain in gram staining reaction. Bayero Journal of Pure and Applied Sciences, 5(2): 56 – 60. http://dx.doi.org/10.4314/bajopas.v5i2.10

Ihuma, J., Asense, G., Abioye, K. and Dick, S. (2012). "Application of Methanolic Extracts from *Hibiscus Sabdariffa.* As a Biological Staining Agent for some Fungal species: International Journal of plant, animal & Environmental Science. .

Korade, D., Lalita, S., Deepika, M., (2014). Application of aqueous plant extracts as biological stains. International Journal of Scientific & Engineering Research.

Kumar, S., Singh, N., Singh, A., Singh, N., Sinwa, R. (2014). Use of *curcuma longa L*. extract to stain various tissue samples for histological studies. An international quarterly journal of research in Ayurveda.

Ma'aruf S. Y., Mohammed M. O., Muhammad A. T., Okechi O. O., Tsamiya R. I., Abubakar U., Mohammed I., Umar A., Sani S. M., Kabir H., Bello B. A., Garba S., Abubakar S. D. (2020). Hibiscus sabdariffa Extract in Demonstration of Selected Bacteria in Tissue Sections of Wistar Rats. Microbiology Research Journal International. 30(2): 26-33 DOI:10.9734/MRJI/2020/v30i230195

Meenakshi, etal. (2021). A comparative cytological study of Eosin & eco-friendly cytoplasmic stains –A Pilot Study."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), 20(04), 2021, pp. 44-49.

Ochei, J. and Kolhatkar, J. (2005). Medical Laboratory Science Theory & practice 3rd reprint pp 441.

Ragaswami, G and Bagyaraj, D.J. (1993). Agricultural Microbiology, 2nd edition, Practice-Hall of India private Limited, New Delhi, India. Pp. 18-29.

Rubina MP, Ashida M Krishnan, Riyas Basheer KB, Mohammed Safeer TK, Soumya V. (2020). Assessment of Staining Quality of Curcumin as a Substitute for Eosin in Hematoxyline and Eosin Staining in Histopathology, J Res Med Dent Sci, 2020, 8(5): 146-150

Seong-il E, Dong-yoon S, Keo-jong Y. (2001). Improving the dye ability of natural colourants on cotton by cationization. Indian journal of fibre and textile research, 26(4)425-431

Shehu, S.A., Sonfadaa, M.L., Danmaigoroa, A., Umaru, A.A., Henaa, S.A., Wiamb, I.M. (2012) kola nut (cola acuminata) extract as a substitute to Histological tissue stain

eosin. Scientific Journal of Veterinary Advances.

Suryawanshi, H., Naik, R., Kumar, P., Gupla, R. (2017). *Curcuma longa* extract-Haldi: a safe, eco- friendly natural cytoplasmic stain. Journal of Oral and Maxillo Facial pathology. Staining Property of Alcoholic and Aqueous