

Green Histopathology: A Global Systematic Review of Natural Staining Agents as Sustainable Alternatives to Synthetic Dyes (2003 to 2025)

Chinwebudu M. Melford^{1*}

¹Department of Medical Technology, College of Allied Medical Sciences, Cebu Doctor's University, Mandaue City, Cebu, Philippines

*cmmelford@cdu.edu.ph

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Abstract

Despite increasing publications on the potential of natural dyes in histology and laboratory sciences, there is limited comparative synthesis of available literature, which hampers the development of consolidated guidance for practice, regulation and innovation. This study presents a global systematic review of natural histological staining agents reported between 2003 and 2025, evaluating their sources, staining performance, methodological approaches, and sustainability attributes. Following PRISMA 2020 guidelines, peer-reviewed studies assessing plant- and biologically derived dyes for routine and specialized histological applications were analyzed. The findings show that while early investigations reported inconsistent or poor staining outcomes for some extracts, growing evidence supports the eosin-like potential of several natural dyes, particularly *Curcuma longa*, *Hibiscus sabdariffa*, *Lawsonia inermis*, *Zingiber officinale*, betacyanin-rich plants, and *Tectona grandis*. Moreover, staining effectiveness was strongly influenced by extraction methods, pH adjustment, mordant use, and staining duration. Despite demonstrated benefits in safety, biodegradability, and cost, challenges related to standardization, reproducibility, and nuclear staining remain. Overall, the review highlights the promise of natural dyes as complementary, sustainable options in histopathology and underscores the need for further optimization and large-scale validation. These findings support the cautious integration of optimized natural dyes as sustainable adjuncts in histopathology rather than direct one-to-one replacements for synthetic stains.

Keywords: Green histopathology, natural dyes, histological staining, eosin alternatives, sustainable laboratory practices

Introduction

Green histopathology refers to the integration of environmentally sustainable, occupationally safe, and resource-efficient principles into histological staining and tissue processing while maintaining diagnostic accuracy. This concept aligns with green chemistry, which promotes the reduction or elimination of hazardous substances in chemical practices (International Union of Pure and Applied Chemistry [IUPAC], 2019). In histopathology, this translates into

reducing reliance on toxic, non-biodegradable, petrochemical-derived dyes and reagents through the use of natural, renewable staining agents and safer alternatives in tissue processing.

Histological staining approaches are generally categorized according to purpose and tissue affinity. Routine stains, especially hematoxylin and eosin (H&E), remain the foundational method for evaluating tissue morphology, while special stains and histochemical techniques help identify connective tissue elements, microorganisms, carbohydrates, and pathological deposits.

Although hematoxylin is naturally derived, eosin and several ancillary processing reagents are synthetic and have been linked to occupational hazards and environmental persistence. Natural staining agents, often derived from plants, insects, and minerals, contain pigments such as anthocyanins, betalains, curcuminoids, tannins, and carotenoids, which may display selective affinity for nuclear or cytoplasmic structures. Evaluation of these stains commonly involves qualitative microscopic comparison with conventional stains, scoring of staining intensity and uniformity, assessment of reproducibility and stability, and increasingly, digital image analysis and inter-observer agreement measures for diagnostic concordance (College of American Pathologists, 2020; World Health Organization, 2022).

At the laboratory and health-systems level, histopathology laboratories contribute substantially to chemical consumption and biomedical waste generation. Prolonged exposure to synthetic dyes, solvents, and fixatives may present dermatologic, respiratory, and systemic risks for laboratory personnel, supporting global calls for safer work environments (International Labour Organization, 2019). Additionally, improper chemical disposal contributes to environmental contamination, especially in low- and middle-income countries with weak waste management systems. Consequently, sustainable laboratory practices have been encouraged as part of broader environmental responsibility efforts within healthcare delivery (United Nations Environment Programme, 2021).

In resource-limited settings, green histopathology also offers economic and logistical relevance. Synthetic dyes and reagents often require costly importation and are vulnerable to supply chain disruption, while natural stain sources may be locally available and renewable. Although exploratory studies in many regions report promising indigenous stains, routine adoption remains limited due to concerns about standardization, quality assurance, and acceptance by regulatory and professional bodies (Deepthi et al., 2025; The Royal College of Pathologists, 2022).

While previous reviews have discussed the potential of natural dyes in histology and laboratory sciences (Hartika et al., 2021; Mulla

et al., 2024), most have adopted narrative methodologies, focused only on limited stain categories, or emphasized isolated regional studies. To date, no comprehensive global systematic review synthesizing methodological approaches, staining performance, and sustainability attributes of natural histological stains across tissue applications has been conducted using PRISMA 2020 reporting standards.

Despite increasing publications over the last two decades, research on natural histological stains remains constrained by small sample sizes, inconsistent evaluation criteria, limited assessment of long-term stability, and narrow focus on single stains or tissue types. There is also limited cross-regional comparative synthesis across stain classes and diagnostic settings, and prior reviews have often been narrative or geographically restricted. These gaps reduce the ability of stakeholders to develop consolidated guidance for practice, regulation, and innovation in sustainable staining.

Accordingly, this study conducts a global systematic review (2003–2025) to synthesize evidence on natural staining agents used in histopathology by assessing their sources, classification, application, evaluation methods, diagnostic performance, and sustainability attributes, with the aim of informing future research, standardization initiatives, and environmentally responsible laboratory practice.

Methodology

This study used a global systematic review design guided by PRISMA 2020 to ensure transparency and reproducibility (Page et al., 2021). Published studies from January 2003 to December 2025 were searched in PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect, and Google Scholar. The electronic search strategy combined controlled vocabulary and free-text terms using Boolean operators, truncation, and database-specific filters. An example of a search string used in PubMed was: (green histopathology or natural histological stain, or plant-based dye) and (histology or histopathology). Searches were limited to studies published in English between January 2003

and December 2025. Reference lists of eligible articles were also manually screened to identify additional studies.

Included studies were original experimental, comparative, or validation research that applied natural or biologically derived stains to human or animal tissue sections and reported outcomes related to staining quality, diagnostic adequacy, safety, cost, or sustainability. Narrative reviews, editorials, conference abstracts without full texts, and studies not involving histologic tissue staining were excluded.

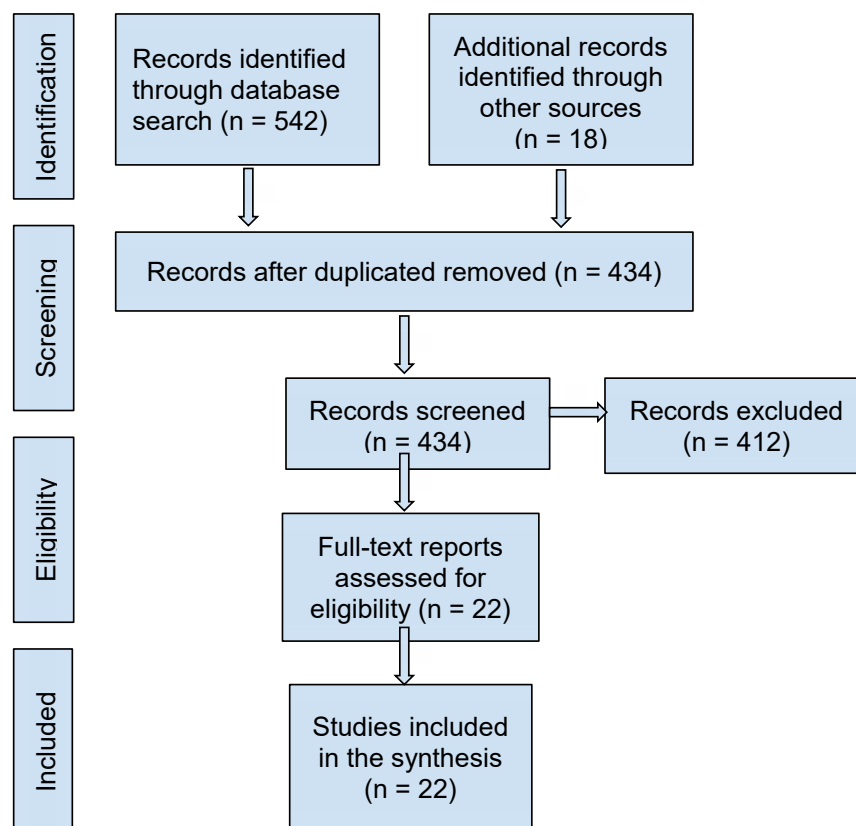
Titles and abstracts were screened by a single reviewer using predefined eligibility criteria. Full-text articles were subsequently assessed using a standardized data extraction form. As the review was conducted by a single investigator, dual independent screening was not performed. Data were extracted using a standardized form (author/year/country; natural stain source/class; tissue type; staining protocol; assessment

method; diagnostic and sustainability findings). Because staining agents and evaluation metrics were heterogeneous, findings were narratively synthesized and organized by stain type and application. Ethical approval was not required because the study used published data, following international guidance for secondary research (World Health Organization, 2021).

Due to substantial heterogeneity across the included studies, particularly in experimental design, staining protocols, outcome measures, and reporting styles, formal risk-of-bias or quality appraisal tools (e.g., GRADE or JBI) were not applied. Instead, a structured narrative methodological appraisal was undertaken. Studies were qualitatively assessed based on transparency of staining protocols, comparability with conventional stains (e.g., eosin or hematoxylin), reproducibility of staining outcomes, clarity of diagnostic evaluation, and reporting of limitations. This approach enabled

Figure 1

PRISMA 2020 Flowchart of Identification, Screening, and Inclusion of Studies



contextual interpretation of the strength of evidence while acknowledging methodological variability across studies.

The database search yielded a large number of records, with 542 identified through electronic databases (PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect, and Google Scholar) and 18 obtained through manual reference screening and other sources. After the removal of 126 duplicate records, 434 records were screened based on their titles and abstracts, leading to the exclusion of 412 articles. Twenty-two full-text articles were subsequently assessed for eligibility, and no additional exclusions were made at the full-text stage, resulting in 22 studies included in the qualitative synthesis. The study selection process is illustrated in the PRISMA 2020 flow diagram above.

Results

The review demonstrates that natural staining agents have been investigated across different tissues and laboratory contexts and have come out with variable outcomes. The findings are presented under three thematic domains: (1) natural dyes demonstrating eosin-like cytoplasmic potential, (2) natural dyes investigated for nuclear staining ability, and (3) studies reporting limited or inconclusive staining outcomes. Across these categories, staining performance was influenced by extraction methods, pH adjustment, mordant use, and tissue type. A comparative summary of included studies is provided in Annex 1.

Natural Dyes with Eosin-Like Cytoplasmic Properties

Several studies identified plant-based dyes with promising eosin-like properties, which means having consistent cytoplasmic affinity, adequate contrast with nuclear stains, reproducibility across tissue types, and preservation of morphological detail comparable to conventional eosin. Avwioro et al., (2007) demonstrated that a 1% aqueous extract of *Curcuma longa* (turmeric) effectively stained collagen fibers, red blood cells, muscle fibers, and cytoplasm within five minutes, producing staining results comparable to

eosin in routine H&E preparations, albeit with a yellow hue. Phytochemical analysis supported turmeric's affinity for acidic tissue components, and the authors emphasized its affordability and suitability for use in resource-limited settings.

Beyond routine histology, natural dyes were also evaluated for specialized tissues and plant histology. Tousson and Al-Behbehani (2010) reported that *Morus nigra* (black mulberry) fruit extract effectively stained neurons and astrocytes in rat brain sections, showing a particular affinity for astrocytes and providing bright, stable coloration without carcinogenic azo compounds, although seasonal availability remained a limitation. In plant histology, Jan et al., (2011) demonstrated that *Berberis pachyacantha* wood extract stained lignified tissues, particularly sclerenchyma, in both monocot and dicot stems, with ethanol proving to be the most effective solvent. Similarly, Okpidu et al., (2012) showed that aqueous extracts of *Ceratonia siliqua* bark produced intense cytoplasmic staining on liver, lung, and kidney tissues, especially under acidic conditions, without the need for mordants, highlighting its potential as a biodegradable and eco-friendly counterstain.

From 2014 onward, studies increasingly focused on optimizing staining protocols and expanding tissue applications. Ibnouf et al., (2014) found that *Hibiscus sabdariffa* stained formalin-fixed paraffin-embedded renal tissues comparably to eosin when used at optimal concentration and duration, although shorter staining times resulted in poor quality. Ajileye et al., (2015) reported that ethanolic extracts of *Zingiber officinale* (ginger) produced effective cytoplasmic and muscle fiber staining when used as a counterstain to hematoxylin, preserving tissue detail despite a distinct greenish-yellow coloration.

Comparative and refinement studies strengthened the evidence base for certain natural dyes. Abraham et al. (2017) showed that turmeric extracted by maceration yielded significantly clearer staining than soxhlet extraction, and that the use of a mordant further enhanced staining quality to levels comparable with eosin. Bondoc (2018) similarly reported that turmeric rhizome extract produced staining effects comparable to commercial synthetic stains across a range of plant and animal specimens, regardless of concentration. Kelesoglu (2019) highlighted

turmeric's broader potential in histology and bioimaging.

Evidence from 2022 onward indicates that optimized conditions significantly improve the performance of natural stains. Alshamar et al. (2022) demonstrated that betacyanins derived from beetroot and pokeweed produced eosin-comparable cytoplasmic staining, with pokeweed prebetanin yielding more intense coloration due to its chemical structure. Sarode et al. (2022) reported that food coloring agents could serve as safe and inexpensive eosin substitutes with comparable staining quality, although conventional H&E retained superior crispness. Strong support for teak-based dyes emerged in 2023, as Smith-Togobo et al. showed that *Tectona grandis* young leaf extracts, particularly methanolic extracts, produced eosin-like cytoplasmic staining on formalin-fixed paraffin-embedded tissues, while Barbosa et al. (2023) identified multiple Atlantic Forest plant species capable of effectively staining collagen and vascular structures.

By 2025, higher-level comparative studies suggested increasing feasibility of natural dyes when properly optimized. Karlina et al. (2025) demonstrated effective staining by multiple plant dyes rich in secondary metabolites, and Deepthi et al. (2025) identified *Lawsonia inermis* as the closest eosin alternative, showing no significant difference in staining intensity. Supporting biochemical evidence from Joyroy et al. (2025) further reinforced the relevance of *L. inermis* through its high phenolic and flavonoid content, highlighting its potential for continued development as a sustainable histological stain.

Natural Dyes Investigated for Nuclear Staining Ability

Attempts to replace hematoxylin were initially limited, as Okolie et al. (2021) reported that several plant extracts produced cytoplasmic staining but failed to stain nuclei effectively when substituted for hematoxylin, likely due to pH and chemical constraints. However, optimized conditions have demonstrated improved outcomes. Osman et al. (2022) showed that *Hibiscus sabdariffa* extract, when acidified and combined with an iron mordant, produced effective nuclear staining in buccal and cervical smears. Mohammed et al. (2025) found that aqueous *Hibiscus*

sabdariffa extracts provided superior nuclear clarity and overall staining quality compared to ethanolic extracts.

Beyond nuclear staining alone, Elowni et al. (2020) further demonstrated that *H. sabdariffa* pigment could differentiate viable from non-viable protozoa similarly to eosin, extending its application to parasitological viability testing. In microbiology, Kamel and Najmaddin (2016) demonstrated that extracts from plants such as *Isatis*, *Myrtus*, *Hibiscus*, and *Juglans* could successfully replace Gram stain for differentiating Gram-positive and Gram-negative bacteria, producing clear results at lower cost.

Njinga et al. (2020) evaluated the toxicity of *Hibiscus sabdariffa* calyx extract and reported acceptable tolerance in acute and sub-acute exposure, although prolonged administration produced biochemical changes, highlighting the importance of safety evaluation.

Studies Reporting Limited or Inconclusive Staining Outcomes

Not all natural dyes demonstrated satisfactory performance. Olise et al. (2018) reported that crude extracts from several indigenous Nigerian plants produced inconsistent and generally weaker staining compared to conventional synthetic dyes, highlighting the necessity for improved purification and protocol standardization. Rusadha (2019) reported that fruit-based dyes such as dragon fruit and raspberry, even with mordant enhancement, failed to match the staining intensity of the Papanicolaou stain in cytological applications. Abu et al. (2020) observed that several plant extracts stained buccal cell nuclei and cytoplasm but did not achieve diagnostic equivalence with the Papanicolaou stain due to lower color intensity.

Early evidence from the Philippines also showed limited success for some plant extracts. Sarmiento et al. (2003) evaluated 100% *Ipomoea batatas* (camote) leaf extract as an alternative to eosin using 60 tissue samples. While eosin consistently stained all tissue sections, the camote leaf extract failed to produce adequate staining, leading to the conclusion that it is not a viable substitute for eosin in histopathological techniques.

Discussion

The findings of this review indicate that the performance of natural staining agents varies according to pigment chemistry, tissue affinity, and methodological optimization rather than merely plant origin. Across the included studies, some plant-derived dyes demonstrated consistent cytoplasmic or specialized staining potential, while others showed limited or inconsistent diagnostic adequacy. For example, the failure of *Ipomoea batatas* leaf extract to produce satisfactory staining highlights the critical importance of chemical composition, extraction technique, pH conditions, and mordant application in determining staining success. These findings collectively emphasize that natural dyes require rigorous scientific validation and protocol refinement before consideration for routine diagnostic use, rather than adoption based solely on accessibility or traditional familiarity.

At the biochemical level, the staining limitations of many natural dyes can be explained by their ionic and coordination chemistry. Pigments such as anthocyanins, flavonoids, and betalains are typically weakly acidic and exhibit pH-dependent charge states, resulting in limited electrostatic interaction with negatively charged nucleic acids. In contrast, hematoxylin forms stable metal-dye complexes (commonly with aluminum or iron mordants) that strongly bind DNA and RNA, accounting for its superior nuclear affinity.

Repeated evidence supporting *Curcuma longa* across multiple studies suggests that certain plant pigments, particularly curcuminoids, possess physicochemical properties well suited for cytoplasmic staining. The consistent eosin-like performance of turmeric, especially when extraction methods and mordants were optimized, demonstrates that natural dyes can achieve diagnostic comparability when methodological variables such as solvent choice, pH, and staining duration are carefully controlled. These findings reinforce the notion that optimization, rather than mere substitution, is central to the successful integration of green histopathology into routine practice.

The effectiveness of other plant-based dyes such as *Hibiscus sabdariffa*, *Zingiber officinale*, *Morus nigra*, and *Tectona grandis* further supports the growing feasibility of natural stains.

However, their performance was often context-dependent, varying with tissue type, fixation method, and staining protocol. The improved nuclear staining achieved with *Hibiscus sabdariffa* following acidification and mordant application illustrates how limitations observed in earlier studies can be overcome through protocol refinement. Similarly, the strong cytoplasmic staining produced by teak and betacyanin-rich extracts highlights the role of secondary metabolites such as anthocyanins, betalains, and tannins, in enhancing tissue affinity and color stability.

Despite these promising outcomes, the reviewed studies also reveal persistent challenges that limit widespread adoption. Several natural dyes demonstrated adequate cytoplasmic staining but insufficient nuclear detail, particularly when tested as replacements for hematoxylin. This limitation reflects fundamental staining principles, as acidic plant dyes may inherently lack affinity for nuclear components unless chemically modified. Moreover, variability in staining intensity, reproducibility, and long-term stability remains a concern, especially in studies using crude or minimally processed extracts. These inconsistencies underscore the need for standardized extraction, purification, and quality-control protocols before natural dyes can be reliably implemented in diagnostic laboratories.

Safety and sustainability considerations further strengthen the argument for continued research in green histopathology. Toxicological evaluations, such as those conducted on *Hibiscus sabdariffa*, indicate that while many plant extracts are relatively safe, prolonged exposure and systemic effects must be assessed alongside staining performance. Importantly, the demonstrated utility of certain natural dyes in microbiology, cytology, and parasitology suggests that their applications may extend beyond routine histology, potentially reducing dependence on hazardous synthetic reagents across multiple laboratory disciplines.

In general, the evidence synthesized in this review indicates that natural staining agents, particularly *Curcuma longa*, *Hibiscus sabdariffa*, *Lawsonia inermis*, *Zingiber officinale*, betacyanins, and *Tectona grandis*, hold significant promise as sustainable alternatives to eosin and other synthetic dyes. However, their successful translation into routine histopathological prac-

tice will depend on further optimization, standardization, and large-scale validation studies. Addressing these gaps will be essential for aligning diagnostic accuracy with environmental responsibility, thereby advancing the principles of green histopathology in both resource-limited and well-equipped laboratory settings.

In addition to synthesizing available evidence, this review provides a structured global comparison of natural histological stains reported between 2003 and 2025, highlighting plant sources with consistent eosin-like potential and identifying key methodological variables, such as extraction technique, pH adjustment, and mordant use, that influence staining performance. By systematically mapping strengths, gaps, and areas for optimization, the study advances understanding of how natural dyes may be refined and standardized for environmentally responsible histopathological practice.

Nevertheless, several limitations should be acknowledged. Most included studies inferred environmental sustainability based primarily on natural origin rather than quantified degradation kinetics or life-cycle assessment data, and empirical evidence regarding long-term ecological impact remains limited. In addition, the review was conducted by a single investigator, which may introduce selection bias despite the use of predefined eligibility criteria and structured data extraction procedures.

Future research should prioritize standardized extraction and purification protocols, multi-center comparative validation, objective digital image-based assessment of staining quality, and comprehensive toxicological and environmental profiling. Addressing these areas will be essential for strengthening regulatory confidence and supporting the gradual integration of optimized natural staining agents into routine, sustainable histopathological practice.

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Annex 1

Comparative summary of outcomes for 22 studies reviewed

Study (Author, Year)	Natural Dye Source	Pigment class (reported/inferred)	Target stain role	Tissue / specimen	Key outcomes (vs conventional)	Main limitations / notes
Sarmiento et al., 2003	<i>Ipomoea batatas</i> (camote) leaf	Chlorophyll/phenolics (inferred)	Cytoplasmic (eosin substitute)	Mixed tissues (60 samples)	Failed to produce adequate staining; not comparable to eosin	Poor staining affinity; not viable substitute
Avwioro et al., 2007	<i>Curcuma longa</i> (turmeric)	Curcuminoids	Cytoplasmic / collagen, RBC	Collagen fibers, RBC, muscle, cytoplasm	Comparable eosin-like cytoplasmic staining (yellow hue)	Hue difference; optimization needed
Tousson & Al-Behbehani, 2010	<i>Morus nigra</i> (black mulberry) fruit	Anthocyanins (inferred)	Specialized nervous tissue stain	Rat brain sections	Effective staining of neurons/astrocytes; bright stable color	Seasonal availability; protocol dependence
Jan et al., 2011	<i>Berberis pachyacantha</i> wood	Alkaloids/berberine (inferred)	Plant histology stain	Monocot/dicot stems	Stained lignified tissues (esp. sclerenchyma); ethanol best solvent	Solvent dependence; plant histology focus
Okpido et al., 2012	<i>Ceratonia siliqua</i> bark	Tannins/phenolics (inferred)	Cytoplasmic counterstain	Liver, lung, kidney	Intense cytoplasmic staining esp. acidic conditions; no mordant required	Limited standardization; extract variability
Ibnouf et al., 2014	<i>Hibiscus sabdariffa</i> (roselle)	Anthocyanins (inferred)	Cytoplasmic (eosin-like)	FFPE renal tissue	Comparable to eosin under optimal conc./time	Poor quality with short staining time
Ajileye et al., 2015	<i>Zingiber officinale</i> (ginger)	Polyphenols (inferred)	Cytoplasmic / muscle	Muscle fibers/cytoplasm	Effective counterstain preserving detail	Greenish-yellow tone; needs optimization
Kamel & Najmaddin, 2016	Mixed plant dyes (e.g., <i>Isatis</i> , <i>Myrtus</i> , <i>Hibiscus</i> , <i>Juglans</i>)	Mixed natural pigments	Gram-stain alternative	Bacterial smears	Differentiated Gram+/Gram- at lower cost	Not histology; microbiology context
Abraham et al., 2017	<i>Curcuma longa</i> (turmeric)	Curcuminoids	Cytoplasmic (eosin comparator)	Histology sections	Maceration > soxhlet; mordant improved quality; comparable to eosin	Method-sensitive; protocol standardization needed

Bondoc, 2018	Curcuma longa rhizome	Curcuminoids	Broad histology stain	Plant & animal specimens	Comparable staining across conc.; supports versatility	Largely qualitative comparisons
Olise et al., 2018	Multiple indigenous Nigerian plants (crude extracts)	Mixed pigments	Mixed histology/micro	Histology + bacteriology/ mycology	Inconsistent/ weak staining vs conventional dyes	Crude extracts; poor reproducibility
Rusadha, 2019	Fruit flesh dyes (dragon fruit, raspberry)	Betalains/ anthocyanins (inferred)	Cytology stain	Cytology (Pap comparator)	Did not match Pap stain intensity even with mordants	Cytology focus; limited equivalence
Kelesoglu, 2019	Turmeric (Labconscious report)	Curcuminoids	Histology/ bioimaging application	Tissue staining guidance	Highlights broader potential and sustainability framing	Not primary experimental validation study
Elowni et al., 2020	Hibiscus sabdariffa aqueous extract	Anthocyanins (inferred)	Viability stain (eosin-like)	Hydatid protoscolices	Differentiated viable/ non-viable comparably to eosin	Parasitology setting, not routine histology
Njinga et al., 2020	Hibiscus sabdariffa (toxicity study)	—	Safety profiling	Animal exposure	Generally tolerated; biochemical changes with prolonged exposure	Safety evidence indirect to staining performance
Abu et al., 2020	Leaf aqueous extracts (buccal cytology)	Mixed pigments	Cytology stain	Buccal cells	Stained nucleus/ cytoplasm but not equivalent to Pap stain	Lower intensity; diagnostic limitations
Okolie et al., 2021	H. sabdariffa, Lawsonia inermis, Vitex doniana	Anthocyanins/ quinones/ tannins (inferred)	Cytoplasmic emphasis	Liver, kidney	Cytoplasmic staining observed; limited nuclear staining as hematoxylin substitute	Nuclear staining limitations; protocol constraints
Alshamar et al., 2022	Betacyanins (beetroot, pokeweed)	Betalains (betacyanins)	Cytoplasmic (eosin-like)	FFPE tissues	Eosin-comparable cytoplasmic staining; pokeweed more intense	Intensity variability; chemistry-dependent
Sarode et al., 2022	Food coloring agents	Synthetic food dyes (safer class)	Eosin alternative	Soft tissue sections	Comparable staining; conventional H&E slightly crisper	Not plant-derived; classification note needed
Osman et al., 2023	Hibiscus sabdariffa + iron mordant	Anthocyanins + metal complex	Nuclear stain (optimized)	Buccal/cervical smears	Improved nuclear staining under acidified + mordant conditions	Cytology setting; needs histology validation

Smith-Togobo et al., 2023	Tectona grandis young leaf	Tannins/anthocyanins (inferred)	Cytoplasmic (eosin-like)	FFPE tissues	Methanolic extract gave strong eosin-like staining	Solvent optimization required
Barbosa et al., 2023	Atlantic Forest plant species (multiple)	Mixed pigments	Collagen/vascular stains	Tissue sections	Several species effectively stained collagen/vascular structures	Species heterogeneity; standardization limited
Deepthi et al., 2025	Lawsonia inermis (+ others compared)	Lawsonic/quinones (inferred)	Cytoplasmic (eosin comparator)	Oral histopathology	L. inermis closest eosin alternative; no significant difference	Needs multicenter validation
Joyroy et al., 2025	Lawsonia inermis (bioactivity paper)	Phenolics/flavonoids	Supportive mechanistic evidence	Not tissue staining	Reinforces pigment richness relevant to staining potential	Not direct staining validation
Karlina et al., 2025	Multiple plant dyes (natural dye “H&E alternative”)	Mixed pigments	H&E alternative attempt	Histological preparations	Reported feasibility as alternative staining	Protocol/reporting variability
Mohammed et al., 2025	Hibiscus sabdariffa extracts	Anthocyanins (inferred)	H&E alternative (cyto+nuclear)	Histology sections	Aqueous extract reportedly superior nuclear clarity vs ethanolic	Journal details incomplete; verify citation metadata

Notes.

1. FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin.
2. Pigment classes labeled as “inferred” were deduced from known phytochemical composition of the plant source when not explicitly reported in the original study.