REVIEW

Natural Alternatives to Chemical Staining in Routine Histopathology - A Narrative Review

Sayem Anwarhussain Mulla[1], Aishah Ansari[1], Sarita Shrivastava[2], Minakshi Bhattacharjee[3], Ashwini Panchmahalkar[3]

Abstract

Introduction: Histopathology is the study of diseases accomplished by fixing tissue onto a glass plate using dyes or stains under a microscope. The commonly used dyes in routine histopathology are Hematoxylin and Eosin (H and E) stain, Gram stain, silver nitrate, Mason's trichrome stain, Periodic acid–Schiff (PAS) stain, etc. However, one major drawback of these stains are toxicity due to chemicals used.

Objectives: The present article reviews the various natural stains that can be used as an alternative to the routinely used stains.

Materials and Methods: Data was collected by electronic search of databases including PubMed and Google Scholar for "natural stains", "natural staining" and "natural stains AND histopathology".

Results: Routinely used stains demonstrate excellent tissue coloring capacity. However, the major drawbacks of routine stains and dyes include use of chemical ingredients, high cost, and some extent of tissue damage. These issues can be resolved by using natural substitutes like turmeric, ginger, henna, beetroot, wood dust etc. Over the past few years, interest in natural options to chemical dyes has grown.

Conclusion: This article has highlighted major natural staining alternatives with their advantages and disadvantages along with their efficacy in staining routine histopathological tissue specimens.

Keywords: Histopathology, Staining, Dyes, H and E Stain, Hematoxylin, Eosin, Turmeric, Ginger, Natural, Histology, Pathology.

Introduction

Histopathology is the microscopic examination of a biopsy or tissue specimen that has been dyed and fixed on a glass slide to examine diseases and their characteristics. Typically, the dye aids in visualization of the tissue specimen under a microscope. The main goal of this procedure is to visualize the suspected cells for any alterations and to reveal the disease's characteristic features. This is usually accomplished through staining and counterstaining.[1]

In healthcare systems, histopathology is an important diagnostic tool. It combines the principles of normal with abnormal thereby, enabling healthcare professionals to observe changes in normal tissues during, or as a result of the pathological process. It also allows the identification of the etiological or causative agent in cases where unaided vision is insufficient.[2]

Clinicopathological correlation is a crucial component of health sciences that can be carried out by health care personnel. The medical staff can search for cellular alterations with the aid of an adequate clinical examination and fine histopathology. The probable cause, diagnosis, and therapy of the condition may then be determined by using this information to provide scientific reasons for the symptoms a patient is experiencing. Histopathology thus expands and develops therapy choices, enabling doctors to provide better patient care.

The main protocol followed in histopathology is staining and counterstaining the tissue specimen using certain stain-

[1]Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Sector-7, CBD-Belapur, Navi Mumbai, Maharashtra, India – 400614; [2]Department of Pathology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Sector-7, CBD-Belapur, Navi Mumbai, Maharashtra, India – 400614; [3]Department of Microbiology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Sector-7, CBD-Belapur, Navi Mumbai, Maharashtra, India – 400614.

Corresponding author: Aishah Ansari, Student, Bachelor of Dental Surgery, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Sector-7, CBD-Belapur, Navi Mumbai, Maharashtra, India – 400614, Email: ansariaisha377@gmail.com

How to cite the article: Mulla SA, Ansari A, Shrivastava S, Bhattacharjee M, Panchmahalkar A. Natural Alternatives to Chemical Staining in Routine Histopathology - A Narrative Review. Oral Maxillofac Pathol J 2024; 15(1). Page number 99-104.

Source of support: Nil

Conflict of interest: None

ing agents. While the stains impart colour to the cell, the counterstain dyes the background. Staining is used to highlight important features of the tissue and to enhance the tissue contrast. Key steps in the procedure are fixation, dehydration, clearing, processing, embedding, sectioning and staining.[3]

Commonly Used Stains in Routine Histopathology:

Some of the commonly employed stains in routine histopathology are mentioned in Figure 1[3].

Nearly all the tissue specimens today are stained using H and E stain.[4] It is inexpensive, quick, efficient, and adaptable. Gram staining is typically used to stain biopsies of infected tissues and produces results quickly, even when there is a significant difference in disease prognosis and treatment. In modern histology, it is frequently used in conjunction with paraffin fixatives for tissue sectioning.[5] Silver nitrate has been used in staining techniques for a long time and is still used in modern pathology. It was initially used to improve the visibility of tissue structure while studying it, which was accomplished by applying solid silver nitrate to a tissue and then studying it under a microscope.[5]

Drawbacks of Commonly Used Stains and Need for Natural Stains in Routine Histopathology:

Despite the benefits of H and E stain, it cannot reproduce all the minute details and features in a slide, necessitating the use of a special stain.[6] Staining using silver nitrate can lead to an argyrophilic reaction.[7] Gram staining is ineffective against certain bacteria and has limited application in environmental microbiology.[7,8]

The majority of stains currently in use are chemically synthesized from less expensive petroleum byproducts and have been found to be hazardous to human health.[9,10,11] Some synthetic dye components, such as Picric acid in Masson's trichrome and von Geison's stains,[12] have been found to be carcinogenic or allergenic. Their widespread use has resulted in air, water, and land pollution, which is proven to be extremely harmful to the ecosystem and the Earth. Synthetic dyes are non-biodegradable, flammable, and non-renewable.[13]

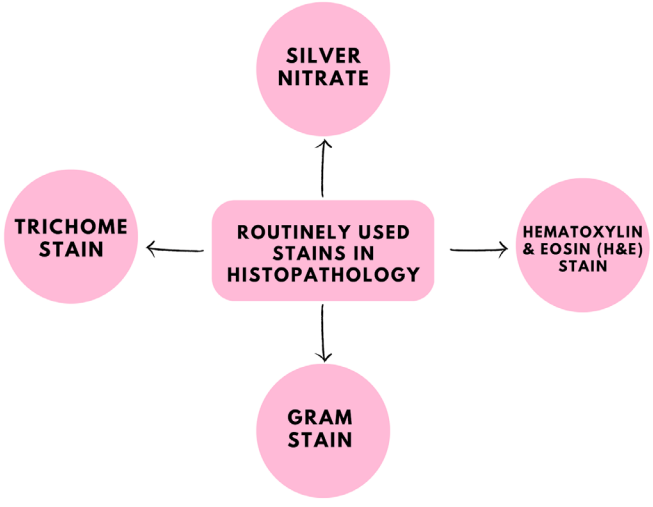

Fig. 1: Routinely used stains in histopathology, including; Silver Nitrate, Hematoxylin and Eosin, Gram and Trichome stains.

Formaldehyde used in routine histopathology is a known carcinogen and allergen to the human body.[14] It can be inhaled during the staining procedure leading to damage to internal organs. In addition to this, Congo red is known to be carcinogenic because of the presence of aromatic amine groups.[15] Toluene and xylene are commonly used solvents which help in fixation of tissue specimens as well as rinsing of the stains. Pathologists, researchers, laboratory workers, laboratory assistants, etc. exposed to toluene and xylene for a long period of time are prone to develop Raynaud's phenomenon which can later develop into systemic sclerosis.[16]

Hence, the utilization and implementation of natural staining techniques in standard histology is urgently needed. A natural stain should have all of the properties of a synthetic dye. Several studies have demonstrated the use of natural dyes in botanical and animal histological studies, with satisfactory results when compared to synthetic stains.[17,18,19] Natural stains can be used in the same manner as conventional stains despite their drawbacks.[20] According to Adeyemo *et. al*., natural dyes are less expensive, more reliable, and easier to use. Their extraction and application do not necessitate the use of skilled hands. They have also been discovered to be eco-friendly and biodegradable, and do not cause harm to the handler.[13]

Different Natural Stains Used as an Alternative in Routine Histopathology:

Numerous studies have used a variety of natural stains as an alternative to the chemical-based synthetic stains used currently (Figure 2).

***Zingiber officinale* Roscoe (Ginger) as a Natural Alternative:**

Ginger has been used to stain sections of the lungs, heart, liver and spleen.[19,21] It is also being tested to stain lichen planus, fibroma, salivary glands and squamous cell carcinoma.[22]

It is freshly extracted from rhizomes of *Z. officinale* and washed off to remove the impurities. This is followed by peel-

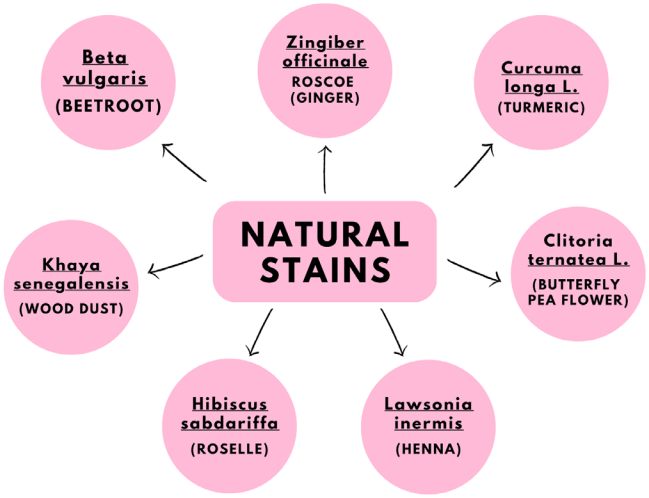

Fig. 2: Natural stains used as an alternative to chemical staining, including; Zingiber officinale Roscoe (Ginger), Curcuma longa L. (Turmeric), Clitorea ternatia L. (Butterfly Pea Flower), Lawsonia inermis (Henna), Hibiscus sabdariffa (Roselle), Khaya senegalensis (Wood dust), Beta vulgaris (Beetroot).

ing and cutting it into smaller pieces. 25 grams of these pieces are mixed with 100 ml of 90% alcohol and set aside for 24 hours. After this, the mixture is filtered to obtain 80 ml ethanolic extract of *Z. officinale* which is then used to stain tissue sections as per routine staining techniques.[18,19]

In the heart tissue sections, muscle fibers were stained yellow and the nuclei took up a deep green color. Respiratory bronchioles consisting of a small number of alveoli and alveolar sacs, portal tract and collagenous tissue in lung and liver tissue specimens were similarly stained. In a spleen tissue section, the central artery was again stained yellow; however, the white pulp and red pulp were not seen or stained clearly.[19] *Z. officinale* contains flavonoids which are phenolic (acidic) compounds.[23] This enables it to stain basic parts of the cell when used as counterstain for hematoxylin where it stains the cells deep yellow. Fibroma tissue sections when stained with *Z. officinale* showed crisp staining.[22]

***Curcuma longa* L. (Turmeric) as a Natural Alternative:**

Turmeric has been demonstrated to stain sections of collagen fibers, blood vessels and red blood cells. Fresh rhizomes of *Curcuma longa* are collected and cut into small pieces which are sun-dried and milled to form fine powder. 20-25 grams of the powder is weighed and dissolved in 100 ml of 70% alcohol, followed by centrifugation at 3000 rpm (rotations per minutes) for 5 minutes. The resultant supernatant fluid is collected and used to stain the tissue sections.[24]

Studies have shown that *Curcuma longa* stains the epithelium and keratin a deep yellow-orange colour, collagen and muscle dull yellow, RBCs exhibit bright yellow and the bone is stained deep yellow. Striations in muscle fibers were well appreciated. Melanocytes appeared bright brown against the light yellow background of the epithelium.[18,25,26] Affinity of *C. longa* for cytoplasm indicates that it is an acidic dye. It is composed primarily of flavonoids and has the same mechanism of action as that of *Z. officinale*, wherein *C. longa*, a typical polyphenol with acidic nature (due to its ability to release hydrogen from the hydroxyl group), enables it to stain the basic parts of the cell.[27]

***Beta vulgaris* (Beetroot) as a Nature Alternative:**

Beetroot has been found to stain oral smears[28] along with tissue components like muscles, mucins, red blood cells, keratin and nerve fibers.[29] It has also been tested to develop a mycolic stain.[30] *Beta vulgaris* has been used to stain liver, cerebral and lung sections31 as well as breast cancer tissue sections.[32]

Fresh *B. vulgaris* is procured and washed with water, peeled off and sliced into small pieces. 50 grams of these pieces are mixed in 100 ml of double distilled water and heated to 40 °C for 30 minutes in a water bath. The red extract is separated and cooled, and then purified by a filtration process (Whatmann filter paper No. 1) followed by centrifugation at 3000 rpm for 15 minutes. The supernatant extract is collected and stored for further use[33]. It may be mixed with glycerol, and citric acid to buffer the solution. This is done to obtain a pH level of 5, which has been proven to be more stable.[34]

The staining intensity and preservation of cellular morphology and details by *B. vulgaris* is said to be similar to routine histopathological stains.[28] In the cerebral cortex, it does not show good staining properties for nucleus even though *B. vulgaris* has good staining capacity for nerve fibers. The nucleus stains brown with distinct alveolar spaces in the lung sections. In kidney sections, the nucleus yet again stains brown, but deeper, and the ground substance along with cytoplasm stains light brown. The cytoplasm is poorly stained light pink, whereas the central vein is stained deep brown in liver sections. Brown stained nuclei and cytoplasm can be observed in distinct layers comprising the mucosa, submucosa, muscular layer and serosa in large intestine sections. In the small intestine, nuclei and cytoplasm of the mucosa, submucosa, muscularis and serosa lay-

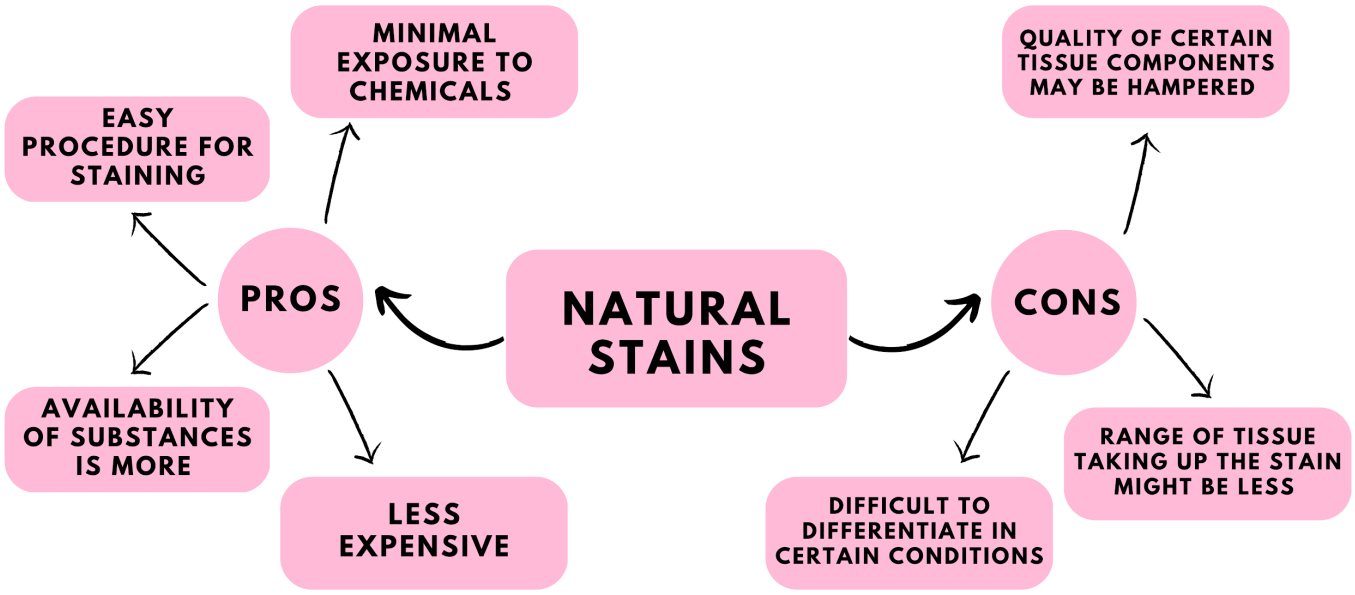

Fig. 3: The Pros and Cons of natural stains in routine histopathology.

ers are stained light pink. In tissue sections of skin, deep brown stained bands of epidermal keratin, light pink stained dermis and brown stained nuclei are seen. In heart tissue sections, brown stained nuclei and light pink cardiac muscle fibers can be noted.[29] The macrospore of *Microsporum gypseum* is stained red with clear appearance of its fusiform structure with typical 5 cells.[30]

B. vulgaris contains betalain pigment and betalamic acid35 which allows it to stain basic structures like cytoplasm, nerve fibers, muscle fibers, mucins, keratin, and red blood cells. Keratin contains high sulfur, cysteine plus half cysteine, arginine and lysine which are responsible for its demonstration. The nuclei stains pale which indicates *B. vulgaris'*s pH selectivity i.e. the nuclei may stain at a low pH (less than 4.0) suggesting staining with adequate acidification.[36]

***Khaya senegalensis* (Wood Dust) as a Natural Alternative:**

Wood Dust has been demonstrated in experimental animals' skin, heart, lung, kidney, liver and intestine sections.37 The roots of *K. senegalensis* are peeled off, cut, dried, and crushed. The powder obtained (50 g) is added to 750 ml distilled water and then boiled for 20 min, cooled, and filtered through Whatmann filter paper no. 1 paper. The mixture (742.5 ml) is then evaporated in an oven (55 °C), generating 7.4 g of dry extract, producing a yield of 14.8%. The concentration of the stock solution is 10 mg/ml38, which is then used to stain the tissue sections.

It has good affinity towards the cellular cytoplasm and stains the tissues in various shades of red and brown. It stains both; acidic and basic components of the tissue, but exhibits a strong affinity for basic structures.[37] Such dyes are usually termed acidic dyes.[39] *KS* wood extracts are rich in flavonoids and tannins, both phytochemicals, which explains their staining abilities.[40,41] These are known to be acidic in nature as they lose a positive hydrogen ion from their hydroxyl group. Hence, in dye-tissue reactions, an electrostatic attraction of unlike ions exists whereby the anions of an acidic dye interact with tissue structures that are rich in cations.[42] Plausibly, it may be inferred that the stain from *KS* wood extract is acidic in nature due to its strong affinity for the basic components of the tissue.

***Clitoria ternatea L.* (Butterfly Pea Flower) as a Natural Alternative:**

Butterfly Pea Flower has been shown to stain human spermatozoa[43], canine mast cell tumors[44], animal blood smears[45], bacteria (Staphylococcus aureus and Escherichia coli)[46], amongst other samples. Its blue coloured petals are thought to be a ternatin storage facility. Ternatins are a class of delphinidin glycosides, which are anthocyanin pigments that dissolve easily in water and change color depending on the pH. The crude extract is made by soaking the petal powder in distilled water overnight at 4 °C and filtering it through gauzes and filter paper. Before staining, the filtrate is adjusted to pH 0.2 and treated with a mordant. Methanol-fixed blood smears are commonly used.

Preliminary findings show that faint acidophilic staining is found in the nuclei of nucleated cells of all species' blood smears. The cytoplasm of red blood cells stains grayish pink with shading variations. Furthermore, dull acidophilic staining is found in the granules of chicken heterophils as well as eosinophils of all species.[44] Bacteria, on the other hand, do not completely absorb the stain and thus require further investigation.[46,47]

***Hibiscus sabdariffa* (Roselle) as a Natural Alternative:**

Hibiscus Sabdariffa is a plant that is grown in many countries around the world, including Sudan.[48] In Sudan, it is known as Karkade, and the extract is typically consumed as a drink, hot tea-like in the winter and cold in the summer. *Hibiscus Sabdariffa'*s watery extract is red in color and has an acidic taste. The plant has a variety of well-known industrial, medical, and nutritional applications.[49,50,51] It has been used as a natural stain for specimens of skin[52], kidney, liver[53], testis[54] and fungi.[8]

A measured amount of Roselle powder (1g, 5g, 10g, or 100g) is brought to a boil in 100 ml of distilled water with constant mixing and shaking. It is then allowed to stand for 30 minutes, and filtered to obtain the coloured extract at the appropriate concentration.[55]

Results revealed that the extract can be used as cytoplasmic stain in place of eosin in routine H and E staining. However, it has been reported that squamous epithelial tissue (skin) is more resistant to Hibiscus solution penetration than glandular tissues (renal and intestinal).[53]

***Lawsonia inermis* (Henna) as a Natural Alternative:**

Lawsonia inermis, also known as Henna, is a shrub in the Lythraceae family. It is primarily used in cosmetics as a pigment for coloring hair and nails, imparting a red-yellow tint. Aside from that, it is widely used for dyeing wool and nylon, in textile industries, and for medicinal purposes.[8,56] It has recently been used as a biological stain for plants and microorganisms.[57]

Henna has been demonstrated as a biological stain for oral tissues, such as normal oral mucosa as well as squamous cell carcinoma[58], liver specimen[59], kidney, intestine, tonsil and lung specimens.[60]

2g of Henna dye extract and 5 ml of ethanol is dissolved in 50 ml of distilled water to make the staining solution. This solution is vigorously shaken for 1 minute and allowed to stand for 30 minutes, allowing the henna dye to dissolve properly. The solution is then diluted to 100 ml with distilled water and mixed with 4 ml of glacial acetic acid. Following, the solution is filtered through a Whatman filter paper. The staining solution's pH should be adjusted to 7.5. A few thymol crystals can be added to prevent fungal growth. Polyethylene bottles are used to store the solution. In order to prepare the mordant solution, 10g of potassium alum is added before dilution to 100 ml.[58]

According to animal tissue studies, Henna is an acidic stain with a high affinity for sclerotic proteins such as collagen, keratin, and elastin, which make up connective tissue and muscle fibers. It has been proposed that the Lawsonia inermis staining mechanism is based on ionic interactions between the phenolic group in dye, and the amino end-group in tissue proteins- a major component of connective tissue. Such ionic interactions are accompanied by non-ionic interactions such as polymerization, giving the dye metachromatic properties.[61]

Lymphoid follicles, glomerulus, normal tubules and surface epithelium cells are perfectly stained when Henna is used as a hematoxylin substitute.[60] Henna stains the tissues by coloring cytoplasm of cells, keratin, collagen fibers, and red blood cells brown. The use of Henna in conjunction with hematoxylin shows clear morphologic identification of epithelium and connective tissue elements. Because Henna stain does not mask the

hematoxylin color, there is a good contrast between the two. However, the time required for Henna and hematoxylin staining is significantly longer than for hematoxylin and eosin staining.[58]

Pros and Cons of Natural Stains:

The use of Natural Stains in routine histopathology has its own set of advantages and disadvantages. They are markedly less expensive, easy to procure and rarely, if ever, cause harm to the handler. Although natural stains may sometimes produce results with less visibility or hampered tissue quality, the full effects of these stains under various circumstances have yet to be studied. The highlights and challenges of natural stains are listed below in Figure 3.

Conclusion

Stains extracted from natural products have equally comparable staining properties to routinely used stains in histopathology. In fact, natural stains are cheaper and safer in comparison to chemically synthesized stains and show considerably positive results. While it may be impossible to completely replace the use of chemical stains by natural alternatives, further research involving a thorough analysis of these stains can lead to safer and more economical practices. Presently, natural stains can be recommended in routine histopathological processes, together with chemical stains. This combines the benefits and harmless components of natural dyes, with the assured results of chemical stains.

References

1. Gurcan, Metin N, Boucheron L, Can A, Madabhushi A, Rajpoot N, Yener B. Histopathological Image Analysis: A Review. 2010;147–71.
2. Johnson B. Histopathology as a Diagnostic Tool. J Interdiscip Histopathol [Internet]. 2022;10(1):1. Available from: https://www.ejmjih.com/ejmjih-articles/histopathology-as-a-diagnostic-tool.pdf
3. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological Stains: A Literature Review and Case Study. Glob J Health Sci. 2015;8(3):72–9.
4. Suvarna SK, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques [Internet]. 2016. 1–23 p. Available from: https://knustmeltsa.files.wordpress.com/2020/08/bancrofts-theory-and-practice-of-histological-techniques-7th-edition-0702042269autosaved1.pdf
5. Musumeci G. Past, present and future: overview on histology and histopathology. J Histol Histopathol. 2014;1(1):5.
6. Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. Acta Med Indones [Internet]. 2007;39(2):86–93. Available from: https://pubmed.ncbi.nlm.nih.gov/17933075/
7. Titford M, Bowman B. What May the Future Hold for Histotechnologists? Lab Med. 2012;43(suppl 2):e5–10.
8. Ihuma JO, Asenge. GH, Abioye. JO., Dick. SK. Application Of Methanolic Extracts From Hisbiscus Sabdariffa Linn As A Biological Staining Agent For Some Fungal Species. 2012;254–9.
9. Arora J, Agarwal P, Gupta G. Rainbow of Natural Dyes on Textiles Using Plants Extracts: Sustainable and Eco-Friendly Processes. Green Sustain Chem. 2017;07(01):35–47.
10. Bhuyan R, Saikia, C N. Isolation of colour components from native dye-bearing plants in northeastern India. Bioresour Technol. 2005;96(3):363–72.
11. Ratna., Padhi B. Pollution due to synthetic dyes toxicity & carcinogenicity studies and remediation. Int J Environ Sci [Internet]. 2012;3(3):940–55. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:ijes&volume=3&issue=3&article=002
12. Bordoloi B, Jaiswal R, Siddiqui S, Tandon A. Health Hazards of Special Stains. Saudi J Pathol Microbiol. 2017;2(5):175–8.
13. Adeyemo S, Akinloye, Akinwumi J, Adekanmi G. The Use of Plant Dyes for Microbial Staining and Identification: An Eco-friendly and Non-Toxic Alternative Method. J Adv Biol Biotechnol. 2018;16(4):1–10.
14. Protano C, Buomprisco G, Cammalleri V, Pocino RN, Marotta D, Simonazzi S, et al. The carcinogenic effects of formaldehyde occupational exposure: A systematic review. Cancers (Basel). 2022;14(1):1–12.
15. Jalandoni-Buan AC, Decena-Soliven ALA, Cao EP, Barraquio VL, Barraquio WL. Characterization and identification of Congo red decolorizing bacteria from monocultures and consortia. Philipp J Sci. 2010;139(1):71–8.
16. Purdie GL, Purdie DJ, Harrison AA. Raynaud's phenomenon in medical laboratory workers who work with solvents. J Rheumatol. 2011;38(9):1940–6.
17. Akinloye AJ, Illoh HC, Olagoke AO. Screening of some indigenous herbal dyes for use in plant histological staining. J For Res. 2010;21(1):81–4.
18. Kumar S, Singh N, Singh A, Singh N, Sinha R. Use of Curcuma longa L. extract to stain various tissue samples for histological studies. AYU (An Int Q J Res Ayurveda). 2014;35(4):447.
19. Ajileye AB, Iteire AK, Arigi QB. Zingiber officinale (ginger) extract as a histological dye for muscle fibers and cytoplasm. Int J Med Sci Public Heal. 2015;4(10):1445.
20. Ananthalakshmi R, Ravi S, Jeddy N, Thangavelu R, Janardhanan S. Natural alternatives for chemicals used in histopathology lab-A literature review. J Clin Diagnostic Res. 2016;10(11):EE01–4.
21. Udo-affah G, Obeten E, Obasee PP, Isaac VN. Extract of Ginger (Zingiber Officinale) on the Histology of the Spleen Using Adult Male Rats. J Biol Agric Healthc. 2014;4(25):259–68.
22. Sudhakaran A, Hallikeri K, Babu B. Natural stains Zingiber officinale Roscoe (ginger) and Curcuma longa L. (turmeric) – A substitute to eosin. AYU (An Int Q J Res Ayurveda). 2018;39(4):220.
23. Ghasemzadeh A, Jaafar HZE, Rahmat A. Identification and concentration of some flavonoid components in Malaysian young ginger (Zingiber officinale Roscoe) varieties by a high performance liquid chromatography method. Molecules. 2010;15(9):6231–43.
24. Bondoc CC. Curcuma longa Linn rhizome extract as an alternative stain for histological studies. J Pharmacogn Phytochem. 2018;7(5):3010–7.
25. Suryawanshi H, Naik R, Kumar P, Gupta R. Curcuma longa extract – Haldi: A safe, eco-friendly natural cytoplasmic stain. J oral Maxillofac Pathol. 2017;21(3):244–51.
26. Avwioro OG, Onwuka SK, Moody JO, Agbedahunsi JM, Oduola T, Ekpo OE, et al. Curcuma longa extract as a histological dye for collagen fibres and red blood cells. J Anat. 2007;210(5):600–3.
27. MP R, Krishnan AM, Basheer RK, TK MS, V S. Assessment of Staining Quality of Curcumin as a Substitute for Eosin in Hematoxylin and Eosin Staining in Histopathology. J Res Med Dent Sci | [Internet]. 2020;8(5):146–50. Available from: www.jrmds.in
28. Singnarpi S, Ramani P, Natesan A, Sherlin HJ, S G, Giffrina., et al. Vegetable Stain as an Alternative to H&E in Exfoliative Cytology. J Cytol Histol. 2017;08(03).
29. Udonkang MI, Inyang IJ, Ukorebi AN, Effiong F, Akpan U, Bassey IE. Spectrophotometry, Physiochemical Properties, and Histological Staining Potential of Aqueous and Ethanol Extracts of Beetroot on Various Tissues of an Albino Rat. Biomed Hub. 2018;3(3):1–10.
30. Sutradhar P, Bhattacharya C. Use of the Natural Pigments of Red Beet Root Pomace (Beta vulgaris L.) to Develop a Mycological Stain: An eco-friendly Alternative Substitute. J Sci Res. 2021;65(03):73–7.
31. Finbarrs-Bello E, Ojo OP, Agu CK. A Bioprospective Study on Histological Staining Potential of. J Exp Res. 2019;7(4):1–5.
32. Udonkang M, Ene C, Archibong A, Egbe A, Inyang I. Aqueous Beetroot Dye As an Alternative To Haematoxylin and Eosin in the Diagnosis of Breast Tumours Mfoniso Udonkang, Collins

Ene, Anietie Archibong, Agala Egbe, Imeobong Inyang. Glob J Pure Appl Sci Vol [Internet]. 2021;27:417–23. Available from: www.globaljournalseries.com,

33. Cheng CW, Saad SM, Abdullah R. Alternative staining using extracts of hibiscus (Hibiscus rosa-sinensis L.) and red beet (Beta vulgaris L.) in diagnosing ova of intestinal nematodes (Trichuris trichiura and Ascaris lumbricoides). Eur J Biotechnol Biosci. 2014;1(5):14–8.
34. Sturzoiu A, Stroescu M, Stoica A, Dobre T. Betanine extraction from Beta vulgaris - Experimental research and statistical modeling. UPB Sci Bull Ser B Chem Mater Sci [Internet]. 2011;73(1):145–56. Available from: https://www.semanticscholar.org/paper/BETANINE-EXTRACTION-FROM-BETA-VULGARIS-—-RESEARCH-Sturzoiu-Stroescu/8e90ccc2c7a6a6933094af339cc604f50f8944af#citing-papers
35. Dumbrav◎ A, Enache I, Oprea CI, Georgescu A, Gîrțu MA. Toward a more efficient utilisation of betalains as pigments for Dye-Sensitized solar cells. Dig J Nanomater Biostructures. 2012;7(1):339–51.
36. Lillie RD., Conn HJ, Stotz EH, Emmel VM. H. J. Conn's biological stains : a handbook on the nature and uses of the dyes employed in the biological laboratory. Balt Md by Williams Wilkins [Internet]. 1977; Available from: https://www.worldcat.org/title/hj-conns-biological-stains-a-handbook-on-the-nature-and-uses-of-the-dyes-employed-in-the-biological-laboratory/oclc/623131409?referer=di&ht=edition
37. Azubuike NC, Ikele IT, Onyemelukwe AO, Maduakor UC, Onwukwe OS, Maduakor-Oparah CL, et al. Histological staining properties of Khaya senegalensis wood dust extracts: a preliminary study. Anat J Africa. 2018;7(2):1314–21.
38. Kandeda AK, Lewale S, Djeuzong E, Kouamouo J, Dimo T. An aqueous extract of Khaya senegalensis (Desv.) A. Juss. (Meliaceae) prevents seizures and reduces anxiety in kainate-treated rats: modulation of GABA neurotransmission, oxidative stress, and neuronal loss in the hippocampus. Heliyon. 2022;8(5).
39. Ochei J, Kolhatkar A. Medical Laboratory Science, Theory and Practices, Tata McGraw-Hill, New York. 2008.
40. Jondiko IJO, Pattenden G. Terpenoids and an apocarotenoid from seeds of Bixa orellana. Phytochemistry. 2001;28(11):3159–62.
41. Adeel S, Ali S, Bhatti IA, Zsila F. Dyeing of cotton fabric using pomegranate (Punica granatum) aqueous extract. Asian J Chem. 2009;21(5):3493–9.
42. Prentø P. Staining of macromolecules: Possible mechanisms and examples. Biotech Histochem. 2009;84(4):139–58.
43. Chomean S, Nantabut M, Chomean. S, Nantabut. M, Kongtia W, Saenguthai K, et al. Evaluation of natural dyes for human spermatozoa morphology assessment. Acta Histochem. 2019;121(2):227–33.
44. Suebkhampet A, Sotthibandhu P. Effect of Using Aqueous Crude Extract from Butterfly Pea Flowers (Clitoria ternatea L.) as a Dye on Animal Blood Smear Staining. Suranaree J Sci Technol. 2012;19(1):15–9.
45. Suebkhampet A, Sotthibandhu P, Laoharatchatathanin T. Butterfly pea flower extract as an alternative dye in cytological canine mast cell tumor staining. Thai J Vet Med. 2020;50(4):473–8.
46. Triol CB, Dionela AT V., Ecube AID, Medioda CJA. The use of Clitoria ternatea (blue ternate) ethanolic extract as a potential stain for bacteria. Publiscience. 2020;3(1):42–7.
47. Weerasinghe T, Perera D, De Silva N, Poogoda D, Swarnathilaka H. Butterfly pea: An emerging plant with applications in food and medicine. Pharma Innov J [Internet]. 2022;11(6):625–37. Available from: www.thepharmajournal.com
48. Mohamed BB, Sulaiman AA, Dahab AA. Roselle (Hibiscus sabdariffa L .) in Sudan, Cultivation and Their Uses. Bull Environ Pharmacol Life Sci. 2012;1(6):48–54.
49. Ahmed AHR, Nour AAM. Some physico-chemical and nutritional characteristics of the oil and proteins of Sudanese Hibiscus sabdariffa seed. Univ Khartoum J Agric Sci (Sudan). 1994;1(1):74–81.
50. Herbal remedies using Roselle (Hibiscus Sabdariffa). Trust Herb Anti-aging [Internet]. Available from: https://ageless.co.za/roselle.htm
51. Odigie IP, Ettarh RR, Adigun SA. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. J Ethnopharmacol. 2003;86(2–3):181–5.
52. Raheem EMA, Ibnouf abd AO, Shingeray OH, Farah HJ. Using of Hibiscus Sabdariffa extract as a natural histological stain of the Skin. Am J Res Commun [Internet]. 2015;3(5):3. Available from: www.usa-journals.com
53. Okolie N, Aa N, Ib E. Staining Potentials of Indigenous Hibiscus sabdariffa Calyx, Lawsonia inermis Leaves and Vitex Doniana Stem Barks on Liver and Kidney Tissues. J Cytol Histol. 2021;12(3):1–9.
54. Egbujo EC, Adisa OJ, Yahaya AB. A Study of the Staining Effect of Roselle (Hibiscus sabdariffa) on the Histologic Section of the Testis. Int J Morphol. 2008;26(4):927–30.
55. Ibnouf AA, AbdulRaheem E, SeedAhmed M, Dahab D. Assessment Of Staining Quality Of Roselle (Hibiscus Sabdariffa) On Formalin- Fixed Paraffin-Embedded Renal Tissue. IJCRR. 2014;6(21):26–8.
56. Jan HU, Shinwari ZK, Khan AA. Staining effect of dye extracted from dry leaves of Lawsonia inermis Linn (henna) on angiospermic stem tissue. Pak J Bot. 2011;43(1):383–9.
57. Hafiz H, Chukwu O, Nura S. The potentials of henna (Lawsonia inamis L.) leaves extracts as counter stain in gram staining reaction. Bayero J Pure Appl Sci. 2013;5(2):56–60.
58. Chukwu O. O. C., Odu CE, CHukwu DI, Hafiz N, Chidozie VN, Onyimba IA. Application of extracts of Henna (Lawsonia inamis) leaves as a counter stain. African J Microbiol Res. 2011;5(21):3351–6.
59. Raju L, Nambiar S, Augustine D, S.V. S, Haragannavar VC, Babu A, et al. Lawsonia inermis (Henna) extract: A possible natural substitute to Eosin stain. J Interdiscip Histopathol. 2018;6(2):54–60.
60. Adisa JO, Musa KK, Egbujo EC, Uwaeme IM. 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(3):1058–65.
61. Odigie EB, Ehimigbai ROA, Aliu HS. Lawsonia inermis Linn (Henna) Lyophilized Extracts as Alternate Stains May Deter Staining Efficacy in Histology Sections. Dutse J Pure Appl Sci (DUJOPAS). 2022;8(3):1–7.